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BIOMANIPULATION OF SHALLOW WETLANDS IN SOUTHERN MINNESOTA USING ROTENONE RECLAMATION: ROTENONE MAXIMUM CONCENTRATION AND DEGRADATION

by

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EXECUTIVE SUMMARY

Due to the presence of black bullhead *Ameiurus melas* in study ponds, a target rotenone concentration of 4 mg/L was established prior to treatment. However, in the seven treated ponds rotenone concentrations ranged from 0.210 to 3.346 mg/L in the 360 h following treatment. The variability of rotenone concentration measurements within ponds post-treatment coupled by the higher variability at early sample times (e.g., 1-h post treatment) relative to later sample times (e.g., 36-h post treatment) indicated that mixing of rotenone in ponds likely took time and was not instantaneous. Thus, the lower than expected rotenone concentrations observed during this study may have been due to rapid degradation of rotenone following application. Attempts to include environmental covariates in the regression models were unsuccessful.

Table of Contents

List of Figures

List of Tables

INTRODUCTION

Optimal walleye *Sander vitreus* fingerling production has historically been attained in water basins with limited existing fish populations (Bandow 1989; Smith and Moyle 1945; Ward et al. 2007). However, many of the available basins in southern Minnesota contain persistent populations of undesirable fish species such as black bullhead *Ameiurus melas*, fathead minnow *Pimephales promelas*, and common carp *Cyprinus carpio*. The proliferation of undesirable fish species can be attributed to elevated water levels and high interconnectivity among water basins (Blann et al. 2009). Thus, winterkill events, which had been relied upon to occasionally eliminate undesirable fish species from walleye rearing ponds have become less frequent during recent years. Ultimately, the lack of winterkill can limit production capability of rearing ponds through direct predation or indirectly through competition for similar resources (Bandow 1989; Smith and Moyle 1945; Ward et al. 2007).

Biomanipulation has been used by the Minnesota Department of Natural Resources (MNDNR) to mimic the effects of winterkill with varied success. For example, Herwig et al. (2004) reported a temporary reduction in fathead minnows through stocking of walleye fry, and suggested that repeated maintenance stocking would be needed to maintain a clear water state. Shroyer (2007) attempted to replicate the effects of winterkill in walleye rearing ponds through the use of reverse aeration. Reverse aeration was successful in eradicating oxygen sensitive species such as walleye, sunfishes *Lepomis* spp., and white suckers *Catostomus commersonii*; however, reverse aeration failed to eliminate fathead minnow and black bullhead. Another biomanipulation tool is the use of the piscicide rotenone to eradicate existing fish populations, essentially chemically mimicking a winterkill event.

Successful rotenone treatments require sufficient and sustained concentrations of rotenone that effectively eliminate tolerant species, such as black bullhead and fathead minnow. During early experimentation with rotenone it was reported that successful reclamation could be achieved with rotenone concentrations of 0.5 mg/L (Ball 1948; Leonard 1938). However, the low concentrations of rotenone often failed to achieve the desired complete mortality of fish. Gilderhus (1972) found that black bullhead death was not guaranteed unless a minimum rotenone concentration of 2.5 mg/L was maintained for at least ten hours. In that study, bullheads held in 12°C water at 1 mg/L rotenone did not perish until after 100 hours. The Cooperative Extension Service at Mississippi State University recommended a 1-2 mg/L concentration (5% formulation rotenone) to eradicate bullheads (www.aac.msstate.edu/pubs/pub1954.htm). However, DNR Fisheries typically target no less than 4 mg/L when bullheads are present (unpublished data, MNDNR).

Study Design and Data Analysis

This study evaluated the use of rotenone in walleye rearing ponds. Specifically, this study compared actual and targeted rotenone concentration in ponds, and determined relationships among rotenone degradation rates and pond physical characteristics. Shallow lakes and wetlands (hereafter referred to as "ponds") that had historically been utilized for walleye rearing by the MNDNR were considered for inclusion in this study. Study ponds were selected from the Western Corn Belt Plains and Northern Glaciated Plains ecoregions of southern and southwestern Minnesota. Ponds in these regions were characterized as having relatively shallow depth (≤ 9 feet), many had been affected by sedimentation, and most had been influenced by agricultural drainage. Landscape use in watersheds of nearly all study ponds was agriculture in nature, which included a mixture of tilled fields and grazed pasturelands. Selection of ponds was based on similarities in physical and biological parameters to limit confounding effects of non-uniformity among a total of 16 ponds (Table 1).

Temporally, field sampling was divided into two overlapping phases during which 8 ponds were sampled during each phase. Field sampling of ponds during the initial phase of this study began in 2002 with subsequent sampling in 2003, 2004 and 2005. The second phase of the study began in fall of 2004 with field sampling conducted in 2005, 2006, and 2007. Armstrong Lake, which was a scheduled treatment pond in the second phase, was removed from this study because high water levels and excessive runoff prevented reclamation during fall 2004. Thus, this study encompassed a total of 15 ponds including seven treatment ponds and eight control ponds.

Prior to reclamation, substrate composition was determined from 30 randomly selected locations within each basin using an Eckman dredge. Substrate composition samples were visually inspected and categorically classified as being composed mostly of gravel, sand, clay, detritus, or muck (silt). In addition, water samples were collected and analyzed for various properties (see Physical and Chemical water properties below). A small sample of powdered rotenone was provided to the Minnesota Department of Agriculture (MDA) for the identification of rotenone high performance liquid chromatography spikes (Dawson and Allen 1988) and a newer technique known as a GC-MS method that was less hindered by interferences in the sample. The MDA provided estimates of the actual rotenone concentration formulation so that the appropriate quantity of rotenone was applied during reclamation efforts.

Treatment basins were reclaimed using synergized powdered rotenone (Prentox Prenfish, Prentiss Incorporated, Floral Park, NY) administered at a target formulation concentration of 4-mg/L (0.2 mg/L actual rotenone). Rotenone was administered to basins using a boat and a gasoline powered water pump connected to a Venturi pump. Water was pumped from the intake at the back of the boat, through the Venturi pump connected to a suction tube used to draw the rotenone powder from barrels. The water and rotenone were mixed in the Venturi pump and the concentration was then pumped out a hose to a nozzle mounted at the bow of the boat. Thus, the boat was utilized as the initial mixing device during treatment. Wind and wave action provided additional mixing within reclaimed ponds. Application of rotenone was completed in a zigzag pattern within each basin until the rotenone supply had been depleted.

Table 1. Study pond information including treatment group (Pond Type), administrative area (Area), Division of Water identification number (DOW), surface area (Acres), county, years in study, latitude and longitude. Acreages are those listed by the Waters Section (1968) unless noted.

Pond Type	Administrative Info.		Pond Location			
Pond	DOW	Acres	Area	County	Latitude	Longitude
Control						
Boot	32-0015-00	89	Windom	Jackson	43.68390	-95.08258
Bohemian	41-0109-00	111	Ortonville	Lincoln	44.62822	-96.39459
Butterfield	83-0056-00	52	Windom	Watonwan	43.96032	-94.81029
Clam	46-0111-00	72	Windom	Martin	43.73054	-94.67474
Clear-Dundee	17-0041-00	222	Windom	Cottonwood	43.85187	-95.41042
County 13	17-0048-01	71	Windom	Cottonwood	43.95109	-95.37126
Oak Leaf	52-0010-00	181	Waterville	Nicollet	44.30769	-94.01545
South Wilson	51-0081-00	164	Windom	Murray	43.99161	-95.94045
Treatment						
Armstrong[1]	07-0125-00	125	Waterville	Blue Earth	44.15315	-94.34487
Clear	17-0008-00	76	Windom	Cottonwood	43.90051	-95.07700
Kinbrae	53-0018-00	38	Windom	Cottonwood	43.81923	-95.48633
Little Twin	46-0130-00	68	Windom	Martin	43.74256	-94.74008
Lower Case	83-0012-00	13	Windom	Watonwan	43.99585	-94.38505
Oak	41-0062-00	107	Ortonville	Lincoln	44.53671	-96.24171
Toners	81-0058-00	127	Waterville	Waseca	44.16520	-93.59959
Upper Case	83-0010-00	43	Windom	Watonwan	44.00020	-94.38750

[1] Removed from study when conditions in 2004 prevented reclamation.

Following treatment, water samples were collected by subsurface grab from an offshore station and two littoral stations located on opposite shorelines in each basin at 1 h, 24 h, 3 d, 7 d, and 14 d post-treatment. Water samples were placed on ice and transported immediately to the MDA laboratory for analysis. In the lab, one liter of sample water was passed through a glass fiber filter (GF/D; 2.7 µm pore size) to remove larger particulates. Filtrate was then extracted on a Horizon automated solid phase extraction system using a pre-conditioned JT Baker Hydrophillic DVB disk. The sample extract was then reduced by evaporation to 0.7 ml on a TurboVap concentrator system. Rotenone measurement was conducted using the Gas Chromatography/Mass Spectrometry (GC/MS) method. Secchi depth, pH, and water temperature also were recorded at the time each water sample was collected post-treatment.

Water samples were collected using subsurface grabs in September prior to treatment (treatment ponds only) and again in June and September during three post treatment years (all ponds). The samples were placed on ice and taken to the Minnesota Department of Agriculture (MDA) laboratory in St. Paul, where they were analyzed for the following parameters: pH, total alkalinity ($CaCO_3$), conductivity (µohms), total dissolved solids (TDS), total suspended solids (TSS), total phosphorus (PO_4), total Kjeldahl Nitrogen (TKN), nitrite (NO2) and nitrate (NO3), ammonia (NH_3), and chlorophyll-a (Chl *a*) using standard protocols. In addition, water temperature and secchi depth were recorded each time water samples were collected.

Statistical Analysis

Rotenone concentration estimates were $\log_{10}$ transformed to control variance and improve normality prior to analysis. Linear regression was utilized to assess rotenone maximum concentration and persistence in study ponds. Akaike's information criterion (AIC) was assessed and significant models with the lowest AIC were selected as the 'best fit.' Covariates were added to final regression models to determine the effects of environmental characteristics (e.g., water temperature, secchi, etc.) on the rotenone maximum concentration and persistence. The correlations between maximum rotenone concentrations (back transformed model intercept), degradation rates (change in rotenone concentration from 1 to 48 hours), and pond parameters were calculated and evaluated using Pearson Correlation Coefficients with an *a priori* significance level of 0.05.

RESULTS

Rotenone concentrations during this study in treated ponds ranged from 0.210 to 3.346 mg/L in the 360 h following treatment (Figure 1; Table 2). The maximum observed concentrations of rotenone were achieved within 48 h following treatment in all ponds. Average rotenone concentrations ranged from 0.838 – 3.346 mg/L, 1.012 – 1.613 mg/L, and 0.944 – 1.650 mg/L at 1-h, 24-h, and 48-h post treatment, respectively. Subsequently, the concentration of rotenone declined.

Initially, a general linear model of environmental effects on rotenone concentration suggested positive main effects of pond (df = 6, F = 3.17, P = 0.009), time post-treatment (df = 7, F = 33.30, $P < 0.001$), and a significant pond by time interaction (df = 16, F = 3.09, $P < 0.001$). Within pond comparisons generally showed significant differences in rotenone concentration from the 1 - 48 h samples to the 72 – 360 h samples indicating a decline in rotenone as time elapsed. Again, the concentration of rotenone peaked at 24 to 48 hours and then gradually declined. Only a single zero rotenone concentration was obtained during this study, thus some rotenone concentration persisted through the end of sampling at 360 h post treatment in all cases. Uniquely, the rotenone concentration in Oak Pond did not demonstrate a decline over time based on the slope not being different from zero (P = 0.1487). The Oak Pond rotenone concentration was initially 1.2 mg/L and never declined below 0.71 mg/L during the 360 h post-treatment sampling duration.

Various models were regressed to determine the maximum concentration (intercept) and persistence (slope) in treated ponds and to assess a predictive model for rotenone concentration in ponds (Table 3). The original model regressed time post-treatment on rotenone concentration. From the base model, the addition of a pond effect adjusted the intercept of the predictive model, while addition of a pond by time interaction effect adjusted the slope. Regression models substantiated a significant overall effect of time on concentration of rotenone in study ponds. Initially, regression analysis indicated significant main effects of time, pond, and also a significant pond by time interaction (Table 3). However, inspection of predictive models suggested that Oak Pond was different from other ponds (Figure 1). In fact, removal of Oak Pond from the regression analysis improved the fit of the overall model and resulted in a lower AIC. With Oak Pond removed from the model there was no significant difference in slope of predictive models among ponds, which further suggested that the rotenone declined similarly in all ponds. However, there was a significant difference in the intercept among ponds meaning the maximum concentration (back transformed to time zero) was different among ponds.

The back-transformed intercepts of the regression models provided a theoretical mean concentration at time = 0, after which time concentrations would steadily decline (Table 4). Apparently, the uniform concentration represented by the intercept could not be realized because the rotenone was not equally distributed instantaneously within the ponds. Furthermore, it appeared that mixing took time based on the variability in measurements within a lake post-treatment (e.g., variability is greater at earlier sampling times; Figure 1; Table 4). Thus, obtaining concentrations of rotenone at adequate levels to kill fish (based on expected concentrations) would require the treatments to overshoot the intended uniform concentrations at first. In addition, proper mixing would have been necessary to attain a uniform distribution before the rotenone degraded below the target concentration.

During this study attempts to include covariates in the regression models were unsuccessful because covariates were correlated. There was a weak correlation of the percent composition of gravel in the benthic substrate with rotenone concentration maximum concentration (Table 5); however, no physical or chemical property other than percent gravel was significantly correlated with either the maximum concentration or the degradation of rotenone in treated ponds. The lack of any correlation of environmental variables and rotenone maximum concentration and degradation was unexpected and most likely reflected highly variable data. The best overall model describes exponential rotenone decay with a half-life of 179 h (i.e., log (0.5) / -0.00168). Pond-specific values ranged from 137 to 630 h.

Figure 1. Rotenone concentration (mg/L) and degradation in seven study ponds treated with powdered rotenone during Fall 2002 and 2004. Samples were collected beginning 1-hour post treatment up to 360 hours post treatment and transported to the Minnesota Department of Agriculture laboratory in St. Paul, MN, for immediate analysis. Each data point (+) represents the concentration of rotenone from a single pond at a given hour post-treatment. Regression models are depicted for each pond (dotted lines) and an overall predictive model is shown (solid line). Regression analysis indicated a significant difference in intercept (maximum concentration) among ponds (df = 70, F = 25.77, $P < 0.001$) but no difference in slope based on an overall lower Akaike's information criterion (AIC) using the main effect model (AIC = -58.02) relative to a model with interaction term (AIC = -56.81). Formulation concentration was estimated based on an active ingredient (rotenone) of 7.4%.

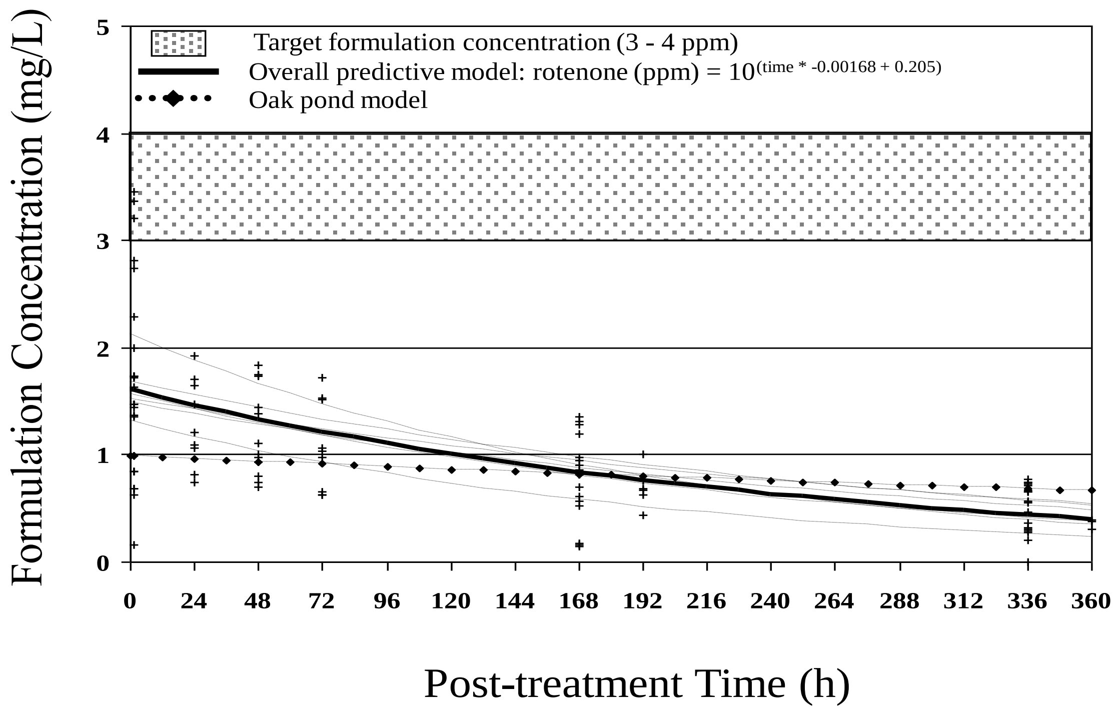

Table 2. Mean rotenone concentration (mg/L) and standard error (SE) in study ponds following treatment. Samples were collected beginning 1-hour post treatment up to 360 hours post treatment and transported to the Minnesota Department of Agriculture laboratory in St. Paul, MN, for immediate analysis. Formulation concentration was estimated based on an active ingredient (rotenone) of 7.4%. Values within column with similar letters were not significantly different (P < 0.05). Missing values are indicated by "---."

	Clear		Oak		Little Twin		Kinbrae		Toners		Upper Case		Lower Case	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	1.195^{a}	0.171^{a}	1.178^{a}	0.562	1.614^{a}	0.079	1.721^{a}	0.160	1.320^{a}	0.494	3.346^{a}	0.072	2.077^{a}	0.686
24	1.264^{a}	0.191^{a}	1.012^{a}	0.232	---	---	---	---	1.613^{a}	0.210	---	---	---	---
48	---	---	---	---	1.333^{a}	0.280	1.650^{a}	0.135	---	---	0.944^{b}	0.107	0.880^{b}	0.090
72	1.022^{a}	0.025^{a}	---	---	---	---	---	---	1.587^{a}	0.067	---	---	---	---
168	0.568^{b}	0.025^{a}	0.774^{a}	0.037	---	---	---	---	0.917^{ab}	0.049	1.013^{b}	0.133	1.286^{ab}	0.047
192	---	---	---	---	0.689^{b}	0.040	0.707^{b}	0.163	---	---	---	---	---	---
336	0.256^{c}	0.029^{a}	0.707^{a}	0.027	0.605^{b}	0.119	0.670^{b}	0.055	0.563^{b}	0.058	---	---	---	---
360	---	---	---	---	---	---	---	---	---	---	0.359^{c}	0.027	0.315^{b}	0.006

Table 3. Regression analysis including degrees of freedom (df), residual standard error (RSE), F-statistic (F), P-value, multiple r-squared, and Akaike's information criterion (AIC) for rotenone concentrations in ponds treated with powdered rotenone in Fall 2002 and 2004. Overall models included all study ponds. Adjusted models did not include Oak Pond because the slope was different from the other ponds. A lower AIC score assumes a better-fit.

	df	RSE	F	P-value	r^2	AIC
Overall Model						
Time	86	0.1707	111.80	< 0.001	0.5653	-57.42
Time + DOW	80	0.1632	19.49	< 0.001	0.6303	-59.69
Time + DOW + Time*DOW	74	0.1515	13.62	< 0.001	0.7053	-67.62
Adjusted Model						
Time + DOW	70	0.1569	25.77	< 0.001	0.6883	-58.02
Time + DOW + Time*DOW	65	0.1538	15.34	< 0.001	0.7220	-56.81
Model comparison	5	---	1.57	0.1802	---	---

Table 4. Predictive models results from regression analysis for rotenone concentrations in ponds treated with powdered rotenone in Fall 2002 and 2004. Overall models included all study ponds with main effects of time post treatment (time, h), pond, and time x pond interaction. Original rotenone concentrations were log10 transformed prior to regression analysis. Back-transformed maximum concentration (Max Conc) were model intercepts (time = 0) and observed maximum concentration were the maximum mean concentration observed in study ponds at any time post treatment. The range of actual rotenone concentrations at the time post treatment that the maximum mean concentration was observed is indicated (Range at Max). All models were significant ($P < 0.05$).

Pond	Model $10^{\text{(intercept + slope*time)}}$	Back-transformed Max Conc	Observed Max Conc	Observed Range at Max
Overall Model				
Clear	0.1195 – 0.002118 *time	1.317	1.264	1.058 – 1.644
Oak	-0.0045 – 0.000478 *time	0.990	1.178	0.616 – 1.741
Little Twin	0.1717 – 0.001351 *time	1.485	1.614	1.147 – 1.744
Kinbrae	0.2252 – 0.001397 *time	1.680	1.721	1.446 – 2.001
Toners	0.1834 – 0.001246 *time	1.525	1.613	1.214 – 1.926
Upper Case	0.3277 – 0.002196 *time	2.127	3.346	3.212 – 3.457
Lower Case	0.1959 – 0.001732 *time	1.570	2.077	0.686 – 2.808
Overall	0.1741 – 0.001507 *time	1.493	---	---

Table 5. Correlation (Pearson Correlation Coefficient) between rotenone maximum concentration (back-calculated at time = zero using predictive model; Maximum Concentration) and degradation (measured as change from time = 1 hour to time = 48 hours; Degradation) for rotenone measured in ponds in 2002 and 2004. Samples were collected beginning 1-hour post treatment up to 360 hours post treatment and transported to the Minnesota Department of Agriculture laboratory in St. Paul, MN, for immediate analysis. Formulation concentration was estimated based on an active ingredient (rotenone) of 7.4%. Values in bold were significant with an *a priori* $P = 0.05$.

	Maximum Concentration		Degradation	
Environmental Variable	Correlation Coefficient	*P*-value	Correlation Coefficient	*P*-value
Surface area	-0.5374	0.2135	0.4370	0.3269
Temperature				
Begin	-0.1668	0.7207	0.1592	0.7332
Maximum	-0.2587	0.5753	0.0717	0.8787
Secchi	-0.6820	0.0914	0.6650	0.1031
pH	0.2516	0.5863	-0.2303	0.6193
Total P	0.1800	0.6994	0.0272	0.9539
Chlorophyll	0.1598	0.7322	0.1119	0.8112
Total Alkalinity	0.4661	0.2918	-0.3603	0.4273
TDS	-0.6823	0.0913	-0.0111	0.9811
Conductivity	-0.5405	0.2103	0.4080	0.3636
TSS	0.2006	0.6663	-0.0371	0.9370
$NO_2 + NO_3$	-0.0666	0.8872	0.1427	0.7602
TKN	-0.2778	0.5464	0.1603	0.7313
NH_3	0.1130	0.8094	0.1601	0.7317
Substrate				
Clay	0.3364	0.4606	-0.5672	0.1842
Detritus	0.4502	0.3107	-0.1134	0.8087
Gravel	**-0.8064**	**0.0285**	0.4812	0.2743
Mud/Silt	0.6849	0.0896	-0.3636	0.4228
Sand	-0.7426	0.0559	0.3785	0.4024
Macrophytes				
Emergent	-0.0785	0.8671	-0.1650	0.7238
Submergent	-0.5545	0.1964	0.5429	0.2079
Floating	-0.0998	0.8314	0.2532	0.5837
Terrestrial	-0.4023	0.3709	0.2762	0.5488

DISCUSSION

Overall, the rotenone concentrations obtained during this study were lower than expected. The targeted concentration in all treatments was 4 mg/L due to the presence of black bullhead; however, no water sample collected during this study attained the target mean concentration based on MDA lab results. Potential treatment related causes for the lower-than-expected rotenone concentrations included inaccurate estimates of water volume in the planning stages, inadequate mixing in the water basin, rapid degradation at the moment of application, degradation during shipping to the lab, and settling. The underestimation of water volume was considered unlikely because the magnitude of such a miscalculation was large (4x) and the mistake would have to be repeated for each of the seven treated ponds. Thus, planning stage errors were excluded from consideration in explaining the low rotenone concentrations. Instead, the lower than expected rotenone concentrations in treated ponds may have been a function of sampling and improper mixing. For example, because samples were taken beginning 1-hour post-treatment a conservative assumption would be to expect values ranging above and below the 4-mg/L-target concentration due to incomplete mixing. Conversely, lab results indicated a rotenone concentration ranging from 0.2 to 3.5 mg/L at the 1-hour post treatment sample interval. Again, it is unlikely that all 24 1-hour post treatment samples would have failed to attain 4 mg/L because random sampling should have included "hot spots" in the pond due to incomplete mixing along a random littoral-pelagic-littoral transect.

Ruling out these potential explanations for the lower than expected rotenone concentrations led to hypotheses of rapid degradation, degradation during shipping, or a combination of both. The maximum concentration of rotenone was typically attained within 48 hours of treatment and never exceeded 3.5 mg/L. The mean concentration represented by the intercept was lower than the target most likely because the rotenone was not instantaneously distributed within the ponds. Apparently, mixing took some time based on the high initial and subsequently declining variability in measurements within a pond over time. These results suggested that the lower than expected maximum concentrations observed during this study were likely due to rapid degradation. Thus, these results suggest it may be beneficial to plan treatment to initially overshoot the intended concentration to warrant against degradation prior to complete mixing in ponds.

The lower-than-expected rotenone concentrations encountered during this study may not be unique. Other studies (e.g., Dawson et al. 1991) have also reported actual concentrations of rotenone slightly less than the calculated treatment concentration. Dawson et al. (1991) suggested that the lower than expected rotenone concentrations was most likely due to adsorption to sediments and decomposition during the time between actual application and collection of the water samples. For example, a rotenone treatment in the Green River was targeted for even distribution at 5 mg/L; however, 2.5 to nearly 10 mg/L were actually attained in different sections of the river (Wyoming Game and Fish Commission 1967). Based on the deposition and persistence study of Gilderhus et al. (1988), rotenone that is properly applied has a 10 d half-life in cold waters, which should allow sufficient time to eradicate fishes. Our estimation of half-lifes ranged from 5.7 to 26.25 days in various ponds, with an overall value of 7.46 days. Other than temperature, it appears that light (Jones et al. 1933), substrate composition (Dawson et al. 1991) and water clarity influence rotenone degradation rates (Engstrom-Heg and Colesante 1979). The only environmental parameter that demonstrated a significant relationship with rotenone concentration in treated ponds during this study was the percentage of gravel substrate, which was negatively correlated with rotenone maximum concentration. High variability among ponds likely influenced assessment of the effect of environmental variables on rotenone maximum concentrations and persistence.

Physical, chemical, and biological properties of ponds have been shown to influence the toxicity through variable maximum concentration and persistence of rotenone. For example, Gilderhus (1982) reported that the aquatic macrophyte Canadian waterweed had no effect on the toxicity of rotenone to fathead minnows. However, the rotenone quantity would have to be increased by 7 times to effectively eliminate fathead minnows in the presence of suspended bentonite clay at just 0.5 g/L relative to a treatment in the absence of clay. Clemens and Martin (1953) also suggested that concentration of rotenone declined quickly at the bottom of treated ponds likely due to the higher dissipation potential in the lower water column. In fact, Dawson et al. (1991) reported that rotenone disappeared two to three times faster in earthen ponds than in concrete ponds with all other aspects constant indicating rapid absorption of rotenone by substrate. Finally, water temperature at the time of rotenone application can also influence maximum concentration and persistence and it has been reported that rotenone persistence can be inversely related to water temperature (Gilderhus and Dawson, 1986; Dawson et al. 1991). In the absence of oxygen, rotenone degradation did not occur; thus, dissolved oxygen concentration could also be correlated to rotenone degradation rates (Jones et al. 1933; Gunther 1943).

The ultimate cause for the lower-than-expected rotenone concentrations remains unexplained. Literature suggests that complete kills of black bullhead are not attained at concentrations such as reported during this study; however, complete kills were experienced in all treated ponds. This suggests inaccurate lab results, immediate binding of rotenone in treated ponds, or changes to samples during shipping. Hypothetically, since particulate organic matter was filtered from the samples at the lab it is possible that rotenone may have been bound to these particles during transport and rotenone subsequently removed prior to lab processing. Studies should be initiated to examine how filtering in the field prior to shipping affects results. It is undesirable to add more rotenone than is needed; however, due to the expenses involved in a reclamation, it is important to accomplish the desired effect (typically a complete fish kill). Applications of rotenone should not exceed 5 mg/L of 5% active rotenone formulation, as that would be a violation of labeling restrictions (Prentox 2002). The actual concentrations of even a well-calculated rotenone application can be highly variable though. Undoubtedly, further research is needed if lab results are used to verify rotenone concentrations during reclamation efforts. In addition, further studies should address the ability to uniformly achieve a target rotenone concentration under various mixing conditions in earthen ponds.

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