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PROJECT: Use of low-concentration rotenone for biomanipulation of Iowa lakes

PROJECT LEADER: Mark Flammang and Gary Sobotka

LOCATION: Multiple Southern Iowa Reservoirs, and Rathbun Research Facility

PERIOD OF RESEARCH: 11/1/2010 to 11/15/2014

ABSTRACT- *Illegal Gizzard Shad Dorosoma copedianum introductions have occurred with increasing frequency in recent years in Iowa. These introductions typically precede large-scale declines in angling quality for important centrarchid species including Bluegill (Lepomis macrochirus), Largemouth Bass Micropterus salmoides, and crappie Pomoxis spp. Traditional management of such introductions was usually preceded by years of poor angling and resulted in the eventual renovation of the entire fishery. To avoid these cycles, the Iowa Department of Natural Resources Fisheries Bureau has taken a proactive approach and is attempting complete, but selective, Gizzard Shad removal with minimal non-target loss. Five-percent rotenone was applied at nominal concentrations ranging from 5.0-9.5µg/l active ingredient at 13 lakes in November 2010, 2011, and 2012, and late October and early December 2013. Rotenone solution was applied to each lake zone (e.g., shallow upper lake, deep pelagic) with several passes to minimize localized differences in rotenone concentration (e.g., hot spots) and ensure even chemical distribution. Post-application water sample analysis demonstrated active rotenone concentrations were met with precision, but were typically higher than nominal concentrations in 24 h post-treatment samples. Subsequent fish sampling demonstrated 100% removal of Gizzard Shad and Yellow Bass (Morone mississippiensis) at nominal treatment concentrations of 8.0 µg/l. Lakes targeted with higher concentrations of rotenone also resulted in the elimination of Gizzard Shad and Yellow Bass; however significant negative impacts to game fish populations were often identified. In lakes targeted with lower nominal concentrations, Gizzard Shad elimination was more variable. Experimental evaluation of rotenone toxicity in hatchery ponds suggested complete Gizzard Shad and Yellow Bass removal can be attained at a treatment level of 8.0 µg/l. Grass Carp Ctenopharyngodon idella and Silver Carp Hypophthalmichthys molitrix demonstrated susceptibility to rotenone that was similar to other game fish species, suggesting partial reductions of population density are possible, but only complete renovation will eliminate these species. Overall, our study provides continued insight into the use of low-concentration rotenone as a management tool for quality fisheries with specific undesirable components.*

INTRODUCTION

Within the last two decades, the illegal introduction of Gizzard Shad *Dorosoma copedianum* into public waters has occurred with increasing frequency in Iowa. These introductions typically precede declines in biomass and size quality of game fish (i.e. Hill 1983) and declines in angling quality for important centrarchid species including Bluegill *Lepomis macrochirus*, Largemouth Bass *Micropterus salmoides*, and crappie *Pomoxis* spp. (i.e. Flammang 2007). Gizzard Shad have been directly and indirectly implicated in declines in abundance and quality of several gamefish populations in small impoundments (DeVries and Stein 1990). The Gizzard Shad is an omnivorous fish species native to the Midwestern and southern United States. In eutrophic constructed lakes and reservoirs, Gizzard Shad can attain high biomass and often predominate the fish assemblage (Stein et al. 1995; Gido 2001). Overpopulation of Gizzard Shad leads to competition with other species during early life history stages (Garvey and Stein 1998; Aday et al. 2003; Schaus et al 2010). Gizzard Shad abundance has also been correlated with increased turbidity (Miller 1960; Aday et al. 2003; Schaus et al. 2010). At high abundance, Gizzard Shad can have strong ecosystem effects (e.g., alter zooplankton and phytoplankton assemblages, bioturbation and nutrient loading) and are commonly targeted for removal or control at low abundances (e.g., Catalano and Allen 2009).

Management agencies have used rotenone applications to remove or reduce unwanted fish populations for many years, (Leonard 1939; Ball 1948; Bettoli and Maceina 1996). Rotenone is the most widely used fish poison for assessment, management, and eradication of fish, including undesirable species (McClay 2000; Ling 2003). Rotenone induces mortality by inhibiting cellular respiration within the mitochondria and preventing the electron transport system (Singer and Ramsay 1994). In the later stages of toxicosis, fish experience respiratory paralysis (Perry and Conway 1977; Hyatt 2004) then death due to tissue hypoxia (Ling 2003). The removal of undesirable fish species can ameliorate their negative impacts on the physiochemical conditions (e.g., bioturbation, nutrients) and other fish species. However, the use of rotenone to restore aquatic ecosystems often is preceded by declines in angling pressure and negative impacts to local economies. Biomanipulation using piscicides (e.g., rotenone) is costly, time consuming, and often used after fish assemblages have been substantially altered (e.g., few sport fish).

Previous studies of rotenone toxicity have indicated variable susceptibilities for different species. Gilderhus (1972) suggested that different species required different effective contact times (ECT) to induce mortality. Specifically, the ECT was 2 hours for Rainbow Trout *Oncorhynchus mykiss*, 8 hours for Largemouth Bass *Micropterus salmoides*, 18 hours for White Sucker *Catostomus commersoni*, and 24 hours for Common Carp *Cyprinus carpio* when exposed to 50 ppb liquid 5% rotenone (Gilderhus 1972). Additionally, Brown (2010) observed that tolerances to rotenone can vary for individuals of the same species as there was a significant relationship between time of death and size.

The use of low concentration rotenone applications to manage Gizzard Shad was developed because it was observed that Gizzard Shad were among the first species to die during complete system renovations (Bowers 1955). However, efficacy of targeting Gizzard Shad with low concentrations of rotenone and the non-target effects of such applications warranted further research due to the undesirable economic and social constraints of lake-wide fishery renovations. Some agencies have attempted to exploit these differences in rotenone tolerance to specifically reduce biomass or eliminate Gizzard Shad from important fisheries with some success (e.g., Wisener 2005).

In fall of 2009, the Iowa Department of Natural Resources Fisheries Bureau (IDNR) successfully eradicated Gizzard Shad from Mt Ayr Reservoir, a 12- acre lake with the application of 5 µg/l active rotenone (Sobotka 2009). However, this lake was small relative to many systems in which Gizzard Shad have been introduced, lake morphometry was relatively simple, and the existing fishery was relatively poor. Little was understood regarding the potential for these methods as applied to medium and large reservoirs (i.e. >20 acres) that have only recently become infested with Gizzard Shad and thus maintain a high-quality fishery. The goal of this study was to experimentally evaluate the feasibility of selective Gizzard Shad removal using low-concentration rotenone (i.e., <12 µg/L active rotenone) and quantify the non-target effects of such applications. Specifically, The objectives of this study were to: **1)** evaluate the efficacy of low-concentration rotenone applications for the targeted removal of Gizzard Shad populations from lakes where they were unintentionally stocked, **2)** assess the impact of these treatments on the remaining fish community, and **3)** experimentally evaluate the toxicity of rotenone to Gizzard Shad and several other sport and undesirable fishes that are commonly observed in many Iowa systems (i.e., Bluegill, crappie, Largemouth Bass, Yellow Bass *Morone mississippiensis*, Grass Carp *Ctenopharyngodon idella*, and Silver Carp *Hypophthalmichthys molitrix*, in a controlled hatchery pond environment.

METHODS

General methods

The use of rotenone for the removal of fish from impaired systems is a common management practice employed by IDNR biologists. Multiple manufacturers provide this chemical and annual purchases by the IDNR are determined based on low-bid response. The IDNR requires analytical evidence of rotenone concentration upon delivery and thus, has noted sizable variation in supplied active rotenone concentration in these various shipments. In fact, it is not uncommon for concentration of active rotenone to vary by as much as 20% from manufacturer's labeled concentration. We establish an analytical grade stock rotenone solution through chemical assay prior to any "low-concentration" treatment by submitting samples for laboratory analysis of the delivered 5% rotenone product. From this analytical determination, we can then calculate nominal dosages more accurately. Thus, this analysis facilitates the highest level of precision possible for determining application rates.

Both Prentiss Prenfish® and Tiffa Chemfish-Regular® 5% rotenone were utilized in this evaluation. Pre-use determination of active rotenone was assessed analytically by reverse-phase high-pressure liquid chromatography (HPLC) with UV absorption detection (Warner et al. 1982). Active rotenone concentrations in rotenone shipments varied from 4.46% active rotenone to 5.70% active rotenone.

Water samples for all post-treatment assessments were taken using a 3 foot integrated sampler. This sample was transferred to 20 mL amber vials, samples were chilled to approximately 34° F, kept in a lightless container, and immediately delivered to the University of Iowa Hygienic Laboratory for analysis of active rotenone. The laboratory determined active rotenone concentration using a Waters Alliance 2695 HPLC® system coupled with a Waters Micromass Quattro tandem quadruple mass spectrometer.

Field trial methods

Thirteen lakes were treated with low-concentration rotenone from November 2010 through December 2013 (Table 1). Lake size ranged from 10 to 575 acres. Temperature ranged from 55 °F to 34 °F. To identify the most effective method of ensuring Gizzard Shad eradication with minimal non-target loss, nominal rotenone concentrations have varied across years. One system was treated at 5.0 µg/l, five were treated at 6.5 µg/l, three were treated at 8.0 µg/l, and four were treated at 9.5 µg/l active rotenone.

All lakes were mapped with Odom single beam sonar mapping. Survey data were collected and edited using Hypack Hydrographic Survey Software. Lake volume was calculated using ArcGIS 9.3 ArcMap and Golden Software Surfer 7.0. Lakes were subdivided into zones, each corresponding to 10 to 20 surface acres for individual treatment. In addition, zones were subdivided further into shallow (<12.0 ft) and deep (>12 ft) zones. Rotenone quantities were calculated based on the assayed result of active rotenone, prior to application. Surface application of rotenone utilized

Table 1. Pertinent treatment information for low-concentration rotenone treatments in Iowa lakes.

Lake	Date of Treatment	Acres	Secchi (in)	Temperature (°F)	Nominal concentration (µg/l active rotenone)	Gizzard shad eliminated
Sugema	11/16/2010	575	20	45	5.0	Yes
Badger Creek	11/29/2010	270	20	39	6.5	No
Fisher	11/1/2011	110	31	55	6.5	No
Lacey	11/2/2011	22	43	53	6.5	Yes
Humeston	11/3/2011	40	24	54	6.5	No
Don Williams	11/11/2011	148	12	46	6.5	No
Badger Creek (retreatment)	11/17/2011	270	20	39	9.5	Yes
Don Williams (retreatment)	11/1/2012	148	31	50	9.5	Yes
Hanen	11/20/12	38	NA	41	9.5	Yes
Binder	11/26/2012	36	NA	36	9.5	Yes
Humeston (retreatment)	10/30/2013	54	24	54	8.0	Yes
Indian	10/31/2013	51	39	51	8.0	Yes
Atlantic Pond	12/3/13	10	NA	NA	8.0	Yes

NA = not available

a calibrated ATV sprayer mounted to a boat. Rotenone was applied at a rate that corresponded to approximately 5 hours of total application time for each section. Pumps were calibrated at approximately 0.25 gallons / minute. Rotenone was diluted in 15 gallons of lake water and injected directly into the prop wash from the outboard motor of each boat. The total amount of undiluted rotenone to be mixed in each tank was determined based on dividing the total quantity of chemical to be applied to that zone by 5 (number of tanks to be applied over five hours). A boat mounted GPS system was utilized to monitor the application coverage route. Each zone's surface area was covered several times to minimize localized "hot spots". A graphic description of a "typical" GPS track recorded during an application is pictured in Figure 1. Sub-surface application was used in reservoir zones where water depth exceeded 12 feet. Equipment consisted of a gas powered water pump designed to blend the contents of an on-board holding tank with lake water and expel its outflow approximately 12 feet below the surface through a trailing hose through a diffusion nozzle. Chemical flow rates were proportionally similar to surface application rates.

The success of applications was evaluated in subsequent comparative surveys of game and nongame fish populations to pretreatment surveys. The collection of any Gizzard Shad in post-treatment surveys would indicate a failure of the application effort if Gizzard Shad were a target species.

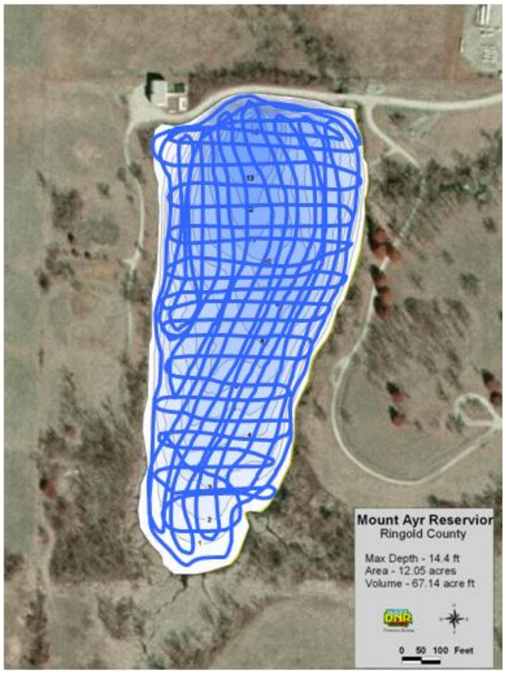

Figure 1. Typical GPS track of application path for low-concentration rotenone.

All fish sampling was conducted using spring pulsed DC electrofishing. Pre- and post-treatment catch per unit effort (CPUE) of stock-length fish were indexed as fish captured per hour electrofishing. All CPUE data were log10 transformed to meet assumptions of normality with non-transformed means and standard errors reported for clarity. Analysis of variance (ANOVA) was used to detect differences within a species across lakes. When differences among the main effects of lake, species, or their interaction were detected, the Tukey's honest significance test was used to determine where differences occurred. All statistical analyses were performed using SAS 9.3 (SAS Institute, Inc., Cary, North Carolina). Statistical significance was determined at α = 0.05 for all analyses.

Experimental pond evaluation of low-concentration rotenone toxicity

In this study, 96 h static acute-toxicity bioassays were conducted using Prentiss

Prenfish® (5% active ingredient) rotenone to determine the comparative mortality of several species of game and non-game fish typical of Iowa lake systems. Trials were conducted at the Rathbun Hatchery Research Facility. Specifically, we were interested in the reaction of target species (Gizzard Shad and Yellow Bass) as well as the impact of the treatment on game fish such as Largemouth Bass, Bluegill and crappie. In addition, there was interest in examining the response of Grass Carp and Silver carp to these treatments. Redear Sunfish and Common Carp were utilized in two replicates at low densities. These species were added as available and were not extensively analyzed.

Grass Carp were formerly widely utilized in many Iowa lakes and their longevity in these systems is of concern. A change in management philosophy in recent years has lead to plans to reduce or eliminate many of these Grass Carp populations. In addition, recent flooding has resulted in the range expansion of Asian carps into several Iowa lentic systems, raising concerns about their impacts on fish assemblages and other waterborne recreation.

In these toxicity evaluations, fish were exposed to nominal concentrations of 0.0, 4.0, 6.0, 8.0, 10.0, and 12.0 µg/l. These concentrations were selected to bracket rotenone concentrations that have resulted in successful Gizzard Shad elimination in field trials. Our goal was to more precisely identify the impacts to other fishes within Iowa lakes.

Static acute toxicity tests were conducted in six, 0.1 surface ac plastic-lined ponds. Pond dimensions were 100 feet by 50 feet with a maximum depth of 6 feet tapering to a minimum depth of 3 feet at the far end. Ponds held a total of 87,600 gallons of water when full. Water was provided from Rathbun Lake, Iowa and was filtered to exclude extraneous fish. Each pond was equipped with a collection kettle that was two feet deep where fish were held during pond harvest. Ponds were also equipped with an airlift system that circulated 300 gallons of water per minute from the pond bottom to the surface in a way that would result in complete circulation of the pond. Six levels of rotenone were tested over three repetitions. Water chemistry data is noted in Table 2. Trial rotenone concentrations were randomly assigned to individual ponds prior to each replication. One replication of each trial was completed weekly for three replicates total. While temperature declined across replications, all treatments within a repetition were consistently uniform in hardness, dissolved oxygen, temperature, and pH. Water temperature was always below 60º F.

All fish utilized in pond trials (except Grass Carp) were wild-caught using electrofishing gear and were transported to the Rathbun Fish Hatchery where they were stocked directly into study ponds. Grass carp were purchased directly from a private fish hatchery and were delivered each week prior to initiation of each trial. Stocking density was consistent across trials within repetitions but varied across repetitions for some species. Bluegill, Yellow Bass, Gizzard Shad, and crappie densities ranged from 35 to 70 /pond/ trial. Largemouth bass were stocked at 25 fish/pond/trial. Redear Sunfish and Common Carp were

Table 2. Measured water quality metrics from research ponds at start of each repetition. Initial data are from the start date, final values were collected 96 h post-treatment at the completion of the trial.

Initial Start of Trial	Pond	Initial hardness (mg/l $CaCO_3$)	Final hardness (mg/l $CaCO_3$)	Initial dissolved Oxygen (mg/l)	Final dissolved Oxygen (mg/l)	Initial temperature (F°)	Final temperature (F°)	Initial pH	Final pH	Nominal treatment concentration (µg/l)
10/23/14	1	81	84	10.4	11.5	56	59	8.12	8.60	10.0
	2	87	85	10.5	11.6	55	59	8.76	8.61	6.0
	3	89	90	10.1	10.7	56	59	8.13	8.99	12.0
	4	89	87	10.2	10.2	56	59	8.14	8.36	4.0
	5	87	88	10.1	12.4	57	59	7.93	8.91	8.0
	6	87	87	10.4	10.7	56	59	8.69	8.43	0.0
10/30/14	1	88	87	10.4	10.8	54	49	8.06	7.61	12.0
	2	88	90	10.5	11.1	53	48	8.01	7.70	10.0
	3	89	90	10.4	11	54	47	7.95	7.70	6.0
	4	93	87	10.4	11.1	54	47	7.89	7.77	0.0
	5	88	89	10.4	11	55	48	7.89	7.88	4.0
	6	90	87	10.4	11	54	48	7.87	7.83	8.0
11/6/14	1	90	85	10.5	11	51	48	7.81	7.58	12.0
	2	88	90	10.8	11.1	51	47	7.80	7.74	8.0
	3	90	90	10.7	11	51	47	7.78	7.73	0.0
	4	88	91	10.8	10.9	51	48	7.81	7.80	4.0
	5	90	89	10.7	10.9	52	48	7.76	7.76	6.0
	6	86	89	10.7	10.9	51	48	7.82	7.85	10.0

stocked at approximately 4 fish/pond/trial. Insufficient numbers of Bighead Carp *Hypophthalmichthys nobilis* were routinely available and thus only Silver carp were evaluated in this study. Ten Silver carp were stocked in each pond for all trials. Grass carp were also stocked at a rate of 10 fish per trial. Variability in fish size by species across treatments and repetitions remained relatively consistent (Table 3).

Ponds were filled each week on Monday. Fish stockings into test ponds began on Monday afternoons and were completed by Wednesday morning. All fish were allowed to acclimate for a minimum of 24 h prior to treatment. Ponds were examined for post stocking mortality. Mortalities were removed when possible and were not considered as part of the treatment-related mortality. Trials began each Thursday at 9:00 AM. The air lift system was activated each Wednesday morning, 24 h prior to treatment. A chemical assay of the rotenone utilized yielded an active ingredient concentration of 4.46%. The appropriate quantity of rotenone was determined for each treatment and was diluted in 15 gallons of water. The solution and was pumped directly into the air lift system that turned these ponds over a 1 h period. The air lift system was allowed to operate for 6 h post-treatment to ensure complete mixing. After this period was completed, the use of the air lift system was discontinued and the ponds allowed to rest.

Table 3. Mean, minimum, and maximum length of fish utilized in low-dose pond trials.

Species	Mean length	Minimum length	Maximum length
Asian Carp	22.3	16.7	26.6
Bluegill	5.9	3.1	9.0
Crappie (species combined)	6.9	3.5	10.9
Grass Carp	11.1	8.0	14.5
Gizzard Shad	4.6	3.2	13.4
Largemouth bass	6.3	4.5	18.5
Yellow Bass	7.2	4.0	9.1

Fish mortality was monitored throughout a 96 h period. Mortality was quantified as a percentage for each species by day and by trial. However, only Day 4 (96 h) cumulative results are reported. Moribund fish were allowed to remain in the pond until they expired. Fish were identified, measured, and weighed upon collection. Mortalities were collected each morning and afternoon until completion of the trial. There was some loss of dead fish to scavengers at night. Overall loss to scavengers was enumerated at the end of each 96 h trial and averaged 4%. At 96 h post-treatment, the ponds were drained; living and dead fish were identified, enumerated, and weighed. The ponds were thoroughly washed using a high pressure, high volume water supply and allowed to refill.

We used logistic regression in SAS v9.3 to examine the relationship between rotenone concentration and the probability of fish mortality as a function of two model structures – (1) an additive model of rotenone concentration and species and (2) an interaction model between rotenone concentration and species. We chose the best supported model by evaluating the difference in AIC between model structures (the model with lower AIC is better supported from the data). From the most parsimonious model, we estimated species-specific predicted probabilities of mortality (and 95% confidence intervals) as a function of rotenone concentration between 0 and 12 µg/l. Length frequencies were compared with the Kolmogorov-Smirnov test to determine if there was a size-related impact of mortality within each species. A significance level of α=0.05 was established *a priori* for all tests.

RESULTS AND DISCUSSION

Field trials – reservoir evaluation of low-concentration rotenone application

In general, water samples from Iowa lakes displayed similar trends in rotenone decomposition (Figure 2). This figure demonstrates sample concentrations of active rotenone across four different nominal treatment concentrations. The overall trend is a relatively fast decline in active rotenone concentration; however, detectable quantities are commonly noted 20 or more days post-treatment. Figure 3 demonstrates the measured:nominal ratio of active rotenone for all lakes. A value of 1.0 would indicate exact agreement between calculated and measured rotenone concentrations in study lakes. Typically, the measured:nominal ratio ranges between 1.2 and 1.5 at 24 h (1 d) post-treatment. This ratio steadily declines with sample concentrations over time. Ratios approaching one to one were only observed at days five and seven. However, variability on day five

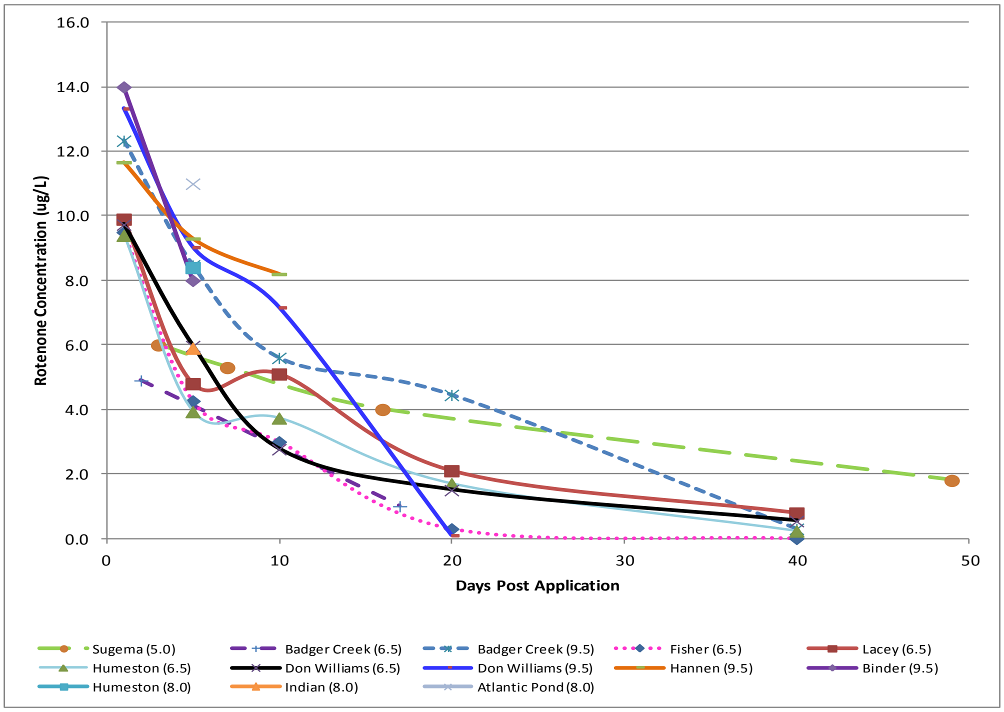

Figure 2. Active rotenone concentration across time at lakes treated from fall 2010 through fall 2013. Nominal targeted concentration is listed in parentheses.

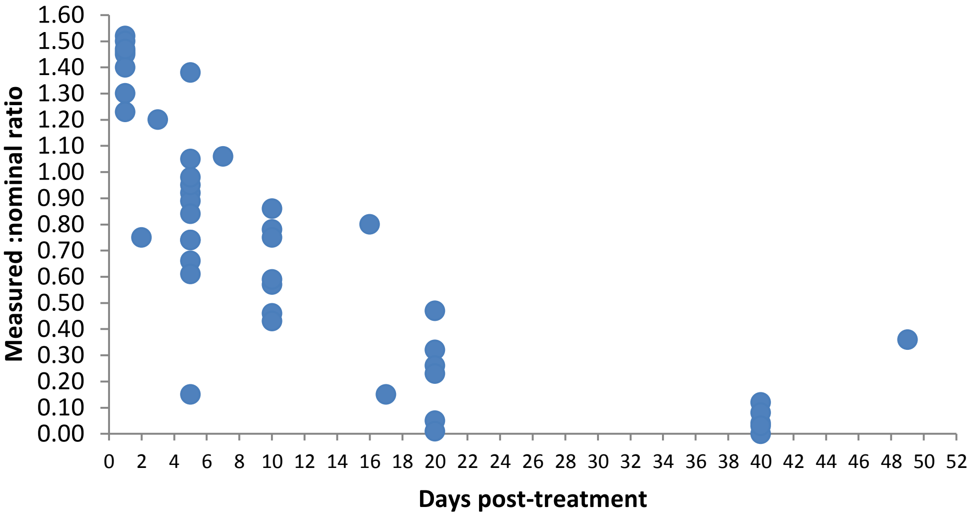

Figure 3. Measured to nominal ratio of active rotenone by time post-treatment.

was still substantial and the ratio ranged from 0.15 to 1.4. Most values on day five corresponded to measured:nominal ratios of 0.85 to 1.05. It is likely that higher ratios on day one are associated with incomplete mixing of lake environments. Measurements approaching 1.0 were more commonly observed on Day 5 may be the result more complete mixing of the lake. However, it should be noted that degradation of rotenone toxicity is time-dependent (Finlayson et al. 2001) and; thus, cannot be eliminated as a potential contributing factor to decreases in rotenone concentration. Chapman et al. (2003) attributed changes in rotenone concentration in just 0.5 h to chemical degradation where observed concentrations were just 28% of nominal values in a laboratory experiment using relatively small tanks. However, in light of our results, we suggest that in lake environments, at temperatures below 60º F, day five provides an acceptable indicator of true active rotenone levels.

Figure 4 demonstrates the relationship between measured rotenone values and the successful elimination of Gizzard Shad in study lakes. It is possible to make generalized predictions on the individual success or failure of lake treatments (as expressed by elimination of Gizzard Shad) based on these values. If measured rotenone toxicity measures 10.0 µg/l active rotenone at 24 h post treatment (irrelevant of nominal target concentrations), the treatment was always associated with success. At day five, success was always observed when measured concentrations were 8.0 µg/l or higher, and in one case, 5.0 µg/l. At day 10, 5.0 µg/l was associated with the successful elimination of Gizzard Shad. Similar predictions can be made for later dates using this figure.

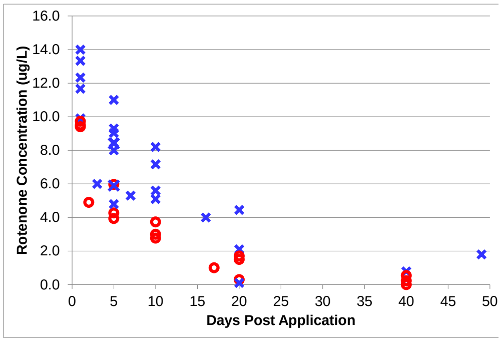

Figure 4. Active rotenone concentration across time. Successful applications are indicated by an “X” and treatments that did not eliminate Gizzard Shad are denoted by a “O”.

Gizzard Shad CPUE was affected by the interaction of lake and sample period (Table 4). Gizzard Shad were eradicated in 9 of 13 lakes treated from 2010 through 2013 (Table 1). Gizzard Shad CPUE was affected by the interaction of lake and sample period (Table 4) and, thus, gizzard shad pre and post-treatment data were analyzed by treatment (lake; Figure 5). For all lakes treated at a nominal concentration of at least 8.0 µg/l, Gizzard Shad were always successfully eradicated. Gizzard Shad CPUE was significantly reduced post-treatment in five of eight study lakes (Figure 5 (a)). However, Gizzard Shad CPUE increased in two of the post treatment samples. Both of these lakes were treated at 6.5 µg/l. We believe that in cases where Gizzard Shad abundance is substantially reduced, but not completely eliminated, increased CPUE is a response to a shift in stock recruitment brought on by reduced biomass and abundance of adults. In fact, most of this increase in CPUE was observed for age-0 Gizzard Shad.

Bluegill CPUE was affected by the interaction of lake and sample period (Table 4). CPUE was lower in post-treatment samples (Figure 5(b)). However, these differences were only significant in lakes where nominal rotenone concentrations were 9.5 µg/l. Two non-treatment control lakes (Keomah and Red Haw) were included for comparative purposes only. In these systems there was no significant difference in Bluegill CPUE in time periods corresponding to the pre and post-treatment sampling evaluated for

Table 4. Analysis of variance (ANOVA) for catch per unit effort (CPUE) of important species pre and post low-dose rotenone treatment. Sampling was performed in spring by day time electrofishing. Sample period is pre or post-treatment.

Source of Variation	DF	F Value	P
Largemouth bass			
Period	1	1.92	0.1698
Lake by year	14	13.55	<0.0001
Period*Lake by year	14	0.97	0.4952
Yellow bass			
Period	1	46.59	0.0001
Lake by year	2	2.26	0.1663
Period*Lake by year	2	2.26	0.1663
Gizzard shad			
Period	1	350.00	<0.0001
Lake by year	10	41.05	<0.0001
Period*Lake by year	8	31.12	<0.0001
Bluegill			
Period	1	22.41	<0.0001
Lake by year	14	6.97	<0.0001
Period*Lake by year	14	4.37	<0.0001
Redear sunfish			
Period	1	1.04	0.3157
Lake by year	9	7.11	<0.0001
Period*Lake by year	9	2.30	0.0582
Crappie			
Period	1	0.08	0.7830
Lake by year	14	5.77	<0.0001
Period*Lake by year	14	2.07	0.0270

the study lakes. In Don Williams Lake in 2012, Bluegill were completely eradicated at a treatment level of 9.5 µg/l, an issue that is contradictory to project goal of game fish preservation.

Crappie CPUE was influenced by the interaction of lake and sample period (Table 4). We observed few differences in CPUE across pre- and post-treatment samples within lakes (Figure 5(c)). This relationship held true across data from Red Haw Lake, the control lake sample. While we recognize electrofishing is not a preferred gear for indexing crappie abundance (Pope et al. 2009), it was the most convenient method of estimating population trends for this particular study. Shoreline observations of crappie mortality at 24 h post-treatment were relatively rare at field trial lakes. Thus, we believe only limited crappie mortality resulted from these low-concentration treatments. We believe these results are indicative of a lack of change in crappie abundance as a result of these treatments.

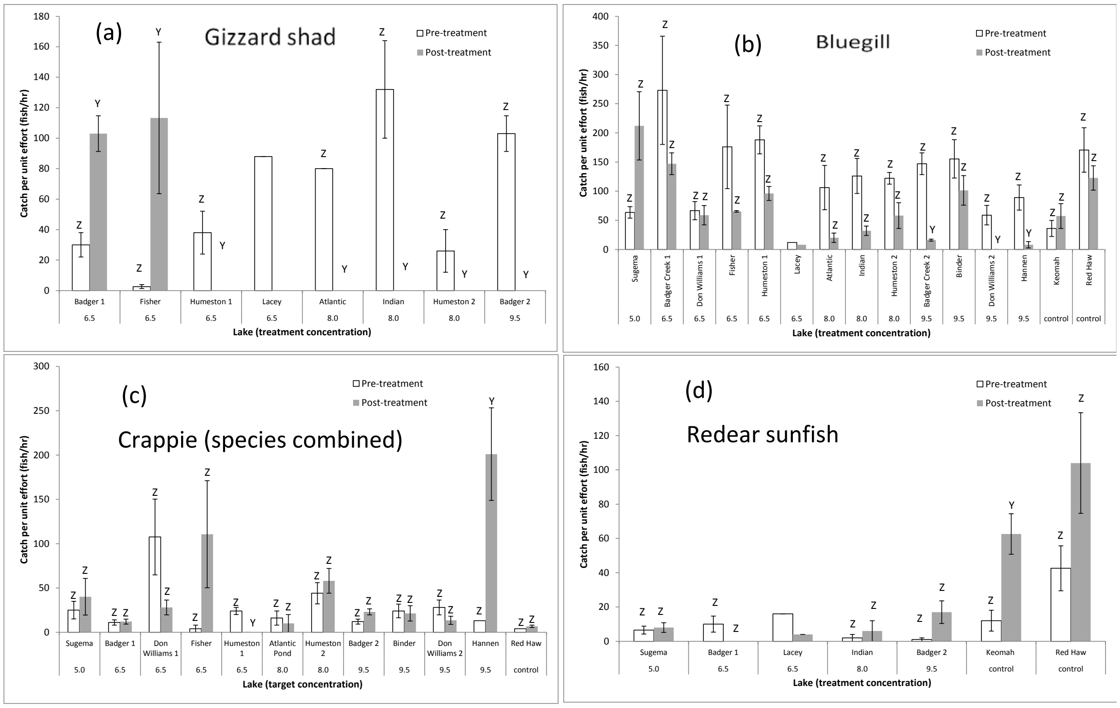

Figure 5. Pre- and post-application CPUE (with standard error in parentheses) for target and non-target species in study lakes. Differing letters correspond to differences across pre and post CPUE within a lake. Nominal rotenone concentrations are noted below lake names.

Redear Sunfish *Lepomis microlophus* CPUE did not demonstrate any significant differences in pre and post-treatment Redear Sunfish CPUE in any of the test lakes (Table 4; *P*=0.3157).

Pre and post-treatment Largemouth Bass CPUE was not impacted across lakes (Table 4; *P*=0.4952) and thus was not negatively impacted by any level of treatment (Table 4). Yellow Bass CPUE was negatively impacted by treatment (*P*=0.0001; Table 4). However, Yellow Bass were only present in three of the lakes treated from 2010 to 2013. In all cases, Yellow Bass were eliminated. Two of these lakes were treated at 6.5 µg/l and one was treated at 8.0 µg/l.

Research pond trials - experimental evaluation of low-concentration rotenone toxicity

Measured concentrations of active rotenone varied across repetitions (Figure 6). A measured : nominal ratio of 1.0 would indicate exact agreement between calculated and measured rotenone concentrations. While there is variability within each trial concentration, nearly all values are within 20% of the 1.0 ratio. A chi-square test indicated that the proportion of samples above the 1:1 ratio was not statistically different from the number of samples below the 1:1 ratio (X^2=0.5930) and thus no specific miscalculation in treatment levels is likely. Variability in treatment levels is a random effect. These concentrations are much closer to desired levels than those observed in a similar study in 2013 (Flammang 2013). The measured : nominal ratios tend to demonstrate less variability about the

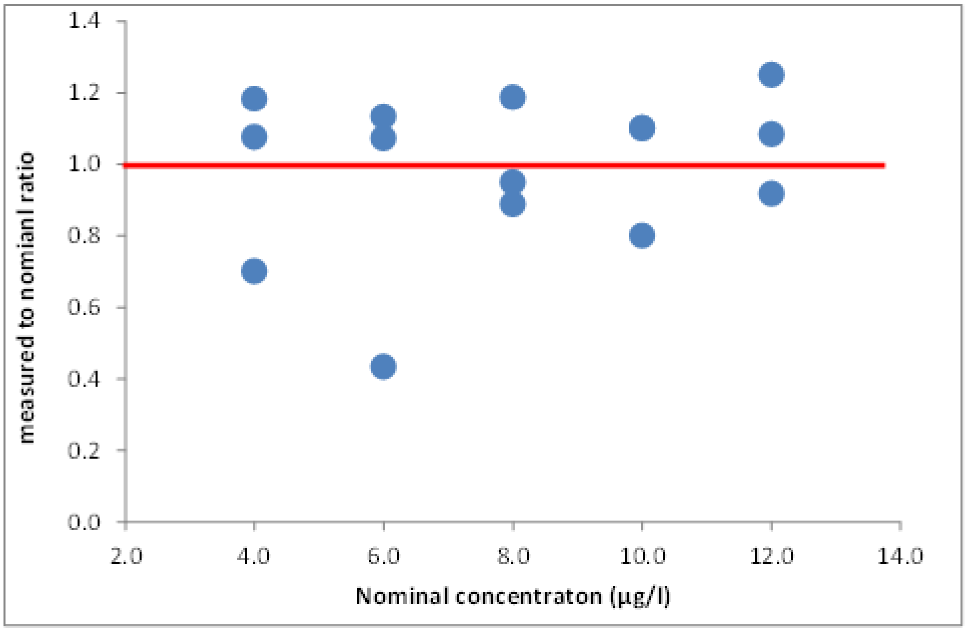

Figure 6. Measured : Nominal ratio of rotenone treatments by nominal concentrations. The red line indicates a 1:1 relationship, or perfect agreement between treatment (nominal) and measured values.

nominal values for pond trials versus field trials. It is important to note that pond trials are much more tightly controlled, volumes are likely more accurately estimated, and the air lift system causes complete mixing of the treatment area, which is not possible in such a short time in field trials. Our concentration results were closer to theoretical levels than other experimenters. Chapman (2003) observed measured : nominal ratios of rotenone in a bioassay that consistently were less than 0.5, despite a relatively small scale of experimentation, thus attaining agreement in nominal and measured concentrations remains problematic and some level of variability is likely, whether it be in controlled experiments or field trials.

Table 5 lists the significance of rotenone-based mortality for all study fishes. Mortality relationships were positively related to rotenone concentration for all species. There was no significant length-related bias associated with mortality within a species except for Largemouth Bass (Table 6). Mean length of dead Largemouth Bass was approximately 0.75 inches smaller than Largemouth Bass that survived. In fact, very few Largemouth Bass 9.0 inches and greater (TL) died as a result of rotenone exposure compared to those that survived (Figure 7). Whereas, there was a very similar response of mortality and

Table 5. Results of logistic regression evaluating the effect of rotenone concentration on mortality probability of study fishes.

Source of Variation	DF	Wald Chi-Sqare	P
Asian Carp	1	51.92	<0.0001
Bluegill	1	286.85	<0.0001
Crappie (species combined)	1	238.24	<0.0001
Grass Carp	1	52.08	<0.0001
Gizzard Shad	1	221.56	<0.0001
Largemouth Bass	1	120.74	<0.0001
Yellow Bass	1	196.34	<0.0001

Table 6. Kolmogorov-Smirnov test of length of dead and living fish by species following 96-h of low-dose rotenone exposure. Standard errors of length estimates are reported in parentheses.

Species	DF	F Value	Mean Length Dead (inches)	Mean Length Alive (inches)	Mean Length All (inches)
Largemouth bass	1	0.0030	5.78 (0.11)	6.52 (0.17)	6.27 (0.12)
Yellow Bass	1	0.4390	7.20 (0.03)	7.23 (0.03)	7.22 (0.02)
Gizzard Shad	1	0.3762	4.69 (0.08)	4.61 (0.05)	4.63 (0.04)
Grass Carp	1	0.4749	11.03 (0.09)	11.13 (0.11)	11.07 (0.07)
Bluegill	1	0.1256	5.91 (0.04)	5.82 (0.05)	5.88 (0.03)
Crappie (species combined)	1	0.4322	6.84 (0.03)	6.89 (0.06)	6.85 (0.03)
Asian Carp	1	0.5611	22.39 (0.13)	22.27 (0.14)	22.34 (0.10)

survival in smaller fish less than 8.0 inches TL.

Control mortality was low for all species examined. Only Gizzard Shad mortality in controls was notable and averaged 32% across all three trials (Figure 8). Gizzard Shad mortality reached 100% at the treatment level 8.0 µg/l. Similarly, Yellow Bass mortality also reached 100% in all three repetitions of each trial dosage at 8.0 µg/l. Gizzard Shad and Yellow Bass are the two species for which complete elimination is the threshold for project success. Given the high levels of fecundity these species are capable, anything less than complete eradication will quickly be outpaced by increased recruitment. The logistic models for Gizzard Shad and Yellow Bass do not predict complete eradication until a rotenone concentration of 10 µg/l is attained (Figure 8, E and G). However, these models are imperfect and are an approximation of these data. In addition, the model is constrained by its form. For both Gizzard Shad and Yellow Bass, complete eradication was reached at 8.0 µg/l and thus we suggest that this be the level targeted in field trials aimed at eliminating one or both of these species.

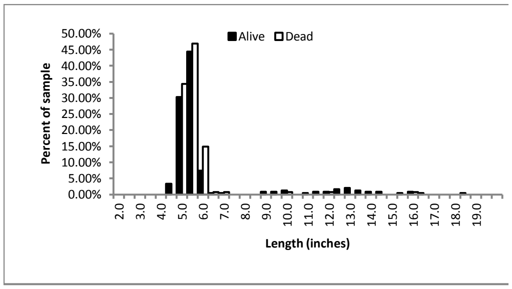

Figure 7. Comparative length frequency of dead and surviving Largemouth Bass 96-h post treatment.

Figure 8 (H) demonstrates that Gizzard Shad and Yellow Bass models predict higher levels of mortality at lower concentrations of rotenone than all other species examined. Several species, including Asian Carp, Bluegill, Grass Carp, and Largemouth Bass were shown to exhibit similar levels of mortality at the same range of rotenone concentration. The most tolerant of the primary species examined were crappie (Figure 8 H). In fact, at 8.0 µg/l crappie mortality was only modeled at approximately 8%.

Excessive gamefish mortality during low-dose treatment is counter to project objectives. Largemouth Bass and Bluegill mortality is modeled in Figures 8 F and B, respectively. These models suggest that both species are subjected to approximately 60% mortality at a treatment level of 9.5 µg/l. This is supported by field trial CPUE data that demonstrates substantial decreases in Bluegill and Largemouth Bass CPUE in post-treatment surveys (Figure 5). However, in field trials, the reduction in CPUE is often greater than 60%. However, differences in CPUE in pre and post-treatment samples was not often significant (Figure 5). There is some evidence that mortality declines as Largemouth Bass mean length increases; thus, high-density, slow-growing

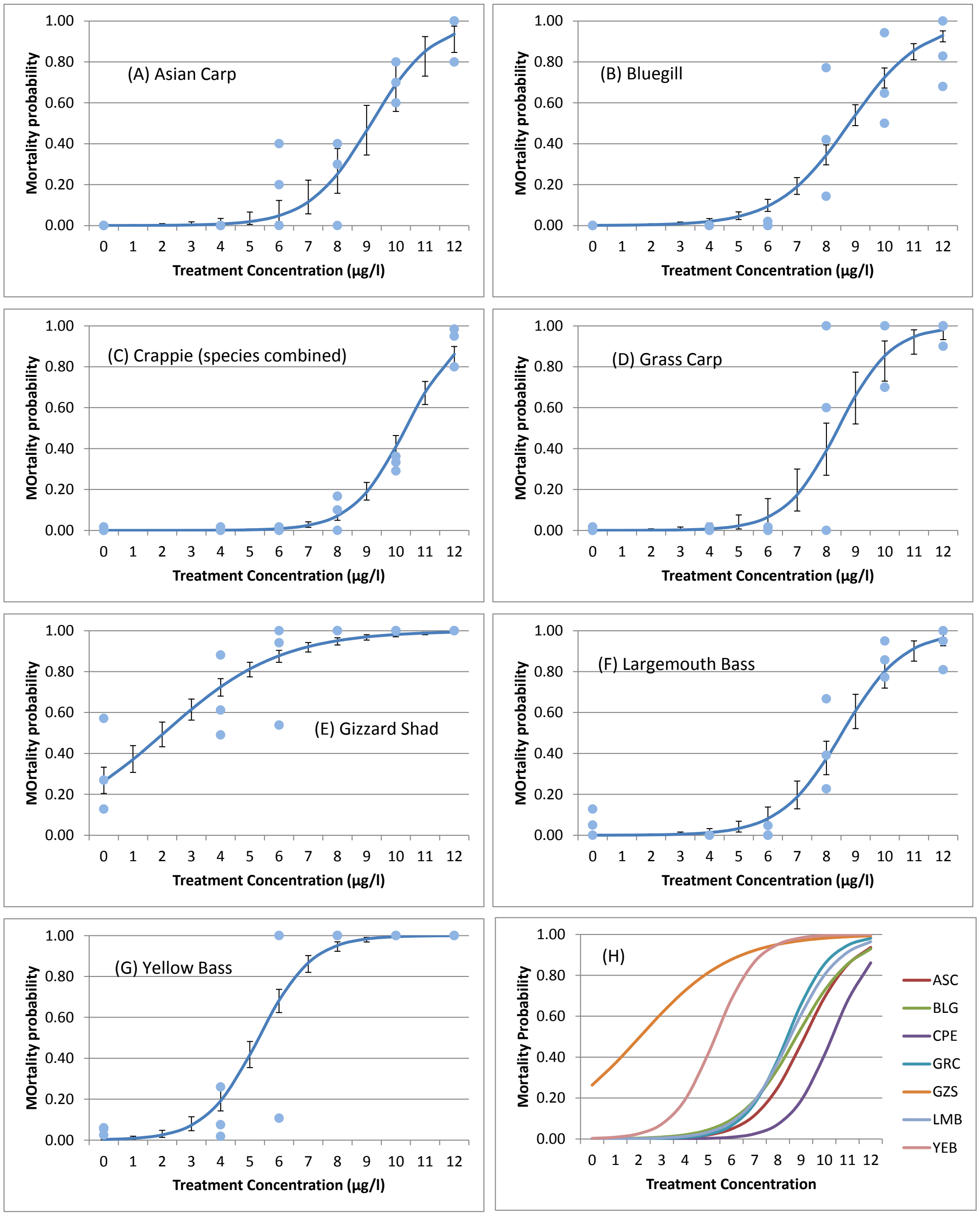

Figure 8. Predicted probability of mortality among study fishes at various treatment concentrations of rotenone with 95% Confidence Limits. Individual point estimates are noted by dots (A-G). Figure H demonstrates mortality at the full range of rotenone treatment, relative to all fishes.

populations of Largemouth Bass may demonstrate higher mortality rates than those dominated by larger, faster growing individuals (Figure 7). It is possible that differences in field applications may result in a higher level of mortality; however, we suggest that the logistic models created from the pond trials are more supported by the data and allow us a level of predictive ability for biomanipulation of many of the species examined.

For managers who wish to alter the population structure of abundant, but slow growing Bluegill populations, it is possible to predict mortality rates of based on these logistic models. For instance, Figure 8 (B) demonstrates that a treatment level of 8.0 µg/l will result in a Bluegill mortality rate of approximately 40%. A similar response was observed in Largemouth Bass. Thus, treatment at this level will have a similar impact on the major predator species, Largemouth Bass, in these systems. The reduced predator density may result in more short-lived positive impacts of Bluegill restructuring. Therefore, we suggest that in cases where Bluegill reduction is a priority, a treatment level of 7.0 µg/l will still result in a 20% reduction in Bluegill density, while reducing the impact on Largemouth Bass. We suggest that multiple treatments at the 7.0 µg/l level may be required to obtain the desired results. We discourage the treatment of Bluegill at concentrations over 8.0 µg/l as Largemouth Bass demonstrate greater rates of mortality over Bluegill at rotenone concentrations of 8.0 µg/l and greater (Figure 8).

Grass Carp manipulation is of increasing interest to fisheries managers. Grass Carp are known to alter aquatic trophic conditions and eliminate important aquatic macrophytes (Mitzner 1978). The use of Grass Carp has been an important management tool in Iowa since 1973. However, a recent change in management philosophy suggests that the removal of this long-lived species is preferable to the perturbed environs resulting from their presence. There is no documented evidence of natural recruitment of this species in Iowa lakes, and thus the complete removal of the species is not necessitated as a management practice. Instead, mangers may be able to utilize low concentration rotenone over multiple treatments to induce chronic mortality among these populations. In cases where Grass Carp density is to be decreased in an effort to affect change in macrophyte communities, a treatment of 8.0 µg/l rotenone will result in approximately a 35% decline in Grass Carp density. However, similar reductions in important game fishes will also be observed at this concentration. By treating at 7.0 µg/l there will be substantially less negative impact on important game fishes (Figure 8) yet a 20% reduction in Grass Carp density will still be observed. As these species do not recruit in lentic systems, multiple treatments over years may be utilized until the desired effect on the macrophyte community is obtained.

Flammang (2013) observed high levels of mortality in Silver Carp treatments in a similar study. The magnitude of this mortality was not repeated in this evaluation. However, we speculate that rotenone treatments in 2013 actually stressed Silver Carp, without inducing mortality. Instead, fisheries personnel likely removed these carp from the ponds following treatment prematurely. In 2014, all fish remained in the pond until mortality was definitive. Chapman et al. (2003) observed 100% mortality of Bighead and Silver Carp after four hours of exposure to 15µg/l rotenone and above. In that study they evaluated both juvenile and adult Asian carps. Our logistic model suggests we obtained similar results (Figure 8 (A)). Marking and Bills (1981) reported 96 h LC50s for Bighead and Silver Carp of 2.2 µg/l and 2.8 µg/l, respectively. Rach etal. (2009) observed Silver Carp mortality in low-concentration trials. However, their reported concentrations to achieve 24 h and 96 h LC50s were 114 µg/l and 62 µg/l 5% rotenone solution, respectively. Corresponding calculations of nominal active rotenone concentrations for their results are 5.7 µg/l and 3.1 µg/l, considerably less than the concentration suggested by our logistic model (Figure 8 (A)) for 50% mortality.

It seems likely that many Silver Carp affected by the rotenone treatment in 2013, would have recovered over time. As such, it is not likely that Asian carps can be removed from lentic systems without complete elimination of the fishery.

CONCLUSIONS

Gizzard Shad and Yellow Bass are problem species that managers typically prefer to eliminate from small impoundments in Iowa. Both species have been effectively eliminated at various levels of rotenone treatment in field trials. However, complete removal of Gizzard Shad has not been attained in all treatments until active rotenone concentration is at least 8.0 µg/l. In both field trials and static pond trials, 8.0 µg/l was effective at eliminating both species in all repetitions. Lower dosages of rotenone did not always eliminate these species (Figure 8).

Bluegill CPUE consistently declines by more than 70% in field trials following the use of low-dose rotenone (Figure 5). However, logistic regression models from our pond study suggest that at 8.0 µg/l Bluegill mortality is approximately 35%. Given that both Yellow Bass and Gizzard Shad can be effectively removed at a treatment concentration of 8.0 µg/l, we suggest that this level of Bluegill mortality is sustainable.

Largemouth Bass, Bluegill, and Crappie are not as heavily impacted by rotenone treatment levels that typically eliminate problem species, such as Gizzard Shad and Yellow Bass (Figure 8). However, in all cases, at any treatment level of rotenone, density of these fishes may be substantially reduced from untreated populations.

Grass Carp and Asian Carp populations can be manipulated through the use of low-dose rotenone; however, to completely remove these species, a complete renovation is required. To reduce Grass Carp populations by 50% a rotenone concentration of 8.5 µg/l would be effective. A treatment of 9.0 µg/l would reduce grass carp density by approximately 65%. Any project to remove Grass Carp should be undertaken with the concept that multiple treatments may be required to return the aquatic macrophyte community to the desired level.

The general public, natural resource managers, and government agencies have become increasingly concerned about the continued spread of Silver Carp and Bighead Carp in the Mississippi River basin and their potential spread into the Great Lakes. Recent flooding along the Des Moines River and Missouri River basins in Iowa has led to their establishment in multiple riverine wetland systems within these floodplains. However, there is little available toxicity information indicating the potential for rotenone control of Asian carps, particularly literature that would establish the ability of managers to selectively remove these invaders while maintaining important game fish fisheries. Marking and Bills (1976) reported that Asian carps were less sensitive than Bowfin, Coho or Chinook Salmon, Rainbow, Brook, or Lake Trout, Northern Pike, Longnose or White Sucker, or Walleye. We suggest that Asian carps similarly vulnerable to rotenone as many game fish, including Grass Carp, Bluegill and Largemouth Bass. Thus, the potential for selective elimination of this species is limited, and in fact, complete renovations will likely be required in those systems where they are targeted.

We suggest that sufficient data exists for the wide-scale use of low-dose rotenone for the selective manipulation of fish communities in Iowa. While the primary purpose of this study was to investigate the potential for the elimination of Gizzard Shad, multiple other species can be eliminated or reduced in density as a management tool. We stress the importance for accurate estimates of volume in target lakes and the need for rotenone concentration verification prior to treatment. However, the methods utilized thus far are supported by both the field and static pond studies.

MANAGEMENT RECOMMENDATIONS

Gizzard Shad Removal: We suggest a target concentration of 8.0 µg/l for all field trials aimed at eliminating Gizzard Shad. This has resulted in 100% success in all field and static pond trials.

Yellow Bass Removal: We suggest a target concentration of 8.0 µg/l for all field trials aimed at eliminating Yellow Bass. This has resulted in 100% success in all field and static pond trials.

Bluegill population structure manipulation: Managers interested in reducing high-density bluegill populations may consider multiple concentrations of rotenone. However, we suggest initial attempts should aim on the side of caution. A treatment of 8.0 µg/l will reduce bluegill density by 30-40% and will likely have desirable impacts on Bluegill growth and size structure in the years immediately following treatment. A treatment of 9.0 µg/l will have a greater impact but may result in heavier than intended mortality of Bluegill and other species.

Grass Carp reduction: Managers interested in affecting macrophyte communities should consider the use of low-dose rotenone for partial removal of Grass Carp. We recommend a treatment concentration of 8.0 µg/l, which will result in a reduction in Grass Carp Density of 30% - 50%. Multiple treatments may be necessary over multiple years to allow for the restoration of the desired aquatic macrophyte community.

Largemouth Bass preservation: Largemouth Bass are the primary predator in most Iowa lakes. It is recommended that treatment levels not exceed 9.0 µg/l to ensure sufficient predator density in post-treatment environments.

Asian Carp reduction: Managers wishing to eliminate Asian Carp with minimal impacts to the existing fish community will not be satisfied with the use of low-dose rotenone. Rotenone concentrations for complete elimination of Asian carps mirror concentrations for the removal of many game fish species. It may be possible to reduce densities of these fishes in lentic systems, which may prove useful as these species should not be able to reproduce successfully in lentic systems (Lohmeyer 2009).

Low-concentration rotenone applications can be a useful management tool for eliminating Gizzard Shad and Yellow Bass in Iowa lakes. In addition, substantial evidence points to low-dose rotenone as an important management tool for the management of Bluegill and Grass Carp. We suggest that these results are sufficient for managers to employ low-dose rotenone as a common management tool in Iowa.

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