Control of the common carp through species-specific toxin delivery systems and

biocontrol by bluegill sunfish

A THESIS SUBMITTED TO THE FACULTY OF THE UNIVERSITY OF

MINNESOTA

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

Dr. Przemyslaw G. Bajer

March 2018

Comprehensive Abstract

The Common carp (*Cyprinus carpio,* or 'carp') is an invasive fish native to Eastern Europe and Asia and is one of the world's most ecologically harmful species. It is known to cause issues with water clarity, increase nutrient levels, reduce aquatic vegetation, and impact waterfowl. There are many existing strategies to control carp, however, each has various issues. This has led to the search for more practical, sustainable, or broadly applicable control strategies. I investigated two emerging control strategies for carp: biocontrol of carp by bluegill sunfish, and selective toxin delivery systems. Biocontrol has been used successfully for decades in the agriculture industry; however, biocontrol for aquatic pest control is still rudimentary. Previous evidence has suggested that bluegill sunfish (*Lepomis macrochirus)* may be capable of controlling carp recruitment by consuming eggs and larvae of carp, however, this has never been tested experimentally in natural, whole-lakes. To test if bluegill were capable of limiting carp recruitment in natural lakes, I conducted a two-year experiment where carp were stocked into natural lakes, and bluegill were stocked in half of the lakes. The recruitment success of carp was assessed at various stages in first growing season of development: (1) the egg stage, (2) the post-larval juvenile stage, and (3) the end-of-season juvenile stage. The results indicate that bluegill predation had a clear effect on the abundance of post-larval carp, but the abundance of end-of-season carp was also affected by other processes (such as density-dependence). This, to my knowledge, is the first experiment on biocontrol of an invasive fish conducted using whole-lake manipulations. Its results are applicable to management of carp in shallow, productive lakes.

Next, I performed an analysis to examine lake characteristics that cause bluegill abundance to be low, and thus define conditions where bluegill density is too low to control carp through biocontrol. To conduct this analysis, I used a large dataset on bluegill abundance and lake characteristics provided by Minnesota Department of Natural Resources. My model incorporated 12 lake and watershed variables that were used to explain variation in bluegill sunfish catch-per-unit-effort (CPUE). Of those variables, depth and water clarity had the largest effect. Specifically, bluegill abundance declined rapidly in lakes with maximum depths of less than 7 m, and a Secchi disk depth less than 0.7 m. These conditions are indicative of lakes that often winterkill, thus aeration may be a feasible way to stabilize bluegill populations in some of these systems.

Last, I incorporated antimycin-a (ANT-A), a known fish toxicant, into a corn-based bait and conducted a series of experiments to determine its toxicity, leaching rate, and species-specificity. My results showed that ANT-A was lethal to carp at doses ≥ 4 mg/kg and that the amount of ANT-A that leached out of the bait in 72 h was not lethal to carp or bluegill, which was desired. Species-specificity trials were conducted in 227 L tanks, in which carp were stocked with three native species representing families that occur sympatrically with carp in my study region: the fathead minnow (*Pimephales promelas*), yellow perch (*Perca flavescens*) and bluegill. These trials showed high mortality of carp (46%) and fathead minnows (76%) but no significant mortality of perch or bluegill. Finally, a pond study, which used the same species composition except for fathead minnows, resulted in 37% morality among adult carp and no mortality among perch or bluegill. My results suggest that corn-based bait that contains ANT-A could be

used to selectively control carp in lakes dominated by percids or centrarchids, such as

across the Great Plains of North America, where carp are especially problematic.

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Chapter 1: Thesis Introduction

The common carp (*Cyprinus carpio,* or 'carp') is a large, benthivorous cyprinid from Eurasia that has become invasive throughout North and South America, Australia, and Africa (Balon 1995; Moreau & Costa-Pierce 1997). In the United States, it was intentionally introduced in the late 1800s due to demand from European immigrants who prized it as a sport and food fish (Cole 1905). An extensive stocking program introduced carp to many river systems and the Laurentian Great Lakes, aiding carp's spread. Today, carp have spread across the United States, invading every state expect for Alaska (Nico & Fuller 1999).

The effects carp have on lakes have been well documented. Carp are an ecosystem engineer, meaning they alter the habitat where they live (Weber and Brown 2009). Carp are known for rooting in lake bottoms while searching for food, which leads to sediment re-suspension, reduced water clarity, and release of sediment-bound nutrients into water column (Vilizzi et al. 2015). Carp also uproot aquatic vegetation and cause lakes to shift alternate stable states: from a clear, vegetated state, to a state characterized by lack of vegetation and turbid water (Zambrano et al. 2001, Bajer et al. 2009). The shift in lake conditions has a negative impact on native biota, including migratory waterfowl, whose numbers often decline by an order of magnitude in lakes dominated by carp (Haas et al. 2007). A century after its introduction, the carp is estimated to have contributed to the decline of aquatic vegetation in approximately 70% of lakes within the Great Plains region of southern Minnesota (Bajer et al. 2016).

Methods exist to control carp that can be used with varied success over varied landscapes. Physical removal has been used to control carp populations, particularly in

temperate North America. Here, carp form tight winter aggregations that can be located by tracking radio-tagged fish and removed via netting (Bajer et al. 2011; Armstrong et al. 2016). This strategy is believed to be sustainable primarily in systems with abundant egg and larval predators that diminish carp's reproductive success (Lechelt & Bajer 2016). In systems with weak predatory communities, removal has not been very effective due to density-dependent, compensatory recruitment (Colvin et al. 2012; Weber et al. 2016). Non-specific toxicants dispersed into lake water, paired with water draw-downs, have also been used to eradicate carp populations. This method has been used sporadically because it is expensive, impacts native biota, and can primarily be used in lakes that are isolated with barriers to prevent reinvasion (Hanson et al. 2017). Viruses and genetic technologies have been proposed for carp control; however, carp might develop resistance to viruses within a few generations, (McColl et al. 2014), and genetic technologies remain at the developmental stage and are associated with social concerns and uncertainties (Thresher et al. 2014a; Thresher et al. 2014b).

Further techniques need to be developed and added to the repertoire of current carp control methods. Particularily, natural, sustainable, and practical schemes are needed, which can be part of an intregrated pest management (IPM) approach. IPM is where natural weaknesses of an invasive species physiology or behvaior are exploited by applying multiple techniques to lessen the pests' ability to cause economic injury (Kogan, 1998). In this thesis, I will describe experiments and studies testing new techniques for the integrated management of carp populations. Specifically, I will address (1) biocontrol of carp by bluegill sunfish (*Lepomis macrochirus*), (2) lake conditions that cause low

bluegill abundance (i.e., conditions where biocontrol is unlikely to occur, and where other control strategies might be needed, such as toxins), and (3) species-specific toxin delivery systems to control carp.

In chapter 2, I dicuss results from experiments conducted in natural lakes to test biological control of carp recruitment by bluegill sunfish. In these experiments, four small lakes were selected in 2016, and six in 2017 to test if bluegills were capable of controlling carp recruitment (the ability of an individual to survive to become part of the population). Half of the experimental lakes were stocked with bluegill sunfish and all were stocked with pre-spawning adult carp. This created a simple treatment-control design, where carp recruitment could be compared in lakes with bluegills and without bluegills. Carp were observed spawning and then different stages in recruitment (eggs, post-larval juveniles, end-of-season juveniles) were monitored throughout the growing season. Differences between lakes with bluegills and without bluegills were analyzed. These results determine if biocontrol of carp by bluegill is practical on a large-scale, natural system level. I also use this experiment as an example of biocontrol being feasible for other species of fishes, and set up a framework that outlines reasons certain species may be more or less difficult to control using biocontrol.

In Chapter 3, I outline an analysis of bluegill abundance based on habitat characteristics of lakes, such as maximum lake depth, lake area, Secchi disk depth, macrophyte species richness, and watershed characteristics (e.g., land use, watershed area). Primarily, I focus on determining characteristics that result in low bluegill

abundance. In lakes that have these characteristics, bluegills are incapable of acting as a biocontrol agent for controlling carp recruitment. Thus, my results help characterize lakes that are likely to function as carp nurseries within the landscape, based on their physical characteristics. It also suggests lakes and lake types in which management efforts, such as aeration, may be necessary to support biocontrol of carp by bluegills. In many of these lakes, aeration will not be practical or feasible, and other management approaches, such as use of carp-specific toxin delivery systems, might be pursued.

In Chapter 4, I discuss results from four experiments conducted in collaboration with the United States Geological Survey. The contributors to this work include Blake Sauey, Jon Amerg (both USGS), and Przemyslaw Bajer, my advisor. This is a post-peer-review, pre-copyedit version of an article published in *Biological Invasions*. The final authenticated version is available online at: http://dx.doi.org/ 10.1007/s10530-018-1662-y. Experiments were designed to determin if a toxicant could be incorporated into a species-specific bait. Ideally, this bait would affect mortality of carp but not native species. First, a corn-based bait was designed, which incorporated antimycin-a (a known fish toxiciant). Trials were then conducted to determine the potency of the bait, examine leaching of the toxicant from the bait, and test species-specifictiy in mixed-species tank and pond experiments. My results determined a lethal concentration of antimycin-a, demonstrated limited leaching of the toxicant into water, and showed mortality of carp and fathead minnows (*Pimephales promelas*), but not yellow perch (*Perca flavescens*) or bluegill sunfish when exposed to the toxic bait.

Overall, this thesis provides information on potentially practical methods to control carp. Included for each is background, the logic and reasoning behind these methods, the results from my studies, discussions of their implications, limitations of my experiments, future directions, and how my findings can be used in management of carp. Each chapter was written individually for publication in scientific journals, and stands alone without this introduction section. As such, there is some overlap between chapters.

Chapter 2: Natural lake experiments indicate that bluegills may be capable of controlling

early stages of common carp recruitment

Introduction

Biological control (or 'biocontrol') of invasive species has been used successfully for decades in the agriculture industry. Early examples of successes using biocontrol are the control of the citrus grove pest, the cottony cushion scale (*Icerya purchasi*) by the ladybird beetle (*Rodolia cardinalis;* Bosch et al. 1982), or the control of boll weevil (*Anthonomus grandis)* in southern United States by an introduced parasitic wasp (*Catolaccus grandis*; Lange et al. 2009). Historically, natural enemies, introduced from the pests' native range, have been used as biocontrol agents (Hokkanen & Pimentel, 1984). More recently, new association approaches for selecting biocontrol agents have been used, where a predator native to the invasive range is used to control an introduced pest (Hokkanen & Pimentel, 1984). This approach is thought to have fewer unintended, harmful consequences (Bosch et al. 1982). Though biocontrol has often been successful in controlling agricultural pests, its application to aquatic pests, especially fishes, is practically non-existent. To date, there are no management strategies for invasive fish that intentionally use biocontrol.

The hypothesis that invasive fishes might be controlled using biocontrol is supported by several lines of evidence. First, a long history of studies documenting the strong effect of predation on structuring freshwater fish assemblages lends credence to the possibility that predation might also have similar effects on invasive fish success (Tonn et al. 1992). Second, invasive fish become abundant in only a fraction of locales with otherwise favorable abiotic conditions, suggesting that biotic processes (like

predation) might be limiting their success in may locales (Hansen et al. 2013; Bajer et al. 2016). Finally, small-scale (laboratory, mesocosm, pond) experiments and correlative observations have suggested the possibility of biocontrol by native predatory fishes for several invasive fishes (Davies and Britton 2015, Iguchi and Yodo 2004; Bajer et al. 2015). Despite this mounting evidence, no tests of biocontrol have ever been conducted using large, natural experiments (e.g., whole lakes over multiple seasons).

Biocontrol strategies would be most effective if weaknesses in the pest species' life history were exploited (Iguchi & Yodo 2004). For example, small body size as an adult would make an invasive fish susceptible to predation during multiple life stages, or lack of parental care (e.g., nest building, guarding eggs, etc.) would leave fishes vulnerable to predation in their first weeks of life when they are small and defenseless (Lechelt 2016). Local habitat and predator composition would also affect success of biocontrol strategies. Structurally complex habitat would limit predators' foraging success and access to prey (Huffaker 1958; Savino & Stein 1982), and abundance of the predator would influence the rate at which the invasive species is controlled. As such, integrated pest management (IPM) strategies should be created for invasive fishes that enable the success of biocontrol by manipulating local habitat and predator composition, and also employ techniques that exploit aspects of the pests' life history that are not targeted by biocontrol.

In this study, biocontrol was tested in whole-lake systems using the common carp (*Cyprinus carpio,* hereafter, 'carp')*,* as a model for a highly fecund invasive fish that

lacks parental care. Carp are vulnerable in that their eggs are small and adhere to vegetation (Balon 2004) where they could be consumed by predators adapted to foraging within vegetation stands. Carp larvae are free swimming and are not know to possess defense mechanisms. Observational studies, lab experiments, and mesocosm experiments have suggested that carp recruitment can be limited by a small native fish, the bluegill sunfish (*Lepomis macrochirus),* which act as a micropredator— a predator capable of consuming eggs and larvae of carp (Bajer & Sorensen 2010; Bajer et al. 2012; Silbernagel and Sorensen 2013; Bajer et al. 2015; Lechelt 2016). Despite bluegills' propensity to act as a micropredator, carp are still able to successfully recruit in many interconnected systems by migrating into seasonally unstable habitat that frequently experiences winterkill events (Bajer and Sorensen 2010; Bajer et al. 2015). Due to the frequency of winterkills in these marsh systems, bluegills, which are especially sensitive to hypoxia (Farwell et al. 2007), are not abundant, and carp spawn free from micropredation. Later, carp recruits migrate from the unstable habitat they were born, to the lake where they live their life, returning again as adults to spawn (Bajer and Sorensen 2010). If bluegills could be stabilized in these marsh systems, they might be able to control carp recruitment through micropredation. One such strategy to prevent winterkill and stabilize bluegill populations, is winter aeration. Aeration works by creating a refuge area of continuous oxygen in water bodies that would otherwise experience winterkill due to oxygen loss (Lackey and Holmes 1972; Cornelius 2006). Winter aeration may work in many locations; however, it may not work in all locations, such as lakes less than 1.5 m deep (Cornelius 2006). Regardless, if bluegill populations could be stabilized in at least

some areas carp use as nursery habitat through winter aeration, IPM strategies could be developed that use biocontrol to control carp recruitment. Additionally, efforts to strengthen biocontrol could be combined with physical removal of adult carp by targeting their winter aggregations (Lechelt & Bajer 2016). Despite many pieces of evidence, the hypothesis that bluegill sunfish might control carp recruitment in shallow lake habitats has not been tested using whole-lake experiments.

To test if bluegills could control carp recruitment, I conducted a two-year experiment where adult carp were stocked into natural lakes in Minnesota, and bluegill were stocked in half of these lakes. The recruitment of carp was assessed at various stages: (1) the egg stage, (2) the post-larval young-of-year (YOY) stage, and (3) the end-of-season YOY stage. The results indicate that bluegill predation had a significant effect on the abundance of post-larval YOY carp, but the abundance of end-of-season YOY carp were secondarily affected by other processes (such as density-dependence) as well.

Methods

This study consisted of an experiment conducted in several small lakes over two consecutive years. It included four lakes in 2016 (two treatment lakes and two control lakes), which were used again in 2017, with two more lakes (one treatment lake and one control lake) added (Table 1). Two lakes were present at of each of the three sites (Crown College, Albert Lea, Metro), allowing a paired design (treatment and control) at each site. Lakes at Crown College and Albert Lea sites were directly adjacent, while Metro site lakes were both located in the Twin Cities metro area, approximately 30 km apart. During

the experiment, recruitment of carp at three stages of development was assessed. First, I assessed the survival of carp eggs in control and treatment lakes. This experiment was labor intensive and could only occur when carp were spawning, requiring daily checks of the study lakes; thus it was conducted in only one pair of lakes (Crown College) each year. Second, in each lake, I quantified carp recruitment at the first post-larval stage that could be reliably sampled (approximately one month after spawning, when carp were 30 - 60 mm in length). This was the first life stage where carp outgrew predation by bluegill (i.e., bluegills can only forage on carp eggs and larvae). As such, I considered this the most important data for quantifying the overall impact of bluegills on carp recruitment. Finally, I assessed the recruitment of carp at the end of the season (4-6 months after spawning; when carp were 100-160 mm in length) by conducing mark-recapture estimates in each lake. This life stage was not directly influenced by bluegill predation, but it was of most management importance (these juveniles are considered large enough to recruit to next year class). I hypothesized that lakes which had high abundance of post-larval juveniles will also have high abundance of end-of-season juveniles.

In addition to quantifying the abundance of various life stages of carp, I also monitored the survival and recruitment of bluegills, and presence of other native fish species in each lake (Supplement A, Table 1); while I strived to conduct this experiment in lakes that were initially "fishless", such systems could not be found and most lakes contained small numbers of native species that often occur in small lakes prone to hypoxia, such as fathead minnows (*Pimephales promelas)* and black bullheads (*Ameiurus melas).*

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Study Lakes

A physical description of the study lakes, their location, and habitat characteristics are presented in Table 1. Briefly, the lakes ranged in size from 0.35 ha to 1.8 ha and had maximum depths of < 3 m (Table 1). All systems were eutrophic to hypereutrophic, which was determined by collecting water samples monthly from May-July and analyzing them for total phosphorus. The lakes' dissolved oxygen level, temperature, water clarity, and aquatic vegetation cover were also monitored.

Stocking lakes with adult carp and bluegill

Bluegill sunfish were collected from lakes near the study sites using trapnets in April and May and transported to every other experimental lake (one in each pair). Bluegills were stocked at a density of 100 kg/ha, a representative density for bluegill in the littoral zone (where carp spawn) of many Minnesota lakes (Carlander, 1955; Bajer et al. 2012). Carp stocking occurred in all lakes approximately two weeks later, to allow time for bluegills to acclimate. Adult carp (pre-spawning) were collected from local lakes using boat electrofishing and transported to the study lakes. Prior to being stocked, the gender of carp was determined by extrusion of eggs or sperm. Each carp was measured and the number of eggs each female contained was estimated from the total body length (Bajer et al. 2012). Enough females were stocked to achieve a theoretical propagule pressure of 20 million eggs/ha, because carp recruitment is often highest at such egg density (Bajer et al. 2012). To achieve this, approximately 10-15 females were stocked per hectare. Male carp were stocked at a density of 1.5-2 males per female.

Removal of fish at the end of the 2016 season was not feasible, thus, carp and bluegill were left in lakes over the winter (i.e., the same treatments continued from one year to the next). Before re-stocking in 2017, mark-recapture estimates were conducted to estimate the number and biomass of bluegills that survived over winter and determine how many new bluegills needed to be stocked in 2017 to achieve 100 kg/ha (Supplement A, Tables 2 & 3). Adult carp were stocked in 2017 at the same density as in 2016, because the number of carp that survived the winter could not be determined; adult carp are notoriously difficult to capture to estimate their abundance.

Egg Predation Experiment

This experiment occurred in Crown College lakes (one treatment and one control) each year. After carp were stocked, the lakes were monitored daily between the hours of 0600 and 0900 for carp spawning. When spawning occurred, its intensity (number of splashes per minute) was recorded and locations of spawning sites were mapped. The following day (carp eggs usually take 3-4 days to hatch), I collected aquatic vegetation with carp eggs adhered to it from one of the main spawning areas and used it to conduct the egg predation experiment. Sub-samples of the collected vegetation (~ 30-70g) were placed inside experimental arenas (1 m x 0.5m x 0.5 m) constructed using PVC pipes and a green mesh. The arenas consisted of two halves: open (allowing bluegill access to the vegetation) or closed (excluded bluegills using 1 cm mesh; Supplement A, Figure 1). An equal amount of vegetation was placed in each half of each arena. In addition, a sample of vegetation of similar size was placed in a re-sealable bag, and taken back to the lab

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immediately to determine the initial egg density, and act as a control (i.e., to make sure eggs didn't hatch or fall off of vegetation during experiment) Ten experimental arenas were placed in each lake, with the exception of control lake in 2016 where only 5 arenas were used. The arenas were distributed along the shoreline of each lake, approximately equidistantly. After 24 h, the arenas were removed from the lakes; vegetation was placed on ice and taken back to the lab where the vegetation was blotted dry and weighed (nearest 0.1 g). Carp eggs present in each sample of vegetation were then counted to determine egg density (eggs/g of vegetation).

Egg densities in each treatment were analyzed using analysis of variance (ANOVA) at an alpha level of 0.05. When the ANOVA was significant, a pairwise t-test with Bonfferoni adjustment was used to determine which treatments differed from one another.

Abundance of post-larval juveniles

Approximately one month after spawning, backpack electrofishing surveys were conducted in each lake to quantify the abundance of post-larval juveniles. Three such surveys were conducted in 2016, and four in 2017. Surveys were conducted approximately every two weeks. Each survey consisted of four 15 min transects. In each transect, I waded slowly along the shoreline of the lake and electrofished in zigzagging motion. All stunned fish were netted and total length was measured to the nearest mm. In cases where greater than 50 individuals were captured for a single species, the first 50 specimens were measured, and remaining fish were counted. These surveys generally

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captured post-larval carp, juvenile bluegills (only in treatment lakes), and fathead minnows (Supplement A, Table 1).

For analysis, a mean catch-per-unit-effort (CPUE; number of post-larval carp per hour) was calculated for each survey, by averaging CPUEs among transects. Next, overall mean CPUE for each lake in each year was calculated. Mean CPUEs were analyzed using an ANOVA to test how CPUE was affected by the treatment (whether bluegills were present or not), year (2016 or 2017), and site location (Albert Lea, Crown College, Metro). Due to small sample sizes needed to conduct natural, whole-lake experiments like this (four or six lakes), I tested significance using an alpha of 0.10.

Abundance of end-of-season juvenile carp

The end-of-season juvenile carp abundance was assessed using mark-recapture analysis. Carp were captured via trapnets or by seine net. Five trapnets (69 X 99 cm frame, four 61 cm hoops, 1 cm mesh; Duluth Net Co. Duluth, MN) were placed in the littoral zone of each lake and left for 24 h, after which fish were removed. The seine net (30 X 3 m, bag seine, 1 cm mesh; Duluth Net Co. Duluth, MN) was used from shore or boat to capture fish. Captured carp were marked with a fin clip. The population of the end-of-season juvenile carp was estimated using the Schnabel method of mark-recapture, which allows for multiple capture periods (Ricker, 1975). Different numbers of surveys were used in different locations to get as accurate population estimates as possible. In most lakes, three or four surveys yielded greater than 20 recaptures, and surveying was deemed complete. In few locations, sampling continued for up to 11 surveys, to get as many recaptures as possible. In lakes were no YOY carp were captured in the first three

surveys, the population was assumed to be zero (Table 2). The FSA package in R statistical software was used for the population estimate calculation and estimation of the 90% confidence intervals (R Development Core Team 2013; Ogle, 2016). The abundance of end-of-season juvenile carp was considered statistically significant when the 90% confidence interval didn't overlap between control and treatment lakes.

Abundance and survival of bluegills

The number of adult and fry bluegill sunfish present at the end of the season in treatment lakes was also assessed using mark-recapture, concurrently with end-of-season mark recapture estimates for carp. In 2016, bluegill fry present at the end of the season was assessed using relative catch-rate compared to adult bluegill instead of mark-recapture.

Results

Egg Predation Experiment

In 2016, there was no significant difference between either of the two treatments (df = 5; F = 1.50; P=0.21; Figure 1). In 2017, the ANOVA showed a significant difference in at least one of treatments (df = 5; F = 7.89; P < 0.01). The pairwise t-test revealed egg density in the closed portion of the experimental arena was significantly greater than in the treatment lake but not in the control lake (Figure 2). In either year, the control group was never significantly different from the closed treatment.

Abundance of post-larval juveniles

In 2016, the CPUEs of post-larval juvenile carp were consistently higher in control than treatment lakes at both sites (Figure 3). At Crown College lakes, CPUEs ranged from 26 to 56 in the control lake and 10 to 15 in the treatment lake. At Albert Lea lakes, CPUEs ranged from 44 to 73 in the control lake and between 1 and 3 in the treatment lake (Figure 3). In 2017, a similar pattern occurred. At Albert Lea lakes, CPUEs ranged from 32 to 360 in the control lake and between 7 and 13 in the treatment lakes. At Metro lakes, CPUEs ranged from 5 to 13 in the control lake while no post-larval carp were caught in the treatment lake (Figure 3). At Crown College, no post-larval carp were captured in either treatment or control lake, however, the results in the control lake were abandoned, because this lake became invaded by bluegills. Bluegills were first detected in the control lake in early July, 2017; all bluegills captured by backpack electrofishing were juveniles (mean size = 50.23 mm). Over the four surveys, 44 bluegills were captured per hour. Bluegill were not found until after the egg experiment was complete; thus, results for this lake were included for the egg experiment but not thereafter. The ANOVA showed that treatment effect was significant (df = 1; F = 6.57; P = 0.06), but year (df = 1; F = 0.09; P = 0.78) and site (df = 2; F = 1.73; P = 0.29) were not.

Abundance of end-of-season juvenile carp

In 2016, the estimates for YOY carp at the end of the season were higher in control lakes than in treatment lakes. The end-of-season juvenile carp estimate for the

control lake was approximately 7 times higher than the estimate for the treatment lake at the Albert Lea location and approximately 6 times higher at the Crown College location. The 90% confidence intervals for control lakes and treatment lakes did not overlap at either location.

In 2017, the estimates for YOY carp at the end of the season were typically lower than estimates from 2016. In two of the treatment lakes (Metro and Crown College), no YOY carp were ever captured. The Metro control lake was estimated to have 1,036 YOY carp/ha, and data from the Crown College control lake was abandoned due to invasion by bluegill (see above). In the Albert Lea location, similar numbers of juvenile carp were estimated in the treatment lake and the control lake at the end of the season, but both were low. Population estimates, CIs, and mark-recapture statistics for each lake in each year can be found in Table 2.

Abundance and survival of bluegills

Approximately 600 to 1,000 bluegills/ha survived through the end of the season in treatment lakes. Bluegills recruited between 230 and 6,800 individuals in treatment lakes, and their biomass was included in the biomass present in each lake. The estimates for adult and fry bluegill abundance at the end of the season can be found in Supplement A, Table 2. It was estimated that 51 to 78% of the bluegill biomass was present at the end of each season relative to the initial biomass.

Discussion

This is the first experiment to my knowledge that has tested biocontrol on an invasive fish using multiple whole-lake manipulations. The results indicate that bluegill sunfish can significantly reduce the recruitment of carp, because recruitment of carp was consistently low in locations where bluegill were present and high recruitment rates occurred only in lakes without bluegills. Despite this, bluegills were not able to eliminate carp recruitment in all locations, even at the high density they were stocked for this experiment. Further, I observed the largest effects of biocontrol on the post-larval carp, whereas the effect was less consistent at the end-of-season YOY carp (i.e., the catch-rate of post-larval juveniles was always higher in control lakes than treatment lakes, but the end of the season estimates for YOY carp were not always higher in control lakes than treatment lakes). This suggests that processes other than bluegill predation affected carp at later stages of recruitment. IPM strategies that seek to control carp will need to recognize that while bluegills can significantly reduce carp recruitment they cannot always eliminate it, even at high densities. Nevertheless, reductions in carp recruitment via bluegill predation will make IPM strategies that involve other tools (e.g., adult removal) more sustainable.

Aeration is one feasible method to stabilize bluegill populations (and other micropredators) in habitat where carp spawn, and enhance the ability to control carp through biocontrol. Managers will need to weigh the costs and benefits of using aeration systems. For example, the costs of purchasing, operating, and maintaining aeration

systems should be compared to the effect aeration has on bluegills, whose population should be monitored before and after aeration. Additionally, aeration isn't feasible in all systems; for example, it's often not feasible in water bodies less than 1.5 m in depth (Cornelius 2006). In almost all of our experimental lakes, bluegills produced abundant offspring; thus, if bluegills could be kept alive in the marsh areas (through aeration), they would likely survive and propagate, and there would be no need to repeatedly stock bluegill into marshes for the purpose of biocontrol. This study is unique in that I was able to stock a known number of an invasive fish, and use mark-recapture to estimate the number of recruits that were produced. This allowed me to estimate the number of recruits produced per female in the study lakes. In the lakes, up to 2,200 recruits were produced per female, which is well above previous estimates (~800 per female, Bajer et al. 2015). This new information should be incorporated into carp population dynamics models that are used to assess potential effectiveness of carp management strategies.

In the egg predation experiments, egg density decreased significantly in the open halves of the experimental arenas even in lakes without bluegills. I attribute this effect to the presence of and consumption of eggs by other fish species, particularly the fathead minnow, which was present in both lakes in moderate to high abundance (30-75 fathead minnows/hour of backpack electrofishing; Supplement A, Table 1). In the egg predation experiment, fatheads were seen around experimental arenas, presumably consuming eggs. This shows that even in this relatively controlled environment, bluegills exclusively weren't responsible for all the reduction of eggs. Regardless, the reduction of eggs I observed (~58% reduction where bluegills were present, ~49% when absent) was much

less than the reduction of carp observed at the post-larval stage (~9-fold fewer post-larval carp in treatment than control lakes). From this, it appears egg predation accounts for a relatively small decline in recruitment, and that predation in other stages of recruitment (larval or fry) are important for biocontrol of carp. The egg survival documented in this study was similar to what was documented within natural carp spawning areas in two Minnesota lakes (Silbernagel and Sorensen, 2013) and in a mesocosm experiment (Lechelt 2016); however, it was substantially lower than what was observed by Bajer et al. (2012) who used artificial spawning substrate (green yarn) placed in relatively open patches among stands of aquatic macrophytes. This suggests that greater habitat complexity (vegetation density) might diminish the ability of bluegills to forage on carp eggs.

Bluegill had a large effect on carp recruitment in the early, post-larval stages, but it appears that later stages of recruitment were also affected by other factors, particularly, the density of other carp. In Albert Lea in 2017, capture rates of post-larval juvenile carp were over 10 times higher in control lake than in treatment lake, but at the end of the season, abundance of YOY carp was similar in both (both were relatively low). Many of the YOY carp captured in the control lake at the end of the season were small, appeared emaciated, and had external parasites. The initial high density of post-larval juvenile carp, the presence of yearling carp (from previous year), along with high density of adult carp (which were left in the lake in 2016, and more were stocked in 2017), appears to have reduced survival of end-of-season YOYs. I also observed water and habitat quality changes in Albert Lea lakes between 2016 and 2017. Both lakes switched from a clear-

water, vegetated state in 2016 to a turbid state with little aquatic vegetation in 2017. Density dependence has been shown previously to influence carp recruitment dynamics (Weber & Brown 2013), and removal of carp from lakes often leads to an increase in recruitment in subsequent years (Lechelt and Bajer 2016). In addition, density dependence at early stages of recruitment has been well established in many species of fishes (Lorenzen & Enberg 2001). Management strategies need to account for density dependent factors, and consider the possibility that when fish are removed through biocontrol or other means, surviving fish will likely have higher survivability and growth rates.

These results may provide the most robust evidence to date that biocontrol of fishes can be used successfully in natural systems. Biocontrol likely has a broad significance not just for carp, but for other invasive fishes as well. Species that employ no parental care, or have a small body size may be particularly susceptible to biocontrol; species that employee parental care, or grow quickly into large-bodied adults may be more difficult to control through biocontrol. Small-bodied invaders, such as the Mosquitofish (*Gambusia spp.)* could be targeted by predators even as adults (Godinho et al. 1997; Horth 2004), whereas large-bodied invaders like the largemouth bass (*Micropterus salmoides)* are probably only subject to predation in early stages of life (Miranda & Hubbard 1994). Species that employ little or no parental care, like the carp, are relatively common among fishes, allowing for potential weakness that could be exploited by biocontrol strategies. Of the 495 families of bony fishes, only around 20% of families contain species that employ any behavioral parental care (Helen 1940; Gittleman

1981; Crawford and Balon 1996; Smith and Wootton 1995). Invasive northern Pike (*Esox lucius*) and goldfish (*Carassius auratus*) both adhere their eggs to vegetation and have no parental care, similar to carp (Nilsson 2006). These fishes, and others like it, may be able to be targeted by the same or a similar biocontrol technique used here. Species such as the silver carp (*Hypophthalmichthys molitrix)* and bighead carp (*H. nobilis*), whose eggs are dispersed in rivers (Garcia et al. 2013), or invasive lionfish (*Pterois spp.),* whose eggs are released as buoyant masses that float on the surface (Morris et al. 2008) may require different biocontrol strategies, or different control strategies altogether. Overall, it seems plausible that biocontrol strategies could be successfully used for a variety of fishes.
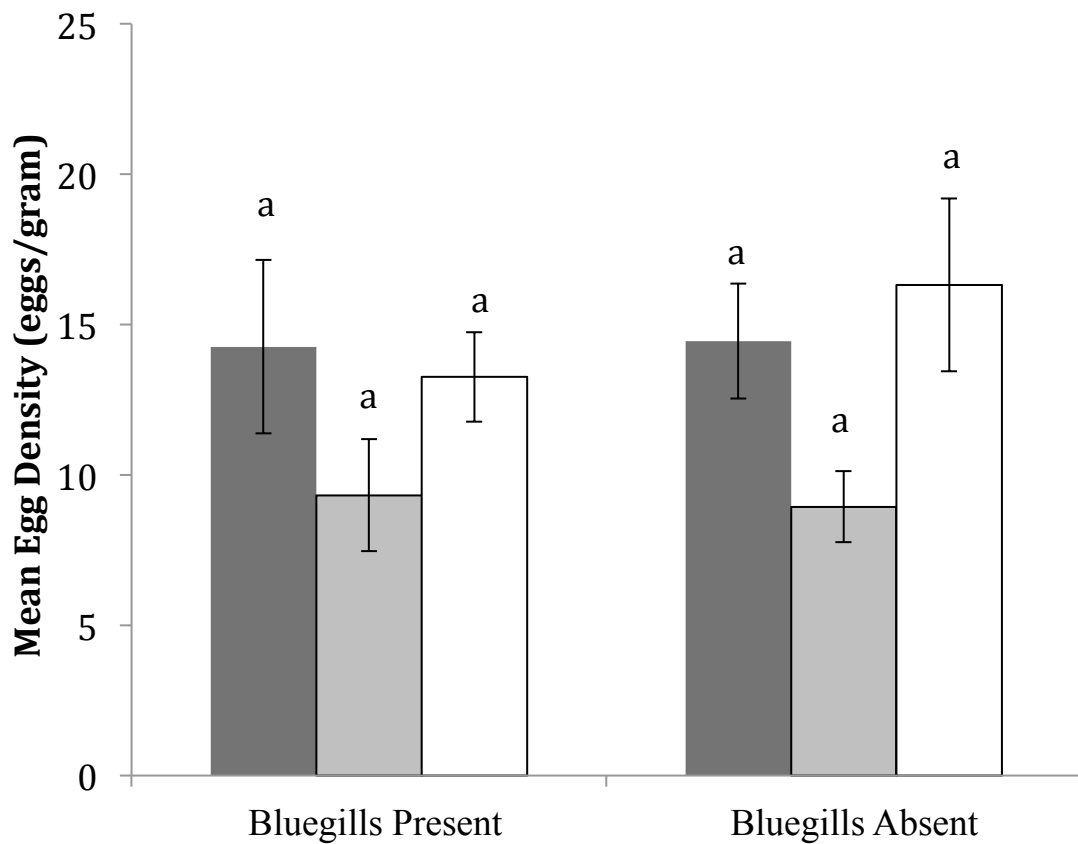
Figure 1. Results from the egg predation experiment in 2016. Dark grey bars represent egg density inside the experimental arena (excluding fish predation), light grey represents outside the experimental arena (allowing fish predation), and white bar is the control. Error bars represent the standard error. Letters above the bars represent statistical differences between treatments. "Bluegill Present" is lake with bluegills, "Bluegill Absent" is lake without bluegills.
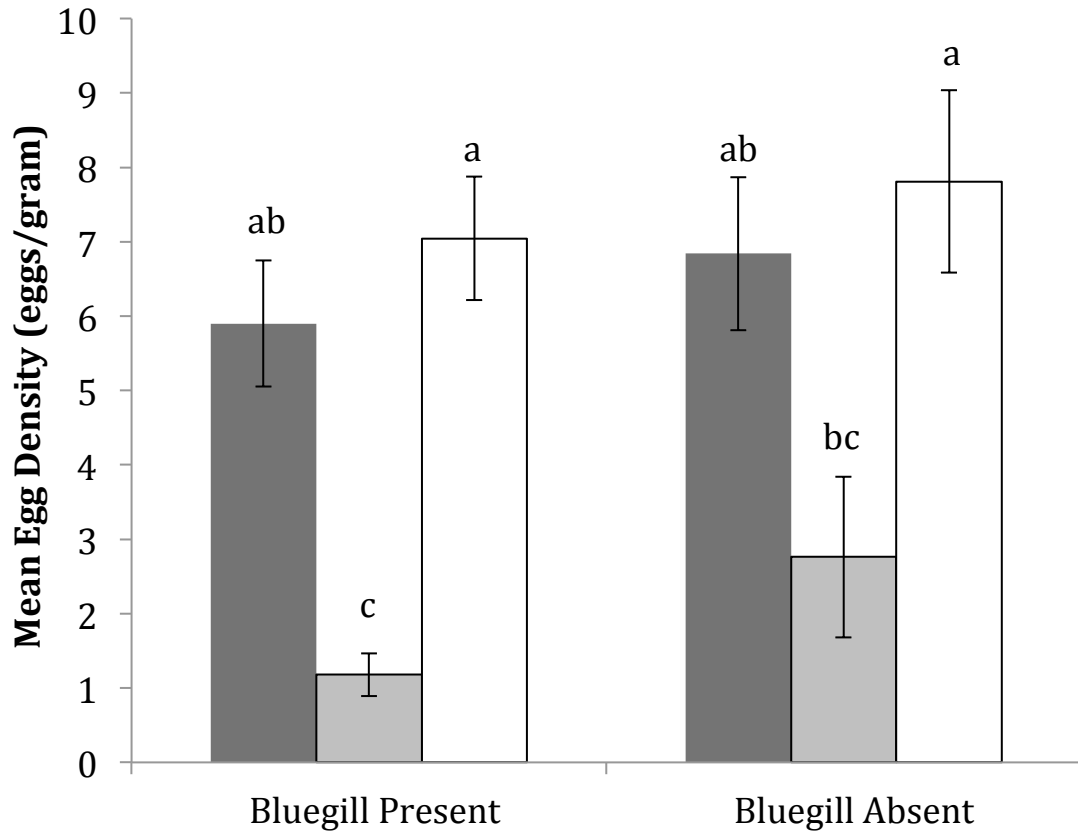
Figure 2. Results from the egg predation experiment in 2017. Dark grey bars represent egg density inside the experimental arena (excluding fish predation), light grey represents outside the experimental arena (allowing fish predation), and white bar is the control. Error bars represent the standard error. Letters above the bars represent statistical differences between treatments. "Bluegill Present" is lake with bluegills, "Bluegill Absent" is lake without bluegills.
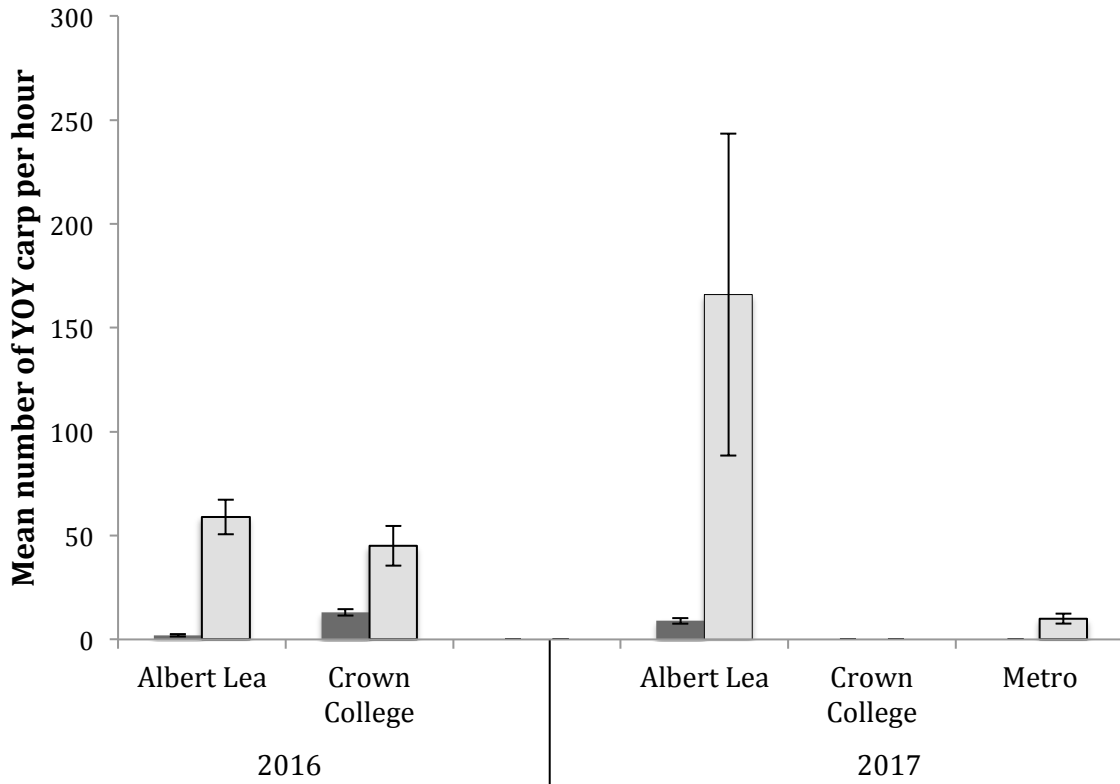
Figure 3. Mean number of post-larval early-season YOY carp caught per hour of backpack electrofishing +/- standard error. Dark bars are treatment lakes (lakes containing bluegills), and light bars are control lakes (lakes lacking bluegills). Three surveys were conducted in each lake in 2016 and four in each lake in 2017.
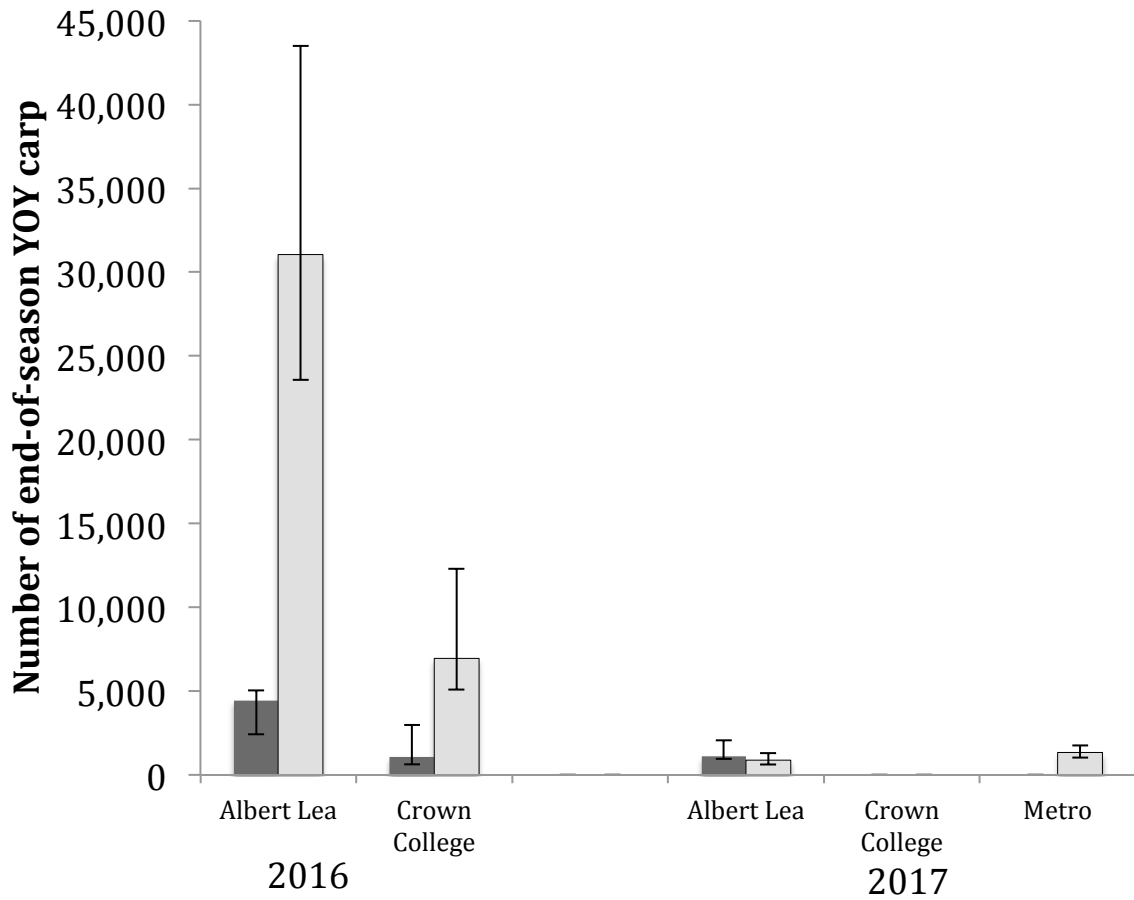
Figure 4. Mark-recapture estimates and 90% confidence intervals for end-of-season YOY carp in each lake for 2016 and 2017. Dark bars represent treatment lakes (lakes containing bluegill), and light bars represent control lakes (lakes lacking bluegill).

Table 1. Physical characteristics of the study lakes. Lakes are marked 'T' or 'C' to designate them as treatment or control lakes, respectively. TP is total phosphorus. Crown College lakes are located at 44.884956, -93.745116; Albert Lea lakes are located at 43.669828, -93.386296; the Metro treatment lake was located at 44.702426, -93.488563, and the control lake at 45.114023, -93.184685. Dominant vegetation in lakes was Coontail (*Ceratophyllum demersum*) and Elodea (*Elodea Spp)*.

Lake	Treatment	Year	Area (ha)	Maximum Depth (m)	Mean TP (ug/l)	Dominant Vegetation	Vegetative Cover (%)
Crown College	T	2016	0.85	1.6	86.5	Coontail	63.8
Crown College	C	2016	0.35	1.4	123.3	Coontail	42.5
Albert Lea	T	2016	0.65	1.6	47.7	Elodea	70.5
Albert Lea	C	2016	1	1.7	97.0	Elodea	92.0
Crown College	T	2017	0.85	1.6	88.6	Coontail	26.0
Crown College*	C	2017	0.35	1.4	185.3	Coontail	25.5
Albert Lea	T	2017	0.65	1.6	120.0	None	>1%
Albert Lea	C	2017	1	1.7	293.7	None	>1%
Metro	T	2017	0.55	1.5	87.0	Elodea	3.2
Metro	C	2017	1.8	2.3	33.7	Coontail	58.3

* Lake invaded by bluegills; data were not used in analyses.

Table 2. Mark-recapture statistics for end-of-season YOY carp in each lake in 2016 and 2017. Different numbers of surveys were used in different locations. In most lakes, three or four surveys yielded greater than 20 recaptures, and surveying was deemed complete. In few locations, sampling continued for up to 11 surveys, to get as many recaptures as possible. In lakes were no YOY carp were captured in the first three surveys, the population was assumed to be zero.

Location	Year	Size (ha)	Treatment	No. surveys	Total marked	Total recaptured	Density (Ind./ha)	90% CI
Crown College	2016	0.85	Bluegills	11	109	7	1,090	635 to 2,999
Crown College	2016	0.35	Control	8	230	14	6,973	5,096 to 12,319
Albert Lea	2016	0.65	Bluegills	3	842	21	4,449	2,429 to 5,035
Albert Lea	2016	1	Control	3	1,084	30	31,031	23,574 to 43,499
Crown College	2017	0.85	Bluegills	3	0	0	0	-
Crown College *	2017	0.35	Control	3	0	0	-	-
Albert Lea	2017	0.65	Bluegills	4	307	149	1,126	946 to 1385
Albert Lea	2017	1	Control	7	184	21	888	626 to 1297
Metro	2017	0.55	Bluegills	3	0	0	0	-
Metro	2017	1.8	Control	4	126	36	1,336	1029 to 1762

* Lake invaded by bluegills; data were not used in analyses.

Chapter 3: Ecological conditions at which biocontrol of common carp falls apart: factors

limiting the abundance of bluegill sunfish in lakes of south-central Minnesota

Introduction

Common carp (*Cyprinus carpio,* or 'carp') is an invasive fish native to Eastern Europe and Asia and is one of the world's most ecologically harmful species (Lowe et al. 2004). Carp is known for rooting in lake bottoms while searching for food, which leads to sediment re-suspension, reduced water clarity, and release of sediment-bound nutrients into water column (Vilizzi et al. 2015). Due to this foraging behavior, carp also uproot aquatic vegetation causing lakes to shift from a clear, vegetated state, to a state characterized by lack of vegetation and turbid water (Zambrano et al. 2001, Bajer et al. 2009). These shifts in lake conditions have negative impacts on native biota, including migratory waterfowl, whose numbers often decline by an order of magnitude in lakes dominated by carp (Haas et al. 2007). It is estimated that a century after its introduction, the common carp has contributed to the decline of aquatic vegetation in approximately seventy percent of lakes within the Great Plains region of southern Minnesota (Bajer et al. 2016).

Biocontrol, defined here as the use of a native predator to control an invasive species, has been suggested to play an important role in controlling carp populations in lakes of south-central Minnesota (Bajer et al. 2012). Carp spawn in shallow areas, broadcasting eggs that adhere to vegetation (Balon, 2004). Unlike many fishes native to North America, carp do not guard their eggs or provide any other parental care (Marchetti et al. 2004). This leaves carp vulnerable to egg and larval predators. Bluegill sunfish (*Lepomis macrochirus*), a common native fish, has been shown to play a key role in controlling carp recruitment by foraging on carp eggs and larvae in lakes of south-central

Minnesota (Bajer & Sorensen 2010; Bajer et al. 2012; Silbernagel & Sorensen 2013; Bajer et al 2015a). However, while bluegills appear to be an important biocontrol agent in many lakes, their abundance often declines in lakes prone to hypoxia (Bajer et al. 2012). Adult carp exploit this scenario by migrating out of lakes, where they spend most of the year, to hypoxia-prone, predator-free basins to spawn (Bajer et al. 2015b).

Lake surveys showed a threshold in bluegill abundance associated with the occurrence of carp recruitment. Specifically, carp recruitment did not occur in lakes in which bluegill catch rates exceeded one individual per trapnet, but it occurred commonly in lakes with lower bluegill catch rates (Bajer et al. 2012). Apparently, this threshold represents conditions at which bluegills are no longer able to function as biocontrol agents for the early life stages of carp. Understanding lake characteristics that limit bluegill abundance below this threshold is important in determining sites within the landscape that are particularly likely to function as carp nurseries. It is also important in directing management actions to stabilize bluegill populations. For example, winter aeration systems might be used to eliminate hypoxic events in some lakes, or reduce their frequency in others, and by doing so make carp management efforts more achievable. Ecological and water quality benefits that result from reduced carp abundance are likely to outweigh the costs of using aeration systems in many lakes (Bartodziej et al. 2017).

As a common sport fish, bluegills have been studied extensively throughout Minnesota and the Midwest (Aday et al. 1999; Cross & McInery 2001; 2005; Tomcko & Pierce 2005; 2011; Michaletz et al. 2012). However, extant studies have focused on attributes that make bluegills attractive to anglers such as growth, size structure, and age-

at-maturation (Clark and Lockwood 1961; Aday et al. 1999; Tomcko & Pierce 2005, 2011; Michaletz et al. 2012). Few studies have addressed bluegill abundance (Cross & McInery 2001, 2005), which is of main interest for biocontrol. Further, studies that have examined bluegill abundance focused on a specific subset of "bass-panfish" lakes (lake Class 24) (Cross & McInery 2005). These lakes represent relatively deep (mean maximum depth of 18 m) (Schupp, 1992), ecologically-stable lakes and are not representative of the shallow lakes that are prone to hypoxia and where bluegill populations are often low. Processes that regulate bluegill abundance in shallow, hypoxia-prone lakes where carp recruit is poorly documented.

The purpose of my study is to examine characteristics that regulate bluegill abundance in lakes of south-central Minnesota, a region abundant in shallow, productive, hypoxia-prone lakes. I focus primarily on conditions that might be driving low bluegill abundance in those systems. Because low abundance of bluegills or local extinctions of their populations are unlikely to be driven by predators or competitors (i.e., largemouth bass are unlikely to drive bluegills to local extinction), I focus on physical lake characteristics, including those that might be associated with occurrence of hypoxia, such as lake depth and productivity (Rahel 1984). My results help define lakes that are likely to function as carp nurseries within the landscape based on their physical characteristics. It also suggests lakes in which management efforts, such as aeration, are likely to be necessary to enhance biocontrol of carp by bluegills.

Methods

A dataset was obtained from the Minnesota Department of Natural Resources

(MNDNR) containing catch-per-unit-effort (CPUE) for bluegills collected using standard lake surveys and re-surveys from 1993 to 2012. The CPUE data were provided for both gillnets and trapnets, however, I only used trapnet CPUEs in this analysis, which are thought to be a more accurate indicator of bluegill abundance (Cross & McInery 2005). Habitat characteristics of lakes, such as maximum lake depth, lake area, Secchi disk depth, and macrophyte species richness, and watershed characteristics such as land use and watershed area were also obtained from MNDNR. I used data only when fish surveys occurred in the same year as vegetation surveys and where landscape characteristics were available. Only the most recent survey was included for each lake to avoid pseudo-replication. The dataset encompassed two ecoregions of Minnesota: the Great Plains (N=41) and the Eastern Temperate Forests (N=149). Northern forests, the third ecoregion in Minnesota, was not included because carp invasion is not frequent in these lakes (Bajer et al. 2015a), and data in this region were limited (N=11).

Bluegill CPUEs were transformed [$\log_2$ (x + minimum x value)] to achieve linear (or piecewise, see below) relationships with predictor variables and to allow for the analysis of zero CPUE values. Initial evaluation of the data set revealed a small number of observations (<10 %) that were one-half of the mean value or greater away from the nearest observation (e.g., lakes with extremely large watershed sizes, etc.). These data were excluded from the analysis to avoid fitting linear models across conditions that were infrequently represented in the data set.

I used piecewise multiple regression to explain bluegill CPUE because most relationships showed 'hockey stick' patterns. I evaluated whether a piecewise model was

needed for each predictor variable separately (Supplement B, Figures 1-5) by comparing

AIC scores for piecewise vs. linear model. Piecewise regression was performed using the

'segmented' package in R (Vito & Muggeo 2003, 2008; R Development Core Team

2013). I began the analysis by including 12 variables: (1) maximum lake depth, (2)

submerged aquatic macrophyte species richness, (3) Secchi disk depth, (4) watershed

area, (5) lake area, (6) the shoreline development index, (7) proportion of developed land

in the watershed, (8) proportion of forested land in watershed, (9) proportion of bare land

in watershed, (10) proportion of grassland in watershed, (11) proportion of land used for

agriculture in the watershed, and (12) proportion of land occupied by wetlands in the

watershed. Since variables may be closely correlated, variables were checked for

multicollinearity using the 'car' package (Fox and Weisberg 2011) in R based on the

variance inflation factor so that no variable was included that was too closely correlated

to another. Akaike's information criterion (AIC) scores were used to assess the support

for competing models to select the most parsimonious model (model with AIC score

closest to zero). The 'AICcmodavg' package (Mazerolle 2013) in R was used to conduct

model selection. The mean and standard deviation for the variables used in the most

parsimonious model are shown in Table 1.

Variables included in the most parsimonious model were then analyzed using a

regression tree to better visualize lake and watershed conditions in which biocontrol

might or might not be likely (Figure 1). For this, I used a threshold in bluegill CPUE =

0.1 kg/net (i.e., one bluegill per trapnet) after Bajer et al. (2012) to classify lakes into two

categories: biocontrol likely (above the threshold), biocontrol unlikely (below the

threshold). Regression tree analysis was performed using the 'party' package (Hothorn et al. 2006) in R.

Results

The most parsimonious model that explained bluegill CPUEs included (1) maximum lake depth, (2) Secchi disk depth, (3) submerged aquatic macrophyte species richness, (4) percent of developed land in the watershed, and (5) percent of forested land in the watershed. The model explained 50.26% of the variation in bluegill CPUE. All of these variables showed a threshold response, where bluegill CPUEs declined rapidly below threshold value specific for each variable but remained high and relatively flat above those values; slopes were 2-30 times steeper below than above the thresholds (Table 2). Pertinent to biocontrol, CPUEs declined rapidly below a Secchi depth of 0.68 m, maximum depth of 7.4 m, macrophytes richness of 7.1, proportion of forested land in the watershed below 7.1%, and the proportion of developed land in the watershed below 48.1% (Table 2).

The regression tree analysis, in which bluegill CPUE was analyzed as a categorical variable being above or below the threshold (biocontrol likely or unlikely), included only one split at a maximum depth of 3.0 m. The regression tree indicated that 61% of lakes with a maximum depth less than or equal to 3.0 m had low potential for biocontrol, while 90% of lakes with a maximum depth greater than 3.0 m had a high potential for biocontrol.

Discussion

This study examined bluegill abundance in lakes of central and southern Minnesota to determine lake and landscape characteristics that are associated with low bluegill CPUEs, indicative of conditions where biological control of carp is unlikely to occur. Maximum lake depth appeared to be the main driver of this relationship. The model selection analysis suggested that bluegill abundance declined rapidly in lakes with maximum depths of less than 7.0 m, and, more specifically, the regression tree suggested that more than half of lakes with maximum depth below 3.0 m had low potential for biocontrol. Low Secchi depth (<0.68 m) also had a strong negative effect on bluegill CPUE. Lakes with low maximum depth and low Secchi depth are typically associated with higher fish biomass and have been associated with higher growth rates for bluegills (Tomcko & Pierce, 2011), thus I do not believe that these factors limit bluegill abundance *per se*. However, these types of lakes are indicative of systems in which winter hypoxia is especially likely to occur (Barica & Mathias, 1979). Rahel (1984) has shown that hypoxia is the main driver of fish assemblages in the region causing shifts from centrarchids to minnows. It is also known that bluegills are especially sensitive to hypoxia (Farwell et al. 2007). Therefore, the reason that bluegill CPUEs decline in lakes with low Sechhi and low maximum depth might be primarily related to winter hypoxia.

Of many strategies that have been examined to reduce the occurrence of winter hypoxia, aeration is the only strategy that has been used widely (Ellis & Stefan 1989). Aerators function to increase oxygen concentrations in the water by creating a continuous flow of compressed air throughout the water column, or by pumping oxygen into the

surface water (Cornelius 2006; Chowdhury et al. 2014). Aeration may be feasible in lakes as shallow as 1.5 m, but it is likely not economical in shallower lakes (Cornelius 2006). Though there are mixed results indicating if aeration alone can change the species composition in a lake, it is clear that aeration does create areas with substantial dissolved oxygen that could be used as refuge for fish (Lackey & Holmes 1972). It therefore has potential as a management option to keep bluegill abundance above the biocontrol threshold in some (but not all) shallow lakes. Cost associated with using aeration systems need to be weighed against potential benefits that result from controlling carp populations in lakes. Recent whole-lake experiments suggest that managing carp, and the associated recovery of aquatic macrophytes, might be one of more economical ways of reducing phosphorus concentrations and improving water quality in small, shallow lakes (Bartodziej et al. 2017). Thus, costs of winter aeration might often be justifiable.

The analysis also suggested that bluegill CPUE decreased in lakes that had fewer than seven species of macrophytes. Aquatic macrophytes have important roles in providing cover and habitat for juvenile fishes (Bryan & Scarnecchia 1992), and similar effects have also been shown for bluegills (Cross & Mcinerny 2005). Further, because plant cover tends to decline faster than species richness, systems with few species of macrophytes may represent lakes in turbid state that generally lack any significant macrophyte cover (Bajer et al. 2016). Restoring plant communities might have a positive effect on bluegill abundance and might therefore increase biological resilience of lakes to carp invasion. Coincidentally, removing carp is often one of the most effective ways to restore macrophytes in lakes. Thus, positive feedbacks might develop in systems where

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carp are removed: macrophyte communities are restored, water quality improves, and bluegill populations increase, which would hinder the ability of carp to re-invade. These positive feedbacks might only develop in systems where hypoxia does not occur, or occurs infrequently, however.

Watershed characteristics can have major influence on water quality and habitat (Michaletz et al 2012). Watershed development has potential to increase the exploitation of fish by anglers, increase runoff and nutrient loading, and heighten the chances that a lake is invaded by non-native species (Schindler et al. 2000; Brezonik & Stadelmann 2002). Still, given these common issues associated with watershed development, in my model, I saw the lowest bluegill CPUEs where urban development was the least. This discrepancy may be explained by agricultural land use because lakes in this region that are not within urban areas are located within agricultural watersheds (i.e., urban land use was negatively correlated with agricultural land use; Pearson's correlation coefficient = -0.72; $p < 0.01$). Thus, low bluegill CPUEs at low levels of development may be due to high agriculture use and associated issues such as increased biological oxygen demand, poor water clarity, and seasonal hypoxia. Bluegill CPUEs were also lowest in watersheds where forested land was less than 7.1%; again this is probably due to agricultural and urban land use in those areas. Similar findings were reported by Cross & McInerny (2005).

This analysis helps define lake and watershed characteristics that are associated with low bluegill CPUEs in a region where bluegills often dominate lake communities. This will help to define lakes that are likely to function as carp nurseries within the

landscape. While some of the identified drivers of low bluegill abundance might be improved (macrophytes richness and water clarity by carp management), others might not (lake depth and watershed use). Aeration might be needed to stabilize bluegill populations in some lakes, especially those deeper than 1.5 m (Cornelius 2006). In shallower systems, carp should be managed by other methods such as carp-specific toxins (Chapter 4), barriers or deterrents to prevent migrations, and physical removal.
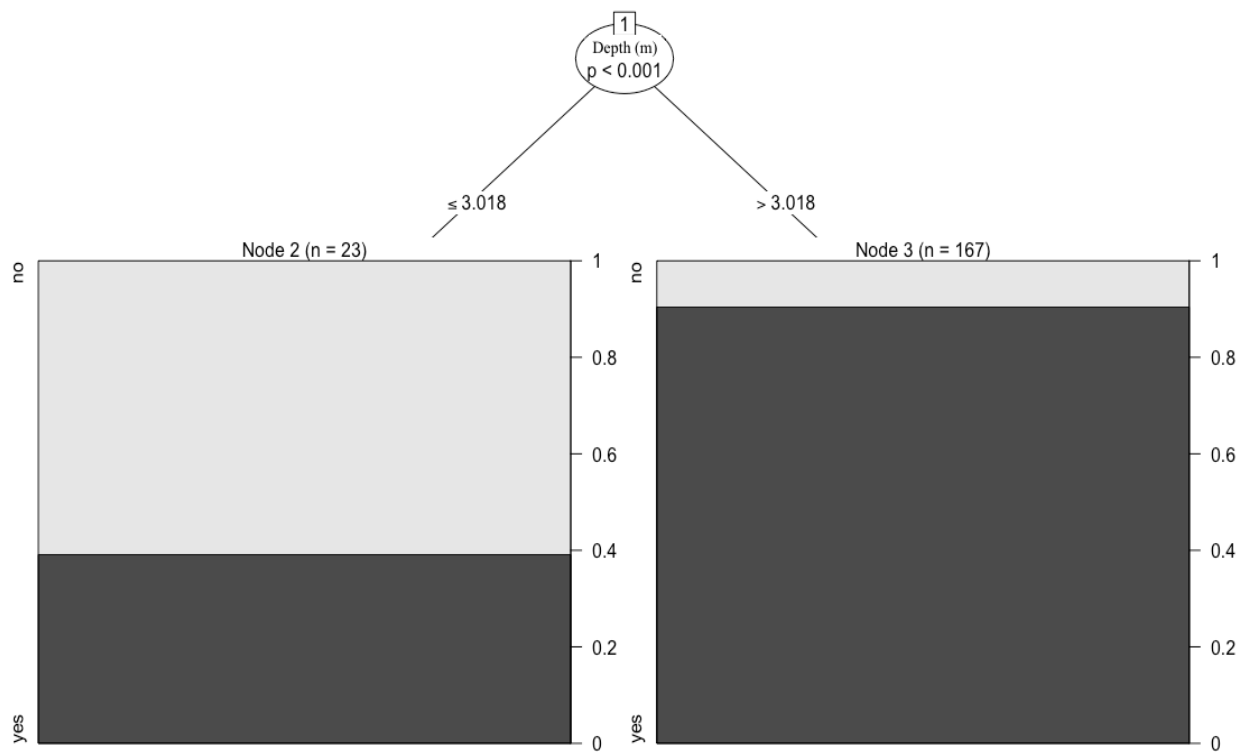
Figure 1. Regression tree based on the five factors included in the best model.

Table 1. The mean and standard deviation for the five variables used in the best model.

Variable	Mean	Standard Deviation
Maximum Depth	8.92	5.52
Secchi Disk Depth	1.34	0.75
Submerged Aquatic Macrophyte Species Richness	8.18	5.22
Percent of Developed Land in the Watershed	0.14	0.21
Percent of Forested Land in the Watershed	0.13	0.12

Table 2. Description of breakpoints, slopes for both segments, and the p-value as it relates to the slope from segment 1 for each factor included in the final model.

Factor	Breakpoint	Slope (Segment 1)	Standard Error (Segment 1)	Slope (Segment 2)	Standard Error (Segment 2)	p-value
Secchi Disk Depth (m)	0.685	2.4195	0.4032	-0.8367	0.8792	0.2800
Maximum Lake Depth (m)	7.381	0.3132	0.1633	-0.0028	0.1663	0.0309
Proportion of Forest in the Watershed	0.071	33.0545	11.8833	-1.3580	11.8644	0.0047
Proportion of Developed Land in the Watershed	0.481	5.3708	1.5071	1.3114	5.6363	0.0131
Submerged Aquatic Macrophyte Species Richness	7.114	0.2683	0.0989	0.1023	0.1148	0.0118

Chapter 4: Assessing the efficacy of corn-based bait containing antimycin-a to control

common carp populations using laboratory and pond experiments

Introduction

The Common carp (*Cyprinus carpio,* or 'carp') is one of the world's most invasive and ecologically harmful species (Lowe et al. 2004). Invasions of freshwater ecosystems by carp are commonly associated with severe declines in aquatic macrophytes, causing a loss of habitat for waterfowl and other biota (Crivelli 1983; Haas et al. 2007; Bajer et al. 2016). Due to their feeding behavior, carp also stir up sediment, reduce water clarity, and increase nutrient concentrations, which often promote nuisance blooms of cyanobacteria (Weber and Brown 2009; Vilizzi et al. 2015). The search for sustainable control strategies for carp has continued for the last several decades, first in North America and later in Australia (Marking 1992; Koehn 2004). Physical removal has been used frequently to control carp populations, especially in temperate North America, because carp form tight winter aggregations that can be located by tracking radio-tagged fish and removed via netting (Bajer et al. 2011; Armstrong et al. 2016). This strategy is believed to be sustainable mainly in systems with abundant egg and larval predators that control carp's reproductive success (Lechelt and Bajer 2016). In systems with poor predatory communities, removal has not been very effective due to density-dependent compensatory responses in recruitment (Colvin et al. 2012; Weber et al. 2016). Non-specific toxicants dispersed into lake water and water draw-downs have also been used to eradicate carp populations, but they have been used sporadically because they are expensive, impact native biota, and can primarily be used in lakes that are isolated with barriers to prevent reinvasion (Hanson et al. 2017). Viruses and genetic technologies have been proposed for carp control in Australia; however, carp are likely to develop

resistance to viruses within a few generations, (McColl et al. 2014), and genetic

technologies remain at the developmental stage and are associated with social concerns

and uncertainties (Thresher et al. 2014a; Thresher et al. 2014b).

Strategic use of toxicants has been instrumental in developing arguably the only

successful integrated pest management strategy for an aquatic invasive species to date,

the control of the sea lamprey (*Petromyzon marinus*) in the Great Lakes (Hubert 2003).

Toxicants might similarly be used to manage common carp populations in a selective and

effective manner. Currently, four compounds are registered in the United States (U.S.) for

use as piscicides: 3-Trifluoromethyl-4-nitrophenol (TFM) and niclosamide, which are

used to control sea lamprey, and rotenone and antimycin-a (ANT-A), which are used in

the control of bony fishes (Bettoli & Maceina, 1996; McDonald & Kolar, 2007). ANT-A

shows substantial promise over the other piscicides for the purposes of controlling

populations of common carp. It is highly toxic to fishes (more so than rotenone; Marking

& Bills 1981; Finlayson et al. 2002), but much less toxic to higher vertebrates (Herr et al.

1967; Finlayson et al. 2002). In the aquatic environment, ANT-A degrades into

compounds that are not known to pose a risk (Turner et al. 2007; Environmental

Protection Agency 2007), which might be particularly desirable to prevent the

accumulation of unused toxin in the environment. Finally, unlike rotenone, it appears that

fish, including carp, are unable to detect and avoid ANT-A (Bonneau & Scarnecchia,

2001; Gehrke 2003; EPA 2007; Rach et al. 2009). Although ANT-A is often applied

directly to water to affect fish mortality, existing evidence suggests that ANT-A could be

incorporated into bait and delivered to carp as an oral toxicant, which would make its

application more targeted (Rach et al. 1994; Kroon et al. 2005). Feeding experiments conducted in laboratory arenas and in natural lakes showed that carp possesses the ability to quickly learn and remember the location of a food reward (Karplus et al. 2007; Zion et al. 2007; Bajer et al. 2010), which might allow for innovative strategies to apply the toxicant by exploiting cognitive aspects of carp's foraging behavior. For example, in a small lake in Midwestern U.S., Bajer et al. (2010) showed that carp (75% of the population) were attracted to plant-based bait (corn) within six days, whereas native fishes were not. Overall, it seems plausible that ANT-A could be delivered to carp as an oral toxicant in a corn-based bait by first training carp to consume corn at selected times and locations, after which time the bait would be replaced (for brief periods of time) with one that contains lethal doses of ANT-A. This strategy might result in relatively high mortality of carp with minimal impact on native biota. However, no proof-of-concept experiment has examined if a corn-based bait containing ANT-A could selectively target carp and not native species.

In this study, corn-based bait containing ANT-A was developed and experiments were conducted to (1) determine the lethal dose of ANT-A to carp, (2) quantify the leaching rate of ANT-A from the bait, (3) test species-specificity of the bait in mixed-species lab trials, and (4) test species-specificity in mixed-species pond trials. This study has important implications for developing novel and practical management strategies for the common carp.

Methods

Four experiments were conducted to test if ANT-A could be incorporated into a

corn-based bait to selectively kill carp. First, the lethal dose was examined in gavage trials. This information was then used to develop bait that would be lethal to carp after consuming a single pellet. A leaching trial was then conducted to examine how much ANT-A leached into the water from bait containing a lethal dose of ANT-A and whether leaching caused any fish mortality. This assay involved carp as well as bluegill (*Lepomis macrochirus*), which are particularly sensitive to ANT-A. Following the leaching experiment, a mixed-species laboratory species-specificity test was conducted, in which toxic bait was provided (the same amount as in the leaching trial) to carp and the following three native species from families commonly found in lakes where this type of control is likely to be applied: centrarchids [bluegill], percids [yellow perch (*Perca flavescens*)], and cyprinids [fathead minnow (*Pimephales promelas*)]. Finally, in a mixed-species pond species-specificity experiment, carp, bluegills, and perch were used to test if carp could be targeted in a selective manner in a larger, more natural environment. Fathead minnows were not used in the pond trial because their small size would make it difficult to assess mortality.

Bait formulation

A batch of ANT-A was fermented and extracted by the University of Minnesota Biotechnology Resource Center (St. Paul, MN) contracted through Aquabiotics, Inc. (Bainbridge Island, WA). Produced ANT-A powder was determined to contain less than 10% impurities that were not characterized but likely consisted of residual fermentation media. ANT-A powder was then encapsulated into a microparticle developed at the U.S. Geological Survey Upper Midwest Environmental Sciences Center (La Crosse, WI;

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UMESC) prior to incorporation into a corn-based bait. Microparticles were produced similarly to the methods described in Hawkyard et al (2011) and Langdon et al (2008). This microparticle was a spray-atomized product of a core with ANT-A, refined beeswax (Sigma-Aldrich, St. Louis, MO, USA), and sorbitan monopalmitate (Sigma-Aldrich, St. Louis, MO, USA). Microparticles had a diameter of ~0.35 μm and a nominal ANT-A concentration of 20% weight by weight (w/w). Microparticles were stored at -20°C in plastic containers until use. Specific concentrations of ANT-A in microparticle, or later in the bait (see below) were not measured beyond this point, thus all concentrations reported below were nominal. However, manufacturer's specifications (storage at -20°C) were followed to minimize the potential breakdown of ANT-A in the microparticle or bait until it was applied. The process of microparticle formulation required ANT-A in a dry powder form; therefore it was decided not to use the commercially available aqueous ANT-A formulation (Fintrol^{TM}) registered by the U.S. Environmental Protection Agency (EPA).

The bait was made using corn meal (Quaker Oats Company, Chicago, IL; 80% by weight), gelatin (Knox Gelatine, Kraft Foods Group Inc., Northfield, IL; 10% by weight), and microparticle (10% by weight). Thus, the bait contained a nominal concentration of 20 mg ANT-A/g. The corn meal and microparticle were mixed by hand using a plastic spatula. The gelatin was prepared according to manufacturer's instructions, cooled to room temperature, poured into the corn meal-microparticle mixture and mixed by hand using plastic spatula to produce a slurry that was then placed into plastic bags and chilled to 4°C, until the mixture became similar to the consistency of cold putty. The mixture was then extruded from a small opening in a plastic bag to form long lines on a glass

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plate. The lines were allowed to fully harden at 4°C until they could be cut with a razor blade to a size that was sufficient to pass the gape of fish used in the trials: a diameter of approximately 4 mm and a length of 8 mm for the carp < 200 mm, and a diameter of approximately 10 mm and a length of 20 mm for the carp > 200 mm. Any fish whose gape was too small to consume the entire pellets could have still fed on the bait because it was friable in the water. Bait was stored at -20°C in plastic containers until use. Non-toxic (blank) bait, which was used in control treatments and during acclimation phases of the experiments (see below), was prepared in the same way, except that the microparticle used to make it contained no ANT-A.

Test animals

Fathead minnows, bluegill, and yellow perch were reared from eggs at the Upper Midwest Environmental Sciences Center (UMESC). Animal husbandry procedures followed UMESC Standard Operating Procedures for fish care and maintenance. Methods used to conduct research for this research protocol (AEH-16-CCT-01) were approved by the UMESC Animal Care and Use Committee. The juvenile carp used in all trials were obtained from Osage Catfisheries, Inc. (Osage Beach, MO). Adult carp used in the pond species-specificity trial were collected from a lake in Minnesota (Long Lake, Ramsey County; University of Minnesota Animal Care Protocol 1601-33424A). All fish used in the experiments were capable of ingesting the bait pellets, either by swallowing them whole, or by ingesting portions of pellets.

Gavage trial

Common carp (94 to 146 mm in total length [TL]; 38 to 128 g) were acclimated

for 5 d to fiberglass, round, flat-bottom, 227-L tanks containing 150 L heated (~24 °C) well water with a pH of approximately 7.9 and continuous water flow (minimum of 1 tank-volume exchange/h). During acclimation, carp were offered daily a diet of bloodworms and the non-toxic bait each at 1% body weight (BW). The bloodworms were used for nutritional reasons because they often dominate carp's diet in natural systems and are highly palatable (Garcia 1985; Kasumyan 1997); in other trials (see below) bloodworms were used to mimic food sources found in natural systems. During the trial, seven tanks were used, each containing five carp. Two tanks were randomly assigned to each of three ANT-A dose-level treatments (N = 10 carp per treatment), while the remaining tank was used as a control (N = 5 carp). The three different ANT-A dose levels were: 4.0, 8.0, 16.0 mg ANT-A/kg BW, equivalent to ingesting the toxic bait at 0.02%, 0.04%, or 0.08% BW, respectively. Percent BW calculations were based on the mean weight of fish in each tank, weighed before being placed in the tanks. Total fish BW varied from 64 to 74 g in all tanks. In the control treatment, non-toxic bait was administered by gavage at 0.08% BW, equivalent to the amount of bait administered at the highest ANT-A dose. To administer a dose, carp were removed from tank and anesthetized to surgical plane (50 mg tricaine methanesulfonate [TMS]/L; Tricaine-S™, Western Chemical Inc., Ferndale, WA). A 5-mL plastic syringe with the tip removed was filled with appropriate amount of bait and inserted into the mouth of the anesthetized fish past the pharyngeal teeth. The plunger was then depressed to deliver the bait. Fish were immediately placed back into their respective tank where mortality was recorded 1, 3, and 24 h post-gavage. Fish surviving at the end the trial were euthanized by TMS-

overdose (200 mg TMS/L). All fish were measured for total length (nearest mm), and wet weight (nearest 0.1 g) at the conclusion of the trial. Water quality parameters (dissolved oxygen [DO], temperature, pH) were measured at 1 and 24 h with a YSI Handheld Dissolved Oxygen Meter (Yellow Springs, OH), and a Beckman-Coulter pH Meter Φ410 (Brea, CA) (Supplement C, Table 1).

Leaching trial

The trial was conducted in fiberglass tanks (n=5) using conditions described in the gavage trial except that the water temperature was 20 °C. Carp (n=6; 75 to 179 mm TL; 7 to 72 g) and bluegill (n=6, 86 to 152 mm TL; 12 to 70 g) were stocked in each tank. Fish were acclimated to the tank conditions for at least 5 d during which they were offered a mixture of bloodworms and non-toxic bait each at 1% BW.

During the trial, 1 g of the 4-mm ANT-A bait was placed at the bottom of each tank. Instantaneous leaching of all ANT-A present in this amount of bait would have resulted in a water concentration of 0.13 mg ANT-A/L, approximately 300 times higher than the LC_{50} for common carp (0.35 µg/L/96 h; Marking 1992). The bait was placed inside an enclosure that allowed water to circulate around the bait while preventing fish from ingesting or disturbing it. The bait was placed inside a polyvinyl chloride (PVC) pipe (0.6 cm diameter, 10 cm long) with 35 mm mesh on both ends, that was then placed inside a plastic container (47 cm x 23 cm x 17 cm; Rubbermaid™) with >20 holes (diameter = 3.2 mm) drilled in each side. An airstone was placed near the container to ensure there was water movement near the enclosure. Water flow to the tank was stopped concurrent with placing the bait in the tank.

Water samples (25 mL) were taken by submerging a 50-mL centrifuge tube (VWR, Radnor, PA) ~1 cm below the surface of the water immediately before the addition of bait and at 1, 4, 8, 24, 48, and 72 h after. These time points were selected to examine ANT-A concentration at frequent intervals immediately after the bait was placed in the water when it was thought most of the leaching would occur (Table 1). Water samples were processed using solid phase extraction (SPE) to concentrate ANT-A 25 fold as described in Bernardy et al. (2013). ANT-A concentration was then quantified using an Agilent 6530 Accurate-Mass Quantitative Time of Flight Liquid Chromatography Mass Spectrometer (Agilent Technologies, Santa Clara, CA, USA), with a detection limit of 8 ng/L and a quantification limit of 0.32 μg/L. Fish mortality was recorded at each water-sampling period. Water quality parameters (DO, temperature, pH) were measured 1, 24, 48, and 72 h after placing the bait in the tank (Supplement C, Table 2). At the end of the trial, all fish were euthanized, measured and weighed.

Laboratory species-specificity trials

The trial was conducted in fiberglass tanks (n=6) using conditions described in the gavage trial. Each tank contained six common carp (54 to 80 mm TL; 5 to 16 g), five fathead minnows (45 to 72 mm TL; 1 to 9 g), six yellow perch (47 to 61 mm TL; 1 to 4 g), and six bluegills (82 to 123 mm TL; 16 to 66 g). Fish were acclimated to test conditions for 7 d during which they were offered the non-toxic bait and bloodworms each at 1% BW. Three tanks were then randomly selected as treatment tanks and three as control tanks. Fish in the treatment tanks were offered 1 g of toxic bait (~0.30% body weight; 59 mg ANT-A/kg BW). The control tanks were offered 1 g of non-toxic bait. It

was decided to offer 1 g of bait to be consistent with the leaching trial. Fish mortality was monitored every hour for the first 6 h, and then at 24 h, at which time water quality parameters (DO, temperature, pH) were measured. Dead fish were removed from the tank during each monitoring point and weighed and measured. Fish that survived in the treatment tanks were euthanized by overdose of TMS and measured and weighed.

Fish in the three control tanks were then offered the acclimation diet (bloodworms and non-toxic bait at 1% BW each) for 3 d. Two of the 3 tanks were then randomly selected as treatment tanks and the test with toxic bait was repeated while the remaining single tank was used as a control. This design resulted in five replicates of the toxic bait treatment and four replicates of the control treatment with all tanks but one being eventually exposed to the toxic bait treatment. Some fish died between the end of the first trial and the initiation of the second trial, thus the second trial contained fewer fish (Table 2). Water quality parameters were measured at 1 h and 24 h post-exposure (Supplement C, Table 3). All fish were measured for weight and length at the conclusion of the trial.

Pond species-specificity trials

Six concrete ponds (10.4 m long x 5.5 m wide x 0.75 m deep; no water flow; ~12 °C) were stocked with 10 adult common carp (265 to 483 mm TL; 570 to 3,000 g), 9 juvenile common carp (98 to 179 mm TL; 34 to 130 g; fewer juvenile carp were available), 20 yellow perch (46 to 136 mm TL; 4 to 33 g), and 20 bluegill (58 to 149 mm TL; 8 to 106 g). Fish were allowed to acclimate for 7 d, during which they were offered a mixture of bloodworms and the non-toxic bait (1% and 3% BW, respectively). Following the acclimation period, three ponds were randomly assigned to either the toxic bait

treatment or the control treatment. Fish in three ponds assigned to the toxic bait treatment were offered the toxic bait at an overall dosage of 1% BW per day, equivalent to an ANT-A dose of 28 mg ANT-A/kg BW/d. Bloodworms (1% BW/d) and cracked field corn (~100 g/d) were offered concurrent with the toxic bait. It was decided to continue offering bloodworms and to add cracked corn to simulate field conditions in which carp would have access to other foodstuffs in the environment and where toxic bait might be mixed with a non-toxic food reward (e.g., cracked corn) to attract more carp and avoid scenarios in which a single carp might consume large amounts of toxic pellets, reducing cost-efficiency. Fish in the control ponds were offered the same foodstuffs except that the non-toxic bait was offered in lieu of the toxic bait. Fish in all ponds were fed in the evenings and remaining food was removed in the morning with a net. The experimental period during which fish were offered the aforementioned diet combinations lasted for 6 days. Mortality was monitored twice daily. All dead fish were removed from the pond and total length and weight were recorded. Water quality parameters (DO, temperature, and pH) were measured daily throughout the experiment (Supplement C, Table 4).

Statistical Analysis

I elected to use the minimum number of tanks or ponds and the minimum number of animals per treatment to convincingly demonstrate that the toxic bait had the capacity to eliminate a biologically meaningful number of carp in the experiments (> 30%). I did this to avoid unnecessarily exposing large numbers of animals to the toxin. This pertains especially to the species-specificity experiments in the laboratory and in the ponds. Given the nature of the experiments (application of a toxin over a short period of time), I

assumed that mortality in treatment tanks would be high (>30% and consistent), while mortality in control tanks would be nil. I also assumed that I would be using a t-test to analyze the results of the experiments. Power analysis using such assumptions (power = 0.8, α= 0.05, mean difference > 0.3, standard deviation in treatment and controls ~0.1) suggested that three replicates or more would be sufficient for treatment and control experimental units (lab tanks or ponds). Thus, I used three replicates for the pond experiment (where space was more limited) and five replicates of the treatment group in the lab experiment where tanks more easily available. A similar approach was employed by Rach et al. (1994) where three ponds were used to conduct early tests of ANT-A as a toxin for common carp.

For the gavage and leaching trials, fish mortality was recorded at each treatment level. For the laboratory species-specificity trials, a one-sided Wilcoxon Rank Sum Test ($P = 0.05$) was used to test the hypothesis that mortality in treatment tanks was greater than mortality in control tanks for each species. Similarly, for the pond species-specificity trial, a one-sided Wilcoxon Rank Sum Test ($P = 0.05$) was used to test the hypothesis that mortality in treatment ponds was greater than mortality in control ponds for each species.

Results

Gavage trials

No carp died in the control tanks. Five of the 10 carp died after gavage of 4.0 mg ANT-A/kg BW; suggesting that the LD_{50} for carp in the experiments was approximately 4.0 mg ANT-A/kg BW. All carp died after gavage of 8.0 mg ANT-A/kg BW. Nine out of 10 carp died after gavage at 16.0 mg ANT-A/kg BW; the reason for the incomplete

mortality in the highest dose treatment was unknown but it might have been caused by regurgitation (i.e., the bait not being inserted deep enough past pharyngeal teeth).

Leaching trials

No fish died in any of the tanks during the leaching trial. ANT-A was not detected in the water at either the 1 h or 4 h time intervals (Table 1). ANT-A was detected in all tanks at 8 h at less than 0.03 µg/L, equivalent to leaching of less than 0.1% of the initial mass of ANT-A present in the bait at the start of the trial (Table 1). This suggests that only minor leaching occurred within the first 8 h. ANT-A was generally not detected at 24 h and beyond (Table 1), possibly due to degradation of ANT-A in water (the half-life is 12 h at 25 °C; EPA, 2007). Accidentally, the water drained almost completely from one of the tanks between the 24 h and 48 h and ANT-A concentration reached 7.48 mg/L (Table 1), however, no fish mortality occurred because of short exposure time. Detailed estimates of the amount of ANT-A that leached out of the pellets are not provided here because they are complicated by natural degradation in the water (EPA 2007), and in the bait, which is unknown.

Laboratory species-specificity trial

Fourteen of 30 (~47%) carp died in treatment tanks whereas none died in control tanks (Table 2; $P = 0.02$; df = 3; W = 2). Twenty of 26 (~77%) fathead minnows died in treatment tanks whereas none died in control tanks; (Table 2; $P = 0.007$; df = 3; W = 20). Four of 26 (~15%) yellow perch died in treatment tanks, whereas one of 21 (~5%) died in control tanks (Table 2; $P = 0.15$; df = 3; W = 5.5). No bluegills died in either treatment or control tanks (Table 2).

Pond species-specificity trial

Eleven of 30 adult carp (37%) died in treatment ponds, while only one of 30 (this fish jumped out of the pond) died in control ponds (Table 3; $P = 0.03$; df = 2; W = 9). No juvenile carp died in treatment ponds and one juvenile carp died in the control ponds (Table 3; $P = 0.91$; df = 2; W = 6). No bluegill died in treatment ponds and 6 of 48 (13%) died in control ponds (Table 3; $P = 0.96$; df = 2; W = 7.5). No yellow perch died in either treatment or control ponds (Table 3).

Discussion

This study is the first to indicate that ANT-A incorporated into a corn-based bait might be used to selectively control populations of carp. The efficacy and selectivity observed in my study indicates that such a strategy might be most effective in lakes where the fish community is dominated by centrarchids and percids. While some mortality of perch was observed in the laboratory trial, it occurred both in control and treatment tanks, was not significant, and most likely was related to disease or stress. No mortality of perch occurred in the pond trial, which lasted longer than the laboratory trial, included repeated exposure to ANT-A pellets, and more closely resembled natural conditions. No mortality of bluegills occurred in either laboratory or pond trials. The laboratory specificity experiment did also show that corn-based bait could impact native cyprinids. These concerns need to be carefully examined. Non-target mortality of native cyprinids may not be a major concern in many lakes in North America where carp populations are especially problematic, including the shallow lakes of the Great Plains ecoregion. For example, 15 species of cyprinids occur in Great Plains lakes of south-

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central Minnesota (Drake and Pereira 2002), but only four of those are omnivorous and might overlap in diet with the carp (Drake and Pereira 2002). Additionally, these native cyprinid species are small, thus, to exclude them, large, hard pellets could be used, which only adult carp could ingest and crush with their pharyngeal teeth. Non-specific mortality could be further reduced by applying the bait at times and within sites where carp, and not native fish, are most likely to consume it. For example, applying the bait at night, when carp forage most actively, and in deeper areas might exclude native cyprinids with diurnal feeding patterns. Cognitive aspects of carp foraging behavior should also be exploited to behaviorally condition those fish before the bait is applied (Bajer et al. 2010). Carp's gustatory preferences could additionally be exploited by, for example, adding amino acids like cysteine to the bait, which carp have been shown to be attracted to (Kasumyan & Morsi, 1996). In this study, corn was chosen because carp readily ingest it and can be conditioned to aggregate in sites baited with it (Bajer et al. 2010). Aquaculture literature also indicates that corn was a reasonable choice because its main amino acids, glutamic acid and proline

(http://www.fao.org/docrep/t0395e/t0395e03.html) are highly palatable to carp (Kasumyan and Morsi 1996). Carp also have relatively high amylase activity that allows them to digest complex carbohydrates, such as starch, which constitutes approximately 70% of corn (Takeuchi et al. 2002; Li et al. 2016). Nevertheless, the potency and specificity of the bait could undoubtedly be improved.

Catostomids are another group of native fish that could be impacted in lakes of North America, because, like carp, they also often feed on plant material (Cooke et al.

2005). However, in lakes invaded by carp, catostomids are represented primarily by bigmouth buffalo (*Ictiobus cyprinellus)* and white sucker *(Catostomus commersonii*). Bigmouth buffalo is planktivorous and not likely to be attracted to benthic bait, and the white sucker feeds predominately on zooplankton and zoobenthos (Saint-Jacques et al. 2000). Though the attraction of native fishes to corn-based bait is poorly documented, Bajer et al. (2010) used telemetry and cameras to show that in a natural lake in Minnesota, approximately two-thirds of the carp population learned to visit a site baited with corn in less than a week, whereas no native cyprinids or catostomids were attracted to corn, even though white suckers were common in the lake (http://www.dnr.state.mn.us/lakefind/showreport.html?downum=10001300). Further, corn-baited traps have been used to lure and remove carp from at least six lakes in south-central Minnesota showing nearly 100% selectivity for carp (P. G. Bajer, unpublished data, University of Minnesota, 2010-2017). Catfishes, including the black bullhead (*Ameiurus melas*), are also commonly found in lakes with high carp abundance in North America. However, they have much higher tolerance levels to ANT-A (LC_{50} = 25-200 µg/L/96 h; Finlayson et al. 2002) and would most likely not be impacted; ANT-A is commonly used in catfish farms to eliminate other fish while maintaining catfish monoculture. Although more studies are needed in natural systems, corn-based bait could offer high selectivity as a carrier for oral toxicants for the carp in many areas of North America. Where little site-specific information exists, I recommend that underwater cameras or traps are used prior to toxin application to assess potential non-target impacts.

It is not well known what mortality levels are needed to control populations of

invasive fish using oral toxicants, but Lechelt and Bajer (2016) suggested that 30% to 50% annual removal rates might be sufficient to control carp populations in systems with abundant predators, like bluegill, who consume carp eggs and larvae, and by doing so limit carp's reproductive success (Bajer and Sorensen 2010; Silbernagel and Sorensen 2013). Weber et al. (2016) suggested that carp removal in large, inter-connected systems with relatively low abundance of egg and larval predators, might be less effective, and exploitation rates of 50% may be needed to control carp abundance. In the experiments, approximately 40% of the carp died after being offered the toxic bait over only short periods of time. I suspect that the experiments provided conservative estimates of carp mortality. In the laboratory experiment, only 1 g of bait was provided to fish to keep the amount of bait consistent with the leaching trial, and bait was only provided once (single feeding). Larger amounts of bait and numerous exposures would likely result in higher carp mortality. The mortality of carp would also likely have been higher in the pond experiment if these tests were conducted earlier in the season. Pond experiments were conducted in November when water temperatures were below 12° C, at which point carp consumption rates are known to diminish (Goolish and Adelmen 1984). Late summer through early fall is probably the best time period to apply oral toxicants to carp, because these fish are highly attracted to corn at that time (Bajer et al. 2010).

ANT-A is currently registered as a restricted use pesticide that can be applied directly to water (Fintrol[TM]) to control nuisance fish populations. Use of ANT-A in an oral delivery formulation for fish in the United States would require an additional approval process. While the fate of ANT-A in aqueous solution (Fintrol[TM]) including the

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rate and products of breakdown is relatively well documented (EPA 2007), the fate of

ANT-A as an ingredient of carp bait is not known. For example, it is not known if ANT-

A that is incorporated into the microparticle and then into the bait might degrade slower

that ANT-A applied directly into water where it can be hydrolysed rapidly. Products of

ANT-A metabolism once it passes through fish digestive system are also unknown. Non-

target, chronic and sub-lethal effects on humans and biota would also need to be carefully

examined. Available information suggests that the risks associated with oral application

of ANT-A to control carp populations might be acceptable, but potential issues would

need to be addressed. ANT-A delivered through oral exposure routes (i.e., toxic bait) is

lethal to fishes in concentrations considerably less than for higher vertebrates (Lennon

and Berger 1970; Finlayson et al. 2002). The acute (48 h) LD_{50} for rats (*Rattus* sp.) was

nearly 100 times higher than that for fish (EPA, 2007) and there was no mortality in rats

offered ANT-A in the diet (dose = 5 mg/kg BW/d for 4 weeks, and 10 mg/kg/d for an

additional 4 weeks; Herr et al. 1967). ANT-A is highly toxic to some water birds, such as

the Mallard (*Anas platyrhynchos*, LD_{50} = 2.9 mg/kg; EPA 2007), thus care would need to

be taken to prevent aquatic birds from feeding on the pellets. This could be accomplished

by designing feeders from which only the carp could consume the pellets. For example,

as a rudimentary solution, researchers commonly use soft mesh bags for that purpose,

where carp can eat the pellets through the mesh, but pellets remain in the bags if uneaten

and can later be removed. The pellets could be applied at night, when carp forage most

actively, and then be retrieved in the morning. Consuming dead carp by predatory birds

or mammals should not pose a significant risk because these organisms have an LD_{50}

greater than that of carp, suggesting that that large quantities of carp would need to be consumed by these animals to affect mortality. For example, LD_{50} values reported for mammals (rats) suggest that a predatory mammal would need to consume an infeasible amount of carp tissue to affect mortality (> 10 kg of carp tissue per one kg of the predators' BW). Further, given ANT-A's short half-life and breakdown into non-toxic metabolites when delivered to water (at least in the case of Fintrol™, it seems likely the toxicant will decay quickly within the body of the carp (EPA 2007) further reducing the risk of non-target impact, though studies need to address this. Carp carcasses could be collected in the morning following an overnight application to mitigate that risk. Some predatory fishes might be impacted, but carp are often large enough to have few predators except during early development. Invertebrate communities are also likely to be impacted within application sites, but broader effects are unlikely (Dinger and Marks 2007). Evidence from streams where Fintrol™ was applied show that invertebrate communities rebound quickly after the application of ANT-A (Dinger and Marks 2007). Human health concerns would also need to be carefully examined and addressed. For Fintrol™ applications, the EPA rules that fish cannot be harvested for 12 months after treatment, drinking water intakes in treatment area are closed until ANT-A levels decline below 0.015 µg/L, and treated areas are restricted from access by the public during treatment and 7 days following. Outflows from systems treated with Fintrol™ are also treated with potassium permanganate to minimize downstream exposure.

The use of toxic bait could help managers control carp populations in systems where conventional management schemes using simple removal techniques are unlikely

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to be sustainable. The toxic bait could target both juvenile and adult carp, since both life stages share a similar diet (Yilmaz el al. 2003). Targeting multiple life stages may be necessary to reach carp management goals in areas where carp recruitment is frequent (Lechelt and Bajer 2016). Since ANT-A appears to be undetectable to fish (Marking 1992), carp are not likely to avoid the bait, and treatment efficiency might be relatively consistent with each application. This is of high practical importance because conventional control schemes, such as removal with nets, often result in reduced efficiency over time due to strong avoidance behaviors (Hunter and Wisby 1964). Nevertheless, future studies should determine the possibility of developing avoidance behaviors due to sub-lethal exposure, which is an important unknown. Biological realism of tests used to assess the efficacy and specificity of toxic baits that incorporate ANT-A also needs to increase. Future experiments should be conducted in larger, more natural systems and need to incorporate a larger diversity of native fishes. Economic factors also need to be examined in comparison to traditional control methods. Currently, the cost of ANT-A is high (approximately $15 per lethal dose for one adult carp) due to limited availability and limited demand, but it is likely to decrease rapidly if this control strategy was popularized. Other aspects, such as the production of pellets, appear to be relatively simple and could be easily scaled-up. While the use of toxic pellets might have its limitations in large and open ecosystems (e.g., the Murray-Darling in Australia or the Mississippi in North America), this approach could offer new and practical management solutions in smaller and more isolated ecosystems, such as lakes and reservoirs.

Table 1. Antimycin-a concentration (µg/L) in the water during leaching trials.

Tank	Time (hours)					
	1 h	4 h	8 h	24 h	48 h	72 h
1	N.D.	N.D.	0.013	N.D.	N.D.	N.D.
2	N.D.	N.D.	0.030	N.D.	0.009	N.D.
3	N.D.	N.D.	0.012	N.D.	N.D.	N.D.
4	N.D.	N.D.	0.018	0.020	7.48*	N.D.
5	N.D.	N.D.	0.019	N.D.	N.D.	N.D.

N.D. = Below the threshold of detection of 8 ng/L.

*Water drained nearly completely from the tank between 24 h and 48 h and was re-filled. Water sample at 48 h for tank 4 was taken before tank was refilled.

Table 2. Results of the laboratory species-specificity trial. Shown is the number of fish that died in each tank over the course of the experiment. Numbers in parentheses show how many fish were placed in each tank at the beginning of the experiment.

Trial #	Bait Type	Number of individuals in tank			
		Carp	Bluegill	Yellow Perch	Fathead Minnow
Trial 1	Blank	0 (6)	0 (6)	0 (6)	0 (5)
	Blank	0 (6)	0 (6)	0 (6)	0 (5)
	Blank	0 (6)	0 (6)	0 (6)	0 (5)
	Toxic	2 (6)	0 (6)	1 (6)	5 (5)
	Toxic	3 (6)	0 (6)	1 (6)	5 (5)
	Toxic	0 (6)	0 (6)	1 (6)	4 (5)
Trial 2	Blank	0 (6)	0 (6)	1 (3)	0 (2)
	Toxic	4 (6)	0 (6)	0 (6)	1 (6)
	Toxic	5 (6)	0 (6)	1 (2)	5 (5)

Table 3. Results of the pond species-specificity trial. Shown are the numbers of fish that survived or died in each control or treatment pond. Fish in treatment ponds were offered toxic bait containing antimycin-a whereas fish in control ponds were offered non-toxic bait without antimycin-a.

	Control 1		Control 2		Control 3		Treatment 1		Treatment 2		Treatment 3	
	Live	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Carp adult	9	1*	10	0	10	0	6	4	6	4	7	3
Carp juvenile	8	1	9	0	9	0	9	0	9	0	9	0
Bluegill	16	0	15	4	17	2	18	0	17	0	18	0
Perch	20	0	20	0	20	0	17	0	19	0	20	0

*fish jumped out of the pond.

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Appendix A: Supplements from Chapter 2

Supplementary Figure 1. An experimental arena (1 m x 0.5m x 0,5 m) used in the egg predation experiment. The arenas consisted of two halves: open (allowing bluegill access to the vegetation with carp eggs) or closed (excluding bluegill access using 1 cm mesh).

Supplementary Table 1. Backpack electrofishing catch-per-unit-effort (CPUE) for non-target species in each experimental lake. Dashes are used when data is unavailable.

| | Electrofishing Mean CPUE (#/hour) | | | | | | | | | | | |
| | Fathead Minnow | | Black Bullhead | | Mud Minnow | | Golden Shiner | | Largemouth Bass (Juvenile) | | Green Sunfish | |
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Crown College Control	57.80	-	5.00	-	2.67	-	0.00	-	0.00	-	0.00	-
Crown College Treatment	29.10	73.67	8.50	6.67	5.33	13.00	1.00	1.00	0.00	0.00	0.00	0.00
Albert Lea Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Albert Lea Treatment	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Metro Control	-	81.75	-	0.00	-	0.00	-	0.00	-	0.00	-	22.00
Metro Treatment	-	0.25	-	0.50	-	0.00	-	0.00	-	45.75	-	0.00

Supplementary Table 2. Mark-recapture statistics for bluegill and bluegill fry (YOY) at the end of the season in each treatment lake in 2016 and 2017. The number of surreys, total marked, and total recaptured refer to adult bluegill. Bluegill fry abundance in 2016 was estimated based on relative abundance compared to adults instead of mark recapture, thus confidence intervals for bluegill fry estimates were not conducted in 2016. No bluegill were captured in control lakes, aside from the Crown College control lake, which was excluded from analyses; thus, control lakes are not included here.

Location	Year	Size (ha)	# surveys	Total marked	Total recaptured	Density of Bluegill (Ind./ha)	90% Confidence Interval	Bluegill Fry (Ind./ha)	90% Confidence Interval
Crown College	2016	0.85	3	178	40	684	565 to 864	1,547	-
Albert Lea	2016	0.65	3	252	87	844	644 to 1226	4,792	-
Crown College	2017	0.85	2	167	24	625	449 to 891	6,169	4378 to 8927
Albert Lea	2017	0.65	3	313	139	1012	831 to 1295	6,855	4820 to 9406
Metro	2017	0.55	3	190	43	995	775 to 1295	229	80 to 455

Supplementary Table 3. The initial biomass of bluegills in treatment study lakes (includes all bluegills that were stocked in 2016 and all bluegills that were stocked plus remaining bluegills from the previous season in 2017), the biomass at the end of the season, and the percent of biomass remaining at the end of the season.

Location	Year	Size (ha)	Initial Biomass (kg)	End Biomass (kg)	% Biomass Remaining
Crown College	2016	0.85	90.2	46.3	51.4
Albert Lea	2016	0.65	67.8	44.5	65.7
Crown College	2017	0.85	88.4	60.5	68.4
Albert Lea	2017	0.65	68.8	54.2	78.7
Metro	2017	0.55	59.0	43.9	74.4
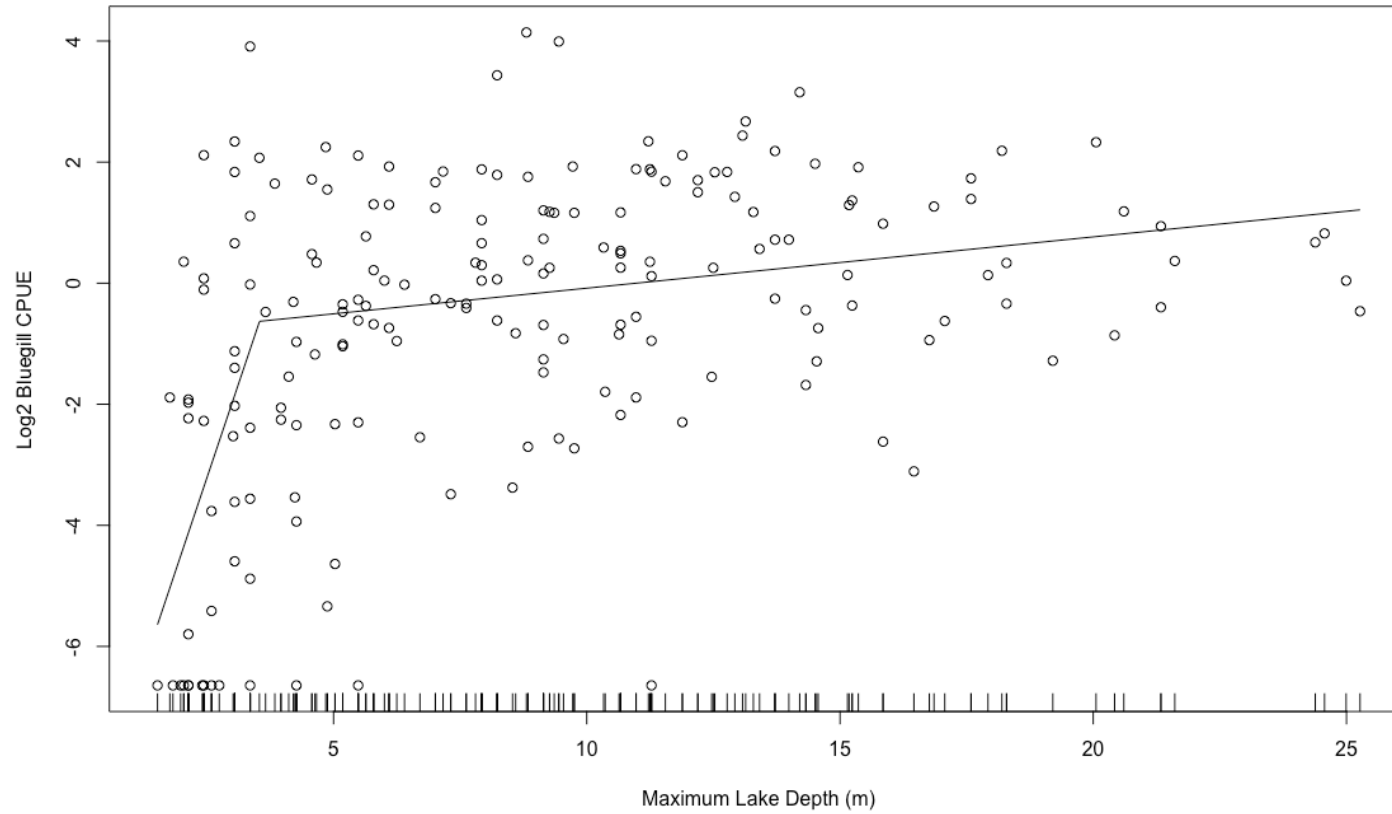
Supplemental Figure 1. Individual segmented regression model of bluegill CPUE related to maximum lake depth.
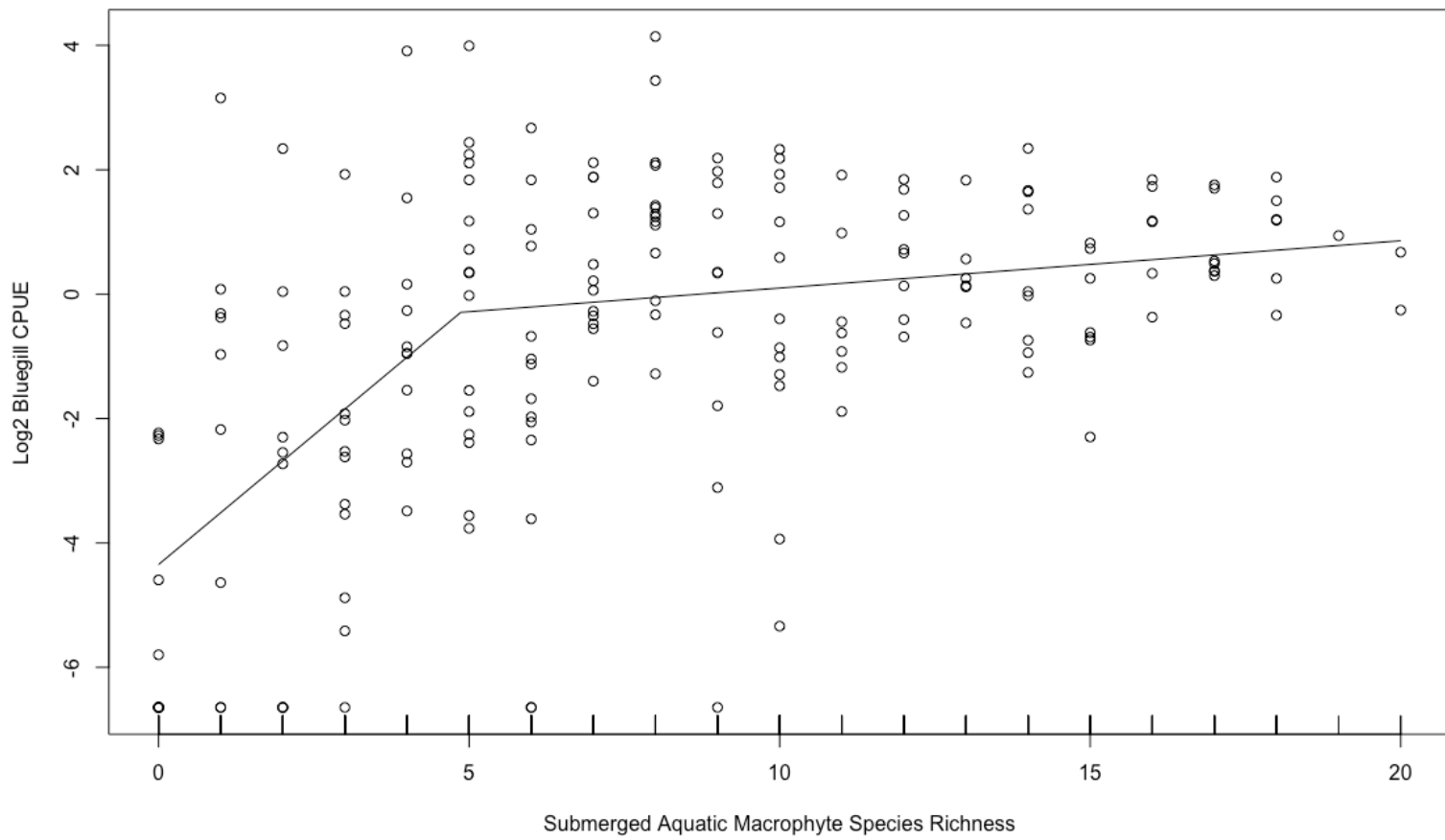
Supplemental Figure 2. Individual segmented regression model of bluegill CPUE related to submerged aquatic macrophyte richness.
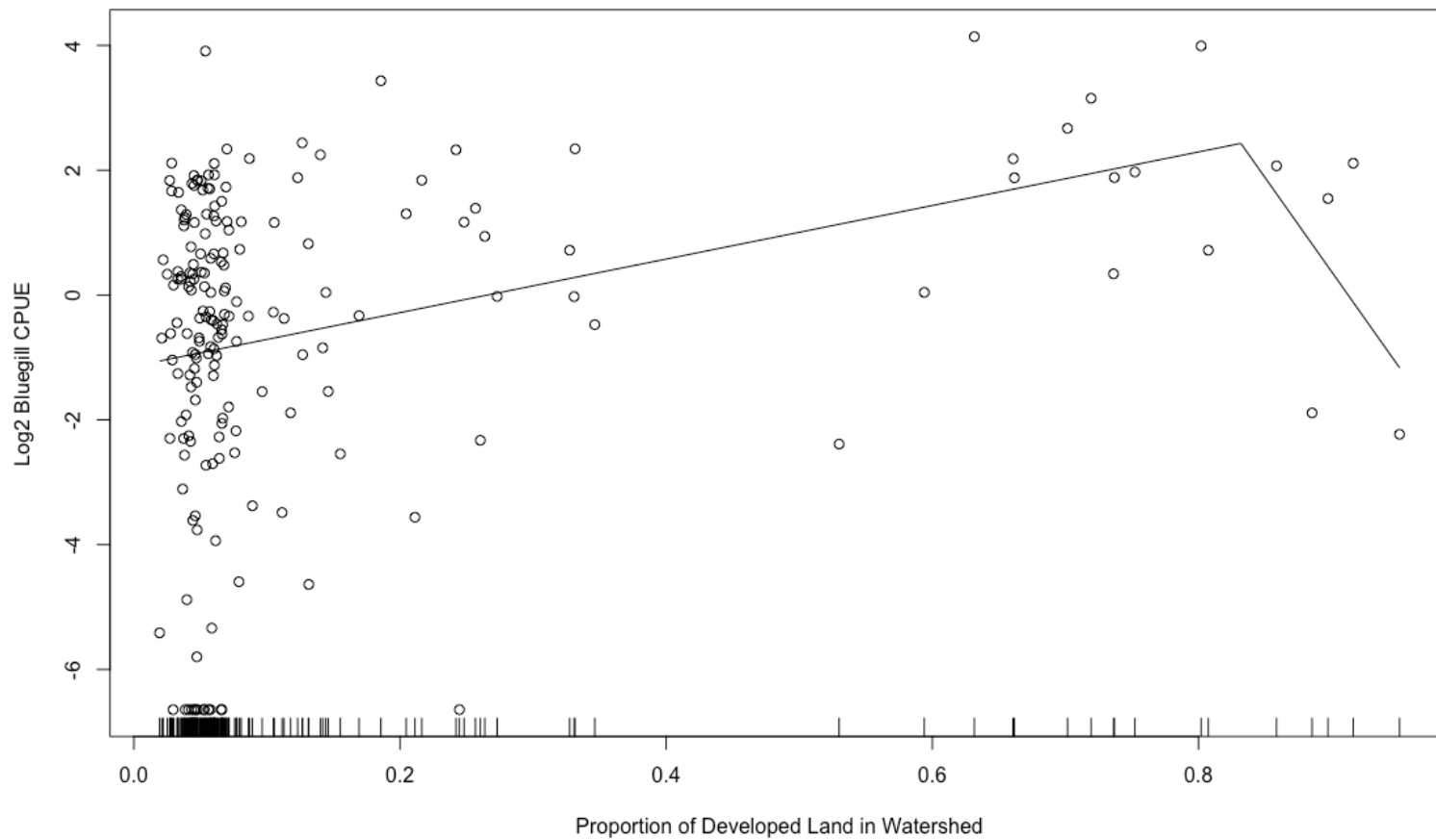
Supplemental Figure 3. Individual segmented regression model of bluegill CPUE related to the proportion of developed land in the watershed.

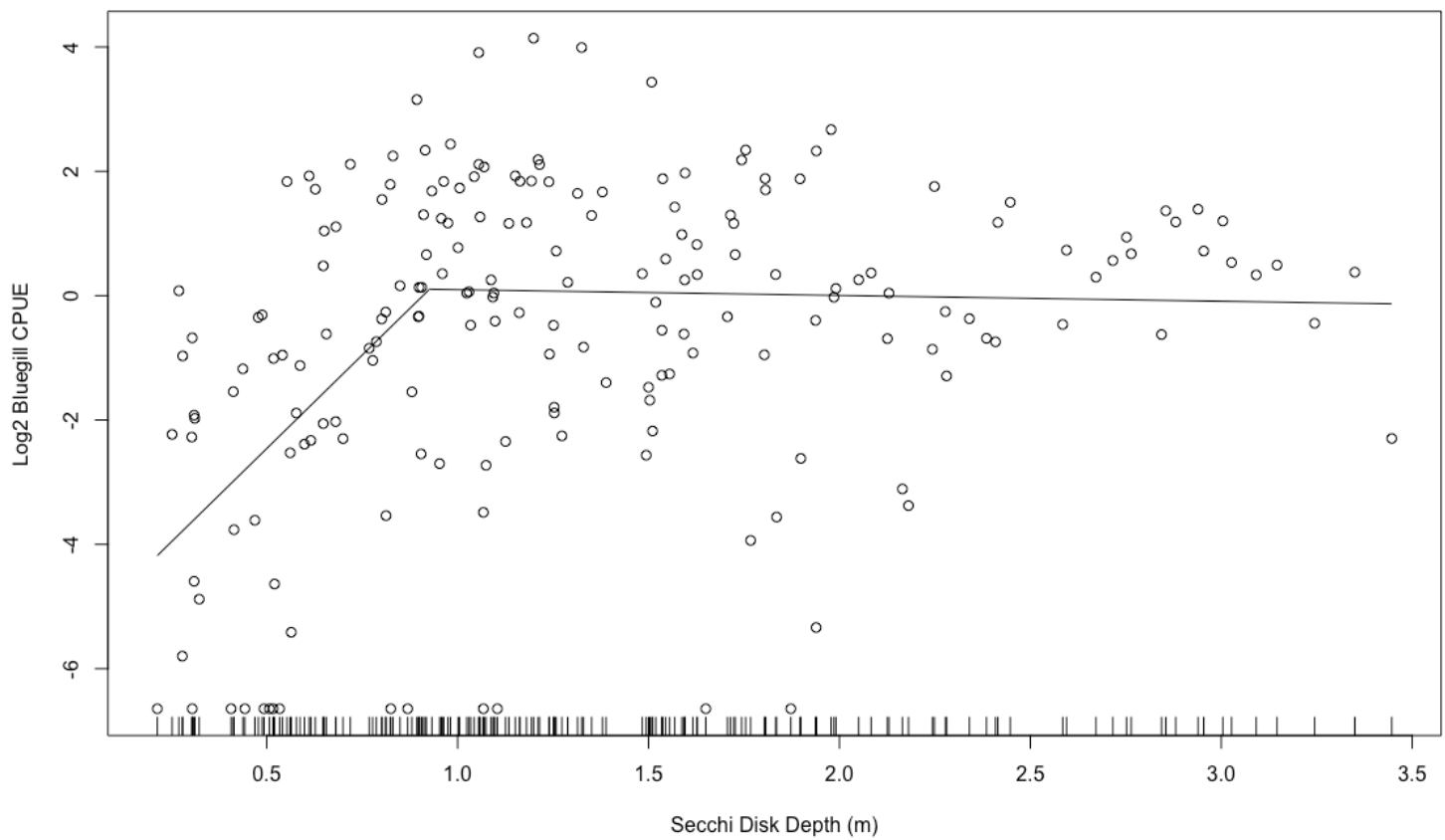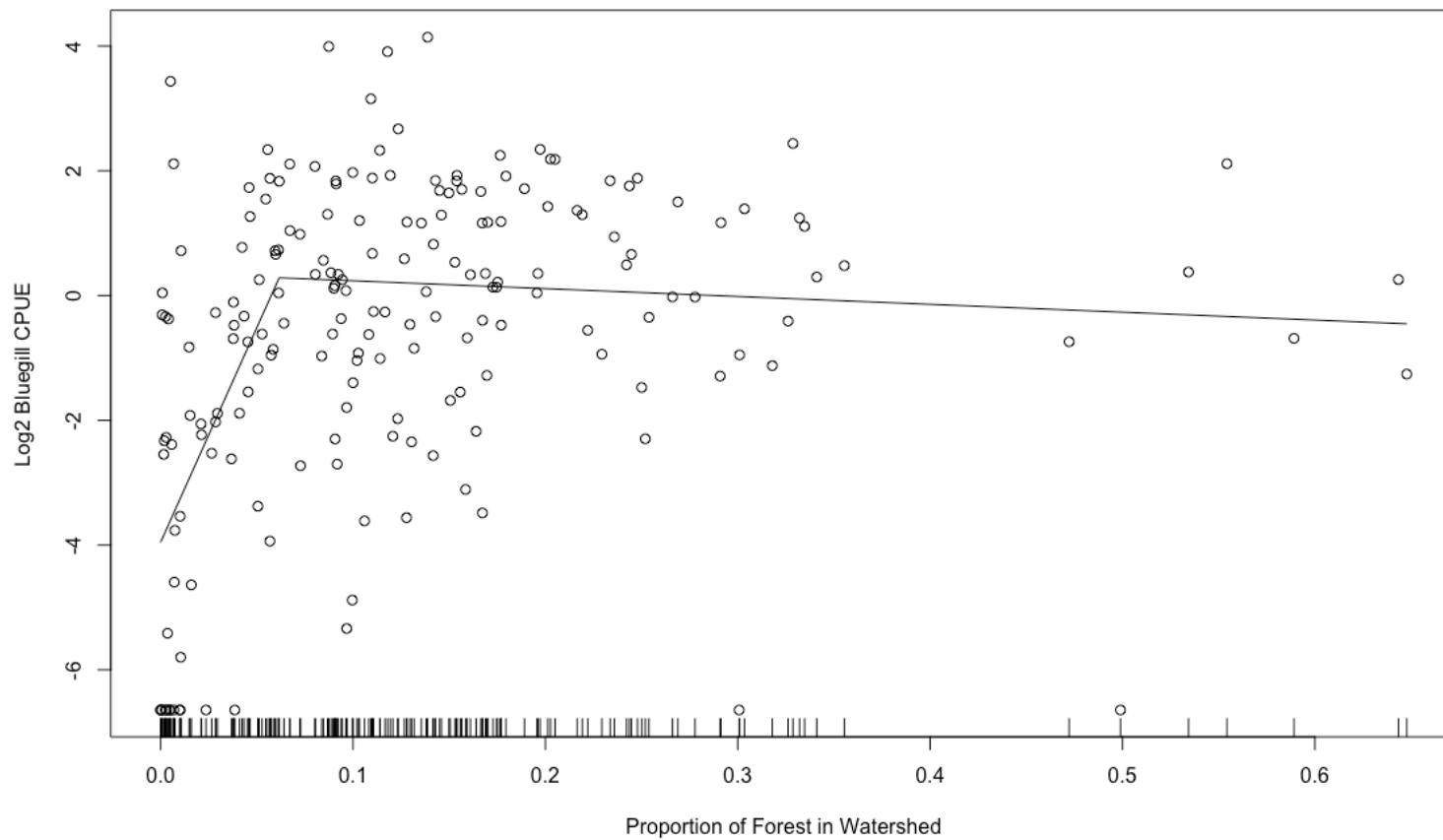Supplemental Figure 4. Individual segmented regression model of bluegill CPUE related to Secchi disk depth.

Supplemental Figure 5. Individual segmented regression model of bluegill CPUE related to the proportion of forested land in the watershed.

Appendix C: Supplements from Chapter 4

Supplemental Table 1. Water quality measurements for gavage trial.

Tank #	Metric	Time	
		1hr	24hr
	pH	7.92	7.89
Tank 1 (Control)	DO (mg/L)	8.32	8.36
	Temp (°C)	19.4	19.4
	pH	7.94	7.94
Tank 2 (4 mg ANT-A/kg)	DO (mg/L)	8.40	8.46
	Temp (°C)	19.5	19.6
	pH	7.94	7.91
Tank 3 (4 mg ANT-A/kg)	DO (mg/L)	8.45	8.46
	Temp (°C)	19.5	19.6
	pH	7.98	7.96
Tank 4 (8 mg ANT-A/kg)	DO (mg/L)	8.43	8.45
	Temp (°C)	19.6	19.6
	pH	7.95	7.94
Tank 5 (8 mg ANT-A/kg)	DO (mg/L)	8.42	8.44
	Temp (°C)	19.5	19.6
	pH	7.92	7.95
Tank 6 (16 mg ANT-A/kg)	DO (mg/L)	8.40	8.44
	Temp (°C)	19.5	19.3
	pH	7.92	7.84
Tank 7 (16 mg ANT-A/kg)	DO (mg/L)	8.42	8.43
	Temp (°C)	19.5	19.6

Supplemental Table 2. Water quality metrics for leaching trial.

Tank #	Metric	Time			
		1 h	24 h	48 h	72 h
Tank 1	pH	8.06	8.16	8.21	7.99
	DO (mg/L)	7.46	8.37	7.94	8.76
	Temp (°C)	19.5	17.9	17.4	17.4
Tank 2	pH	8.08	8.21	8.23	8.18
	DO (mg/L)	7.64	8.47	8.09	8.99
	Temp (°C)	19.6	18.0	17.5	17.4
Tank 3	pH	8.08	8.21	8.18	8.14
	DO (mg/L)	7.51	8.45	7.79	8.74
	Temp (°C)	19.6	17.9	17.5	17.4
Tank 4	pH	8.14	8.26	8.28	8.33
	DO (mg/L)	7.56	8.52	8.04	9.05
	Temp (°C)	19.6	17.9	17.5	17.3
Tank 5	pH	8.09	8.24	8.17	8.30
	DO (mg/L)	7.34	8.41	7.86	8.88
	Temp (°C)	19.6	17.9	17.5	17.4

Supplemental Table 3. Water quality metrics for Trial 1 and Trial 2 of the Species

Specificity Trials in tanks.

Tank #	Metric	Time	
		1hr	24hr
Trial 1			
Tank 1 (Control)	pH	8.01	8.01
	DO (mg/L)	7.64	7.35
	Temp (°C)	23.6	23.5
Tank 2 (Control)	pH	8.03	8.02
	DO (mg/L)	7.66	7.36
	Temp (°C)	23.6	23.4
Tank 3 (Control)	pH	8.04	8.03
	DO (mg/L)	7.72	7.35
	Temp (°C)	23.6	23.4
Tank 4 (Treatment)	pH	8.04	8.03
	DO (mg/L)	7.70	7.31
	Temp (°C)	23.6	23.4
Tank 5 (Treatment)	pH	8.05	8.04
	DO (mg/L)	7.77	7.34
	Temp (°C)	23.6	23.4
Tank 6 (Treatment)	pH	8.05	8.03
	DO (mg/L)	7.68	7.29
	Temp (°C)	23.6	23.5
Trial 2			
Tank 1 (Treatment)	pH	7.97	7.94
	DO (mg/L)	7.20	7.02
	Temp (°C)	23.5	23.2
Tank 2 (Treatment)	pH	8.06	7.98
	DO (mg/L)	7.22	7.07
	Temp (°C)	23.5	23.2
Tank 3 (Control)	pH	8.07	7.99
	DO (mg/L)	7.20	7.01
	Temp (°C)	23.6	23.2

Supplemental Table 4. Water quality metrics for each of the ponds in the species-specificity pond experiment

Tank #	Metric	Date						
		10-26	10-27	10-31	11-1	11-2	11-3	11-4
Pond 1 (Control)	pH	9.38	8.98	8.02	8.26	8.08	8.30	8.44
	DO (mg/L)	18.02	13.01	10.03	12.82	10.80	12.36	15.24
	Temp (°C)	9.6	10.1	11.2	13.5	12.7	13.7	13.1
Pond 2 (Control)	pH	9.12	8.90	8.02	8.31	8.05	8.36	8.53
	DO (mg/L)	16.98	14.33	10.00	12.89	9.77	12.67	15.59
	Temp (°C)	9.8	10.1	11.2	13.9	12.3	13.6	13.0
Pond 3 (Control)	pH	9.23	8.94	8.12	8.24	8.07	8.39	8.55
	DO (mg/L)	13.95	14.50	11.05	13.32	10.80	13.56	16.68
	Temp (°C)	9.7	9.8	11.1	13.7	12.6	13.6	13
Pond 4 (Treatment)	pH	8.80	8.59	8.09	8.44	8.05	8.40	8.66
	DO (mg/L)	14.62	12.52	9.78	13.69	9.44	11.99	14.94
	Temp (°C)	9.6	9.7	11.2	14.0	12.1	13.1	12.7
Pond 5 (Treatment)	pH	8.85	8.33	8.10	8.37	8.13	8.50	8.70
	DO (mg/L)	13.97	11.28	9.83	12.69	9.91	12.78	16.25
	Temp (°C)	9.6	10.1	11.2	14.0	12.3	13.8	13.3
Pond 6 (Treatment)	pH	8.82	8.49	8.17	8.51	8.22	8.57	8.73
	DO (mg/L)	13.64	11.80	10.97	13.66	10.72	13.39	17.08
	Temp (°C)	9.5	9.9	11.0	13.8	12.5	13.2	12.9