

ENVIRONMENTAL CONDITIONS AFFECTING THE
EFFICIENCY AND EFFICACY OF PISCICIDES FOR
USE IN NONNATIVE FISH ERADICATION

by

Peter James Brown

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Fish and Wildlife Biology

MONTANA STATE UNIVERSITY
Bozeman, Montana

May 2010

©COPYRIGHT

by

Peter James Brown

2010

All Rights Reserved

APPROVAL

of a dissertation submitted by

Peter James Brown

This dissertation has been read by each member of the dissertation committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency, and is ready for submission to the Division of Graduate Education.

Dr. Alexander V. Zale, Committee Chair

Approved for the Department of Ecology

Dr. David Roberts, Department Head

Approved for the Division of Graduate Education

Dr. Carl A. Fox, Vice Provost

STATEMENT OF PERMISSION TO USE

In presenting this dissertation in partial fulfillment of the requirements for a doctoral degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library. I further agree that copying of this dissertation is allowable only for scholarly purposes, consistent with “fair use” as prescribed in the U.S. Copyright Law. Requests for extensive copying or reproduction of this dissertation should be referred to ProQuest Information and Learning, 300 North Zeeb Road, Ann Arbor, Michigan 48106, to whom I have granted “the exclusive right to reproduce and distribute my dissertation in and from microform along with the non-exclusive right to reproduce and distribute my abstract in any format in whole or in part.”

Peter James Brown

May 2010

ACKNOWLEDGMENTS

Support for this research was provided by the Wild Fish Habitat Initiative administered by the Montana University System Water Center and a Montana State University teaching assistantship. This project would not have been possible without the access to study sites, field assistance, and hospitality provided by Hilda Sexauer (Wyoming Game and Fish), Carter Kruse (Turner Enterprises), Scott Barndt (U.S. Forest Service; USFS), Pat Clancy (Montana Fish, Wildlife and Parks; MFWP), Jim Dunnigan (MFWP), Matt Boyer (MFWP), Dave Moser (MFWP-USFS) and their associated field assistants. Bioassay fish were provided Ennis National Fish Hatchery (U.S. Fish and Wildlife Service) and Big Spring Hatchery (MFWP). G. Ackerman, C. Foremen, A. Schaner, and H. Hickers analyzed water samples. Field assistance was provided J. Olsen, J. Stewart, T. Buckle, H. Johnson, J. Ard, and P. Odenbeck; their efforts were supplemented by J. Stites, M. Brown, K. Gates, D. Peterson, M. Meeuwig, E. Oldenberg, J. Boyd, and A. Ard.

Al Zale provided the freedom and guidance necessary to formulate, execute and report this research. Carter Kruse, Tom McMahon, and Bob Gresswell greatly influenced this research and my intellectual development. Brian McGlynn, Joel Cahoon, Otto Stein, Trevor Rainey, Mark Greenwood, Megan Higgs, and Dave Roberts provided informal but no less valuable assistance. Fellow graduate students provided assistance with everything from presentations to paperwork; Brad Shepherd and Mike Meeuwig were especially helpful. Martha, Jim, and Case Brown provided valuable support in many forms during my time at Montana State University. Above all, I would not have been able to complete this research or graduate program if it were not for the support provided by Mindy Brown.

TABLE OF CONTENTS

1.	TECHNIQUES FOR NATIVE FISH RESTORATION: AN OVERVIEW	1
	Background Information.....	1
	Overview of Dissertation	14
2.	EFFECT OF FISH SIZE ON RESPONSE TO PISCICIDES	17
	Abstract.....	17
	Introduction.....	17
	Methods.....	19
	Results.....	22
	Discussion.....	23
3.	DETERMINATION OF THE AMOUNT OF ISOLATED EXPOSURE TO SUNLIGHT, ORGANIC MATTER, AND TURBULENCE NECESSARY FOR THE PISCICIDES ROTENONE AND ANTIMYCIN TO BECOME INEFFECTIVE	33
	Abstract.....	33
	Introduction.....	34
	Methods.....	35
	Results.....	41
	Discussion.....	44
4.	ROTENONE PERSISTENCE MODEL.....	61
	Abstract.....	61
	Introduction.....	62
	Methods.....	63
	Study Streams	63
	Environmental Characteristics	64
	Bioassay and Rotenone Application.....	66
	Modeling Mechanics.....	68
	Model Development and Testing.....	69
	Results.....	70
	Discussion.....	72
	Model Application	76

TABLE OF CONTENTS-CONTINUED

5.	TRANSVERSE MIXING OF SIMULATED PISCICIDES IN SMALL MONTANE STREAMS	84
	Abstract	84
	Introduction	84
	Methods	86
	Results	88
	Discussion	89
6.	DISSERTATION SUMMARY	93
	LITERATURE CITED	100

LIST OF TABLES

Table	Page
3.1. Environmental exposures necessary for sunlight, turbulence, and organic matter to cause 7.5 and 15.0 $\mu\text{g/L}$ antimycin to have eradication probabilities of 0.00001, 0.500, 0.950, and 0.999 (EE level). NA indicates that because the true estimate was not provided, a confidence interval is not applicable. No Effect indicates that all of the fish in the exposures died and there was no environmental effect on the chemical	52
3.2. Environmental exposures necessary for sunlight, turbulence, and organic matter to cause 12.5 and 15.0 $\mu\text{g/L}$ rotenone to have eradication probabilities of 0.00001, 0.500, 0.950, and 0.999 (EE level). NA indicates that because the true estimate was not provided, a confidence interval is not applicable. No Effect indicates that all of the fish in the exposures died and there was no environmental effect on the chemical	53
4.1. Reach length, number of bioassay sites used, water chemistry, and discharge of treatment reaches included in this study. Jumping Creek and Graves Creek were used for model testing; all other treatment reaches were used for model development	77
4.2. Covariates selected by stepwise selection when a model based on data from four treatment reaches was built and tested on the fifth test stream. X represents inclusion of a covariate in a model.	77
4.3. Goodness-of-fit statistics for models predicting probability of eradication using rotenone in montane streams. Statistics measure model fit to data from all five streams used in model development (ORP = oxidation reduction potential; TDS = total dissolved solids)	78
4.4. Goodness-of-fit statistics for models tested against validation data from Jumping Creek and Graves Creek. Area under the curve (AUC) was calculated from receiver operating curves	78

LIST OF FIGURES

Figure	Page
2.1. Box plots summarizing the distributions of the time to death of rainbow trout when exposed to 7.5 µg/L antimycin or 12.5 µg/L rotenone. Box represents 25th and 75th percentiles, horizontal lines represent the median value, whiskers represent the 10th and 90th percentiles and dots represent outliers. Median time to death was significantly different between antimycin and rotenone (Wilcoxon Rank Sum test; $Z = 10.34$; $n = 240$; $p < 0.0001$).....	29
2.2. Frequency distributions of time to death (min) of rainbow trout exposed to rotenone and antimycin. The number of fish exposed (n) is included for each chemical	30
2.3. Time to death of rainbow trout by size group. Dot represents mean value, whiskers represent the 95% confidence interval. Distributions in time to death were significantly different among groups antimycin (ANOVA; $F = 7.66$; $p < 0.0001$) and rotenone (ANOVA; $F = 14.48$; $p < 0.0001$). Triangles in the largest size class of fish exposed to antimycin represent the actual time to death of the only two fish in that size class.....	31
2.4. Relationships between time to death (min) and weight of fish (g) exposed to antimycin and rotenone.....	32
3.1. Relationships between rotenone concentration and exposure to sunlight, turbulence, and organic matter. Note differences in scaling of Y axis. Points represent measurements of rotenone concentration by liquid chromatography-electrospray/mass spectrometry/mass spectrometry	54
3.2. Proportions of fish killed after antimycin and rotenone exposure to sunlight. Solid circles indicate the proportions of fish killed at low concentrations of piscicides and open circles indicate proportions killed at higher concentrations. Solid line indicates the probability of eradication as a function of sunlight exposure for the lower piscicide concentrations and dashed line indicates probability of eradication at the higher concentrations. Dotted lines indicate 95% confidence intervals.....	55

LIST OF FIGURES-CONTINUED

Figure	Page
3.3. Accumulation of sunlight under three sunlight exposures. Diagonal lines represent accumulated sunlight exposure; horizontal lines represent the level of sunlight exposure that would cause 15 $\mu\text{g/L}$ rotenone or antimycin to be less than 99.9% effective. Areas below the horizontal lines represent conditions at which 15 $\mu\text{g/L}$ rotenone or antimycin would remain more than 99.9% effective.....	56
3.4. Proportion of fish killed after antimycin and rotenone exposure to turbulence. Solid circles indicate the proportions of fish killed at low concentrations of piscicides and open circles indicate proportions killed at higher concentrations. Solid lines indicate the probability of eradication as a function of sunlight exposure for the lower piscicide concentrations and dashed lines indicates probability of eradication at the higher concentrations. Dotted lines indicate 95% confidence intervals.....	57
3.5. Accumulation of turbulence in four channel types. Diagonal lines represent accumulated turbulence exposure; horizontal lines represent the level of turbulence exposure that would cause 15 $\mu\text{g/L}$ antimycin to be 50% or 99.9% effective. Areas below the horizontal lines represent conditions at which 15 $\mu\text{g/L}$ antimycin would remain more than 99.9% or 50% effective.....	58
3.6. Residual rotenone concentration as a function of dissolved organic matter concentration. Initial nominal rotenone concentration was 15 $\mu\text{g/L}$. Open dots represent measurements of rotenone when exposed to humic acid. Solid dots represent measurements of rotenone when exposed to a composite mixture of DOM.....	59
4.1. Physical characteristics of study streams. Dot represents mean and whiskers represent range of measurements. Filled dots indicate treatment reaches used for model development and open dots indicate treatment reaches used for model testing.....	79

LIST OF FIGURES-CONTINUED

Figure	Page
4.2. Percent coverage of each size class of substrate in study streams. Substrate was classified using a modified Wentworth scale as silt (< 0.2 mm diameter), sand (0.2-6.3 mm diameter), gravel (6.4-76.0 mm diameter), cobble (76.1-149.9 mm diameter), rubble (150.0-303.9 mm diameter), small boulder (304.0-609.9 mm diameter), large boulder (> 609.9 mm diameter) and bedrock (consolidated parent material). Dark shading indicates streams used for model development and light shading indicates streams used for model testing.....	80
4.3. Eradication probability plotted against distance downstream (m) for models calculated in model development. Points represent response of bioassay fish. Solid line represents the predicted probability of eradication and dashed lines indicate 95% confidence interval.....	81
4.4. Eradication predictions and response of fish using a model of travel time, rubble, and oxidation reduction potential for validation data from Jumping Creek and Graves Creek. Points represent actual response of fish, solid lines represent model prediction, and dashed lines indicate 95% confidence intervals.....	82
5.1. Distribution of mixing distances by stream channel type. Points represent means and error bars represent 95% confidence intervals.....	91
5.2. Distribution of mixing distances and mixing distance to width ratios by application location. Points represent means and error bars represent 95% confidence intervals	92

ABSTRACT

Conservation of native fish is a pressing issue for fisheries managers. Conservation efforts often require eliminating threats posed by nonnative fish by eradicating them with piscicides. The piscicides rotenone and antimycin are used for eradication but their application is often inefficient or ineffective. My goal was to increase the efficiency and efficacy of nonnative fish eradication using piscicides. I identified environmental conditions affecting piscicide application, researched methods to overcome these problems, and provided tools that piscicide applicators can use to make piscicide application more efficient and effective. Rotenone and antimycin were exposed to varying levels of sunlight, turbulence, and dissolved organic matter (DOM) to determine the effect these environmental conditions have on piscicides. Bioassay fish were used to determine the toxicity of the piscicides. Sunlight and turbulence affected rotenone and antimycin but DOM did not. Increasing the concentration of chemical can increase the resistance to the effects of these environmental conditions; however, the effects of these conditions are considerable in natural settings. Observations of bioassay fish in stream applications of rotenone were used to develop a statistical model to predict the persistence of the piscicide. The model can be used to predict rotenone persistence in small montane streams and to estimate where rotenone concentrations need to be fortified. I measured the mixing rate of a chemical plume in different channel morphologies and at center or edge applications. Center application had a significantly shorter mixing distance than edge application, but mixing distance was not different among meandering, straight, and riffle/pool morphologies. Application of my findings will increase the efficiency and efficacy of native fish conservation using piscicides.

CHAPTER 1

TECHNIQUES FOR NATIVE FISH RESTORATION: AN OVERVIEW

Background Information

Conservation of native fishes has become an important issue for fisheries managers. Habitat loss, competition, predation, hybridization with nonnative fish, and disease have all contributed to reductions in native fish abundances and ranges (Callicott 1991; Krueger and May 1991; Ross 1991; Dunham et al. 1997; Campbell et al. 2002). Native fish populations are valuable ecologically, economically, culturally, and legally. These values have prompted fisheries managers to undertake projects that restore diminished native fish populations (Stefferdud et al. 1992; DeMong 2001).

Inland salmonids are commonly the subject of fisheries restoration projects. They have been reduced in abundance, geographic range, and genetic integrity largely because of nonnative species. The presence of nonnative species has decreased the abundance and range of many western trout *Oncorhynchus* spp. populations (e.g., Shepard et al. 2005) and has resulted in the listing of several species as threatened or endangered under the Endangered Species Act (Propst et al. 1992; Young 1995b; Shepard et al. 1997; Weigel et al. 2003). As part of recovery efforts, or to preclude listing, state and federal fisheries management agencies have undertaken projects to reduce the risks to which native salmonids are exposed (Propst et al. 1992). Stocking (Hilderbrand 2002), angling restrictions (Strach and Bjornn 1989), and habitat

modification (Binns and Remmick 1994) have been used to increase populations of native fish. However, eradication of nonnative fish is considered the most effective strategy (Hepworth et al. 2002).

Headwater streams and lakes are common locations for restoration projects because populations of native fish are often isolated in headwater areas and these areas are considered easier to manipulate than larger downstream reaches (Stefferd et al. 1992; Young 1995a; Hepworth et al. 2002). For example, all lotic restoration projects in Utah between 1977 and 2002 were carried out in small headwater streams with low discharge ($< 0.3 \text{ m}^3/\text{s}$; Hepworth et al. 2002). Large-scale projects in Lake Davis, California, and in the Strawberry Valley, Utah, failed to eradicate or exclude nonnative fish (Lee 2001; Lentsch et al. 2001).

Mechanical and chemical methods can be used to remove nonnative fish (Finlayson et al. 2000). Mechanical methods include electrofishing and netting. Chemical removal is the use of piscicides including rotenone, antimycin, and lampricides. Complete eradication was first thought impossible and early removal efforts were meant to reduce abundance of undesirable fish (Moore and Ridley 1983; Moore et al. 1986). Today, eradication techniques are improving and complete eradication is the goal of most removal efforts.

Mechanical removal is attractive because fisheries managers can use gears and techniques that they are already familiar with (e.g., electrofishing) and because these techniques often do not require special permits (Finlayson et al. 2005). Unsuccessful attempts at eradication by electrofishing identified the limitations of this technique

(Moore and Ridley 1983; Moore et al. 1986; Habera et al. 1992). Guided by these unsuccessful projects, recent attempts have proven electrofishing successful in eradicating nonnative fish (Thompson and Rahel 1996; Shepard and Nelson 2004). Eradication using electrofishing is successful in streams with low amounts of coarse woody material in the channel and low riparian vegetation density (Habera et al. 1992; Shepard and Nelson 2004). Clearing abundant coarse woody material and dense riparian vegetation is necessary to allow removal crews better access (Shepard and Nelson 2004). Electrofishing removal and chemical removal were comparable in total cost when bank and coarse wood clearing needs were minimal and could be done by hand; costs of electrofishing removal would likely be significantly higher than the cost of chemical removal if heavy equipment was needed for riparian or channel clearing (Shepard and Nelson 2004). Mechanical removal of fish in backcountry areas (e.g., wilderness areas) has not been attempted, likely because of the need for repeated access. Moving the necessary crew and gear into remote areas for mechanical removal, multiple times each year, for several years, would not be cost effective.

Mechanical eradication in water bodies larger than small streams remains to be proven effective. Currently, gill nets and angling restrictions are being used to try to eradicate lake trout *Salvelinus namaycush* from Yellowstone Lake, Wyoming (Koel et al. 2005). That effort will be the case study for mechanical eradication in large water bodies.

Twenty-nine synthetic and naturally occurring chemicals including nicotine, croton seed powder, lime, and malathion have been used historically to kill fish (Marking 1992). Restrictions enacted in 1970 by the Environmental Protection Agency (EPA) reduced the number of chemicals legally used as piscicides. Many of the original 29 chemicals used as piscicides were never registered. Manufacturers have also allowed “grandfathered” registrations to lapse because of the high cost of research necessary for re-registration and the limited market for piscicides (Marking 1992). Antimycin-A (antimycin), rotenone, and 3-trifluoromethyl-4-nitrophenol (TFM) are the only chemicals currently registered with the EPA for use as piscicides (Finlayson et al. 2000). Rotenone and antimycin kill a variety of fish species and TFM is used specifically to kill lamprey.

Rotenone and antimycin are naturally occurring substances. Rotenone is a substance found in the roots of several species of plants in the family Leguminosae (Finlayson et al. 2000). Antimycin is an antibiotic produced by the bacteria of the genus *Streptomyces*. Both rotenone and antimycin interrupt electron transport during mitochondrial respiration causing death to cells. Death of fish results from overall tissue anoxia and associated cardiac and neurological failure (Ling 2003).

Piscicides have been used to collect fish for consumption (Leonard 1939; Ling 2003), for control of unwanted fish (Brown and Ball 1943; Binns 1967), and to sample fish populations (Bettoli and Maceina 1996). Rotenone has been used by indigenous peoples in Asia, Oceania, and South America to collect fish for consumption for hundreds of years (Leonard 1939; Finlayson et al. 2000; Ling 2003).

Rotenone was first used in the USA in 1934 to eradicate fish from two Michigan ponds (Ball 1945) and soon became a common way to remove unwanted “rough fish” (i.e., non-sport fish) from lakes and rivers (Eschmeyer 1938; Leonard 1939; Brown and Ball 1943; Ball 1945; Binns 1967; Bettoli and Maceina 1996; McClay 2000; Lentsch et al. 2001; Hepworth et al. 2002). These projects were considered successful as judged by increases in angler effort or game fish abundance. However, they rarely eradicated all unwanted fish (Spitler 1970; Lee 2001; Lentsch et al. 2001). Complete fish kills were reported in 40.5% of Michigan lakes subjected to rotenone application and adequate post-treatment sampling (Spitler 1970). Rotenone was commonly used by management agencies to sample fish abundance in lakes and reservoirs during the 1960s, but was replaced by less intrusive sampling methods (e.g., hydroacoustic surveys; Bettoli and Maceina 1996). Antimycin was initially used as an antifungal agent on plants (Leben and Keith 1948). Its toxicity was discovered after application to rice fields killed fish cohabitating the fields. A patent for the use of antimycin as a fish toxicant was first applied for in 1962 (Schnick 1974a) and the first studies of antimycin as a fish toxicant were conducted the following year (Derse 1963).

Piscicides have also been used by fisheries scientists for eradication of disease organisms. The parasitic trematode *Gyrodactylus salaricus* was discovered in Norway in 1975 and was cited as the primary factor in the decline of Atlantic salmon *Salmo salar* abundance in several rivers (Johnsen and Jenser 1991). The adult parasite only lives on fish and can not survive in seawater. Therefore, eradication of all fish in

affected water bodies using piscicides was the most effective way to eradicate the parasite (Johnsen and Jenser 1991).

Minnnows are effectively culled from catfish farming ponds using antimycin. This is an effective method because catfish are more tolerant to antimycin than are other fish. Most antimycin is used by the aquaculture industry for this purpose (Finlayson et al. 2002).

Control or complete eradication of introduced fish in lakes, reservoirs, and streams to conserve threatened or endangered fish has become the primary reason for application of rotenone and antimycin by fisheries managers (McClay 2000; DeMong 2001; Ottenbacher and Hepworth 2001; Finlayson et al. 2002; Hepworth et al. 2002). From 1988 to 2000, rotenone was primarily used for removal of undesirable fish species (McClay 2002) and antimycin application outside of aquaculture was used for the removal of nonnative fish (Finlayson et al. 2002).

Applications of piscicides to lentic and lotic systems require separate techniques and equipment. Piscicide application techniques and equipment were previously developed independently or shared informally between applicators. However, instructions for constructing application equipment have recently been published and workshops provide guidance on the effective use of these systems (Finlayson et al. 2000).

Piscicide application to lakes and reservoirs is typically done from a boat. Boat-mounted application systems use a pump and a reservoir of piscicide. These systems often use a surface spray through a garden hose or fire hose and are effective

to a depth of 1 m (Finlayson et al. 2000). Deeper mixing can be achieved by applying piscicides directly into propeller wash or by pumping piscicides through weighted hoses (Finlayson et al. 2000). Formulations of rotenone and antimycin have been developed to penetrate thermally stratified lakes by a slow release of piscicide on sinking granules (Berger et al. 1969; Spateholts and Lentsch 2001). These methods for mixing piscicides into the water column have reduced the problem of incomplete mixing in lentic waters (Gresswell 1991; Finlayson et al. 2000).

Pools isolated from streams at low discharge potentially allow fish to survive piscicide application to proximal streams, and are therefore a significant problem for piscicide applicators. Time-released piscicide formulations can be used to apply piscicides to isolated pools (Spateholts and Lentsch 2001) but backpack spray systems originally designed to apply herbicides or insecticides to terrestrial environments are more typically used (Gresswell 1991; Stefferud et al. 1992; Finlayson et al. 2000; Lentsch et al. 2001). In streams, the need for these special application techniques can be reduced or eliminated by reducing the number of lentic reaches (e.g., beaver dams; Finlayson et al. 2000; Lentsch et al. 2001). Application of piscicides through ice during winter has also been successful (Finlayson et al. 2002).

Application of piscicides to flowing portions of streams typically involves a reservoir to hold the piscicide and an apparatus to deliver the piscicide at a constant rate. These two components together are typically referred to as a drip station. Drip stations are placed along a stream to fortify the concentration of piscicide and the flow of each station is adjusted to apply a set amount of piscicide over an appropriate

time period (typically 4 to 8 h). Leaking fittings, changes in head pressure, and clogging of the uniform-application orifice by debris are problems associated with drip stations (Gresswell 1991) that likely occur because the apparatuses are built using parts that are not intended for the constant application of a liquid. To overcome these problems, many applicators find it necessary to assign a person to monitor each drip station during the application period. This solution results in inefficient use of personnel.

Identifying and addressing issues associated with efficient and effective piscicide use are critical in the conservation of inland salmonids. Social issues surrounding restoration projects include public concerns over the use of piscicides, potential environmental effects, and the perceived loss of angling opportunities (Finlayson et al. 2000; Finlayson et al. 2005). Protests and lawsuits from stakeholders decrease efficiency by increasing the time and monetary expenditures for each project and are a major impediment to the use of piscicides (Stuber et al. 1988; Bettoli and Maceina 1996; Schnick 2001; Hepworth et al. 2002). Legal challenges can interrupt individual projects (e.g., Cherry Creek, Montana), impede statewide native fish conservation efforts (e.g., the ban on piscicide use in New Mexico), and increase legal expenditures of management agencies. The increasingly complex and difficult process necessary to obtain regulatory clearances has been identified as a legal issue that impedes efficient piscicides application (Hepworth et al. 2002; Finlayson et al. 2005).

Practical issues include problems associated with eradication and exclusion of nonnative fish. The goal of fisheries restoration is to provide native species a place to flourish by eradicating and excluding nonnative fishes. Ineffective eradication and exclusion are common reasons for failure of restoration (Gresswell 1991; Lee 2001; Lentsch et al. 2001; Hepworth et al. 2002). Incomplete removal of nonnative fish, barrier failures, surreptitious stockings, and unsuitable habitat caused failed restoration projects during 25 years of salmonid conservation efforts in Utah (Hepworth et al. 2002). These failures could potentially be prevented by perfecting and disseminating effective methodology.

Piscicides have drawbacks associated with their application aside from social, legal, and practical issues. First, rotenone is perceived to have a greater effect on aquatic invertebrate abundance and diversity than antimycin. Applications of antimycin alone had no short term effects on invertebrate assemblages as measured by bioassays, invertebrate drift, and benthic density and biomass (Cerreto 2004). Rotenone and antimycin applied in combination decreased insect bioassay survival, increased invertebrate drift rates, and reduced benthic density and biomass when compared to control reaches (Cerreto 2004). Another drawback to rotenone is the ability of fish to detect and avoid the formulation (Hogue 1999; Finlayson et al. 2000; Ling 2003). It is currently unknown if fish are detecting the actual rotenone chemical or the surfactants used in all formulations (e.g., acetone; Schnick 2001). Because fish can avoid water treated with rotenone, it is possible for fish to survive rotenone application by finding untreated water. Antimycin is favored by some applicators

because fish cannot detect the chemical and do not search for untreated water (Gresswell 1991).

Persistence of rotenone and antimycin in streams is perhaps the most pressing unknown factor associated with piscicide application. This unknown likely plays a large role in the failure to eradicate nonnative fish and the wasteful application of piscicides. Piscicides are expected to persist in a treated water body such that fish remain in contact with the chemical long enough to absorb a toxic dose. The piscicides will be ineffective if they degrade before they come into contact with fish. Currently, applicators can not predict where in a water body piscicides become nontoxic. Therefore, sections of streams or parts of lakes may be nontoxic allowing fish to survive. Applicators often overlap piscicide applications to try to prevent areas of nontoxic water, but this tactic may result in excessive piscicide use. Understanding the dynamics of piscicide persistence is important in making piscicide application efficient and effective.

Both rotenone and antimycin can persist for several days when applied to water (Zilliox 1960; Walker et al. 1964; Schnick 1974a; Schnick 1974b). However, cases of rapid degradation are common (Gilderhus 1982; Dawson et al. 1991; Tiffan and Bergersen 1996). Rotenone can persist in aquatic systems for up to a year but more frequently lasts 1 to 100 d (Zilliox 1960; Schnick 1974b). Rotenone persistence was less than 1 d when the chemical was exposed to high water temperatures (i.e., > 20°C) in earthen ponds (Dawson et al. 1991). Natural degradation of antimycin can take from 1 to 14 d (Walker et al. 1964), but it can possibly take only hours (Tiffan

and Bergersen 1996), and be shorter than the effective contact time (Gilderhus 1972). Factors known or believed to degrade piscicides include pH, water temperature, sunlight, turbulence, and organic matter.

Information on the effects of pH on rotenone toxicity is sparse but sufficient to make application recommendations. Water with a pH of 10 required three times more rotenone than water with a pH of 5-7 (0.33 $\mu\text{L/L}$ and 0.1 $\mu\text{L/L}$, respectively) to kill fish (Brooks 1961 in Schnick 1974b). On the other hand, information on the influence of pH on the effectiveness of antimycin is substantial. Antimycin is rapidly hydrolyzed allowing it to be effective for short periods at a pH of 8.0 but ineffective at pH levels above 8.5 (Marking 1975). The half life of antimycin was over 7 h at pH 4.5 and 5.5, 5 h at pH 7.0 and 8.0, 4 h at pH 8.5, 40 min at pH 9.0, 20 min at pH 9.5, and 6 min at pH 10.0 (Lee et al. 1971). The effective concentration necessary to kill 100% of fish (EC100) increased three-fold from pH 5 to 8, six-fold from pH 8 to 9, and fourteen-fold from pH 9 to 10 (Marking 1975). Three hours of exposure were necessary to achieve 75% mortality at pH 9.5, and no mortality occurred in 3 h at a pH of 10.0 (Berger et al. 1969). Therefore, the manufacturer recommends application of 5 to 7.5 $\mu\text{g/L}$ antimycin if pH is less than 8.5 and 7.5-10 $\mu\text{g/L}$ if pH is greater than 8.5.

The rate of rotenone detoxification increases with increasing water temperature (Post 1955; Engstrom-Heg and Colesante 1979; Gilderhus et al. 1986; Gilderhus et al. 1988). The half life of rotenone is 13.9 d in water less than 8°C but only 83.9 h in 24°C water (Gilderhus et al. 1986; Gilderhus et al. 1988). Fish are also

more susceptible to both rotenone and antimycin at higher water temperatures because of increased metabolic activity (Lennon and Berger 1970; Marking and Bills 1976). For example, two to five-fold differences exist in the antimycin EC100 at 7 and 22°C (Berger et al. 1969). Therefore, antimycin concentrations can be decreased when water temperatures are greater than 15.5°C. Cold water may have been the primary factor responsible for unsuccessful antimycin treatments in the tributary to Government Creek, Montana (Tiffan and Bergersen 1996). Fish showed no signs of antimycin toxicity during two separate antimycin treatments at water temperatures ranging from 5 to 8°C. Antimycin application at colder water temperatures (average 4°C) of a headwater stream did not produce signs of toxicity 120 m downstream from the drip station (Tiffan and Bergersen 1996).

Sunlight detoxifies rotenone. Ultraviolet radiation rapidly degraded rotenone in spray applications to control terrestrial insects (Subba Rao and Pollard 1951). Rotenone-treated water placed in clear plastic bags and submerged in a lake degraded more rapidly than rotenone-treated water placed in opaque bags (Engstrom-Heg and Colesante 1979). The effect of sunlight on piscicide toxicity decreased as depth increased (Engstrom-Heg and Colesante 1979). Sunlight also degrades the toxicity of antimycin (Berger et al. 1969; Lee et al. 1971) especially near the surface of the water as turbidity limits further penetration of sunlight (Lee et al. 1971). Slightly quicker degradation of antimycin occurs in clear water (i.e., greater light penetration) than in turbid water (i.e., less light penetration; Berger et al. 1969). The half life of antimycin in direct sunlight and “open shade” was less than 20 min (Lee et al. 1971).

Sunlight may therefore affect antimycin toxicity more in high elevation lakes where a thinner atmosphere (i.e., less absorbance) would allow greater levels of sunlight than at lower elevations. Premature detoxification because of exposure to sunlight has been suspected as the cause of several incomplete fish kills in Montana (Don Skaar, Montana Department of Fish, Wildlife and Parks, personal communication). No recommendations exist for adjusting concentrations of piscicides in response to exposure to sunlight despite evidence showing sunlight detoxifies both rotenone and antimycin.

Turbulence is suspected to play a role in the degradation of antimycin. Antimycin in turbulent water has more contact with dissolved air and bubbles. These two factors are thought to increase the deactivation rate of antimycin by allowing oxygen to bind to the molecule. However, no difference was observed in antimycin degradation when air or helium were bubbled into water treated with 1 mg/L antimycin (Tiffan and Bergersen 1996). A loss of toxicity was observed when antimycin solutions were subjected to agitation and turbulence with no air contact (i.e., pumped; Pat Clancy, Montana Fish Wildlife and Parks, personal communication) inferring that degradation may be caused directly by turbulence and not by oxidation. Water turbulence may be an important factor in the degradation of antimycin applied to montane streams, a common location for eradication efforts. Water turbulence is not known to cause degradation of rotenone.

Rotenone is absorbed by inorganic compounds such as fine sediment and carbon (Engstrom-Heg 1974; Dawson 1975; Gilderhus 1982; Dawson et al. 1991;

Bettoli and Maceina 1996). Carbon (i.e., activated charcoal) is effective in absorbing rotenone in the laboratory and small streams (Engstrom-Heg 1974) and is recommended as the most efficient and economical absorbent for piscicides (Dawson 1975). Moreover, other materials can absorb piscicides. Suspended clay particles (i.e., bentonite) reduced the effectiveness of rotenone and antimycin (Gilderhus 1982). Rotenone and antimycin were not detoxified by the macrophyte *Elodea canadensis* (Gilderhus 1982). Contradicting these findings, thick vegetative mats decreased piscicide dispersion (Gresswell 1991) and deciduous leaf litter was suspected in the failure of antimycin treatments in Great Smokey Mountain National Park (Moore et al. 2005). The role of dissolved carbon in absorption of piscicides by organic matter has not been studied and remains unclear.

Overview of Dissertation

Conservation of native fish, and specifically eradication of undesirable fish, relies heavily on the efficient and effective use of piscicides. The goal of my research was to improve the efficiency and efficacy of piscicide application for the eradication of undesirable fish. The objectives, described in the following chapters, are meant to move fisheries managers, piscicide applicators, and fish conservationists closer to that goal. Chapter 2 describes the relationship between size of fish and response to piscicides. I examined whether fish of different sizes have an equivalent response to piscicides, an important assumption when using bioassay fish as sentinels during piscicide applications or toxicity studies. Chapter 3 describes research on three

environmental conditions that were suspected, but not yet proven, to reduce piscicide efficacy by degrading the toxicity of the chemicals. Chapter 4 describes development of a model to predict the persistence of rotenone in streams. Lack of understanding of the persistence of both rotenone and antimycin in streams is a major cause of inefficient and ineffective application. Applicators try to prevent areas of non-toxic water in streams by over-applying piscicides. A persistence model will allow applicators the ability to predict where in a stream the chemical will detoxify, rather than relying on intuition to estimate the detoxification location. Chapter 5 describes a study to determine the sites in a stream that are most efficient at mixing piscicides. Piscicide applicators currently place drip stations at locations that are convenient for access (e.g., the edge of the stream) rather than placing a drip station where channel morphology and flow characteristics most efficiently mix piscicides. Together, the research described herein provides piscicide applicators greater certainty when measuring piscicides using bioassay fish, more detailed knowledge of the effects of environmental conditions, a model to predict rotenone persistence, and protocols for efficiently mixing piscicides into a stream using drip stations.

CHAPTER 2

EFFECT OF FISH SIZE ON RESPONSE TO PISCICIDES

Abstract

The piscicides rotenone and antimycin are commonly used to eradicate unwanted fish populations. However, the relationships, if present, between their toxicities and fish sizes are unknown and could be especially important when bioassay fish are used to detect piscicide presence and effectiveness. Size-mediated toxicity could lead to either excessive or inadequate piscicide applications if bioassay fish are larger or smaller than the fish being eradicated. The relationships between time to death and rainbow trout *Oncorhynchus mykiss* weight (0.7 to 574.0 g) at 7.5 µg/L antimycin and 12.5 µg/L rotenone concentrations were determined. Significant positive relationships existed between size and time to death in both rotenone and antimycin; however, these relationships accounted for less than 21% of the variation in time to death. The remainder of variation was likely caused by the phenotypic threshold effect rather than variation in uptake rate.

Introduction

Bioassay fish are the most practical method for rapidly and effectively determining the presence of rotenone or antimycin (Finlayson et al. 2000). Rotenone can be measured at piscicidal concentrations using high pressure liquid chromatography (HPLC) but antimycin is applied at a lower concentration and cannot

be measured. Moreover, HPLC can not be done in real time in the field. An important assumption surrounding the use of bioassay fish in the past is that fish of all sizes will have a similar response to the piscicide. However, bioassay fish are often collected locally before treatment or brought to the site from a hatchery. A local source produces a wide range in fish sizes with abundance skewed toward smaller fish. A hatchery source typically results in a relatively small size range of same-age fish. Both of these scenarios make the assumption that different size fish will respond similarly to piscicides. This assumption may not be valid.

Existing information on the relationship between fish size and piscicide toxicity is incomplete. The most detailed experiments on piscicide toxicity exposed 21 species of fish to a range of piscicide concentrations for 3, 6, 24, and 96 h to determine the lowest concentration lethal to 50% of fish (LC50; Walker et al. 1964; Marking and Bills 1976). However, the fish used in these experiments were small and ranged from only 1.0 to 1.8 g. Experiments using a wider size range of eight species (30 to 305 mm in length) determined the effective contact time at several concentrations of rotenone and antimycin but the relationship between size and time to death was not explored (Gilderhus 1972). Comparisons among previous studies are problematic because experiments used different concentrations of piscicide, exposure times, and usually narrow size ranges of fish (e.g., Walker et al. 1964; Gilderhus 1972; Marking and Bills 1976). Further, the responses of individuals were not reported because experiments were designed to find an effective concentration that killed 50 or 100% of exposed fish. Schick (1974b) suggested that a higher

concentration of rotenone is necessary to kill larger fish. The only information available describing the relationship between size and piscicide tolerance suggests a positive relationship (Rowe-Rowe 1971). Dwarf tilapia *Tilapia sparrmanii* with an average length of 41 mm, had a lower LC50 (7.28 $\mu\text{g/L}$) than fish with an average length of 77 mm (9.75 $\mu\text{g/L}$; Rowe-Rowe 1971). Whereas this relationship suggests that larger fish are more tolerant of piscicides, it provides little guidance to those using fish as bioassay organisms. For example, a piscicide applicator may prematurely conclude that all fish in a stream are dead if larger fish take longer to die than smaller fish and only small fish are used for bioassay. The objective of this research was to determine the relationship between fish size and piscicide time to death. This information provides piscicide applicators certainty regarding the assumption of a similar response to piscicides among different-sized fish.

Methods

Rainbow trout *Oncorhynchus mykiss* were obtained from Ennis National Fish Hatchery in Ennis, Montana. The hatchery raises McConaughy, Shasta, Arlee, Erwin x Arlee, Eagle Lake, and Fish Lake strain rainbow trout; the strains have widely varied spawning dates allowing the hatchery to provide a wide variety of fish sizes at any given time. Fish of several different ages (2 to 36 months) were used to incorporate a spectrum of fish sizes; therefore, all of the strains were used. Fish were transported to the Aquatic Sciences Laboratory at Montana State University, Bozeman, Montana, and maintained for two weeks prior to experiments. During this

period, fish were held in 469-L fiberglass tanks supplied with 13°C filtered water at a 20 min turnover rate (Zale et al. 2005). Fish ranged in weight from 0.7 to 574.0 g.

Fish were randomly removed from holding tanks, anesthetized with 100 mg tricaine methanesulfonate/L water, weighed, and allowed to acclimate to test chambers for 24 h. Fish were assigned to test chambers to maintain a 1.6 g/L tank loading. The U.S. Environmental Protection Agency (USEPA) recommends loading at 1.1 g/L during static, nonaerated, nonrenewal exposure (USEPA (U.S. Environmental Protection Agency) 2002). However, I used a slightly higher loading because I maintained aeration during toxicity testing. Test chambers were isolated from circulating facility system water immediately prior to treatment with piscicides. Fish were divided into five size classes (31 to 75 mm, 76 to 120 mm, 121 to 210 mm, 211 to 300 mm, 301 to 345 mm) and separated with perforated dividers to prevent large fish from antagonizing or consuming small fish. Three trials were carried out for each chemical. There were 67 fish used in the first antimycin trial, 33 fish used in the second trial, and 18 fish used in the third trial. There were 32 fish used in the first rotenone trial and 45 fish used in each of the second and third trials. Test chambers were treated to 7.5 µg antimycin/L water or 12.5 µg rotenone/L water, which are concentrations within the range of those typically used in stream or lake restoration projects (Finlayson et al. 2010). The liquid formulation of Prenfish (Prentiss Incorporated, Floral Park, New York) was our source of rotenone. Fintrol (Aquabiotics Corp., Bainbridge Island, Washington), a commonly used antimycin formulation, was unavailable because of irregularities in the shelf life of Fintrol

manufactured from 2004 to 2006. Fintrol comes in two parts, Fintrol Concentrate (antimycin, acetone, and soy lipids [a byproduct of manufacture]) and Fintrol Diluent (diethyl phthalate, nonoxyl-9, and acetone). Fintrol Concentrate was “recreated” by mixing 90% pure antimycin (Sigma Aldrich, Product #A8674) with 99.5% pure acetone in the appropriate proportions, and replacing the soy lipids with acetone. This concentrate was mixed with Fintrol Diluent and applied as per the label instructions. The piscicides were applied with a pipette that was held over an air stone to allow circulation of the chemical throughout the tank. Application of dye in the same fashion mixed throughout the tank within three minutes.

A total of 122 fish was exposed to rotenone and 118 were exposed to antimycin. Fish were also maintained in untreated but aerated static hatchery system water as a control exposure to ensure that fish death was caused by piscicides and not extraneous factors. Fish were continually monitored until all fish in treatment groups died. Death was defined as no gill movement and no response to touch. When a fish died, it was removed from the exposure chamber, weighed, and time of death was recorded.

For comparison between chemicals, the Kolmogorov-Smirnov two-sample test was used to test for similarity of time to death distributions between fish exposed to each piscicide. Because this test showed that the distributions were not similar ($D = 0.683$; $p < 0.0001$), I used the Wilcoxon Rank Sum test to compare median time to death of fish exposed to rotenone to median time to death of fish exposed to antimycin.

To determine the relationship between fish size and time to death I first used a mixed model ANOVA to compare mean time to death among size classes and trials. In this model, trial was considered a random effect and size group a fixed effect. I used this test to identify potential confounding factors such as position of fish in the tank. I also plotted the distribution of time to death by size class to determine if one of the groups of fish (potentially the fish closest or farthest from the piscicide application) died sooner than the others. Simple linear regression was used to determine if time to death was dependent on fish weight. Fish weights were \log_{10} transformed to meet assumptions of normality. All statistical analyses were performed using SAS software (SAS institute Inc., Cary, North Carolina; version 9.2) and an alpha of 0.05.

Results

Antimycin took significantly longer than rotenone to kill rainbow trout at concentrations typically used in eradication projects (Wilcoxon Rank Sum test; $Z = 10.34$; $n = 240$; $p < 0.0001$). Median time to death was 161 min when exposed to rotenone and 288 min when exposed to antimycin (Figure 2.1). Time to death also spanned a larger range for antimycin than rotenone (Figure 2.1). The range in rotenone time to death (284 min) was almost half that of antimycin (520 min). Fish showed similar signs of distress between piscicides including erratic swimming, pale coloration, and flaring of opercula. Fish began showing these signs of distress 10 min after exposure to piscicides but survived for an additional 90 min. Most fish died

after this initial 100 min exposure period (Figure 2.2). Fish mortalities were more evenly spread over a longer time when exposed to antimycin (Figure 2.2). Eighty-two percent of fish exposed to rotenone died in less than 215 min whereas only 17% exposed to antimycin died in that time.

There were significant differences in mean time to death among size classes of fish for antimycin (ANOVA; $F = 7.66$; $p < 0.0001$) and rotenone exposures (ANOVA; $F = 14.48$; $p < 0.0001$). None of the groups was unexpectedly lower or higher than the others (Figure 2.3). That is, the pattern of box plots shows the same pattern as the linear regression results. The smaller fish tended to die faster but size explained less than 20% of the variation in time to death. Despite dying faster, the smallest two groups of fish were actually farthest from the piscicide application point. When these groups were pooled there were significant positive relationships between rainbow trout size and time to death during exposures to rotenone (linear regression; $t = 6.73$; $p < 0.0001$) and antimycin (linear regression; $t = 4.03$; $p < 0.0001$; Figure 2.4). Despite the significant positive relationships, fish size only explained 20.1% of the variation in time to death in rotenone and 14.1% of the variation in antimycin. Smaller fish appeared to be affected by the chemicals more quickly but they did not consistently die before larger fish.

Discussion

Time to death and behavior of fish observed in this experiment was similar to that observed during previous research on piscicides (Gilderhus et al. 1969; Gilderhus

1972). Size of fish explained only a small amount of the variation in time to death among rainbow trout. The large residual variation could be caused by different rates of chemical uptake (i.e., variation in chemical concentration among fish) or differences in the physiological response among fish (i.e., fish uptake similar piscicide concentrations but exhibit different responses). A large component of hydrophobic organic chemical (e.g., piscicide) uptake by fish is through the gills (Hayton and Barron 1990). Concentration of contaminants or piscicides in fish is a function of gill uptake, gill elimination, fecal egestion, dilution through growth, and metabolic biotransformation (Arnot and Gobas 2006). I assumed that fecal egestion, dilution through growth, and metabolic biotransformation were negligible because of the short duration of this experiment. I also assumed that the concentration of piscicide in the water was higher than the concentration of piscicide circulating through fish making the diffusion gradient across the fish gills positive in the water and negative in the fish. Therefore, gill elimination was also assumed to be negligible. The concentration of piscicides in each fish was therefore a function of piscicide uptake across its gills.

Gill uptake rate is a function of chemical, anatomical, and physiological characteristics. Chemical hydrophobicity (i.e., octanol-water partition coefficient) and diffusion rate affect the rate at which chemicals pass across gills (Sijm and Linde 1995). These characteristics would not cause variation in uptake rate among individuals because they only vary among different chemicals. Octanol-water partition coefficient varies between rotenone and antimycin but was the same among

fish exposed to each chemical. The diffusion coefficient varies according to the physical-chemical properties of the chemical and the liquid it is traveling through (Sijm and Linde 1995). However, this factor varied negligibly among replicates in this experiment because the water used for each replicate was from the same source and all replicates were kept at the same temperature.

Gill uptake rate could be affected by some anatomical characteristics such as gill surface area to weight ratio or distance between gill lamellae (Sijm and Linde 1995; Arnot and Gobas 2006; Cheng and Farrell 2007). For example, gill surface area to weight ratio varies among strains of rainbow trout (Palzenberger and Pohla 1992) and variation in such characteristics could cause uptake rate to vary among strains for fish. Differences among strains in gill surface to weight ratio could cause differences among strains in time to death. However, for gill related strain differences to be responsible for the positive relationship I observed, the gill surface to weight ratio would need to be coincidentally aligned such that earlier hatched strains would have smaller surface area to weight ratio and successive strains would have progressively larger surface area to weight ratio. This relationship could be present within a yearly period, but across fish of several year classes this relationship would be nonlinear showing a repeating annual pattern. I used several age classes of fish, did not observe this pattern, and conclude that gill surface area to weight ratio is not responsible for variation in uptake rate of piscicides.

Physiological characteristics that would affect uptake rate are ventilation rate and metabolic rate (Sijm and Linde 1995; Arnot and Gobas 2006; Cheng and Farrell

2007). Variation in uptake of piscicides should not be attributed to differences in ventilation rate among fish. All fish in the experiment maintained similar activity levels, were in the same water and therefore had similar oxygen demand and ventilation rate. Smaller fish have a higher metabolism (Liao 1971) and consequently higher uptake rates of some chemicals (Sijm et al. 1993; Sijm et al. 1995). For example, the smallest fish in my experiment use more than twice the oxygen per gram of fish than the largest (Liao 1971). Smaller fish draw relatively more water across their gills allowing greater potential for chemical uptake. Whereas this explains the positive trend in the relationship I observed, fish size explained less than a quarter of the variation in time to death. Variation in characteristics other than size accounted for most of the variation in time to death among fish exposed to piscicides.

Toxicity of piscicides may be more closely linked to the biochemical response of a fish than to internal concentration. That is, individuals with the same internal concentration of piscicide may have different responses to piscicide. The action of piscicides varies among fish because of the phenotypic threshold effect (Rossignol et al. 2003). The threshold effect is caused by randomly occurring mutations in mitochondrial DNA (mtDNA). These mutations cause irregularities in transcription, translation, and enzyme complex assembly that result in inhibition of activity at any of the five enzyme complexes in the respiration chain. These complexes have an excess processing capacity that provides a base level of function for the mitochondria despite varied levels of inhibition among individuals. Further, this buffering capacity

allows mitochondria to withstand additional inhibition by complex inhibitors (e.g., piscicides). However, if activity at a given complex falls below a threshold, mitochondria can no longer supply cells with energy and cell damage occurs. The variation in baseline complex inhibition among individuals allows for differences in the amount of additional complex inhibition they can withstand. For example, if the inhibition threshold at complex I is 75%, and mtDNA mutation causes a baseline inhibition of 25% in one fish, then it can withstand an additional 50% inhibition by piscicides before cell damage occurs. If another fish exhibits a baseline inhibition of 5% in activity at complex I, it can withstand an additional 70% inhibition. Mitochondrial inhibition by piscicides may explain the variation in time to death unexplained by fish size.

The phenotypic threshold effect may also explain differences in time to death between fish exposed to rotenone and antimycin. The response of fish exposed to antimycin displayed a more variable trend than the response of those exposed to rotenone (Figure 2.4) suggesting greater variation in complex inhibition among individuals. Also, the average time to death was significantly lower for rotenone than antimycin (Figure 2.1) indicating that the inhibition threshold is lower for rotenone than antimycin.

The significant difference in time to death between rotenone and antimycin may also be explained by the fact that I used a higher concentration of rotenone than antimycin. Differences between the response of fish exposed to rotenone and antimycin can be dramatic. For example, the effective contact

time required for 50% mortality (LT50) of bighead carp *Hypophthalmichthys molitrix* was 6 h at 2.5 µg/L antimycin but it took 20 times as much rotenone (50 µg/L) to achieve the same LT50 (Rach et al. 2009).

Manifestation of the subtle relationship between time to death and fish size should be negligible at commonly used piscicide concentrations and exposure times; however, applicators using lower concentrations of piscicides (or other polycyclic aromatic hydrocarbons) and exposing fish over longer time periods may observe a more pronounced relationship between fish size and time to death. Higher piscicide concentrations have a shorter effective contact time (Rach et al. 2009) making any differences across a size range less pronounced. Piscicide applicators can expect a similar time to death among the sizes of fish commonly used as bioassay organisms during piscicide application. If bioassay fish are of a wide size range (e.g., from a stream source) or a narrow size range (e.g., hatchery origin) applicators can expect that non-bioassay fish will have a similar response. This information removes a level of uncertainty associated with the response of fish of different sizes when exposed to piscicides and will help piscicide applicators maintain a level of certainty when applying piscicides.

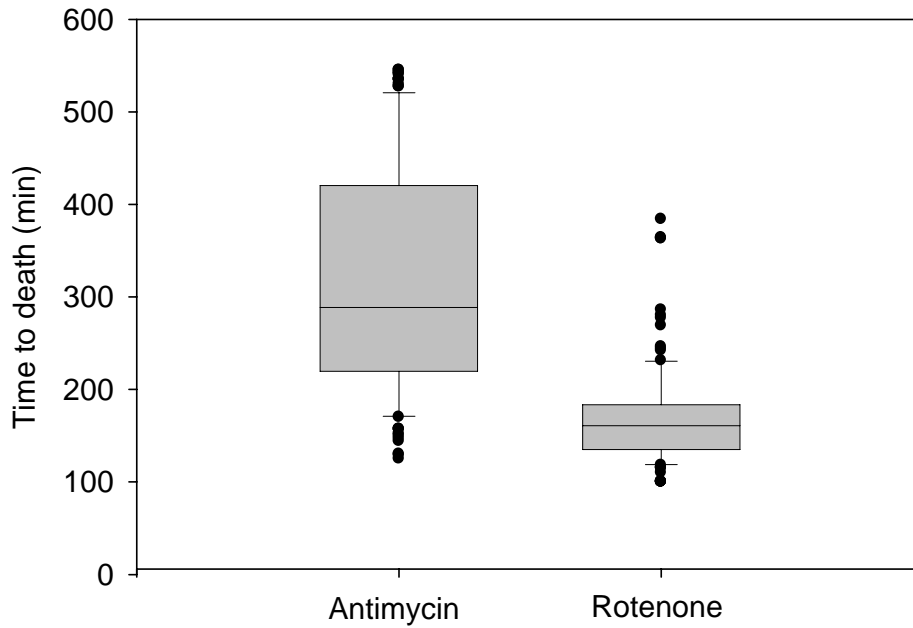


Figure 2.1. Box plots summarizing the distributions of the time to death of rainbow trout when exposed to 7.5 $\mu\text{g/L}$ antimycin or 12.5 $\mu\text{g/L}$ rotenone. Box represents 25th and 75th percentiles, horizontal lines represent the median value, whiskers represent the 10th and 90th percentiles and dots represent outliers. Median time to death was significantly different between antimycin and rotenone (Wilcoxon Rank Sum test; $Z = 10.34$; $n = 240$; $p < 0.0001$).

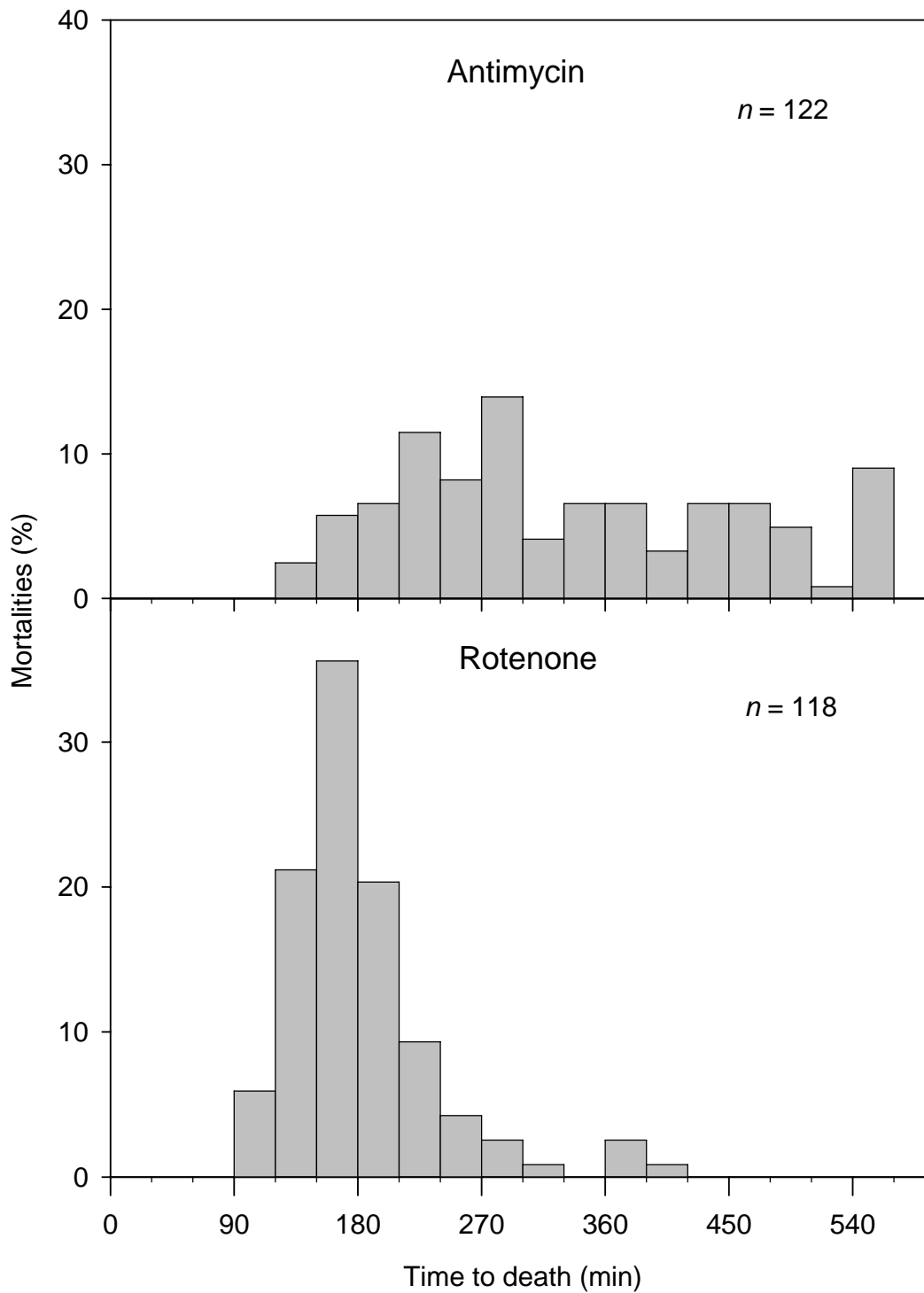


Figure 2.2. Frequency distributions of time to death (min) of rainbow trout exposed to rotenone and antimycin. The number of fish exposed (n) is included for each chemical.

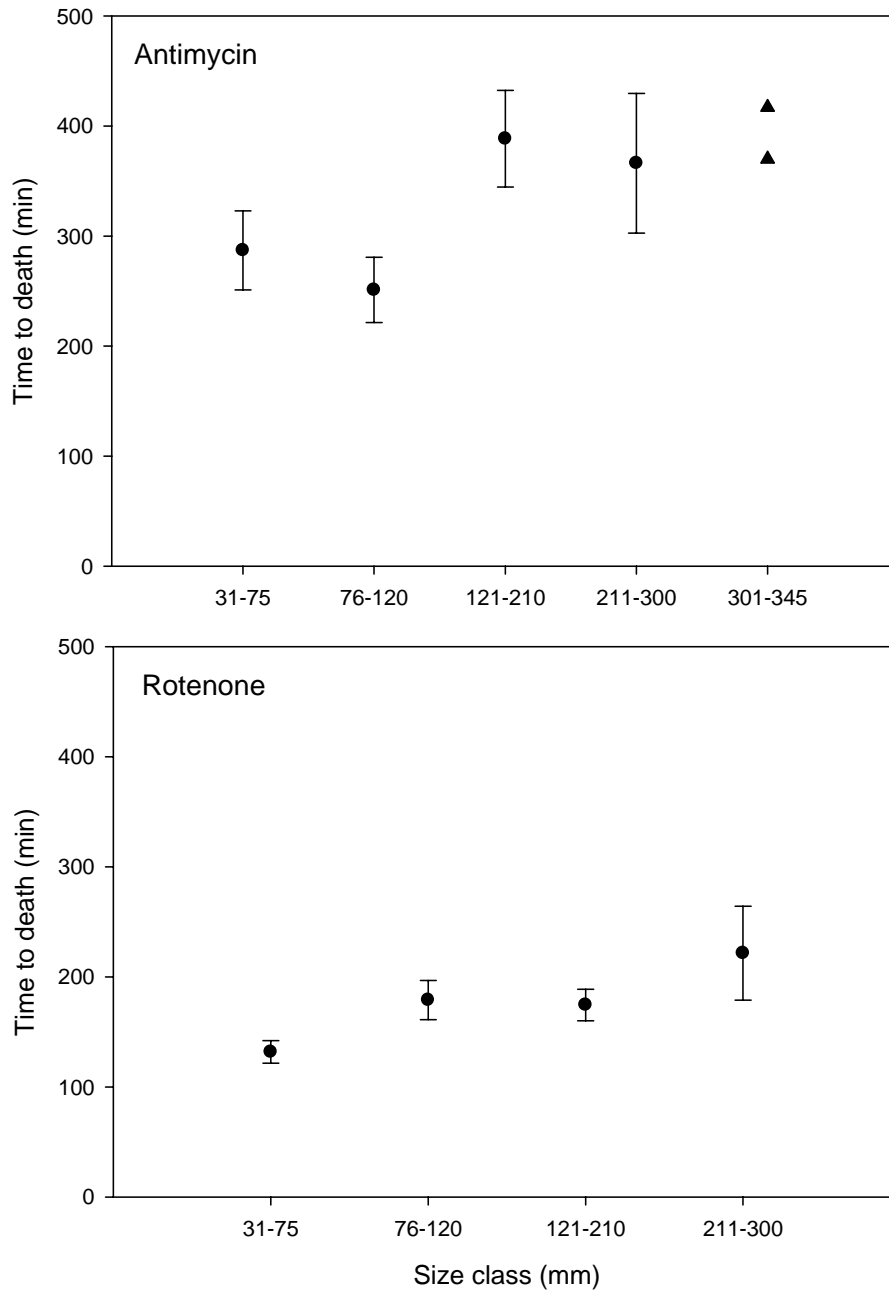


Figure 2.3. Time to death of rainbow trout by size group exposed to antimycin and rotenone. Closed circles represent mean values and whiskers represent 95% confidence intervals. Mean time to death was significantly different among groups antimycin (ANOVA; $F = 7.66$; $p < 0.0001$) and rotenone (ANOVA; $F = 14.48$; $p < 0.0001$). Triangles in the largest size class of fish exposed to antimycin represent the actual time to death of the only two fish in that size class.

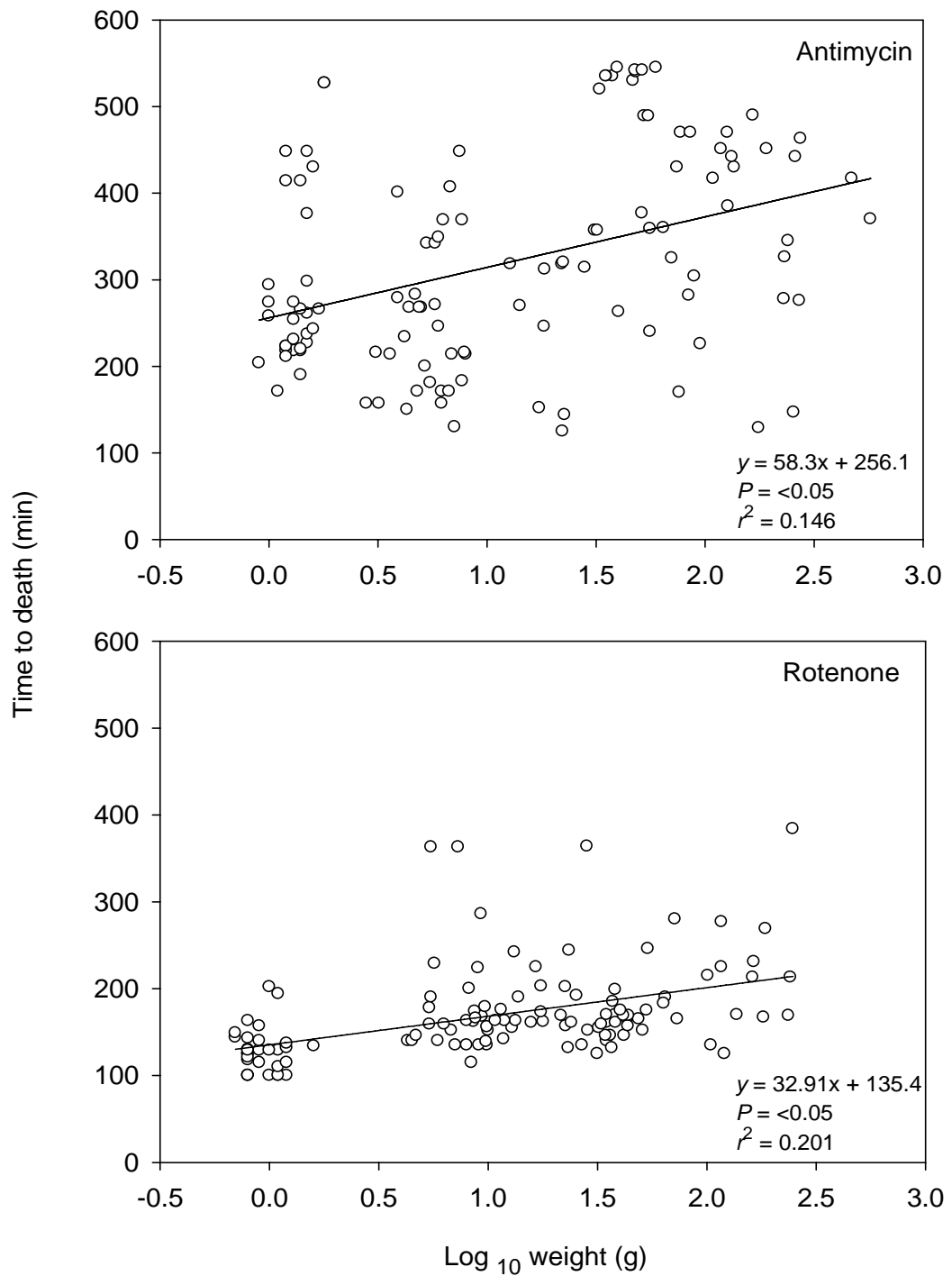


Figure 2.4. Relationships between time to death (min) and weight of fish (g) exposed to antimycin and rotenone.

CHAPTER 3

DETERMINATION OF THE AMOUNT OF ISOLATED EXPOSURE TO
SUNLIGHT, ORGANIC MATTER, AND TURBULENCE NECESSARY FOR THE
PISCICIDES ROTENONE AND ANTIMYCIN TO BECOME INEFFECTIVEAbstract

Piscicide toxicity is known to be affected by pH, water temperature, sunlight, organic matter, and turbulence. However, the effects of sunlight, organic matter, and turbulence have not been studied in enough detail to provide detailed descriptions of the amount of exposure of each of these constituents necessary to detoxify the chemicals. Rotenone and antimycin were exposed to sunlight, turbulence, and dissolved organic matter in controlled experiments to determine the amount of exposure necessary for the chemicals to become nontoxic to rainbow trout *Oncorhynchus mykiss*. Logistic regression was used to calculate the probability of eradication under different amounts of environmental exposure. Sunlight and turbulence degraded rotenone and antimycin; dissolved organic matter did not. Increasing the concentrations of both piscicides increased their resistance to degradation by sunlight and turbulence. Rotenone was more resistant to sunlight and turbulence than antimycin at the same concentration. Both rotenone and antimycin became nontoxic after 2 h of exposure to full sunlight. Whereas increasing concentration is one way to overcome the effects of sunlight and turbulence,

adjustments of treatment protocols such as treatment timing or drip station spacing would be more efficient measures.

Introduction

Sunlight, organic matter, and turbulence detoxify rotenone and antimycin and have been implicated as causes of incomplete fish eradication (Gresswell 1991; Stefferud et al. 1992). Past research determined that these factors affect piscicides by exposing the piscicides to a single exposure level of one of these factors and then testing toxicity (Walker et al. 1964; Gilderhus et al. 1969; Lee et al. 1971; Engstrom-Heg and Colesante 1979; Tiffan and Bergersen 1996). These experiments were successful in answering the “yes or no” question of detoxification, but they did not determine at what level of exposure the piscicide became nontoxic. Current application guidelines cite this body of research when identifying sunlight, organic matter, and turbulence as potential problems (Finlayson et al. 2002; Ling 2003). However, these guidelines fall short of describing the amount of exposure necessary to cause detoxification because this information does not exist. No published research has been conducted that reports on effects of different exposure levels of sunlight, turbulence, and organic matter to determine when rotenone and antimycin become ineffective. I conducted experiments that isolated these environmental conditions in the laboratory and exposed piscicides to different levels of these three conditions to provide a more detailed understanding of piscicide efficacy.

Methods

Toxicity testing is the best way to isolate effects of sunlight, organic matter, and turbulence on the toxicity of rotenone and antimycin toxicity to trout. Standard toxicity testing calls for exposure of test organisms (subjects) to multiple concentrations of a chemical (exposure levels) for 96 h. The survival rates of test organisms at each concentration are then plotted to determine the lowest concentration of chemical that kills 50% of the organisms (LC50). Three modifications to the standard toxicity testing methods were necessary to test the effect of these environmental conditions (exposure level) on piscicides (subjects). First, this experiment exposed replicates of piscicide-treated water to varying amounts of the three environmental characteristics. The duration of exposure of organisms to the chemical was modified from 96 h to 8 h because piscicides are typically applied to water bodies for 4 to 8 h. Allowing 96 h for piscicides to affect fish would be unrealistic. In addition to calculating the LC50 toxicity, thresholds closer to 100% were calculated because piscicide applicators are interested in complete eradication.

Each environmental condition had two associated controls. The negative control used fish exposed only to fresh water (i.e., no piscicides) during the testing. This control was used to ensure that fish mortality was not caused by extraneous factors. The positive control exposed fish to piscicide-treated water taken directly from the stock tank without exposure to an environmental condition. This ensured that initial concentrations were adequate to kill fish.

A two-stage protocol was used to reduce the number of test organisms necessary to determine the lowest environmental exposure that allowed the piscicide to remain effective (Douglas et al. 1986). The first stage used three widely spaced exposures and identified the appropriate range of a more narrow set of exposures. The second stage exposed piscicides to environmental conditions between the levels that caused the piscicides to be ineffective and the level that allowed the piscicide to remain effective (Douglas et al. 1986). For example, a first trial using exposures of 1, 5, and 10 h of exposure might result in the piscicide remaining toxic after 1 h of exposure but not after 5 h of exposure. A second experiment would then use exposures of 2, 3, and 4 h to more accurately determine the exposure that causes the piscicide to become nontoxic. This design causes harm to fewer fish because fish do not need to be exposed to experiments with 6, 7, 8, and 9 h.

Rainbow trout *Oncorhynchus mykiss* ranging in length from 50 to 100 mm, from Ennis National Fish Hatchery, were used as bioassay organisms (fish in this small size range respond similarly to piscicides; Chapter 2). Three fish were randomly assigned to each 30-L aquarium in a recirculating hatchery system. Fish were allowed to acclimate to these aquaria for 24 h before being exposed to piscicides.

Rotenone and antimycin were tested at concentrations of 12.5 µg/L and 7.5 µg/L, respectively, to represent concentrations typically used for eradication. Experiments were also carried out using 15 µg/L of each chemical to determine the effect of piscicide concentration on degradation by each environmental condition. A

stock solution of each concentration was mixed in a 2,000-L tank. It was divided into 30-L units, exposed to the appropriate environmental factor, and then transferred to empty 30-L aquaria adjacent to tanks holding bioassay fish. Bioassay fish were transferred to the static, aerated, piscicide treated water for 8 h. After the 8 h piscicide exposure, fish were returned to the adjacent flow-through aquaria. Dead fish were removed and counted during transfer. The remaining fish were monitored at 24-h intervals until all fish died or until 96 h after the end of piscicide exposure (Gilderhus 1972). These steps for handling fish were identical among experiments. The methods for simulating environmental exposure varied among experiments.

Sunlight exposure experiments were carried out using piscicide treated water, in clear polyethylene bags, exposed to different amounts of sunlight. Treatment levels differed by the density of shade fabric placed over each bag and ranged from 10% to 90% shade. An opaque bag (i.e., 100% shade) acted as a control for sunlight exposures. The shade bags were placed in a 10-cm deep water bath to control temperature and exposed to direct sunlight for 4 h. The amount of sunlight radiation falling on the bags was quantified using a VWR light meter (model 21899-014; West Chester, PA). The sunlight radiation that each bag was exposed to was multiplied by the proportion of light allowed through the shade fabric to calculate the sunlight exposure rate for each bag. This number was then multiplied by the amount of time each bag was exposed to sunlight to determine the total amount of sunlight each bag received (kilolux hours; klxh).

To relate the amount of sunlight exposure in this lab experiment to actual field piscicide application situations, sunlight was measured in three situations. One stream treatment occurred in October under an overcast sky in a stream that averaged 77% canopy cover. The other stream treatment occurred in June under a clear sky and in a stream that averaged 50% canopy cover. The amount of sunlight reaching streams was measured at ten locations spaced at 100-m intervals during two actual piscicide applications (see Chapter 4). A third set of measurements was averaged among measurements taken at mid-day under a clear sky within days of the summer solstice.

Turbulence was created by circulating piscicide treated water through column aerators similar to those described by Marking (1983). The air in these tubes was replaced with helium to eliminate the effect of oxidization on piscicides. Air was initially purged from the tubes and helium was added thereafter at a rate of less than 1 L/min. Turbulence exposures ranged from 5 min to 24 h. The amount of turbulence was quantified as the difference in kinetic energy (E_{Δ} ; kilojoules; kJ) between water falling freely (unobstructed tube) and water falling through an obstructed tube making the water turbulent. This difference was calculated as:

$$E_{\Delta} = E_{k(\text{unobs})} - E_{k(\text{obs})},$$

where E_k , in kilojoules (kJ), was calculated as:

$$E_k = 1/2 mv^2,$$

where m is the mass of the object in kilograms, and v is the velocity in meters per second. The velocity of water falling in an unobstructed tube was calculated as:

$$v_{(unobs)} = \sqrt{2g_o h}$$

where $v_{(unobs)}$ is the velocity of water in an unobstructed pipe, g_o is the gravitational acceleration constant (9.8 m/s), and h is the length of the tube (2 m). The velocity of water moving through an obstructed tube was measured as:

$$v_{(obs)} = d/\bar{t}$$

where d is the tube length and \bar{t} is the average time for 1 L of water to fall through the tube.

To relate the amount of turbulence each treatment received in this experiment to field piscicide application situations, the amount of turbulence was calculated in four turbulent stream reaches. The water velocity was measured in these reaches by introducing a plume of fluorescent dye (Bright Dyes, FLT Yellow/Green; Kingscote Chemicals, Miamisburg, OH) into the stream and recording the time required for the middle of the plume to travel between two points. The mean slope of these reaches was 2.1, 4.5, 7.3, and 11.3% and each of these reaches had rubble and cobble substrates (see Chapter 4 for description of slope measurement). The amount of turbulence (i.e., energy dissipated; E_k) was calculated similarly to that of tubes in the lab experiments (i.e., theoretical velocity/actual velocity) except that Manning's formula was used to estimate the unobstructed water velocity. Manning's formula is:

$$V = \frac{1}{n} R_h^{\frac{2}{3}} S^{\frac{1}{2}}$$

where V is the cross-sectional average velocity, n is Manning's roughness coefficient (i.e., 0.001 for smooth pipes for the unobstructed kinetic energy; Sturm 2001), R_h is the hydraulic radius, and S is slope of the water surface (m/m). The mean Manning's roughness coefficient (n) for these reaches was 0.072 (min = 0.068, max = 0.075).

Dissolved organic matter (DOM) exposures were carried out using rotenone or antimycin treated water mixed with different amounts of dissolved organic matter. Organic matter (i.e., locally collected leaves, sticks, and grass) was soaked in water for 48 h, filtered through a 0.22- μ m filter and stored at 4°C. Treatment levels were 1, 10, and 20 mg DOM/L water and represent the range of DOM levels in montane streams (Thurman 1985). Controls were not exposed to DOM.

The amount of residual rotenone was measured after each experiment. Water samples were collected in amber glass bottles and refrigerated. Rotenone was measured by liquid chromatography-electrospray/mass spectrometry/mass spectrometry (LC/MS/MS) within 24 h of sampling. Dissolved organic matter was measured using a Dohrmann carbon analyzer (model DC-80; Teledine-Tekmar, Mason, Ohio). Antimycin cannot be measured at the low concentrations that are toxic to fish.

Logistic regression was used to develop models that predict the level of exposure to a given environmental condition at which rotenone and antimycin lose toxicity. This analyses was used because the dependent variable (i.e., complete eradication or incomplete eradication) is binary (Cox and Snell 1989). The dependent variable was the proportion of fish in a tank killed by the piscicide. The independent

variable was the level of exposure to a given environmental factor. Logistic regression models were developed using SAS software (SAS version 9.2; SAS Institute Inc., Cary, North Carolina) and used to evaluate the relationship between piscicide toxicity and environmental exposure. The models provided a prediction of the maximum level of exposure to each environmental condition that still allowed for a given probability of eradication.

The environmental exposure level (EE) necessary to establish a given eradication probability was used to evaluate the tolerance of rotenone and antimycin to sunlight, turbulence, and DOM. The EE level is analogous to the LC level in standard toxicity testing. The EE for probabilities of 0.00001, 0.50, 0.95, and 0.999 were calculated. The EE50 allows comparison among environmental characteristics and between concentrations. The EE99.9 provides piscicide applicators with the exposure to a given environmental condition necessary for the chemical to be less than 100% effective.

Results

Sunlight degraded both rotenone and antimycin. The sunlight EE50 and EE99.9 were lower for 7.5 µg/L than for 15 µg/L antimycin (Table 3.1). Similarly, the sunlight EE50 and EE99.9 were lower for 12.5 µg/L rotenone than for 15 µg/L rotenone (Table 3.2). A negative relationship existed between the concentration of residual rotenone and exposure to sunlight at 12.5 and 15 µg/L rotenone (Figure 3.1). Both rotenone and antimycin reached a threshold of sunlight exposure that caused

them to become nontoxic. As expected, these thresholds were at a similar sunlight exposure for 15 µg/L of each chemical but, unexpectedly, were also at similar sunlight exposures when the amount of each chemicals was substantially different (i.e., 7.5 µg/L antimycin and 12.5 µg/L rotenone; Figure 3.2). Doubling the concentration of antimycin increased the chemical's tolerance to sunlight by a factor of 2.41 (Figure 3.2). Increasing the concentration of rotenone by one sixth increased the chemical's tolerance to sunlight by a factor of 2.22 (Figure 3.2). Rotenone was more resistant to sunlight degradation than antimycin at the same concentration (Figure 3.2). The EE99.9 for the lower concentrations of both chemicals (i.e., 7.5 µg/L antimycin and 12.5 µg/L rotenone) was 0 indicating that they were unreliable for eradicating trout; however, control exposures at these concentrations successfully eradicated trout. In all experiments, all fish in the piscicide-only control died, and all fish in the non-piscicide control lived. Comparing the EE values to the sunlight exposure observed in natural settings shows that less than 2 h of exposure to sunlight under a clear sky would make 15 µg/L of both chemicals unreliable for eradicating trout (Figure 3.3).

Turbulence degraded antimycin more than rotenone. The turbulence EE50 and EE99.9 were lower for 7.5 µg/L than for 15 µg/L antimycin (Table 3.1). The turbulence EE50 and EE99.9 were lower for 12.5 µg/L rotenone than for 15 µg/L rotenone (Table 3.2). The toxicity of 15 µg/L rotenone was not affected by turbulence. A negative relationship existed between turbulence exposure and the amount of residual rotenone in both 12.5 and 15 µg/L exposures (Figure 3.1).

Doubling the concentration of antimycin increased the chemical's tolerance to turbulence by a factor of 2.64 (Figure 3.4). Increasing the concentration of rotenone by one sixth increased the chemical's tolerance to turbulence such that it remained toxic at naturally occurring turbulence levels (Figure 3.4). Rotenone was more resistant to degradation by turbulence than antimycin at the same concentration (Figure 3.4). Comparing the EE values to the turbulence exposure observed in natural settings shows that 15 $\mu\text{g/L}$ antimycin will become unreliable for eradicating trout after 45 min of travel in a stream with 11.3% slope and Manning's n of 0.072 or 110 min of travel in a stream with a slope of 7.3% and Manning's n of 0.068 (Figure 3.5). Rotenone at 15 $\mu\text{g/L}$ remained toxic after exposure to all turbulence levels. Concentrations of 7.5 $\mu\text{g/L}$ antimycin and 12.5 $\mu\text{g/L}$ rotenone were unreliable for eradication at even the lowest exposures to turbulence.

Dissolved organic matter at concentrations observed in natural settings did not detoxify rotenone or antimycin. All fish died at all DOM exposure levels. A complex relationship between rotenone and DOM emerged after examining these incongruous results. A linear relationship between the concentration of DOM and its ability to bind rotenone was expected. It was expected that higher concentrations of DOM would absorb more rotenone. However, higher concentrations of DOM did not always absorb more rotenone. Concentrations of 1 mg/L DOM absorbed more rotenone than a concentration of 5 mg/L (Figure 3.1). The rotenone binding capacity of DOM used in this experiment was compared to a standard form of DOM, Aldrich humic acid (HA; product-53680; St. Louis, MO), to determine if the DOM used in

this experiment was somehow confounding measurements or increasing the rotenone concentration. Measurements of residual rotenone were consistently lower for HA than DOM (Figure 3.6). But, these results also showed that 20 mg/L DOM and HA had a higher residual rotenone concentration than 1 mg/L (Figure 3.6). The amount of rotenone measured in most of these replicates was higher than the initial rotenone concentration of 15 µg/L.

Discussion

Sunlight and turbulence affect the toxicity of rotenone and antimycin but DOM does not. Explaining the mechanisms of detoxification is clear in the case of sunlight, speculative in the case of turbulence, and unclear for DOM. Sunlight is known to degrade a wide variety of natural and synthetic materials. Wavelengths from 260-500 nm damage plastics (reviewed by Andradý et al. 1995; Copinet et al. 2004), drugs (Barteles and von Tümpling 2007), and pesticides (Da Silva et al. 2003). In particular, the ultraviolet component of sunlight (UV; < 400 nm) transforms chemicals to excited electron states, which can cause the chemicals to degrade directly or can promote chemical reactions that lead to degradation. Direct photodegradation takes place by homolysis (separation of a chemical bond where a portion retains one of the two bonded electrons), heterolysis (separation of a chemical bond where a portion retains both electrons), or photoionization (removal of one or more electrons from a molecule by absorption of a photon provided by light energy). Each of these processes changes the piscicide without reacting with other molecules

in the water. Indirect photodegradation takes place when piscicides react with other chemicals causing them to be nontoxic. Particularly, reaction with hydroxyl radicals (HO^\cdot) causes indirect photodegradation in an aquatic solution (Burrows et al. 2002). Other chemicals in photosensitized states, particularly acetone, can provide oxygen atoms, protons, or electrons that degrade organic molecules (Larson and Weber 1994). Avoiding the mixture of piscicides into an aquatic solution is impossible, but direct and indirect photodegradation can be avoided by limiting piscicide exposure to sunlight.

Piscicide exposure to sunlight can be limited by canopy cover, sunlight penetration of the waterbody, or application timing. Canopy cover greatly reduces the amount of light reaching a stream (Figure 3.4) and may limit sunlight exposure in littoral zones of lakes; however, pelagic areas of lakes are almost continually exposed to sunlight during daylight hours. Characteristics of the lake surface may limit exposure to sunlight, with a calm surface being more reflective than a surface disturbed by waves. Of the light that penetrates the surface, DOM, suspended sediment, and plankton absorb UV light (Wetzel 2001) limiting the effects of sunlight on piscicides in lakes. Of these factors, the amount of DOM is particularly important in limiting sunlight penetration of lakes. For example, 99% of sunlight is blocked within 10 m when the DOM is less than 1 mg/L but 99% is blocked within 0.5 m when DOM is 2 to 3 mg/L (Williamson et al. 1996). Piscicide applications are typically carried out in the summer during the daytime. With this timing, both rotenone and antimycin would be exposed to enough sunlight to render them nontoxic

in less than 2 h (Figure 3.4). Exposure to sunlight would be greatly limited in the evening or at night.

Sunlight exposure affects the toxicity of piscicides, but those effects can be mitigated with adjustments to typical application techniques. In streams with moderate to full canopy cover, or lakes with high DOM content (e.g., greater than 2 mg/L), sunlight exposure is limited enough that piscicides will remain toxic long enough to be effective. In areas with an open canopy (e.g., lakes, meadow streams) piscicides should be applied at dusk or under cloud cover to limit detoxification by sunlight. If these adjustments are not feasible, treating surface waters (i.e., those with increased exposure to UV light) with a higher concentration than deeper waters would help ensure eradication. Higher concentrations of piscicides can withstand more exposure to sunlight; therefore, increasing the concentration of a piscicide is one way to mitigate for detoxification by sunlight. However, applying piscicides at dusk or under cloud cover would be more cost effective and would moderate undesirable side effects.

I speculate that sorption, volatilization, or a combination of the two removed piscicides from solutions exposed to turbulence. Sorption is the adhesion of a substance to another substance and volatilization is the process of changing from a liquid solution to a gaseous solution. Sorption and volatilization are common processes for polycyclic aromatic hydrocarbons (PAH; Southworth 1979; Chiou 2002). Sorption is a common fate of aquatic PAH pollutants in water-saturated soils. The high surface area to solution ratio in water saturated soils and hydrophobic nature

of PAHs cause the chemicals to sorb to soil particles (Chiou 2002). Similarly, PAHs sorb to synthetic materials such as plastic or metal well casings. This process occurs over the course of days (Jones and Miller 1988) but mixing a PAH solution increases the sorption rate (Sharom and Solomon 1981). Sorption may reduce the concentration, and therefore the toxicity, of a piscicide solution, especially in cases where a high surface area to solution ratio is present and the solution is being mixed. Both the turbulence chambers used in this study and high gradient turbulent reaches of streams provide this environment.

Volatilization may also explain the detoxification of piscicides in turbulent environments. Whereas rotenone and antimycin are not volatile, commercial formulations of the chemicals are mixed with acetone, which is volatile (Hansen and Wilbur 1994). Acetone increases the solubility of rotenone and antimycin in water, allowing the chemicals to be soluble in water at piscicidal concentrations. If acetone is removed from the solution, the solubility of rotenone and antimycin is reduced and they are removed from the solution by precipitation or sorption. Volatilization is a function of a chemical's ability to move through water, the depth of the water, and the concentration of chemical in the air immediately next to the water. Along a continuum of scenarios, volatilization would be minimized in a body of water that is deep and covered with stagnant air and maximized in water with a large surface area and continual airflow. Unmixed water with stagnant air limits volatilization because redistribution of the chemical in the water is limited by molecular forces and because the air becomes saturated with volatilizing chemical. Conversely, highly turbulent

conditions, where water is constantly mixed and often spread in a thin layer across substrates or suspended in airborne droplets, provide an ideal environment for volatilization.

Acetone and other PAHs volatilize from water at rates that are germane to piscicide application (Southworth 1979; Rathbun et al. 1988). For example, the half life of acetone in a quiescent stream 1 m deep, moving at 1 m/s with a wind speed of 3 m/s is in the range of 7.8-16.2 h (Hansen and Wilbur 1994). On the other end of the continuum, the half-life of acetone volatilizing in a home shower is 2.18 s (based on calculations using values from Giardino et al. 1992; Moya et al. 1999). The results of this research fit into this continuum in that piscicide treated water (i.e., positive control) remained toxic because volatilization was minimal in a covered carboy. Conditions for volatilization were better in the turbulence chambers, allowing acetone to volatilize. As the acetone concentration decreased, the amount of rotenone or antimycin remaining in solution also decreased thereby reducing toxicity.

Rotenone is more resilient to the effects of volatilization because it is slightly less hydrophobic (Chapter 1). That is, more acetone would need to volatilize for rotenone to become nontoxic. Better resilience of rotenone compared to antimycin in turbulent environments is consistent with anecdotal observations of piscicide applicators.

Application of the results of the turbulence experiments to the field is tenuous because piscicide degradation in the turbulence chambers was slower than expected. Slow volatilization was caused by the low helium exchange within the turbulence

chambers. The intent of adding helium was to purge air from the turbulence chambers, preventing oxidation. Helium was continually added at the top of the chamber to force out air and any oxygen degassing from the water during the exposure. Volatilized acetone never saturated the surrounding gas, as it did in the carboy, because a continual small flow of helium passed through the turbulence chambers. This flow allowed for a slow rate of volatilization. The volatilization of acetone would have been more rapid if helium were added at higher flow rates. The volatilization rate in turbulence chambers was therefore more comparable to that of a slow, deep stream than to that of a shallow, high-gradient stream. Prediction of piscicide persistence in streams should be made with models based on measurements in actual streams (Chapter 4) instead of the results of this experiment.

Concentrations of antimycin higher than those used in these experiment have been documented degrading at faster rates than the rates observed in these experiments (Tiffan 1992; Tiffan and Bergersen 1996). Volatilization may play an even greater role in streams because air is continually being circulated across the moving water surface. The greater circulation of air provides a practically limitless sink for volatilizing piscicides. Further, water cascading across coarse substrates provides an ideal water condition for volatilization. Increased volatilization and sorption in natural settings may account for the discrepancy between this study and that of Tiffan and Bergersen (1996).

The relationship between DOM and piscicide detoxification is unclear; however, sorption or increased exposure to light may explain problems with piscicide

toxicity in streams with abundant organic matter. Problems with piscicide toxicity in streams with recently-fallen leaf litter suggested that the newly introduced leaves were somehow detoxifying the piscicide (Moore et al. 2005). Leaf litter changes a stream in three ways after falling into a stream: first, leaves leach fulvic acids, humic acids, and sugars (collectively DOM); second, they substantially increase the surface area of the stream; and third, they decrease the canopy cover.

The hydrophobic nature of DOM facilitates binding with other hydrophobic molecules (e.g., organic pollutants, piscicides) reducing their toxicity (Akkanen and Kukkonen 2003; Steinberg 2003). The uptake of organic chemicals by organisms has generally been shown to decrease with increasing DOM concentration (Steinberg 2003). A reduction in toxicity of both piscicides was expected in this study; however, DOM did not affect the toxicity of rotenone or antimycin. Measurements of rotenone and DOM show a confounded relationship (Figure 3.6). Two hypotheses could explain the higher than expected rotenone concentrations. First, DOM and humic acid (HA) were adding rotenone to the solution. This hypothesis can be ruled out because rotenone was not detected in DOM treated water. Second, DOM and HA somehow confound measurement of rotenone by LC/MS/MS. This measurement technique should be able to distinguish between rotenone and DOM and be robust to these sorts of confounding factors; however, no research has been done on the effects of DOM or HA on rotenone measurement by LC/MS/MS. The complex relationship between DOM, adsorption of piscicides, and measurement of rotenone was beyond

the scope of this project and needs to be studied further in order to be fully understood.

The increase in surface area caused by added leaf litter may cause detoxification of piscicides by sorption of piscicides to the leaves. Piscicides are sorbed by activated carbon and suspended sediment (Engstrom-Heg 1974; Dawson 1975; Gilderhus 1982). When exposed to the large amounts of surface area provided by leaves, piscicides may be detoxified by binding to leaf litter. However, piscicide sorption has not been studied; research on piscicide sorption is likely precluded by the inability to detect minuscule amounts of piscicides on a variety of surfaces.

Increased exposure to sunlight after leaf fall may explain the rapid detoxification of piscicides observed by Moore et al. (2005). Expectations of piscicide persistence developed during bioassays under a full canopy would not be valid under an open canopy after leaf fall. Exposure to sunlight dramatically reduces piscicide toxicity and would unexpectedly reduce piscicide persistence if the effects of sunlight were unknown to the applicator.

Piscicide applicators should identify potential exposure to sunlight and turbulence and take steps to minimize degradation of piscicides caused by these factors. The discrepancy between my study and observations of piscicide detoxification in natural settings may also be because of the synergistic interaction of multiple environmental conditions on piscicides. My study measured the effects of isolated environmental condition as a first step in studying their effects. Further study of the effects of these conditions on piscicides should include the interactive effects.

These experiments could be done by expanding the experiments described here to a factorial study design.

Table 3.1. Environmental exposures necessary for sunlight, turbulence, and organic matter to cause 7.5 and 15.0 µg/L antimycin to have eradication probabilities of 0.00001, 0.500, 0.950, and 0.999 (EE level). NA indicates that because the true estimate was not provided, a confidence interval is not applicable. No Effect indicates that all of the fish in the exposures died and there was no environmental effect on the chemical.

7.5 µg/L Antimycin				15.0 µg/L Antimycin			
EE level	(95% CI)	Exposure	(95% CI)	EE level	(95% CI)	Exposure	(95% CI)
Sunlight (klxh)							
0.999	(0.990, 1.000)	< 0	NA	0.999	(0.964, 1.000)	71	(< 0, 97)
0.950	(0.844, 0.985)	12	(< 0, 30)	0.950	(0.750, 0.992)	113	(78, 127)
0.500	(0.275, 0.726)	60	(44, 78)	0.500	(0.308, 0.692)	145	(133, 153)
0.00001	(0.000, 0.001)	172	(139, 235)	0.00001	(0.000, 0.002)	218	(197, 277)
Turbulence (kJ)							
0.999	(0.987, 1.000)	< 0	NA	0.999	(0.569, 0.966)	52	(< 0, 64)
0.950	(0.867, 0.982)	10	(< 0, 16)	0.950	(0.379, 0.819)	69	(42, 76)
0.500	(0.331, 0.674)	30	(27, 38)	0.500	(0.186, 0.632)	82	(76, 87)
0.00001	(0.000, 0.002)	80	(64, 120)	0.00001	(0.001, 0.236)	111	(99, 175)
Dissolved Organic Matter							
0.999		No Effect		0.999		No Effect	
0.950		No Effect		0.950		No Effect	
0.500		No Effect		0.500		No Effect	
0.00001		No Effect		0.00001		No Effect	

Table 3.2. Environmental exposures necessary for sunlight, turbulence, and organic matter to cause 12.5 and 15.0 µg/L rotenone to have eradication probabilities of 0.00001, 0.500, 0.950, and 0.999 (EE level). NA indicates that because the true estimate was not provided, a confidence interval is not applicable. No Effect indicates that all of the fish in the exposures died and there was no environmental effect on the chemical.

12.5 µg/L Rotenone				15.0 µg/L Rotenone			
EE level	(95% CI)	Exposure	(95% CI)	EE level	(95% CI)	Exposure	(95% CI)
Sunlight (klxh)							
0.999	(0.980, 1.000)	< 0	NA	0.999	(0.923, 1.000)	148	(95, 158)
0.950	(0.845, 0.985)	39	(9, 51)	0.950	(0.755, 0.992)	163	(142, 167)
0.500	(0.356, 0.645)	78	(71, 89)	0.500	(0.302, 0.718)	174	(171, 181)
0.00001	(0.000, 0.003)	170	(138, 258)	0.00001	(0.000, 0.040)	200	(188, 262)
Turbulence (kJ)							
0.999	(0.989, 1.000)	< 0	NA	0.999		No Effect	
0.950	(0.882, 0.980)	< 0	NA	0.950		No Effect	
0.500	(0.358, 0.643)	96	(74, 132)	0.500		No Effect	
0.00001	(0.000, 0.001)	406	(308, 625)	0.00001		No Effect	
Dissolved Organic Matter							
0.999		No Effect		0.999		No Effect	
0.950		No Effect		0.950		No Effect	
0.500		No Effect		0.500		No Effect	
0.00001		No Effect		0.00001		No Effect	

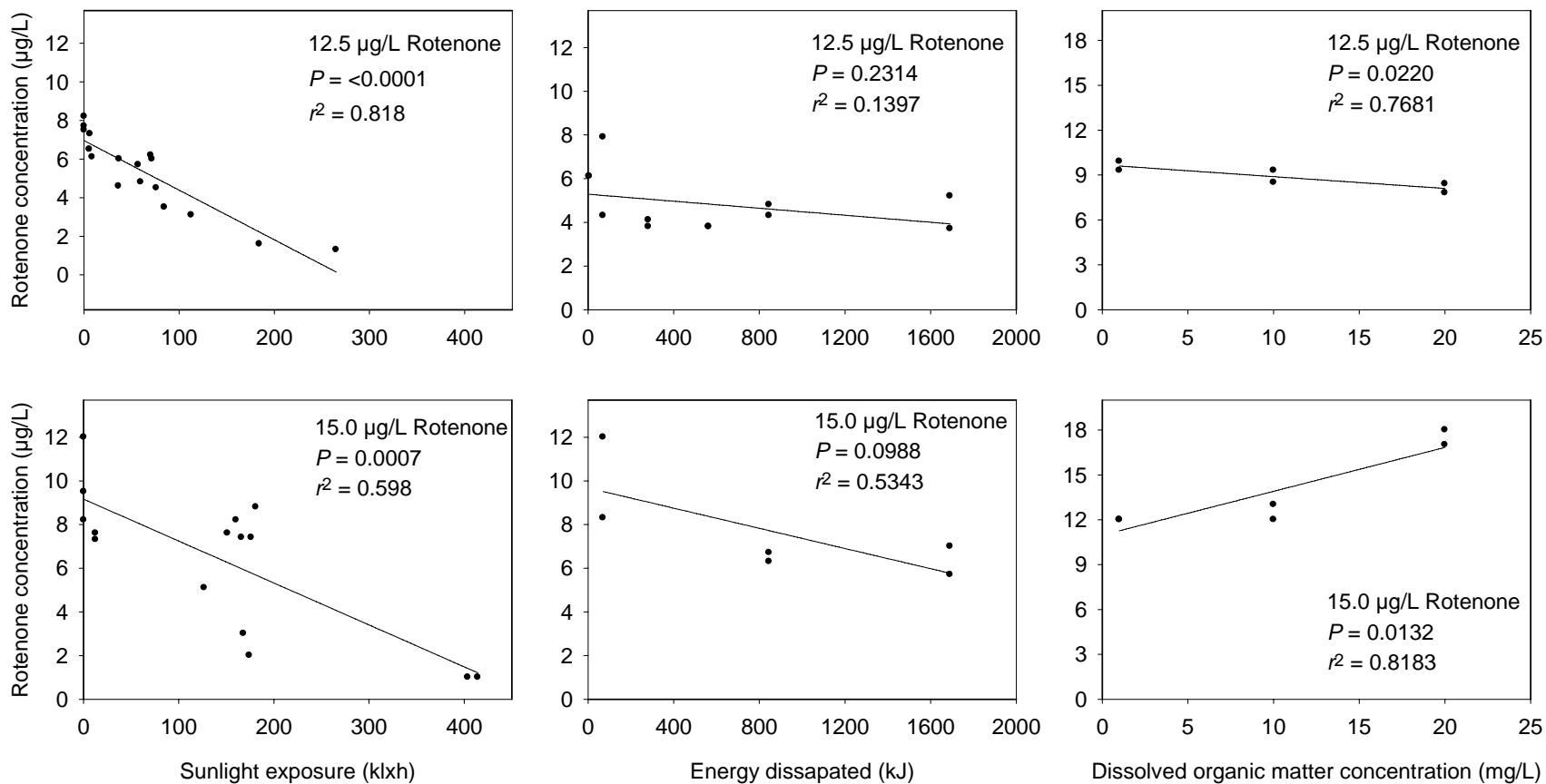


Figure 3.1. Relationships between rotenone concentration and exposure to sunlight, turbulence, and organic matter. Note differences in scaling of Y axis. Points represent measurements of rotenone concentration by liquid chromatography-electrospray/mass spectrometry/mass spectrometry.

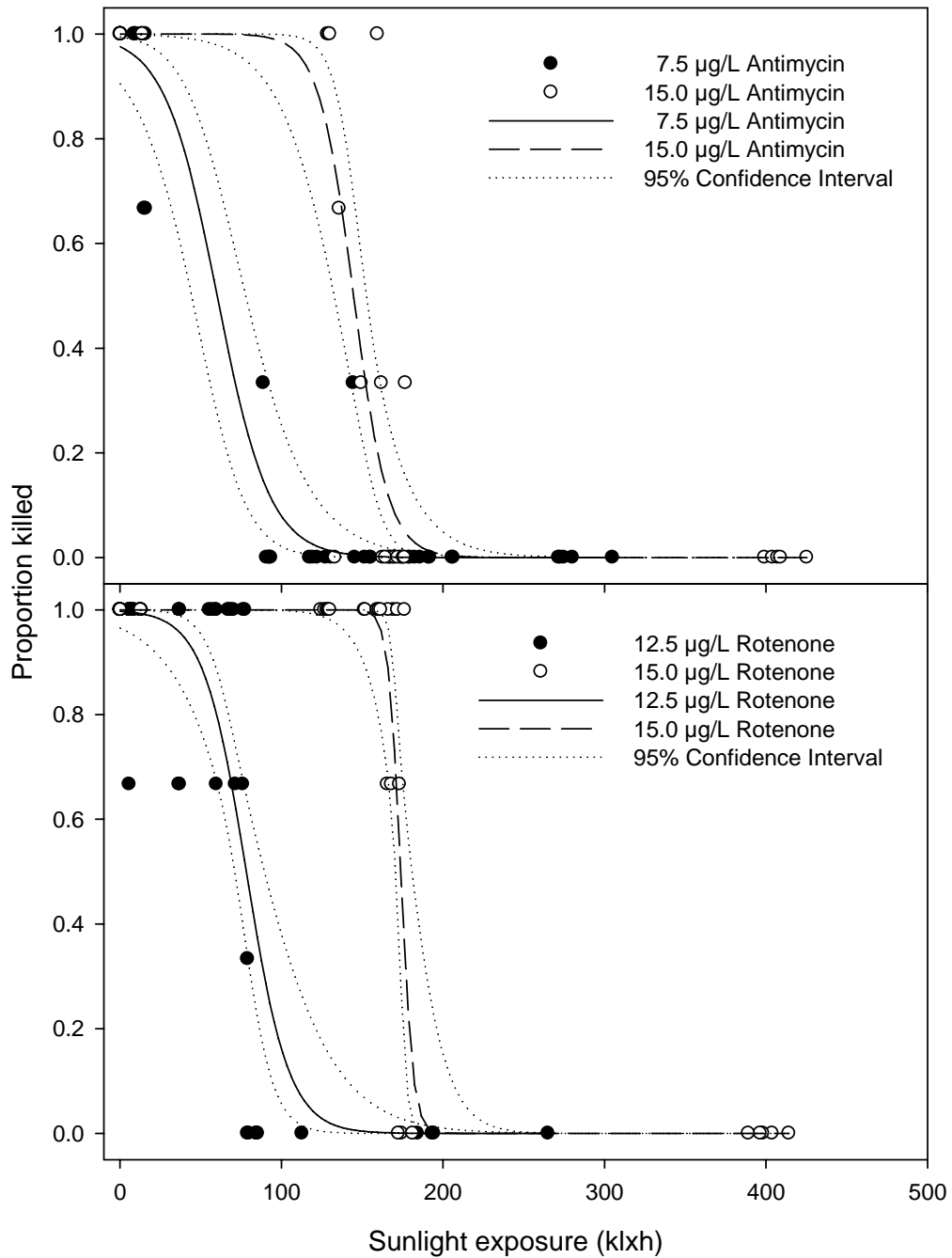


Figure 3.2. Proportions of fish killed after antimycin and rotenone exposure to sunlight. Solid circles indicate the proportions of fish killed at low concentrations of piscicides and open circles indicate proportions killed at higher concentrations. Solid line indicates the probability of eradication as a function of sunlight exposure for the lower piscicide concentrations and dashed line indicates probability of eradication at the higher concentrations. Dotted lines indicate 95% confidence intervals.

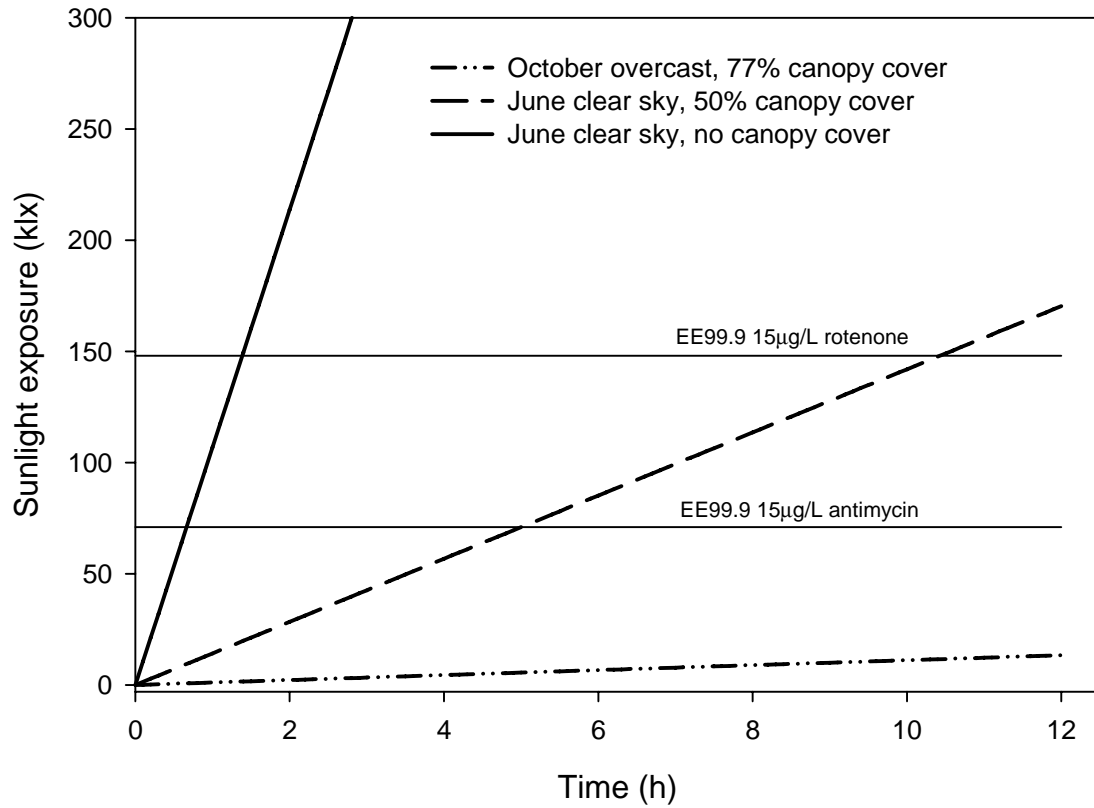


Figure 3.3. Accumulation of sunlight under three sunlight exposures. Diagonal lines represent accumulated sunlight exposure; horizontal lines represent the level of sunlight exposure that would cause 15 µg/L rotenone or antimycin to be less than 99.9% effective. Areas below the horizontal lines represent conditions at which 15 µg/L rotenone or antimycin would remain more than 99.9% effective.

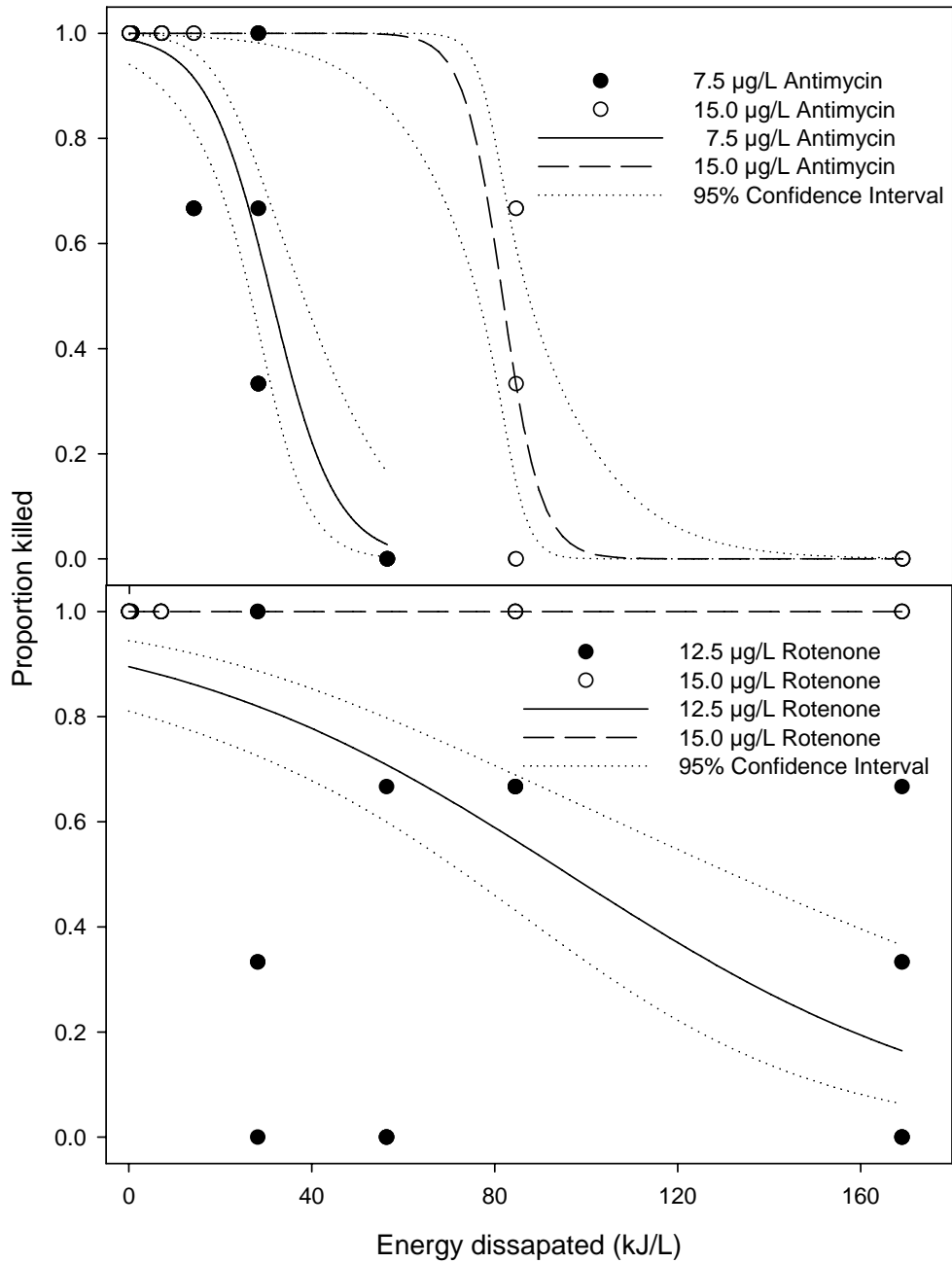


Figure 3.4. Proportion of fish killed after antimycin and rotenone exposure to turbulence. Solid circles indicate the proportions of fish killed at low concentrations of piscicides and open circles indicate proportions killed at higher concentrations. Solid lines indicate the probability of eradication as a function of sunlight exposure for the lower piscicide concentrations and dashed lines indicates probability of eradication at the higher concentrations. Dotted lines indicate 95% confidence intervals.

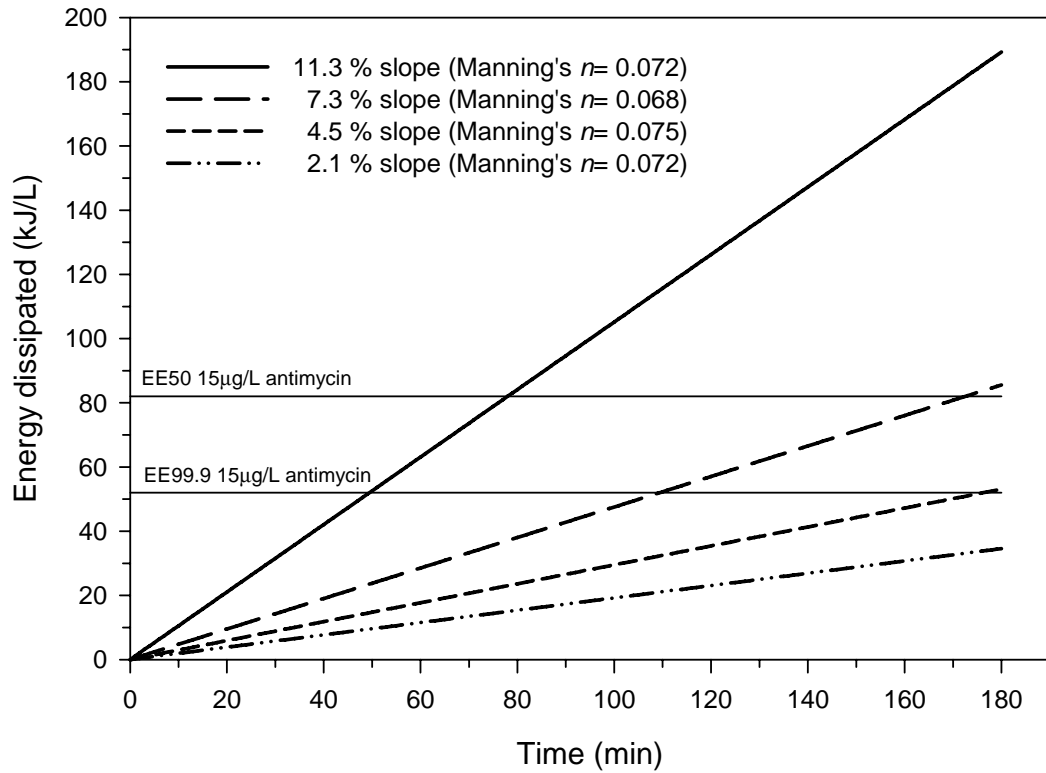


Figure 3.5. Accumulation of turbulence in four channel types. Diagonal lines represent accumulated turbulence exposure; horizontal lines represent the level of turbulence exposure that would cause 15 µg/L antimycin to be 50% or 99.9% effective. Areas below the horizontal lines represent conditions at which 15 µg/L antimycin would remain more than 99.9% or 50% effective.

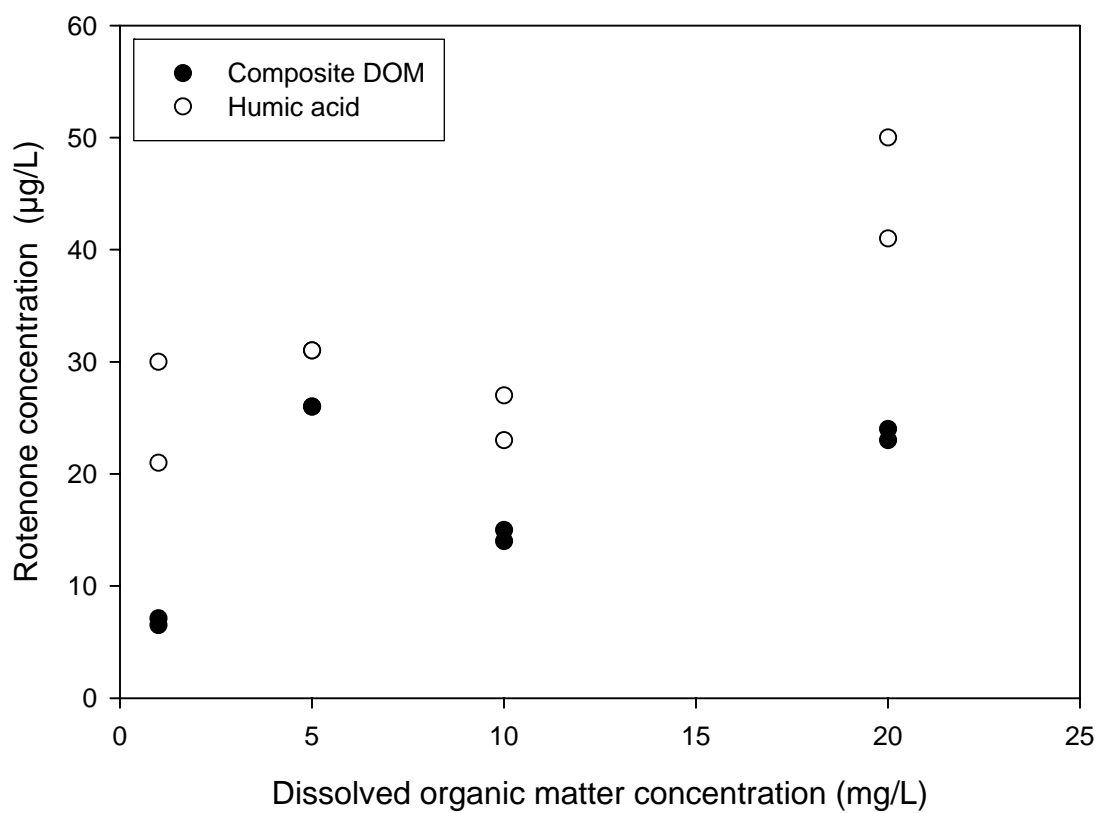


Figure 3.6. Residual rotenone concentration as a function of dissolved organic matter concentration. Initial nominal rotenone concentration was 15 µg/L. Open dots represent measurements of rotenone when exposed to humic acid. Solid dots represent measurements of rotenone when exposed to a composite mixture of DOM.

CHAPTER 4

ROTENONE PERSISTENCE MODEL

Abstract

The efficient and effective use of rotenone is hindered by its unknown persistence in streams. Environmental conditions degrade rotenone, but current label instructions suggest fortifying the chemical along a stream based on linear distance or travel time rather than the environment. My objective was to develop models that use measurements of environmental conditions to predict rotenone persistence in streams. Detailed measurements of UV radiation, water temperature, dissolved oxygen, total dissolved solids, conductivity, pH, oxidation reduction potential, substrate composition, amount of organic matter, channel slope, and travel time were made along stream segments located between rotenone treatment stations and cages containing bioassay fish in six streams. The amount of fine organic matter, biofilm, sand, gravel, cobble, rubble, small boulders, slope, pH, total dissolved solids, oxidation reduction potential, the amount of light reaching the stream, the energy dissipated, discharge, and cumulative travel time were each significantly correlated with fish death. Using logistic regression, measurements of environmental conditions were paired with the response of bioassay fish to develop a model that predicted the persistence of rotenone in streams. This model was validated using data from two additional stream treatment reaches. Rotenone persistence was conservatively predicted by a model that used travel time, rubble, and oxidation reduction potential. When this model predicts a probability less than 0.95, rotenone applicators can expect

incomplete eradication and should plan on fortifying rotenone concentrations. The significance of travel time has been previously identified and is currently used to predict rotenone persistence. However, rubble substrate, which may be associated with degradation by adsorption and volatilization in turbulent environments, was not previously considered. This model is applicable only to montane streams. When applied, it recommends substantially different drip-station spacing than current application guidelines do.

Introduction

Piscicides (e.g., rotenone and antimycin) are commonly used to eradicate nonnative and nuisance fish species from streams. However, variation in the persistence of piscicides is the primary factor leading to ineffective and inefficient eradication projects. The American Fisheries Society Rotenone Stewardship Program and the Rotenone and Antimycin Use in Fisheries Management course identify environmental conditions that potentially contribute to piscicide detoxification; however, these programs provide little guidance for maintaining piscicide effectiveness under exposure to degrading environmental conditions. To maintain effectiveness, project personnel must often use professional experience to identify where piscicides may become nontoxic in streams. Increasing the concentration of piscicides is the most common response of project personnel when faced with incomplete knowledge of piscicide detoxification. However, over-application of piscicides can cause unnecessary mortality of non-target organisms, and wastes piscicides, effort, and resources (McClay 2000; Finlayson et al. 2010; Vinson et al.

2010). Recommendations for application techniques that minimize over-application of piscicides, while maintaining a high probability of fish eradication, have not been developed because the necessary data have not been collected. To date, there has been one published study of antimycin degradation as a function of stream gradient (Tiffan and Bergersen 1996) and no studies of rotenone persistence in streams.

A statistical model that predicts the persistence of piscicides would increase the efficiency of piscicide application. Ideally, this model would use easily measured characteristics to identify detoxification rates along a proposed treatment reach. This information would allow project personnel to apply the proper concentration of piscicides and ensure drip station spacing is appropriate to prevent areas of nontoxic water. Appropriate dosing and spacing increases the efficiency and efficacy of fish eradication using piscicides. The objective of this research was to develop a statistical model to predict the persistence of rotenone in streams.

Methods

Study Streams

Eight stream reaches in the western United States were used to develop and test a rotenone persistence model that was effective under a variety of environmental characteristics. All study streams had either ongoing or planned eradication projects. Streams where piscicide use was already planned provided ideal study sites because they required no additional permitting and bioassay fish could often be collected locally. Additionally, incomplete fish kills associated with this study were acceptable as total eradication was planned in the near future.

Treatment reaches were single channel stream reaches where discharge at the lower end of the reach was 90 to 110% of that at the beginning of the reach to avoid the confounding effects of groundwater or tributary dilution. Treatment reaches included an application site (uppermost point of treatment reach where rotenone was administered) and a series of bioassay sites placed at 100-m intervals downstream from the application station. The number of sites varied among treatment reaches because the lengths of reaches varied (Table 4.1).

Environmental Characteristics

Environmental characteristics of each treatment reach (stream) were measured. Environmental characteristics (e.g., gradient, canopy cover, and substrate) varied among treatment reaches, but treatment reaches were relatively homogeneous. Treatment reaches were marked at 100 m intervals and ten sample sites were spaced 10-m apart within each 100-m interval. Measurements of foliage cover, light penetration, and aspect were made to characterize the influence of ultraviolet (UV) radiation. Foliage cover was measured using a concave densitometer. Densitometer readings were taken while the observer stood in the center of the stream faced upstream, downstream, and toward each bank for a total of four readings. These four readings were averaged to calculate the average canopy cover at each sample site. Light measurements were made at each sample site and averaged across the ten sample sites between each bioassay cage. The stream aspect was measured as the compass heading in an upstream direction at each sample site. These measurements were standardized to measure relative southern exposure of a stream by subtracting all compass headings greater than 180° from 360°. This scales compass headings

from a minimum southern exposure (i.e., north; 0°) to a maximum southern exposure (i.e., south; 180°). After conversion, the ten measurements between each bioassay cage were averaged. Water temperature, dissolved oxygen, total dissolved solids (TDS), conductivity, pH, and oxidation reduction potential (ORP) were measured using a YSI model 556 multi-parameter meter (Yellow Springs Incorporated, Yellow Springs, Ohio) at the rotenone application station.

Substrate was classified using a modified Wentworth scale (Wentworth 1922; Platts et al. 1983) as silt (< 0.2 mm diameter), sand (0.2-6.3 mm diameter), gravel (6.4-76.0 mm diameter), cobble (76.1-149.9 mm diameter), rubble (150.0-303.9 mm diameter), small boulder (304.0-609.9 mm diameter), large boulder (> 609.9 mm diameter) and bedrock (consolidated parent material). Organic matter was classified as dead or alive. Dead organic matter was classified as fine (< 1 mm), coarse (> 1 mm but not wood; e.g., leaves) or wood (> 1 mm and wood; e.g., sticks, logs). Live organic matter was classified as biofilm (i.e., living material on the surface of substrates) or macrophyte (i.e., rooted plant matter). The percent coverage of substrate and organic matter was visually estimated for the 10 m between each sample site (Gibson et al. 1998).

Travel time was measured by introducing a plume of fluorescent dye (Bright Dyes, FLT Yellow/Green; Kingscote Chemicals, Miamisburg, Ohio) into the stream and recording the number of minutes it took the middle of the plume to travel between bioassay sites. Slope was measured using a clinometer. Ten measurements between each bioassay site were averaged to determine average slope between bioassay sites. Travel time and slope measurements were combined to form a

measurement of energy dissipated by turbulence around substrates. Energy dissipated was calculated as:

$$E_{\Delta} = E_{k(unobs)} - E_{k(obs)},$$

where E_k was calculated as:

$$E_k = 1/2 mv^2,$$

where E_k is the amount of energy in joules, m is the mass of the object in kilograms and v is the velocity in meters per second. Manning's equation was used to estimate the velocity of water moving through an unobstructed channel (i.e., at the same slope but without substrates). Manning's equation was denoted as:

$$V = \frac{1}{n} R_h^{\frac{2}{3}} S^{\frac{1}{2}},$$

where V is the cross-sectional average velocity (m/s), n is Manning's roughness coefficient (i.e., 0.001 for smooth pipes; Sturm 2001), R_h is the hydraulic radius (m), and S is slope of the water surface (m/m). Energy dissipation was calculated for the stream section between each bioassay site.

Bioassay and Rotenone Application

Bioassay fish (hereafter fish) were used as bioassay organisms to evaluate rotenone persistence within treatment reaches. Direct measurement of rotenone concentration requires special laboratory equipment, is costly, and logistically difficult. However, fish can often be found on-site or in proximal water bodies and provide the necessary level of resolution (i.e., dead or alive) to determine rotenone toxicity. Use of fish as bioassay organisms is the most appropriate way to determine the persistence of piscicides at remote locations. Using fish to determine the

effectiveness of piscicides is recommended by the manufacturers of rotenone and antimycin (Finlayson et al. 2000) and in the Rotenone and Antimycin Use in Fish Management course.

Two sources of bioassay fish were used. Where eradication was planned for the future, bioassay fish were collected on-site using a backpack electrofisher. Hatchery fish were used in streams where eradication was ongoing and it was critical that any fish entering the stream was of the species being restored. Fish of hatchery origin were acclimated to stream water for 24 h prior to rotenone application. Three bioassay cages were placed out of the thalweg every 100-m within the treatment reach. The total number of cages was usually different in each treatment reach because lengths of treatment reaches varied. Each bioassay cage contained three bioassay fish. Bioassay fish were segregated by size to minimize antagonistic interactions. Rotenone toxicity does not vary much among fish of different sizes (Chapter 2) or between rainbow trout *Oncorhynchus mykiss* and brook trout *Salvelinus fontinalis* (Marking and Bills 1976). Fish in bioassay cages were considered representative of the fish in a given stream reach. Bioassay fish were placed upstream (within 10 m) of the application site to determine if the water quality in the stream was suitable for fish (i.e., negative control). Bioassay fish were placed downstream (within 10 m) from the application site to determine if rotenone minimally affected by environmental characteristics was toxic to fish (i.e., positive control). Rotenone was applied at 25 µg rotenone/L of water for 6 h at the application site (Finlayson et al. 2010). Bioassay fish were checked for mortality 48

hours after rotenone application. The response of each fish was recorded as either dead or alive.

Modeling Mechanics

Measurements of environmental characteristics were combined with the response of bioassay fish to develop a rotenone persistence model. Independent variables were environmental characteristics measured along treatment reaches. The dependent variable was the response of bioassay fish (i.e., rotenone toxicity).

Data from six treatment reaches were used to develop candidate rotenone persistence models. Univariate logistic regression was used to identify independent variables (i.e., environmental characteristics) that had a significant relationship ($p < 0.25$) with the dependent variable. I used a higher p -value than traditional to balance between excluding a potentially helpful variable from model selection (type I error) and including a potentially useless variable in model selection (type II error).

Variables with no significant relationship were excluded from further analyses. A Pearson correlation matrix was used to identify highly correlated independent variables ($R > 0.85$). For each pair of correlated variables, the variable describing a condition known to degrade piscicides was retained. From the remaining variables a candidate model was selected using a stepwise selection procedure (selection criteria $P < 0.10$). First-order interactions were included during the model building procedure. Parameter estimates were calculated using generalized estimating equations in SAS software (PROC GENMOD; SAS version 9.2; SAS Institute Inc., Cary, North Carolina). This procedure corrects for the clustered and correlated nature of longitudinal data providing improved standard errors of parameter estimates over

traditional logistic regression (Allison 1999). Candidate model fit was evaluated using the generalized coefficient of determination (R^2) and Akaike's information criterion (QICu; Zheng 2000; Pan 2001). This identified models that balanced the tradeoff between model fit and parsimony (Mac Nally 2000).

Model Development and Testing

Two steps were used to evaluate models. First, a leave-one-out procedure was used to identify candidate models and assess the potential for model application. Models were developed (using stepwise selection) with data from five treatment reaches then tested on a sixth treatment reach. This procedure was repeated six times leaving out a different treatment reach each time. The feasibility of applying a rotenone persistence model was explored using predictions on the sixth stream.

Second, the predictive ability of candidate models was determined using data from two additional streams. Because these data were not used to develop candidate models, they represent an independent test of each candidate model's predictive ability. Data collected in these two independent reaches represent data that would be collected prior to piscicide application. The eradication probability was calculated for these data using the candidate models and compared to the actual eradication outcome. Model fit to the additional data was compared using the generalized R^2 (Hosmer and Lemeshow 2000). Receiver operating characteristic (ROC) curves were used to measure the predictive ability of the final model. For ROC analysis, the response of fish was converted to a binary outcome (i.e., all fish die or at least one fish survives) and the model predictions were compared to the actual outcomes (Hosmer and Lemeshow 2000; Gönen 2007). These comparisons were made at a

series of prediction probabilities to determine which probability cutoff point provided the most correct predictions. The cutoff with the first false positive was used to determine a conservative estimate of where piscicides will become ineffective. Models were compared using the area under curve (AUC) statistic (Hosmer and Lemeshow 2000; Gönen 2007).

I evaluated how many of the 10 sites within a 100 section needed to be sampled for environmental characteristic measurements that were included in the final model (e.g., % rubble coverage). I randomly selected sets of 2 through 10 sites (i.e., 2 sites, 3 sites, 4 sites, etc.) and calculated the mean of these data sets. I then plotted the mean and confidence interval against the size of the data set to determine the fewest number of sites that can be sampled without increasing the confidence interval around the estimate of the environmental characteristic.

Results

Streams included in this study were all small, montane streams. Streams varied in pH from 7.64 to 8.56, dissolved oxygen from 7.69 to 11.77 mg/L, temperature from 7.00 to 17.21°C, TDS from 0.033 to 1.860 g/L, conductivity from 33 to 227 $\mu\text{S}/\text{cm}$, ORP from 20 to 215 mV, and discharge from 0.0013 to 0.1746 m^3/s (Table 4.1). Most streams were in forested areas with average canopy cover varying from 21 to 74% (Figure 4.1). Also, most streams had moderate slope of less than 4% whereas reaches of Lower Carpenter Creek and Clear Creek approached 8% (Figure 4.1). One hundred meter travel time was less than 30 min in most reaches except in low gradient, meandering reaches (e.g., East Fork Cherry Creek and portions of Kill

Brennan Creek; Figure 4.1). Substrate of most streams was dominated by gravel or cobble with sand and rubble present (Figure 4.2).

Fifteen stream characteristics were significantly associated with rotenone persistence and included in initial model selection. The amount of fine organic matter (-), biofilm (+), sand (-), gravel (-), cobble (+), rubble (+), small boulders (+), slope (+), pH (+), TDS (+), ORP (-), the amount of light reaching the stream (+), the energy dissipated (+), discharge (-), and cumulative travel time (+) were each significantly correlated with rotenone persistence. The amount of biofilm and the amount of rubble were both correlated with the amount of small boulders; the amount of small boulders was removed from further analyses.

East Fork Cherry Creek was physically unlike the other streams in that it is a low-gradient, meandering stream with fine substrate and an open canopy. Models developed by leaving data from East Fork Cherry Creek out and predicting on it performed poorly. East Fork Cherry Creek was deemed an outlier, and removed from further analyses.

Four unique models were developed to predict rotenone persistence using the remaining five streams. Cumulative travel time and the percent rubble substrate were common predictors occurring in all four models (Table 4.2). A model that also included oxidation reduction potential was selected in two streams. Total dissolved solids, cobble substrate, and slope were also used in models (Table 4.2).

Models predicted a lower probability of eradication than actually occurred in three of four cases. Two models appeared to be too conservative, predicting low probability of eradication at locations where rotenone was still effective (Figure 4.3).

The other three models were more accurate, with actual responses falling within, or close to, 95% confidence intervals. A common and conservative trait among models was that at a predicted eradication probability of 0.95 the actual proportion of fish killed was 1.0 (Figure 4.3).

All four unique models were considered as final models. The top three models had R^2 greater than 0.50. The travel time + rubble + ORP model had the lowest QIC_u value and the other three models had similar values when tested against data from all five streams (Table 4.3). The travel time + rubble + slope model was a better fit to data from the five prediction streams ($R^2 = 0.712$) than a model that also included cobble ($R^2 = 0.614$; Table 4.3). The travel time + rubble + ORP model had a lower QIC_u but also lower R^2 (0.587) compared with the travel time + rubble + TDS model.

When the models were validated on data from two additional streams, the travel time + rubble + TDS model had the best fit ($R^2 = 0.875$; Table 4.4). However the travel time + rubble + ORP model had similar fit and narrower confidence intervals. The area under the ROC curve was higher than 0.90 for all models indicating “outstanding discrimination” (Hosmer and Lemeshow 2000). The lowest false positive cutoff was 0.77 for the top model but 0.95 for the travel time + rubble + TDS model (Table 4.4). This indicates that a cutoff of 0.95 is a conservative cutoff for predicting when fish at a site may survive.

Discussion

The probability of eradication of trout in a stream with rotenone can be estimated using travel time, the amount of rubble and the ORP. When making predictions of piscicide persistence, a model prediction of 0.95 is a reasonable cutoff for indicating where some fish will survive. These results provide empirical evidence for using travel time as a predictor of rotenone persistence, and suggest that electron transfer (i.e., oxidation or reduction) is also important in degradation of rotenone, but most importantly show that including environmental characteristics improves predictive ability.

These results are concordant with other studies of piscicide persistence. Controlled experiments showed that rotenone and antimycin degrade with exposure to turbulence (Chapter 3), but the only other study (Tiffan and Bergersen 1996) on the persistence of piscicides in streams tested the effects of high gradient reaches on antimycin. Antimycin degrades in high gradient reaches more quickly than low gradient reaches. Whereas my study used rotenone rather than antimycin, the inclusion of slope in the final model suggests that rotenone also degrades more rapidly in high gradient reaches. Sunlight and temperature also affect piscicide toxicity (Chapter 3; Gilderhus et al. 1986); however, temperature, southern exposure, canopy cover, and sunlight were not included in the highest ranked persistence models. Previous research also identified pH as significantly affecting rotenone (Brooks 1961 in Schnick 1974b) and antimycin toxicity (Berger et al. 1969; Lee et al. 1971; Marking 1975). Oxidation reduction potential is closely linked to pH but is a

better descriptor of the ability to oxidize or reduce a chemical in solution (Wetzel 2001).

Inclusion of rubble substrate in the model suggests that volatilization is the primary factor driving piscicide detoxification in streams. Rubble is most common in high gradient reaches with turbulent conditions. The volatilization rate of chemicals in streams is directly proportional to the oxygenation rate (Tsivoglou and Neal 1976; Rathbun 1990). Several models have been developed to predict reaeration rates (reviewed by Jah et al. 2004) but all include some combination of velocity, slope, depth, and channel roughness. The results of my research are concordant with reaeration models in that the final three models included measurements of velocity (travel time), slope, and channel roughness (e.g., percent rubble; Table 4.4). This similarity suggests that volatilization of acetone is an important factor in reducing piscicide persistence in streams, especially those with high gradients.

Piscicide applicators commonly increase piscicide concentrations, rather than reduce the distance between drip stations, when confronted with a situation where piscicides might become ineffective. This decision unintentionally increases mortality of non-target organisms (Vinson et al. 2010). Currently, rotenone applicators space drip stations 805 to 3220 m (0.5 to 2.0 miles) apart, but not less than 60 minutes or more than 120 minutes apart (label recommendation; Prentiss, Inc., 2002). The average distance rotenone traveled in this experiment, and still killed fish, was 675 m (minimum = 200, maximum = 1700) and 83 minutes (minimum = 15, maximum = 143). Therefore, the shortest values of both linear distance and time were well below the minimum recommended drip station spacing and the variation

among reaches in the distance rotenone persisted was considerable. These two observations suggest that a uniform recommendation for drip station spacing that does not account for local stream characteristics is inappropriate. Rather, a model that incorporates a measure of distance (e.g., travel time), local stream characteristics (e.g., rubble), and water chemistry is a better predictor of rotenone persistence. This point is highlighted by the results of models developed for Upper and Lower Carpenter Creek and Clear Creek (Figure 4.3). Current spacing recommendations (805 m or 60 min) would place stations farther apart than necessary, but a model using distance and stream characteristics was able to accurately predict the point at which rotenone was less than completely effective.

The model of travel time + rubble + ORP should only be applied to small, freestone, montane streams. Application outside of this description will provide misleading results. For example, the model tested on East Fork Cherry Creek, a low-gradient meadow stream, used information from five montane streams to predict rotenone persistence. The model provided nonsensical predictions of eradication probability.

Further research on piscicide persistence in streams should include modeling that incorporates drainage networks, ground water influences, and other stream types. The models described herein were developed in simple, single-channel reaches between stream junctions. Piscicide persistence may not be uniform in stream networks, especially where streams join or in reaches heavily influenced by groundwater inputs. For example, if a rotenone-treated tributary joins a rotenone-treated stream, the travel time of rotenone in the tributary may be much shorter than

of the rotenone in the stream. Whereas the initial rotenone concentration may be the same in both, the rotenone that traveled farther may degrade sooner.

Similar models would be valuable for different types of water bodies.

Additional models may be appropriate for lakes, low-gradient meandering streams, or larger rivers. For example, a model that incorporates depth and dissolved organic carbon may be appropriate for lakes and ponds (Engstrom-Heg and Colesante 1979). A model that incorporates stream pH and the amounts of biofilm and rubble may be more appropriate for low-gradient meandering streams (e.g., East Fork Cherry Creek).

Model Application

Results of this research should be applied to montane stream reaches that are candidates for fish eradication by first collecting information on the stream to be treated, developing predictions on piscicide persistence, and finally comparing the predictions to responses of bioassay fish. The stream reach should be surveyed with detailed calculations of the 100-m travel time and the coverage of rubble substrates. These measurements do not need to be carried out at the same time but doing so would minimize the number of visits to the stream. Travel time information should be collected by applying a plume of dye to a stream and measuring the amount of time the plume takes to travel through every 100-m reach. The percentage of rubble covering the stream bottom should be estimated for 10-m lengths of stream. Whereas only 5 10-m measurements are necessary to accurately estimate the rubble coverage of a 100-m reach as judged by convergence of estimates calculated using different numbers of measurements, more measurements will improve the estimate. Therefore,

estimation of all ten 10-m lengths is prudent if time permits. With practice, rubble estimation in all ten 10-m lengths will likely take less time than it takes for the dye to travel the same 100 m. The ORP of the stream should be measured under stream conditions as close to the application conditions as possible. Data should be organized such that the measurements entered into the model represent the travel time from the beginning of the reach (i.e., from the drip station) to the point where the estimate is being made. For example, rubble estimates from the drip station to the point where the estimate is being made should be averaged and entered into the model. The ORP is measured just once and does not need to be averaged. For an estimate 300 m below a drip station, the travel time (min) from the drip station to the 300 m location, the average rubble coverage (%) for the same area, and the stream ORP (mV) should be entered into the following formula:

$$P(\text{eradication}) = \frac{e^{(7.9116 - 0.1341 \times \text{Rubble substrate} + 0.0012 \times \text{ORP} - 0.0489 \times \text{Travel time})}}{1 + e^{(7.9116 - 0.1341 \times \text{Rubble substrate} + 0.0012 \times \text{ORP} - 0.0489 \times \text{Travel time})}}$$

Predictions of eradication probability should be made in a downstream series. At the first location where the probability of eradication is less than 0.95, it is appropriate to fortify the concentration of rotenone by placing another drip station.

Table 4.1. Reach length, number of bioassay sites used, water chemistry, and discharge of treatment reaches included in this study. Jumping Creek and Graves Creek were used for model testing; all other treatment reaches were used for model development.

Stream	Reach length (m)	Bioassay sites	pH	Dissolved oxygen (mg/L)	Temperature (°C)	Total dissolved solids (g/L)	Conductivity (µS/cm)	Oxidation reduction potential (mV)	Discharge (m ³ /s)
Upper Carpenter	500	6	7.79	9.12	17.19	0.088	109	184	0.0015
Lower Carpenter	500	6	7.79	9.00	17.21	0.095	111	176	0.0014
Clear	1100	12	8.09	11.40	7.73	1.860	180	115	0.0430
East Fork Cherry	400	5	8.56	10.30	16.62	0.156	201	116	0.0013
LaBarge	2100	22	8.44	11.77	11.62	0.199	227	165	0.0308
Kill Brennan	1200	13	6.88	11.24	7.00	0.033	33	215	0.1746
Jumping	1300	14	8.00	11.00	15.00	0.100	200	180	0.0267
Graves	1000	11	7.64	7.69	16.03	0.094	120	20	0.0716

Table 4.2. Covariates selected by stepwise selection when a model based on data from four treatment reaches was built and tested on the fifth test stream. X represents inclusion of a covariate in a model.

Covariate	Test stream				
	Upper Carpenter	Lower Carpenter	Clear	LaBarge	Kill Brennan
Travel time	X	X	X	X	X
Rubble substrate	X	X	X	X	X
Oxidation reduction potential	X	X		X	
Slope					X
Total dissolved solids			X		
Cobble		X			

Table 4.3. Goodness-of-fit statistics for models predicting probability of eradication using rotenone in montane streams. Statistics measure model fit to data from all five streams used in model development (ORP = oxidation reduction potential; TDS = total dissolved solids).

Model	R^2	QIC _u	Δ QIC _u
Travel time + Rubble + ORP	0.587	13.9456	0.0000
Travel time + Rubble + Slope	0.712	21.4378	7.4922
Travel time + Rubble + ORP + Cobble	0.614	26.2097	12.2614
Travel time + Rubble + TDS	0.696	28.2357	14.2901

Table 4.4. Goodness-of-fit statistics for models tested against validation data from Jumping Creek and Graves Creek. Area under the curve (AUC) was calculated from receiver operating curves.

Model	R^2	AUC (95% CI)	First false positive cutoff
Travel time + Rubble + ORP	0.815	0.9895 (0.9603, 1.0187)	0.77
Travel time + Rubble + Slope	0.462	0.9053 (0.7763, 1.0342)	0.95
Travel time + Rubble + ORP + Cobble	0.586	0.9158 (0.7951, 1.0365)	0.82
Travel time + Rubble + TDS	0.875	0.9579 (0.8809, 1.0349)	0.91

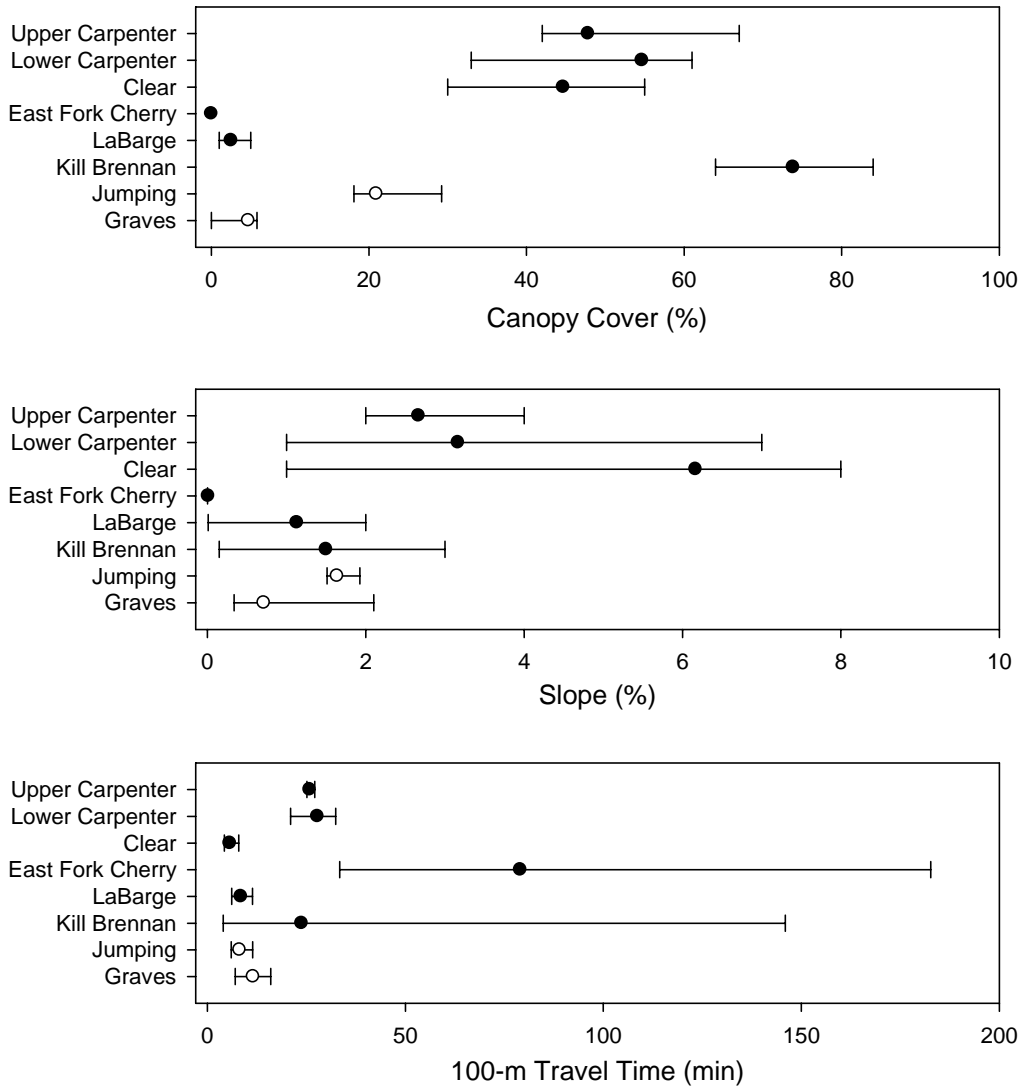


Figure 4.1. Physical characteristics of study streams. Dot represents mean and whiskers represent range of measurements. Filled dots indicate treatment reaches used for model development and open dots indicate treatment reaches used for model testing.

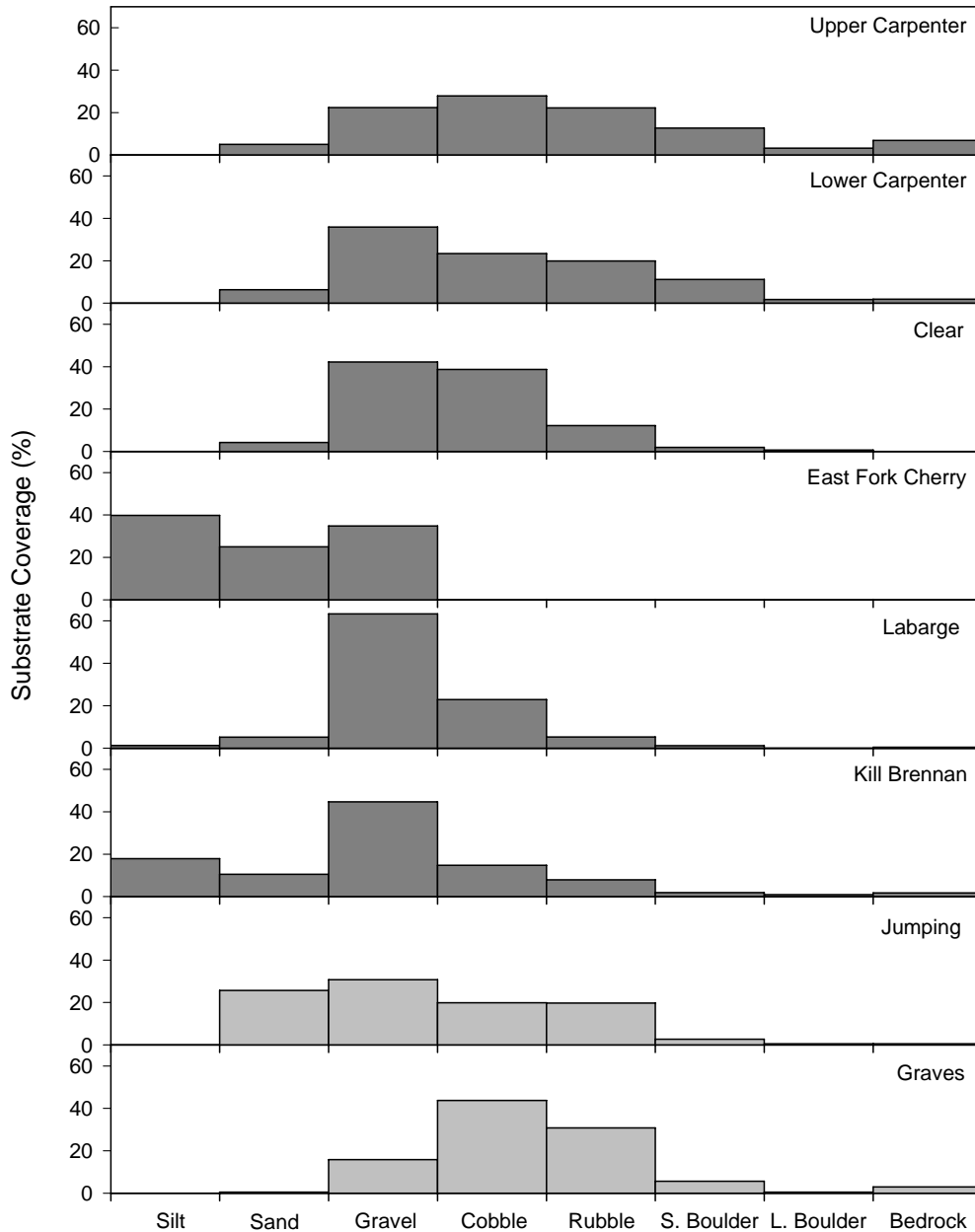


Figure 4.2. Percent coverage of each size class of substrate in study streams. Substrate was classified using a modified Wentworth scale as silt (< 0.2 mm diameter), sand (0.2-6.3 mm diameter), gravel (6.4-76.0 mm diameter), cobble (76.1-149.9 mm diameter), rubble (150.0-303.9 mm diameter), small boulder (304.0-609.9 mm diameter), large boulder (> 609.9 mm diameter) and bedrock (consolidated parent material). Dark shading indicates streams used for model development and light shading indicates streams used for model testing.

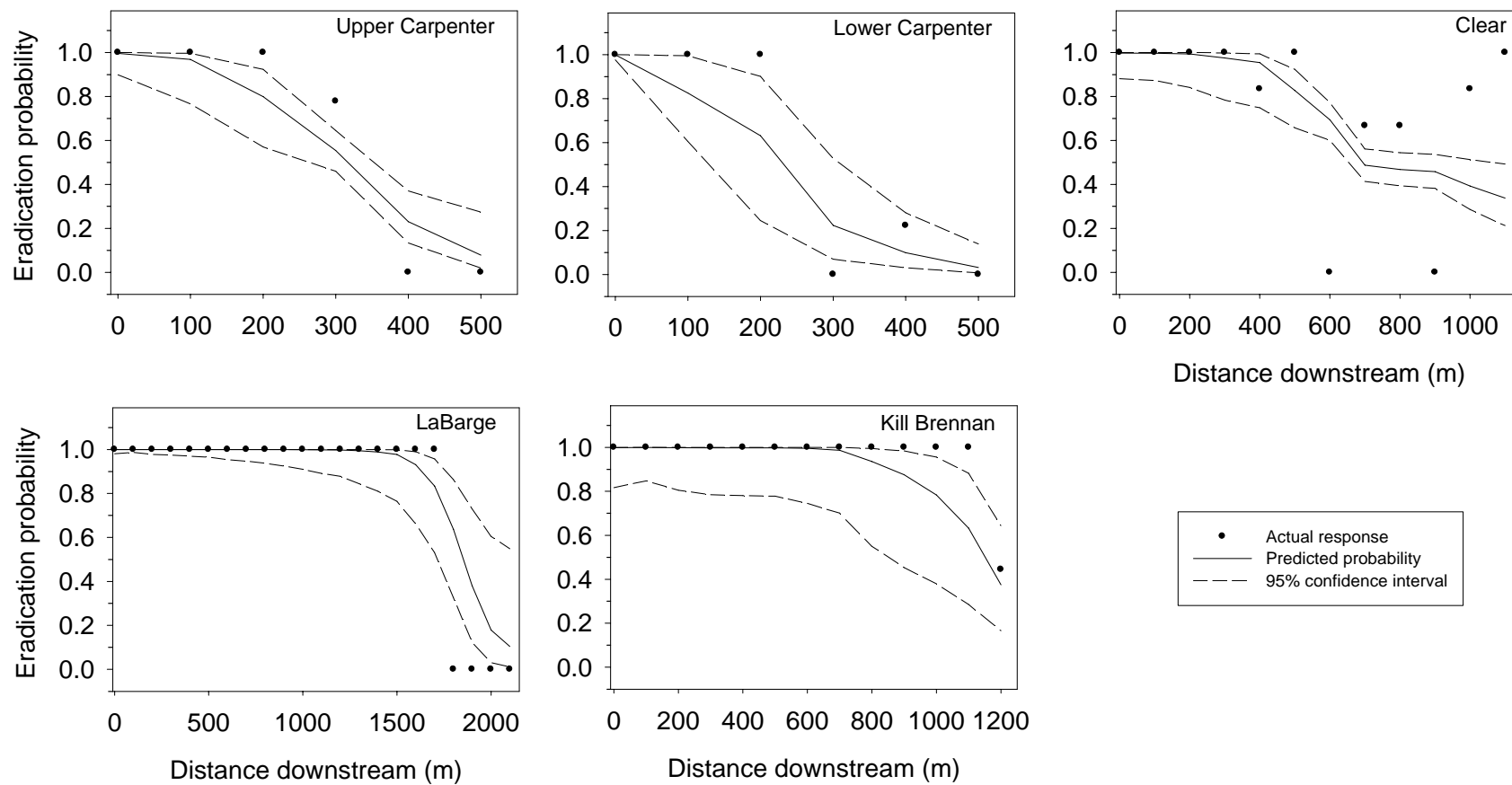


Figure 4.3. Eradication probability plotted against distance downstream (m) for models calculated in model development. Points represent response of bioassay fish. Solid line represents the predicted probability of eradication and dashed lines indicate 95% confidence interval.

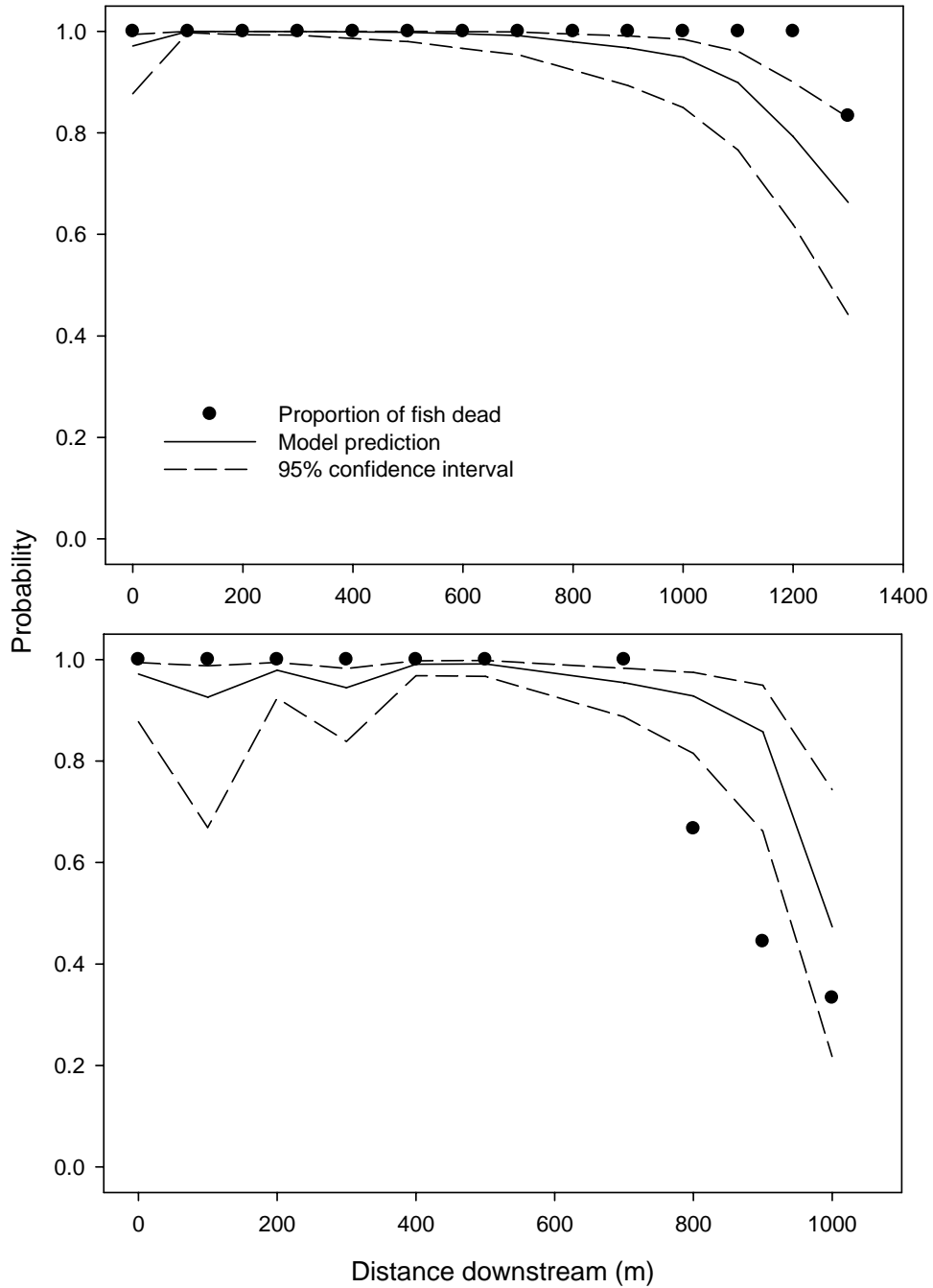


Figure 4.4. Eradication predictions and response of fish using a model of travel time, rubble, and oxidation reduction potential for validation data from Jumping Creek and Graves Creek. Points represent actual response of fish, solid lines represent model prediction, and dashed lines indicate 95% confidence intervals.

CHAPTER 5

TRANSVERSE MIXING OF SIMULATED PISCICIDES IN SMALL
MONTANE STREAMSAbstract

Thorough mixing of piscicides into receiving waters is important for efficient and effective fish eradication. However, no guidance exists for the placement of drip stations with respect to mixing. Salt (NaCl) was used as a tracer to measure the mixing rates of center versus edge applications in riffle/pool, straight, and meandering sections of montane streams. The tracer was applied at either the center or the edge of a channel and measured with a conductivity meter across a downstream grid to determine the distances at which transverse mixing was complete. No advantage was accrued by applying piscicides in specific channel types as transverse mixing distance did not differ among them. However, mixing distance was significantly shorter at center applications. Chemical plumes should be overlapped by 10 stream widths when using center applications and 20 stream widths for edge applications to ensure thorough piscicide mixing.

Introduction

Eradicating fish using piscicides is useful when removing exotic species, restoring threatened or endangered species, and controlling disease (Meronek et al. 1996; McClay

2000; McClay 2002). Factors affecting the performance of piscicides include water chemistry, fish resistance to the chemicals, and application methods (Ling 2003). Piscicide applicators have provided varied and detailed descriptions of piscicide application apparatuses (e.g., Finlayson et al. 2000; Spateholts and Lentsch 2001; Thompson et al. 2001); however, no published guidelines currently exist for placement of these devices to ensure efficient and effective mixing of piscicides into streams.

Complete mixing of chemicals through the water body to be treated is required for complete eradication. Stream piscicide applications use multiple drip stations placed at intervals along a stream to deliver liquid formulations of piscicides. The drip stations are placed at intervals to fortify the concentration of piscicide and prevent areas of nontoxic water. Fish can detect some liquid and powdered formulations of rotenone and avoid treatment by seeking nontoxic water (Hogue 1999). Thus, plumes of piscicide treated water from drip stations must be overlapped such that the piscicide mixes transversely preventing areas of nontoxic water.

Transverse mixing is the horizontal movement of a chemical across a stream channel and is influenced by stream characteristics. It can be predicted using empirical equations derived from data gathered in large rivers (e.g., the Missouri) and laboratory flumes (Rutherford 1994; Boxall et al. 2003; Jeon et al. 2007). The equations use coefficients that are assumed to remain constant transversely and longitudinally (e.g., shear velocity, width, depth, and sinuosity; Rutherford 1994). In natural settings, and particularly in montane streams, the assumption of constant-coefficients cannot be met. Channel morphology, bed form, and bed roughness (e.g., substrates) cause variations in

shear velocity, width, and depth, thereby increasing transverse mixing rates. For example, roughness within the channel and complex channel morphology caused faster transverse mixing than in less complex channels of low gradient streams in Iowa (Heard et al. 2001). Piscicide applications commonly take place in high-gradient montane streams. These streams are characterized by rough beds and frequent changes in channel type (e.g., riffle-pool sequences). However, no studies of transverse mixing have been conducted in montane streams and transverse mixing rate estimates do not exist for them.

Understanding transverse mixing in small montane streams will help make piscicide application more efficient and effective. In the absence of clear guidelines, piscicide drip stations are commonly placed at locations that are most convenient for access by the piscicide applicator. The goal of this research was to better understand transverse mixing in montane streams such that guidelines describing drip station placement can be established. The objectives were to 1) compare transverse mixing distance among channel types, 2) compare transverse mixing distance between center channel and edge applications, and 3) develop a simple technique to rapidly estimate transverse mixing distance.

Methods

This study was carried out on the North Fork of Spanish Creek and the East Fork of Cherry Creek, Madison County, Montana. Three sites were chosen in riffle/pool, straight, and meander channel types for a total of nine study reaches. Riffle/pool reaches alternated between narrow-shallow riffles and wide-deep pools. Straight channels were

characterized by more uniform width and depth profiles. Meander reaches were relatively uniform in width and depth but sinuous in planview. Discharge was measured at each site by measuring water velocities at 0.6 of the total water depth at ten locations across the stream using an electro-magnetic flow meter (Marsh McBirney model 2000, Frederick, Maryland).

A saturated solution of sodium chloride (NaCl) was applied to the stream at the upstream end of each study reach to characterize transverse mixing. The tracer was applied at the edge and then at the center of the stream allowing sufficient time for the solution to flush from the study reaches between trials. Sodium chloride was used as a tracer because NaCl concentration can be measured easily with a conductivity meter. Sodium chloride solution was mixed on-site in a 200-L drum and applied to the stream using a battery-powered pump.

The tracer was measured along a series of 20 downstream cross sections. Cross sections ranged from 1.0 to 4.5 m apart and were spaced one channel width apart, similar to Jeon et al. (2007). Study reaches ranged from 20 m to 90 m. Ten points were evenly spaced across the channel along each cross section to form a grid of measurement points downstream from the application point. Conductivity was measured at each point in the grid using a YSI model 556 multi-parameter meter (Yellow Springs, Inc., Yellow Springs, Ohio) to map the tracer plume. Conductivity measurements along each cross section were compared to determine the distance at which the tracer was uniformly mixed. Uniform mixing was qualified as a less than 1% variation in conductivity measurements within a cross section.

A general linear model was used to determine if significant differences existed in mixing distances among channel types and between center and edge applications. Center and edge applications were considered repeated measures because they occurred within the same stream reach and at the same discharge. Channel type and application location were included as main effects. Stream width was included as a random effect to account for variation in mixing distance caused by stream size. All first order interactions were tested and included in the final model if significant. Student's t-test was used to test for differences in the mixing distance to width ratio between center and edge applications. A significance level of $p = 0.05$ was used in all statistical tests.

Results

Transverse mixing distance was not influenced by channel type but was influenced by application location. Mixing distances varied from 7.5 m for center applications in narrow reaches to 103.0 m for edge applications in wide reaches (Figure 5.1). No significant differences existed among mean transverse mixing distances among channel types (Figure 5.1). Though mixing tended to be faster in meandering reaches.

Transverse mixing distance was significantly shorter at center channel applications (Figure 5.2). The mean transverse mixing distance of center applications (21.0 m; 95% CI = 13.2-28.7) was significantly less than the mean mixing distance of edge applications (41.9 m; 95% CI = 23.8-60.1; Figure 5.2). Edge applications resulted in longer transverse mixing distances than center applications at every site. The mean

ratio of mixing distance to stream width was significantly shorter at center applications (6.8; 95% CI = 4.8-8.8) than edge applications (13.1; 95% CI = 8.9-17.3; Figure 5.2).

Discussion

The secondary currents in montane streams allow chemicals to mix more rapidly than in larger and less turbulent streams and rivers. Meanders and changes in the width or depth of a stream cause secondary currents that disperse chemicals horizontally (Heard et al. 2001; Boxall et al. 2003; Jeon et al. 2007). Therefore, I expected to find longer mixing distances in straight channels where secondary currents are less common and flow is more laminar (Rutherford 1994). However, montane streams are characterized by low width to depth ratios, high gradient, and coarse substrates (Wohl 2000). Substrates often emerged from the water surface in the study reaches, causing turbulent eddies throughout the water column. These secondary currents facilitated transverse mixing even in straight reaches. Secondary currents caused by coarse substrates appear to be more influential to transverse mixing than channel type in montane streams.

Width of the stream and application location directly affect the transverse mixing of chemicals applied to a stream regardless of the amount of turbulence in the channel. Chemicals released at the edge of a stream must travel the full width of the stream to mix evenly. Application of a chemical to the middle of a stream divides in half the horizontal distance the chemical must travel. The mixing distance to width ratio in the center applications was about half that of edge applications (Figure 5.2). Piscicide applicators should apply chemicals at the center of a channel to mix them most efficiently.

Prediction of mixing distance based on stream width allows rapid estimation of transverse mixing distance without complex calculation. Other methods to predict transverse mixing use complex formulae that cannot be calculated without a personal computer. However, multiplying the stream width by 10 at center applications or 20 at edge applications provides a rule of thumb that allows a chemical applicator to rapidly estimate the transverse mixing distance in montane streams. These multipliers provide a margin of error appropriate for estimating transverse mixing when applying piscicides or detoxifying piscicides with potassium permanganate (KMnO_4). Predicting transverse mixing in montane streams has application to research on uptake length of nutrients, influence of groundwater inputs, influence of tributaries, and the area affected by pollution effluent.

A major assumption of tracer applications is that the tracer acts similarly to water and to rotenone or antimycin, both of which are lighter than water (Rutherford 1994). I used a saturated saline solution, which was denser than water, as a tracer. A dense tracer cloud would bias estimates of mixing toward longer distances, providing a larger margin or error. The tracer solution did tend to stay near the bottom within centimeters of the application location but turbulence quickly mixed the solution vertically. No difference in conductivity during ad hoc measurements at the water surface and at the stream bottom was observed except at the application location.

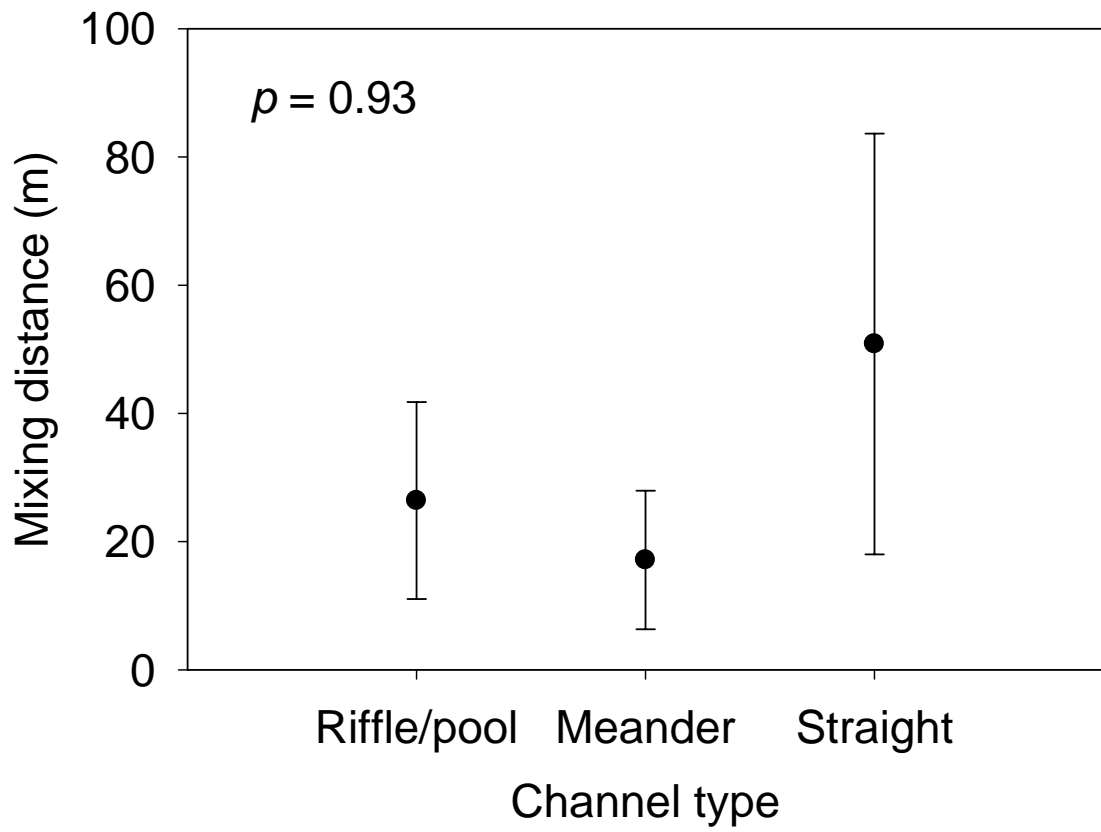


Figure 5.1. Distribution of mixing distances by stream channel type. Points represent means and error bars represent 95% confidence intervals.

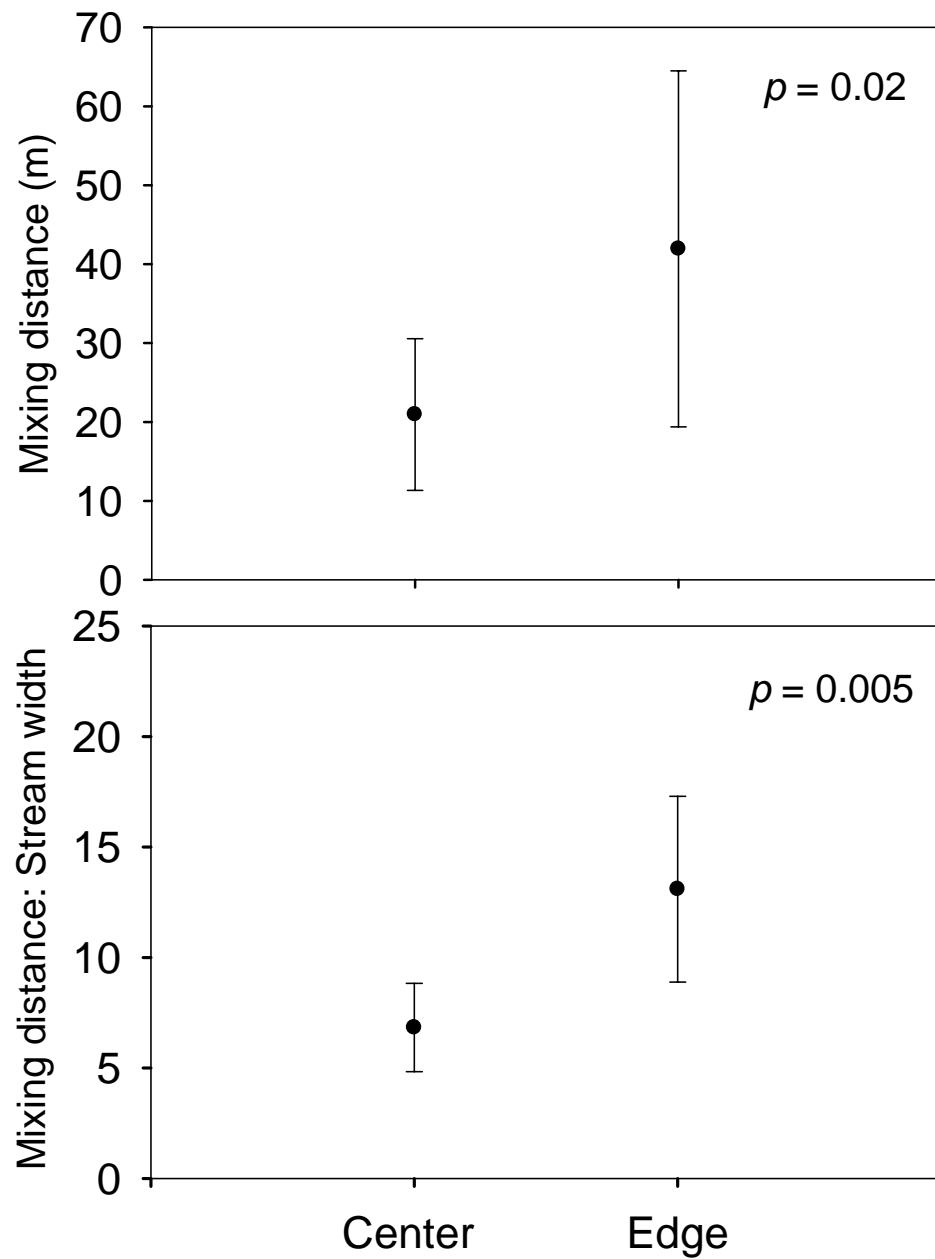


Figure 5.2. Distribution of mixing distances and mixing distance to width ratios by application location. Points represent means and error bars represent 95% confidence intervals.

CHAPTER 6

DISSERTATION SUMMARY

Conservation of native fish often requires eradication of nonnative fish using piscicides (McClay 2000; McClay 2002; Finlayson et al. 2005). Application of these chemicals in the most efficient and effective way is important for the success of conservation efforts but has been hindered by an incomplete understanding of the interactions between the environment and the piscicides. The unknown rate of environmental degradation of piscicides allows for premature degradation and over-application. Premature detoxification allows fish to survive in nontoxic water. Over-application to try and prevent premature degradation is wasteful of resources and causes excessive mortality of nontarget organisms (e.g., invertebrates; Finlayson et al. 2010). Documentation of failed or wasteful piscicide application is rare (e.g., Lentsch et al. 2001; Moore et al. 2005); however, literature reviews on piscicides warn that environmental conditions may cause premature detoxification (Finlayson et al. 2002; Ling 2003). Previous research on antimycin provided guidance for its application in high gradient streams (Tiffan and Bergersen 1996) and in high pH water (Berger et al. 1969; Lee et al. 1971; Marking 1975), but similar information did not exist for sunlight, turbulence, and organic matter. Information describing piscicide persistence after application and exposure to the environment was also lacking. Piscicide applicators knew that the chemicals are affected by the environment and eventually become nontoxic, but lacked a tool for predicting where along a stream environmental exposure

causes the chemicals to become nontoxic. The underlying goal of my research was to fill this gap and provide guidance that will reduce the instances of over-application and premature degradation.

I first used laboratory experiments to isolate the effects of sunlight, turbulence, and organic matter and determine what level of exposure to these environmental conditions affects piscicide degradation (Chapter 3). I showed that dissolved organic matter does not affect piscicide toxicity, but piscicides do degrade with exposure to sunlight and in turbulent conditions. These results are concordant with other research that shows sunlight affects a wide variety of chemicals by exciting electrons directly and destroying the molecule or allowing reactions with other chemicals or water (Andrady et al. 1995; Da Silva et al. 2003; Copinet et al. 2004; Barteles and von Tümpling 2007). The mechanism of piscicide detoxification was not determined, but sunlight likely hydrolyzes or detoxifies piscicides by a photo-assisted reaction with acetone (Larson and Weber 1994). Turbulent mixing of molecules does not affect piscicides; rather, the chemicals are likely detoxified because of acetone volatilization in turbulent environments. Acetone is used to dissolve hydrophobic piscicides into water, but acetone is highly volatile (Hansen and Wilbur 1994). Previous research has shown that acetone volatilizes slowly in quiescent streams (Hansen and Wilbur 1994), but much more rapidly in situations where water is spread into a thin layer or is in droplet form (Giardino et al. 1992; Moya et al. 1999). When commercial mixtures of piscicide (i.e., piscicide and acetone) are mixed into turbulent waters, the acetone volatilizes leaving piscicides to sorb or precipitate from solution. These experiments were helpful in removing the interacting

and confounding effects of multiple conditions to determine if a certain condition affects piscicides and if so at what level of exposure.

Examination of the simultaneous effects of multiple environmental conditions provides a more realistic description of piscicide degradation. I applied rotenone to six streams and monitored the toxicity using regularly spaced bioassay fish (Chapter 4). After determining the stream reach that caused the piscicide to become nontoxic, I made a series of measurements that characterized the environmental conditions in that reach. I then developed a statistical model that describes rotenone degradation in montane streams. The final model uses measurements of rubble, travel time, and oxidation reduction potential to predict the probability that rotenone will eradicate fish. Oxidation reduction potential measures the electron transfer potential of water and is analogous to pH (Wetzel 2001). The inclusion of this measurement into the model is concordant with previous research showing that antimycin toxicity is affected by high pH water. Rubble substrates are most common in high gradient and turbulent stream reaches suggesting that volatilization of acetone degrades rotenone toxicity in natural settings. The importance of high gradient streams is concordant with the only other research on piscicide detoxification in streams; antimycin also degrades rapidly in high gradient streams (Tiffan and Bergersen 1996). The results of my laboratory experiments suggested that, along with turbulence, sunlight would play an important role in piscicide degradation in streams. Consequently, I expected that the amount of canopy cover, aspect, or the amount of sunlight penetration would be included in the model, but they were not.

Most streams included in this study were freestone montane streams with some canopy cover. However, one meadow stream was turbid, low gradient, and with a completely open canopy. Models developed using the montane streams predicted poorly on the meadow stream and it was excluded from further analyses as an outlier. This suggests that it is important to develop models for piscicide persistence that are specific to different stream types. Currently, most eradication efforts are in montane streams; however, future eradication efforts in other types of water bodies (e.g., prairie streams) will require development of additional models. Also, these models were developed for single-channel reaches with limited groundwater influence; models that incorporate stream networks and groundwater influences would provide guidance to piscicide applications in more complex stream systems.

As piscicide-treated water moves downstream, and is affected by the environment, piscicide applicators prevent areas of nontoxic water by fortifying the piscicide concentration with a series of drip stations placed along the stream. The intent of these drip stations is to reapply a toxic concentration of piscicide after the upstream-piscicide has been degraded by environmental exposure. An important application of my model is to identify where it is most appropriate to make the fortifications. Currently distance (either linear distance or travel time) is used to make these predictions. Incorporating measures of environmental degradation into these predictions is more appropriate than placement based on distance alone. The final model incorporated travel time but also included two measures of environmental degradation that have been shown by my research, and others, to degrade piscicides. By placing drip stations where the

eradication probability falls below 0.95, piscicide applicators can appropriately fortify piscicide concentrations.

Piscicide applicators must overlap piscicide plumes from a drip station to effectively eradicate fish. Piscicides applied at a location need some distance to move downstream and mix thoroughly through the channel. However, no guidance existed on the rate piscicides mix into montane streams, nor the most appropriate location (i.e., center or edge) or morphology (i.e., riffle/pool, meander, or straight reach) to maximize piscicide mixing. To determine transverse mixing rates in montane streams, I measured transverse mixing of a simulated piscicide (Chapter 5). I compared mixing rates between center and edge applications and among applications in riffle/pool, meander, and straight reaches. Center applications most efficiently mixed piscicide into a stream, but no one channel type was better at mixing the chemicals through the stream channel. I found that a multiplier of 10 stream widths is a conservative rule of thumb for estimating transverse mixing of piscicides for center applications. This information provides guidance to piscicide applicators so they can efficiently mix piscicides into streams thereby reducing the opportunity for fish to survive in areas where piscicides may not completely mix.

Bioassay fish are an important component of piscicide applications. They are used to determine the toxicity of the piscicide during application and as the chemicals detoxify. Bioassay fish were also important in the laboratory experiments (Chapter 3) and field applications (Chapter 4) of my research. Despite their importance as a measure of piscicides, only one study relates the tolerance to piscicides to the size of fish (Rowe-Rowe 1971). I studied the relationship between piscicide tolerance and fish size by

exposing a wide size range of trout to rotenone or antimycin and measuring time to death (Chapter 2). Larger fish tend to take longer to die, but size explained less than 20% of the variation in time to death. This study provides information on the use of bioassay fish that will provide piscicide applicators and researchers a better understanding of the tolerance of trout to piscicides.

Application of the information gained during my research will make piscicide use to eradicate nonnative fish more efficient and effective. Application of piscicides at dusk will reduce degradation by sunlight. Use of the model described in Chapter 4 will enable drip station placement that incorporates environmental conditions that cause rotenone to become nontoxic. This will reduce the frequency of piscicide over-application and the chance for nonnative fish to survive in water where rotenone has become nontoxic. After the appropriate fortification locations are identified, piscicide applicators can advise personnel monitoring drip stations to place the equipment at the center of the stream channel and overlap the application by 10 stream widths.

Eradication of nonnative fish for the benefit of native fish will be more efficient and effective as the results of this research are applied. Individual projects will require less effort and the incidence of incomplete eradication will be reduced. This will increase the number of conservation efforts that can be carried out and reduce the need for repeating eradication efforts.

LITERATURE CITED

- Akkanen, J., and J. V. K. Kukkonen. 2003. Measuring the bioavailability of two hydrophobic organic compounds in the presence of dissolved organic matter. *Environmental Toxicology and Chemistry* 22:518-524.
- Allison, P. D. 1999. Logistic regression using SAS: theory and application. SAS Institute Inc., Cary, North Carolina.
- Andrady, A. L., M. B. Amin, S. H. Hamid, X. Hu, and A. Torikai. 1995. Effects of increased solar ultraviolet radiation on materials. *Ambio* 24:191-195.
- Arnot, J. A., and F. A. P. C. Gobas. 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environmental Reviews* 14:257-297.
- Ball, R. C. 1945. A summary of experiments in Michigan lakes on the elimination of fish populations with rotenone, 1934-1942. *Transactions of the American Fisheries Society* 75:139-146.
- Barteles, P., and W. J. von Tümpling. 2007. Solar radiation influence on the decomposition process of diclofenac in surface waters. *Science of the Total Environment* 374:143-155.
- Berger, B. L., R. E. Lennon, and J. W. Hogan. 1969. Laboratory studies on Antimycin A as a fish toxicant. U.S. Fish and Wildlife Service, Investigations in Fish Control 26, Washington D.C.
- Bettoli, P. W., and M. J. Maceina. 1996. Sampling with toxicants. Pages 303-333 *in* B. R. Murphy, and D. W. Willis, editors. *Fisheries techniques*, 2nd edition. American Fisheries Society, Bethesda, Maryland.
- Binns, N. A. 1967. Effects of rotenone treatment on the fauna of the Green River, Wyoming. *Fisheries Research Bulletin No. 1*, Wyoming Game and Fish Commission, Cheyenne.
- Binns, N. A., and R. Remmick. 1994. Response of Bonneville cutthroat trout and their habitat to drainage-wide habitat management at Huff Creek, Wyoming. *North American Journal of Fisheries Management* 14:669-680.
- Boxall, J. B., I. Guymer, and A. Marion. 2003. Transverse mixing in sinuous natural open channel flows. *Journal of Hydraulic Research* 41:153-165.

- Brooks, I. C. 1961. Research methods and findings on fish toxicants and their application. S. B. Penick & Co. Research Department, New York.
- Brown, C. J. D., and R. C. Ball. 1943. An experiment in the use of derris root (rotenone) on the fish and fish-food organisms of Third Sister Lake. Transactions of the American Fisheries Society 72:267-284.
- Burrows, H. D., M. Canle L, J. A. Santaballa, and S. Steenken. 2002. Reaction pathways and mechanisms of photodegradation of pesticides. Journal of Photobiology B: Biology 67:71-108.
- Callicott, J. B. 1991. Conservation ethics and fishery management. Fisheries 16(2):22-28.
- Campbell, M. R., J. Dillon, and M. S. Powell. 2002. Hybridization and introgression in a managed, native population of Yellowstone cutthroat trout: genetic detection and management implications. Transactions of the American Fisheries Society 131:364-375.
- Cerreto, K. M. 2004. Antimycin and rotenone: Short-term effects on invertebrates in first order, high-elevation streams. Master's thesis. University of Wyoming, Laramie.
- Cheng, W. W., and A. P. Farrell. 2007. Acute and sublethal toxicities of rotenone in juvenile rainbow trout (*Oncorhynchus mykiss*): Swimming performance and oxygen consumption. Archives of Environmental Contamination and Toxicology 52:388-396.
- Chiou, C. T. 2002. Partition and adsorption of organic contaminants in environmental systems. Wiley, Hoboken, New Jersey.
- Copinet, A., C. Bertrand, S. Govindin, V. Coma, and Y. Couturier. 2004. Effects of ultraviolet light (315 nm), temperature and relative humidity on the degradation of polylactic acid plastic films. Chemosphere 55:763-773.
- Cox, D. R., and E. J. Snell. 1989. Analysis of binary data. Chapman and Hall, New York.
- Da Silva, J. P., L. F. Vieira Ferreira, and A. M. Da Silva. 2003. Aqueous photochemistry of pesticides triadimefon and triadimenol. Journal of Photochemistry and Photobiology A: Chemistry 154:293-298.
- Dawson, V. K. 1975. Counteracting chemicals used in fishery operations: current technology and research. Rehabilitation of fish populations with toxicants: a symposium, St. Louis, Missouri.

- Dawson, V. K., W. H. Gingerich, R. A. Davis, and P. A. Gilderhus. 1991. Rotenone persistence in freshwater ponds: effects of temperature and sediment adsorption. *North American Journal of Fisheries Management* 11:226-231.
- DeMong, L. 2001. The use of rotenone to restore native brook trout in the Adirondack Mountains of New York-an overview. Pages 29-35 *in* R. L. Cailteux, L. DeMong, B. J. Finlayson, W. Horton, W. McClay, R. A. Schnick, and C. Thompson, editors. *Rotenone in fisheries: are the rewards worth the risks?* American Fisheries Society, Bethesda, Maryland.
- Derse, P. H. 1963. Toxicity of antimycin to fish. *Nature* 200:600-601.
- Douglas, M. T., D. O. Chanter, I. B. Pell, and G. M. Burney. 1986. A proposal for the reduction of animal numbers required for the acute toxicity to fish test (LC50 determination). *Aquatic Toxicology* 8:243-249.
- Dunham, J. B., G. L. Vinyard, and B. E. Rieman. 1997. Habitat fragmentation and extinction risk of Lahontan cutthroat trout. *North American Journal of Fisheries Management* 17:1126-1133.
- Engstrom-Heg, R. 1974. Use of powdered activated carbon to eliminate rotenone toxicity in streams. *New York Fish and Game Journal* 21:153-162.
- Engstrom-Heg, R., and R. T. Colesante. 1979. Predicting rotenone degradation in lakes and ponds. *New York Fish and Game Journal* 26:22-36.
- Eschmeyer, R. W. 1938. Experimental management of a group of small Michigan lakes. *Transactions of the American Fisheries Society* 67:120-129.
- Finlayson, B., W. Somer, D. Duffield, D. Propst, C. Mellison, T. Pettengill, H. Sexauer, T. Nesler, S. Gurtin, J. Elliot, F. Partridge, and D. Skaar. 2005. Native inland trout restoration on national forests in the western United States: time for improvement? *Fisheries* 30(5):10-19.
- Finlayson, B., W. L. Somer, and M. R. Vinson. 2010. Rotenone toxicity to rainbow trout and several mountain stream insects. *North American Journal of Fisheries Management* 30:102-111.
- Finlayson, B. J., R. L. Cailteux, W. D. Horton, W. McClay, C. W. Thompson, and G. J. Tichacek. 2000. *Rotenone use in fisheries management: administrative and technical guidelines manual*. American Fisheries Society, Bethesda, Maryland.

- Finlayson, B. J., R. A. Schnick, R. L. Cailteux, L. DeMong, W. D. Horton, W. McClay, and C. Thompson. 2002. Assessment of antimycin A use in fisheries and its potential for registration. *Fisheries* 27(6):10-18.
- Giardino, N. J., N. A. Esmen, and J. B. Andelman. 1992. Modeling volatilization of trichloroethylene from a domestic shower spray: the role of drop-size distribution. *Environmental Science and Technology* 26:1602-1606.
- Gibson, R. J., K. G. Hiller, and R. R. Whalen. 1998. A comparison of three methods for estimating substrate coarseness in rivers. *Fisheries Management and Ecology* 5:323-329.
- Gilderhus, P. A. 1972. Exposure times necessary for antimycin and rotenone to eliminate certain freshwater fish. *Journal of the Fisheries Research Board of Canada* 29:199-202.
- Gilderhus, P. A. 1982. Effects of an aquatic plant and suspended clay on the activity of fish toxicants. *North American Journal of Fisheries Management* 2:301-306.
- Gilderhus, P. A., J. L. Allen, and V. K. Dawson. 1986. Persistence of rotenone in ponds at different temperatures. *North American Journal of Fisheries Management* 6:129-130.
- Gilderhus, P. A., B. L. Berger, and R. E. Lennon. 1969. Field trials of antimycin A as a fish toxicant. U.S. Bureau of Sport Fisheries and Wildlife, Investigations in Fish Control 27, Washington, D.C.
- Gilderhus, P. A., V. K. Dawson, and J. L. Allen. 1988. Deposition and persistence of rotenone in shallow ponds during cold and warm seasons. U.S. Fish and Wildlife Service, Investigations in Fish Control 95, Washington, D.C.
- Gönen, M. 2007. Analyzing receiver operating characteristic curves with SAS. SAS Institute Inc., Cary, North Carolina.
- Gresswell, R. E. 1991. Use of antimycin for removal of brook trout from a tributary of Yellowstone Lake. *North American Journal of Fisheries Management* 11:83-90.
- Habera, J. W., R. J. Strange, and S. E. Moore. 1992. Stream morphology affects trout capture efficiency of an AC backpack electrofisher. *Journal of the Tennessee Academy of Science* 67:55-58.
- Hansen, H., and S. B. Wilbur. 1994. Toxicological profile for acetone. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Atlanta.

- Hayton, W. L., and M. G. Barron. 1990. Rate-limiting barriers to xenobiotic uptake by the gill. *Environmental Toxicology and Chemistry* 9:151-157.
- Heard, S. B., C. B. Gienapp, J. F. Lemire, and K. S. Heard. 2001. Transverse mixing of transported material in simple and complex stream reaches. *Hydrobiologia* 464:207-218.
- Hepworth, D. K., M. J. Ottenbacher, and C. B. Chamberlain. 2002. A review of a quarter century of native trout conservation in southern Utah. *Intermountain Journal of Sciences* 8:125-142.
- Hilderbrand, R. H. 2002. Simulating supplementation strategies for restoring and maintaining stream resident cutthroat trout populations. *North American Journal of Fisheries Management* 22:879-887.
- Hogue, C. C. 1999. Avoidance responses of rainbow trout and Utah chub to rotenone. *North American Journal of Fisheries Management* 19:171-179.
- Hosmer, D. W., and S. Lemeshow. 2000. *Applied logistic regression*, 2nd edition. Wiley, New York.
- Jah, R., C. S. P. Ojha, and K. K. S. Bhatia. 2004. A supplementary approach for estimating reaeration rate coefficients. *Hydrological Processes* 18:65-79.
- Jeon, T. M., K. O. Baek, and I. W. Seo. 2007. Development of an empirical equation for the transverse dispersion coefficient in natural streams. *Environmental Fluid Mechanics* 7:317-329.
- Johnsen, B. O., and A. J. Jenser. 1991. The *Gyrodactylus* story in Norway. *Aquaculture* 98:289-302.
- Jones, J. N., and G. D. Miller. 1988. Adsorption of selected contaminants onto possible well casing materials. Pages 185-198 in A. G. Collins, and A. I. Johnson, editors. *Ground-water contamination: field methods*, ASTM STP 963. American Society for Testing and Materials, Philadelphia.
- Koel, T. M., P. E. Bigelow, P. D. Doepke, B. D. Ertel, and D. L. Mahony. 2005. Nonnative lake trout result in Yellowstone cutthroat trout decline and impacts on bears and anglers. *Fisheries* 30(11):10-19.
- Krueger, C. C., and B. May. 1991. Ecological and genetic effects of salmonid introductions in North America. *Canadian Journal of Fisheries and Aquatic Sciences* 48:66-77.

- Larson, R. A., and E. J. Weber. 1994. Reaction mechanisms in environmental organic chemistry. Lewis Publishers, Boca Raton, Florida.
- Leben, C., and G. W. Keith. 1948. An antibiotic substance active against certain phytopathogens. *Phytopathology* 38:899-906.
- Lee, D. P. 2001. Northern pike control at Lake Davis, California. Pages 55-61 *in* R. L. Cailteux, L. DeMong, B. J. Finlayson, W. Horton, W. McClay, R. A. Schnick, and C. Thompson, editors. Rotenone in fisheries: are the rewards worth the risks? American Fisheries Society, Bethesda, Maryland.
- Lee, T. H., P. H. Derse, and S. D. Morton. 1971. Effects of physical and chemical conditions on the detoxification of antimycin. *Transactions of the American Fisheries Society* 100:13-17.
- Lennon, R. E., and B. L. Berger. 1970. A resume on field applications of antimycin A to control fish. U.S. Bureau of Sport Fisheries and Wildlife, Investigations in Fish Control 40, Washington, D.C.
- Lentsch, L. D., C. W. Thompson, and R. L. Spateholts. 2001. Overview of a large-scale chemical treatment success story: Strawberry Valley, Utah. Pages 63-79 *in* R. L. Cailteux, L. DeMong, B. J. Finlayson, W. Horton, W. McClay, R. A. Schnick, and C. Thompson, editors. Rotenone in fisheries: are the rewards worth the risks? American Fisheries Society, Bethesda, Maryland.
- Leonard, J. W. 1939. Notes on the use of derris as a fish poison. *Transactions of the American Fisheries Society* 68:269- 280.
- Liao, P. B. 1971. Water requirements of salmonids. *The Progressive Fish-Culturist* 33:210-215.
- Ling, N. 2003. Rotenone-A review of its toxicity and use for fisheries management. New Zealand Department of Conservation, Wellington.
- Mac Nally, R. 2000. Regression and model-building in conservation biology, biogeography and ecology: The distinction between - and reconciliation of - 'predictive' and 'explanatory' models. *Biodiversity and Conservation* 9:655-671.
- Marking, L. L. 1975. Effects of pH on toxicity of antimycin to fish. *Journal of the Fisheries Research Board of Canada* 32:769-773.
- Marking, L. L. 1992. Evaluation of toxicants for the control of carp and other nuisance fishes. *Fisheries* 17(6):6-12.

- Marking, L. L., and T. D. Bills. 1976. Toxicity of rotenone to fish in standardized laboratory tests. U.S. Fish and Wildlife Service, Investigations in Fish Control 72, Washington, D.C.
- Marking, L. L., V. K. Dawson, and J. R. Crowther. 1983. Comparison of column aerators and a vacuum degasser for treating supersaturated culture water. *Progressive Fish Culturist* 45:81-83.
- McClay, W. 2000. Rotenone use in North America (1988-1997). *Fisheries* 25(5):15-21.
- McClay, W. 2002. Rotenone use in North America: an update. *Fisheries* 27(6):19-20.
- Meronek, T. G., P. M. Couchard, E. R. Buckner, T. M. Burri, K. K. Demmerly, D. C. Hatleli, R. A. Klumb, S. H. Schmidt, and D. W. Coble. 1996. A review of fish control projects. *North American Journal of Fisheries Management* 16:63-74.
- Moore, S. E., M. A. Kulp, J. Hammonds, and B. Rosenlund. 2005. Restoration of Sams Creek and an assessment of brook trout restoration methods. U.S. National Park Service, Technical Report/NPS/NRWRD/NRTR-2005/342, Fort Collins, Colorado.
- Moore, S. E., G. L. Larson, and B. Ridley. 1986. Population control of exotic rainbow trout in streams of a natural area park. *Environmental Management* 10:215-219.
- Moore, S. E., and B. Ridley. 1983. Standing crops of brook trout concurrent with removal of rainbow trout from selected streams in Great Smoky Mountains National Park. *North American Journal of Fisheries Management* 3:72-80.
- Moya, J., C. Howard-Reed, and R. L. Corsi. 1999. Volatilization of chemicals from tap water to indoor air from contaminated water used for showering. *Environmental Science and Technology* 33:2321-2327.
- Ottenbacher, M., and D. K. Hepworth. 2001. White Creek rotenone treatment project, August 2001. Utah Division of Wildlife Resources, Utah Department of Natural Resources, Salt Lake City.
- Palzenberger, M., and H. Pohla. 1992. Gill surface area of water-breathing freshwater fish. *Reviews in Fish Biology and Fisheries* 2:187-216.
- Pan, W. 2001. Akaike's information criterion in generalized estimating equations. *Biometrics* 57:120-125.

- Platts, W. S., W. F. Megahan, and G. W. Minshall. 1983. Methods for evaluating stream, riparian, and biotic conditions. United States Forest Service, Technical Report INT-138, Ogden, Utah.
- Post, G. 1955. A simple chemical test for rotenone in water. *Progressive Fish Culturist*. 17:190-191.
- Prentiss Inc. 2002. Prenfish toxicant stream and river use monograph. Prentiss, Inc., Floral Park, New York.
- Propst, D. L., J. A. Stefferud, and P. R. Turner. 1992. Conservation and status of Gila trout, *Oncorhynchus gilae*. *The Southwestern Naturalist* 37:117-125.
- Rach, J. J., M. Boogaard, and C. Kolar. 2009. Toxicity of rotenone and antimycin to silver carp and bighead carp. *North American Journal of Fisheries Management* 29:388-395.
- Rathbun, R. E. 1990. Prediction of stream volatilization coefficients. *Journal of Environmental Engineering* 116:615-631.
- Rathbun, R. E., D. W. Stephens, D. J. Shultz, and D. Y. Tai. 1988. Fate of acetone in an outdoor model stream in southern Mississippi, U.S.A. *Journal of Hydrology* 104:181-209.
- Ross, S. T. 1991. Mechanisms structuring stream fish assemblages: are there lessons from introduced species? *Environmental Biology of Fishes* 30:359-368.
- Rossignol, R., B. Faustin, C. Rocher, M. Malgat, J.-P. Mazat, and T. Letellier. 2003. Mitochondrial threshold effects. *Biochemical Journal* 370:751-762.
- Rowe-Rowe, D. T. 1971. Rotenone tolerances of some freshwater fishes of Natal. *The Progressive Fish-Culturist* 33:206-209.
- Rutherford, J. C. 1994. River mixing. John Wiley & Sons, Chichester, England.
- Schnick, R. A. 1974a. A review of the literature on the use of antimycin in fisheries. U.S. Fish and Wildlife Service, Fish Control Laboratory, La Crosse, Wisconsin.
- Schnick, R. A. 1974b. A review of the literature on the use of rotenone in fisheries. U.S. Fish and Wildlife Service, Fish Control Laboratory, La Crosse, Wisconsin.
- Schnick, R. A. 2001. American Fisheries Society rotenone stewardship program. Pages 81-85 in R. L. Cailteux, L. DeMong, B. J. Finlayson, W. Horton, W. Mclay, R. A.

- Schnick, and C. Thompson, editors. Rotenone in fisheries: are the rewards worth the risks? American Fisheries Society, Bethesda, Maryland.
- Sharom, M. S., and K. R. Solomon. 1981. Adsorption and desorption of permethrin and other pesticides on glass and plastic materials used in bioassay procedures. *Canadian Journal of Fisheries and Aquatic Sciences* 38:199-204.
- Shepard, B. B., B. E. May, and W. Urie. 2005. Status and conservation of westslope cutthroat trout within the western United States. *North American Journal of Fisheries Management* 25:1426-1440.
- Shepard, B. B., and L. Nelson. 2004. Conservation of westslope cutthroat trout by removal of brook trout using electrofishing: 2001-2003. Montana Fish, Wildlife and Parks, Helena.
- Shepard, B. B., B. Sanborn, L. Ulmer, and D. C. Lee. 1997. Status and risk of extinction for westslope cutthroat trout in the upper Missouri River basin, Montana. *North American Journal of Fisheries Management* 17:1158-1172.
- Sijm, D. T. H. M., and A. V. D. Linde. 1995. Size-dependent bioconcentration kinetics of hydrophobic organic chemicals in fish based on diffusive mass transfer and allometric relationships. *Environmental Science and Technology* 29:2769-2777.
- Sijm, D. T. H. M., P. Part, and A. Opperhuizen. 1993. The influence of temperature on the uptake rate constants of hydrophobic compounds determined by the isolated perfused gills of rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology* 25:1-14.
- Sijm, D. T. H. M., M. E. Verberne, W. J. de Jonge, P. Part, and A. Opperhuizen. 1995. Allometry in the uptake of hydrophobic chemicals determined *in vivo* and in isolated perfused gills. *Toxicology and Applied Pharmacology* 131:130-135.
- Southworth, G. R. 1979. The role of volatilization in removing polycyclic aromatic hydrocarbons from aquatic environments. *Bulletin of Environmental Contamination and Toxicology* 21:507-514.
- Spateholts, R. L., and L. D. Lentsch. 2001. Utah's rotenone sandmix: a formulation to maintain fish toxicity in seeps or springs. Pages 107-118 *in* R. L. Cailteux, L. DeMong, B. J. Finlayson, W. Horton, W. McClay, R. A. Schnick, and C. Thompson, editors. Rotenone in fisheries: are the rewards worth the risks? American Fisheries Society, Bethesda, Maryland.
- Spitler, R. J. 1970. An analysis of rotenone treatments for elimination of fish populations in southern Michigan Lakes, 1957-1967. *Michigan Academician* 3:77-82.

- Stefferd, J. A., D. L. Propst, and G. L. Burton. 1992. Use of antimycin to remove rainbow trout from White Creek, New Mexico. *Proceedings of the Desert Fishes Council* 22-23:1-12.
- Steinberg, C. E. W. 2003. *Ecology of humic substances in freshwaters: determinants from geochemistry to ecological niches*. Springer, Berlin.
- Strach, R. M., and T. C. Bjornn. 1989. Brook trout removal, stocking cutthroat trout fry, and tributary closures as means for restoring cutthroat trout in Priest Lake tributaries. Idaho Cooperative Fish and Wildlife Research Unit, Federal Aid in Fish Restoration, Project F-71-R-12, Final Report, Moscow.
- Stuber, R. J., B. D. Rosenlund, and J. R. Bennett. 1988. Greenback cutthroat trout recovery program: management overview. Pages 71-74 *in* R. E. Gresswell, editor. Status and management of interior stocks of cutthroat trout. American Fisheries Society, Symposium 4, Bethesda, Maryland.
- Sturm, T. W. 2001. *Open channel hydraulics*. McGraw-Hill, Boston.
- Subba Rao, N. V., and A. G. Pollard. 1951. Photo-decomposition of rotenone in spray deposits-kinetics of the photo-decomposition. *Journal of the Science of Food and Agriculture* 2:462-472.
- Thompson, C. W., C. L. Clyde, D. K. Sakaguchi, and L. D. Lentsch. 2001. Utah's procedure for mixing powdered rotenone into a slurry. Pages 95-106 *in* R. L. Cailteux, L. DeMong, B. J. Finlayson, W. Horton, W. McClay, R. A. Schnick, and C. Thompson, editors. *Rotenone in fisheries: are the rewards worth the risks?* American Fisheries Society, Bethesda, Maryland.
- Thompson, P. D., and F. J. Rahel. 1996. Evaluation of depletion-removal electrofishing of brook trout in small Rocky Mountain streams. *North American Journal of Fisheries Management* 16:332-339.
- Thurman, E. M. 1985. *Organic geochemistry of natural waters*. Martinus Nijhoff/ Dr. W. Junk Publishers, Dordrecht, the Netherlands.
- Tiffan, K. F. 1992. Oxidation and performance of antimycin in high gradient streams. Master's thesis. Colorado State University, Fort Collins.
- Tiffan, K. F., and E. P. Bergersen. 1996. Performance of antimycin in high-gradient streams. *North American Journal of Fisheries Management* 16:465-468.

- Tsivoglou, E. C., and L. A. Neal. 1976. Tracer measurement of reaeration: III. predicting the reaeration capacity of inland streams. *Journal (Water Pollution Control Federation)* 48:2669-2689.
- USEPA (U.S. Environmental Protection Agency). 2002. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. USEPA Report E 729-96, Washington, D.C.
- Vinson, M. R., E. C. Dinger, and D. K. Vinson. 2010. Piscicides and invertebrates: after 70 years, does anyone really know? *Fisheries* 35(2):61-71.
- Walker, C. R., R. E. Lennon, and B. L. Berger. 1964. Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals. U.S. Bureau of Sport Fisheries and Wildlife, Investigations in Fish Control 2, Washington, DC.
- Weigel, D. E., J. T. Peterson, and P. Spruell. 2003. Introgressive hybridization between native cutthroat trout and introduced rainbow trout. *Ecological Applications* 13:38-50.
- Wentworth, C. K. 1922. A scale of grade and class terms for clastic sediments. *The Journal of Geology* 30:377-392.
- Wetzel, R. G. 2001. *Limnology: lake and river ecosystems*, 3rd edition. Academic Press, San Diego, California.
- Williamson, C. E., R. S. Stemberger, D. P. Morris, T. M. Frost, and S. G. Paulsen. 1996. Ultraviolet radiation in North American lakes: Attenuation estimates from DOC measurements and implications for plankton communities. *Limnology and Oceanography* 41:1024-1034.
- Wohl, E. 2000. *Mountain rivers*. American Geophysical Union, Washington, D.C.
- Young, M. K. 1995a. Colorado River cutthroat trout. Pages 16-23 *in* M. K. Young, editor. Conservation assessment for inland cutthroat trout. General Technical Report RM-256. U.S. Forest Service, Fort Collins, Colorado.
- Young, M. K., editor. 1995b. Conservation assessment for inland cutthroat trout. U. S. Forest Service, General Technical Report RM-256, Fort Collins, Colorado.
- Zale, A. V., C. Brooke, and W. C. Fraser. 2005. Effects of surgically implanted transmitter weights on growth and swimming stamina of small adult westslope cutthroat trout. *Transactions of the American Fisheries Society* 134:653-660.

Zheng, B. 2000. Summarizing the goodness of fit of generalized linear models for longitudinal data. *Statistics in Medicine* 19:1265-1275.

Zilliox, R. G. 1960. The use of rotenone for management of New York trout waters. *Canadian Fish Culturist* 28:3-12.