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Assessing the efficacy of corn-based bait containing antimycin-a to control common carp populations using laboratory and pond experiments

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Abstract Strategic use of oral toxicants could allow for practical and sustainable control schemes for the invasive common carp (*Cyprinus carpio,* or 'carp') if a toxicant selectively targeted carp and not native species. In this study, we 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. Our results showed that ANT-A was lethal to carp at doses $\geq$ 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 (*Lepomis macrochirus*). 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 our 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. Our results suggest that corn-based bait that contains ANT-A could be used to selectively control carp in ecosystems dominated by percids or centrarchids, such as lakes across the Great Plains ecoregion of North America, where carp are especially problematic.

Keywords *Cyprinus carpio* · Toxins · Toxicants · Invasive fish · Management · Species-specific

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

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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, b).

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 and Maceina 1996; McDonald and 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 and 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 and 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 common 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 6 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. Our 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, we conducted a mixed-species laboratory species-specificity test, in which we provided toxic bait (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; 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 $\sim$ 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. Our process of microparticle formulation required ANT-A in a dry powder form; therefore we 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 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 (94–146 mm in total length [TL]; 38–128 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 and Adelmen 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 64–74 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^{TM}, 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) (Online Resource 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; 75–179 mm TL; 7–72 g) and bluegill (n = 6, 86–152 mm TL; 12–70 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 ug/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 × 23 cm × 17 cm; Rubbermaid^{TM}) 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 we thought most of the leaching would occur (Table 1). Water samples were processed using solid phase extraction (SPE) to

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

Tank	Time (h)					
	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[a]	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

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

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 (Online Resource 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 (54–80 mm TL; 5–16 g), five fathead minnows (45–72 mm TL; 1–9 g), six yellow perch (47–61 mm TL; 1–4 g), and six bluegills (82–123 mm TL; 16–66 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. We chose 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 and 24 h post-exposure (Online Resource 3). All fish were measured for weight and length at the conclusion of the trial.

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)

Pond species-specificity trials

Six concrete ponds (10.4 m long × 5.5 m wide × 0.75 m deep; no water flow; ~ 12 °C) were stocked with 10 adult common carp (265–483 mm TL; 570–3000 g), 9 juvenile common carp (98–179 mm TL; 34–130 g; fewer juvenile carp were available), 20 yellow perch (46–136 mm TL; 4–33 g), and 20 bluegill (58–149 mm TL; 8–106 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. We chose 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 (Online Resource 4).

Statistical analysis

We 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 our experiments (> 30%). We 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), we assumed that mortality in treatment tanks would be high (> 30% and consistent), while mortality in control tanks would be nil. We also assumed that we would be using a *t* test to analyze the results of our experiments. Power analysis using such assumptions (power = 0.8, $\alpha = 0.05$, 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, we 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. 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 mg ANT-A/kg BW; suggesting that the LD_{50} for carp in our 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).

Table 3 Results of the pond species-specificity trial

	Control 1		Control 2		Control 3		Treatment 1		Treatment 2		Treatment 3	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Carp adult	9	1[a]	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

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

[a]Fish jumped out of the pond

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 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 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 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 ($\sim$ 47%) common carp died in treatment tanks whereas none died in control tanks (Table 2; $P = 0.02$; df = 3; W = 2). Twenty of 26 ($\sim$ 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 ($\sim$ 15%) yellow perch died in treatment tanks, whereas one of 21 ($\sim$ 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 our study indicates that such a strategy might be most effective in lakes where the fish community is dominated by centrarchids and percids. While we did observe some mortality of perch in our 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-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 and Morsi 1996). We chose corn 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 ug/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, we 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– 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 our experiments, approximately 40% of the carp died after being offered the toxic bait over only short periods of time. We suspect that our 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 Adelman 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 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 more 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, we 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^{TM}, 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^{TM} 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^{TM} 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^{TM} 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 to be sustainable. First, the toxic bait could target both juvenile and adult carp, since both life stages share a similar diet (Yilmaz et 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 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), we believe that this approach could offer new and practical management solutions in smaller and more isolated ecosystems, such as lakes and reservoirs.

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