Research Article

Tests in a semi-natural environment suggest that bait and switch strategy could be used to control invasive Common Carp

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Abstract

Common Carp (*Cyprinus carpio* Linnaeus, 1758), is a highly invasive species that has had profound effects on biodiversity and ecosystem services. Many Carp management methods have been applied including physical removal, pesticide treatments of whole lakes, and water drawdowns. Herein, we tested key elements of a potential “bait and switch” approach in which corn could be used to induce feeding aggregations of Carp and then switched for corn pellets with a pesticide Antimycin-A (ANT-A) to selectively target the Carp. First, laboratory experiments were used to determine if addition of lethal concentrations of ANT-A to corn pellets deterred Carp from eating corn-based food pellets. Second, a pond experiment tested if a corn-based bait containing ANT-A functioned as a species-specific Carp management tool in a semi-natural environment with three common native fishes: White Sucker (*Catostomus commersonii* Lacepède, 1803), Yellow Perch (*Perca flavescens*, Mitchill, 1814), and Bluegill (*Lepomis macrochirus* Rafinesque, 1819). The use of baited sites by Carp and native species was monitored using passive integrated transponder (PIT) tags. Mortality of each species and presence of corn in their digestive tracts was also monitored. Our laboratory experiment showed that presence of ANT-A did not deter Carp from consuming the pellets. The pond experiment showed that only Carp perished in significant numbers once toxic bait was applied. Further, only Carp increased their use of baited sites once baiting began, and only Carp had corn in their digestive tracts. Overall, our results indicate that corn might function as a species-specific Carp attractant in systems of North American Midwest and that corn-based bait can be used to effectively conceal a lethal dose of ANT-A. Further research is necessary to refine this potential management tool, specifically investigating the behavioral and social dynamics of Carp aggregating at sites baited with corn to enhance the temporal and spatial specificity of pesticide application.

Key words: *Cyprinus carpio*, Antimycin-A, invasive species, management, corn-based bait, pond

Introduction

Common Carp (*Cyprinus carpio* Linnaeus, 1758; herein Carp) is one of the most widespread and damaging invasive fish (Vilizzi et al. 2015). Due to their high abundance and unique feeding behavior, Carp have been shown to be an important driver of poor water quality and low biodiversity across large geographic areas of North America and elsewhere (Kloskowski 2011; Bajer et al. 2016). While searching for food, Carp root in the sediments increasing water turbidity, releasing sediment-bound nutrients, and uprooting aquatic macrophytes. Lakes dominated by Carp often switch from clear-water, vegetated states to systems characterized by high turbidity, high nutrient concentrations, extensive algae blooms, and lack of macrophytes. Many methods for controlling Carp populations have been attempted. The most common ones include physical removal with nets (e.g., Bajer et al. 2012), water drawdowns (e.g., Hanson et al. 2017), or use of rotenone directly applied to lake water (e.g., Bonneau and Scarnecchia 2001; Gehrke 2003). Management tools such as physical removal, strategic use of barriers to isolate nursery habitats, and use of native predators to control Carp reproduction have also been used in integrated pest management approaches (Bajer 2017). Genetic technologies are also being pursued but are not available yet (Thresher et al. 2014). While examples of success exist, practical and species-specific management strategies are still urgently needed.

Toxic baits have been exploited as a management tool for Common Carp in the USA and Australia (Bonneau and Scarnecchia 2001; Gehrke 2003; Morgan et al. 2014). Early tests included the addition of rotenone to food pellets and were not successful, presumably due to the fact that Carp could detect the rotenone in the pellets and avoided them (Bonneau and Scarnecchia 2001; Mangan 2003). More promising results have been reported using Antimycin-A (ANT-A) as an oral pesticide for Carp (Poole et al. 2018). Poole et al. (2018) conducted a proof-of-concept study in laboratory tanks and concrete raceways stocked with Carp and three native species representing families common to Midwestern lakes: Fathead Minnows, (*Pimephales promelas* Rafinesque, 1820; Cyprinoidei *sensu* Tan and Armbruster (2018) – "minnows"), Bluegill (*Lepomis macrochirus* Rafinesque, 1819; Centrarchidae) and Yellow Perch (*Perca flavescens* Mitchill, 1814; Percidae). In their experiments, ANT-A was incorporated into corn food pellets because Carp ("minnows") consume corn, while centrarchids and percids typically do not. The trials resulted in high mortality of Carp and Fathead Minnows but Bluegill and Yellow Perch were not affected (presumably did not consume toxic pellets although that was not evaluated). Poole et al. (2018) also showed that ANT-A did not leach out of the pellets to cause indirect fish mortality. Overall, Poole et al. (2018) suggested a plausible management strategy for Carp in Midwestern lakes dominated by centrarchids and percids: Carp could be conditioned to aggregate in large numbers at sites baited with corn (cracked corn), which was already shown by Bajer et al. (2010) and Ghosal et al. (2018), and then targeted by replacing corn with corn pellets (corn meal-based pellets) that contain ANT-A. In such "bait and switch" applications, the use of ANT-A would be minimized in space and time. While the eventual use of ANT-A as part of corn pellets to target Carp would require extensive regulatory review, the pesticide itself appears to be of relatively low risk to non-target species (reviewed in Poole at al. 2018). ANT-A has already been used as fish pesticide (but not as an ingredient of food pellets) for decades (Finlayson et al. 2002).

However, to further pursue the potential applications of corn pellets containing ANT-A for Carp management, important gaps need to be addressed. An important limitation of the study by Poole et al. (2018) was that the experiments were conducted in laboratory tanks and small concrete raceways where the corn pellets were easy to locate and where fish did not have access to naturally occurring food, such as invertebrates in natural substrate. It is plausible that in natural systems, only some Carp might be able to find and consume the corn pellets, while others might forage on naturally occurring food. Inter-individual differences among Carp, might also cause some individuals to forego baited sites to avoid competition with other Carp (Huntingford et al. 2010). Consequently, while some Carp might visit baited sites daily, others might visit them sporadically or never. This would reduce the effectiveness of such "bait and switch" strategies. Further, Poole at al. (2018) did not test directly if Carp are able to differentiate between pellets containing ANT-A from those that do not. Lower palatability of pellets containing ANT-A might lead to avoidance behaviors that could undermine "bait and switch" strategies. Finally, the response of native fish to the corn pellets without or with ANT-A was not tested in more natural environments, and Poole at al. (2018) did not examine if native fish consumed corn pellets used in their experiment, either those containing ANT-A or not.

Due to the nature of this work (food pellets containing a pesticide not yet approved for use in open systems as oral fish pesticide), corn pellets containing ANT-A could not be tested in a natural lake to address the knowledge gaps outlined above. Thus, a logical and feasible extension of the Poole et al. (2018) experiment was to conduct an experiment in natural earthen ponds that mimicked natural lakes but where the risks of non-target effects could be minimized. In this study we conducted an experiment in earthen ponds with natural substrate and habitat conditions. All fish (Carp and native species) were implanted with passive integrated transponder (PIT) tags to determine individual fish responses to the bait. We also included a member of the sucker family, which are common in Midwestern lakes and are known to feed in the benthos but were not used by Poole at al. (2018). In addition to monitoring fish mortality, we also directly examined the diet of Carp and native fish used in this experiment to determine which of them consumed corn pellets. Finally, we conducted a laboratory experiment to directly test if Carp show avoidance reactions to corn pellets containing ANT-A. Overall, this study adds several important elements to further advance "bait and switch" as a potential future management strategy for Common Carp.

Materials and methods

Corn pellet and ANT-A microparticle design

The corn pellet and ANT-A microparticle were created by the U.S. Geological Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, Wisconsin, USA as described in Poole et al. (2018). Briefly, microparticles containing ANT-A powder (Sigma-Aldrich, St. Louis, Missouri, USA) were made according to a method adapted from Hawkyard et al. (2011) and Langdon et al. (2008). The ANT-A core of the microparticle was produced by spray atomizing a refined beeswax (Sigma-Aldrich, St. Louis, Missouri, USA) and sorbitan monopalmitate (Sigma-Aldrich, St. Louis, Missouri, USA). Microparticles had a diameter of approximately 35 μm and a nominal ANT-A concentration of 20% weight by weight (w/w) and were stored at (−20 °C) until use. Corn pellets were a combination of corn meal (Quaker Oats Company, Chicago, Illinois, USA; 80% by weight), gelatin (Knox Gelatine, Kraft Foods Group Inc., Northfield, Illinois, USA; 10% by weight), and, in the toxic corn pellets, microparticles (10% by weight). The corn pellets measured ~ 3.5-mm diameter, a size that was sufficient to pass the gape of the Carp. Non-toxic control pellets followed the same formula and storage method as experimental pellets, but included microparticles without ANT-A. Following Poole et al. (2018), we produced toxic corn pellets such that each pellet should contain enough ANT-A (8 mg/kg) to kill the largest single fish, including Carp, in the pond.

Laboratory Experiment

This experiment was conducted to determine if Carp are less likely to consume pellets containing a lethal dose of ANT-A than pellets that did not contain ANT-A, which would indicate that Carp might develop avoidance behaviors towards pellets that contain ANT-A in future management applications.

Young of year Carp (Osage Catfisheries, Osage Beach, Missouri, USA) were acclimated to laboratory conditions and maintained on a diet of floating 3.5 mm Classic Trout© pellets (Skretting, Tooele, Utah, USA) for at least 2 weeks. For the tests, Carp were then moved to 21-L glass tanks (1 Carp/tank) with flowing, heated well water at 20 °C. Carp in individual tanks were acclimated for 7 days and were fed floating 3.5-mm Classic Trout© pellets daily. Opaque plastic foil was placed on the sides of the tank to minimize disturbance from outside of tanks. A black plastic viewing tent was erected around tanks to minimize disturbances and to allow the recording of behavioral observations without being seen by the fish.

Once in glass tanks, Carp were trained to consume non-toxic corn pellets for a minimum of 7 days following protocols by Kasumyan and Morsi (1996). Thirty-four Carp were then randomly assigned to the test (Toxic) group or control (Non-toxic) group (17 per group), without access to food for 48 hrs, and individually tested. During the experiment, a single corn pellet was dropped into each tank and the following data were recorded for 7 minutes: (1) number of times the pellet was approached, ingested into oral cavity, swallowed or rejected (spit out); (2) total duration of time (seconds) the pellet was retained in the oral cavity during the entire 7-minute experiment including instances when the pellet was ingested and rejected more than once, total duration of time pellet was retained in the mouth before it was swallowed, and duration from when the pellet was dropped into the water until it was swallowed or until the 7 minutes ran out. The instant of swallowing a pellet and time it occurred was estimated by watching the movement of oral cavity structures. After ingesting the pellet into the oral cavity, operculi and jaw structures continued moving vigorously as the pellet was being manipulated inside the oral cavity, then the bottom of the oral cavity and operculi suddenly extended, which signified swallowing, and the movement of the oral cavity structures returned to normal. In no instances, did fish that were assumed to have swallowed a pellet, reject it afterwards during the 7-minute experimental period. Also, for fish that swallowed the pellets, we made special effort to examine if the pellet (or its parts) were present on the bottom of the tank when fish were removed, and the tank was cleaned at the end of the trial.

The index of palatability (Ind_{pal}) was calculated as

$$Ind_{pal} = [(R - C) / (R + C)] \times 100$$

where R is the number of consumed test pellets and C is the number of consumed control pellets (Kasumyan and Morsi 1996). A value of 100 indicates that the ANT-A containing pellets were extremely attractive in relation to the control pellets, zero that they were neutral, and −100 that the ANT-A containing pellets were extremely unattractive in relation to control pellets. After the experiment, each Carp was weighed, measured, and euthanized by tricaine methanesulfonate overdose [TMS]/L (Tricaine-S™ Western Chemical Inc., Ferndale, Washington, USA). A chi-square test ($\alpha = 0.05$) was used to determine whether there was a significant difference between the willingness of Carp to consume a test pellet versus a control pellet. A t-test ($\alpha = 0.05$) was used to compare the amount of time the Carp retained test versus control pellets in the oral cavity; this test was conducted only for Carp that approached and ingested the pellet in the oral cavity at least once. Analyses were conducted in R v. 3.4.0 (R Core Team 2017).

Pond experiment

The purpose of this experiment was to test the “bait and switch” strategy in a semi-natural setting with Carp and three species of native fish, while tracking individual fish responses to baiting over time. At the conclusion of the experiment, the digestive tract contents of each individual were also examined for presence of corn.

The pond experiment was conducted from September 21 to October 13, 2017, in six 0.1-ha earthen ponds located at UMESC in La Crosse, Wisconsin. The ponds had a relatively uniform depth of ~ 1 m, a soil overburden on the bottom, sparse aquatic vegetation, and water temperatures between 18.0 and 22.0 °C. Each pond was stocked with 40 Bluegills (92–173 mm Total Length (TL)), 40 Carp (229–510 mm TL), 40 White Suckers (*Catostomus commersonii* Lacepède, 1803; 176–315 mm TL), and 40 Yellow Perch (103–175 mm TL). Carp were collected wild from Albert Lea Lake (Minnesota); Yellow Perch were obtained from UMESC culture facilities; White Suckers were obtained from R.J. Hilger & Sons, Inc (Antigo, Wisconsin, USA); and Bluegill were obtained from Osage Catfisheries. Each fish was implanted with a unique 12 mm-HDX PIT tag (Oregon RFID, Portland, Oregon, USA). A 1-m diameter circular pass-over PIT tag antenna was placed flat on the bottom of the shallow end of each pond and secured to a metal stake to prevent movement. The antennas had a detection range of ~ 30 cm. Two multi-antenna HDX PIT readers (Oregon RFID) were used to record detection data; each reader gathered data from three ponds, 24 hrs/day.

The experiment consisted of three consecutive phases. Phase 1 consisted of a 2-week acclimation period, during which fish were fed ~ 500 g of 4-mm floating Classic Trout© pellets scattered throughout the pond by hand once a day. Phase 2 consisted of a 5-d period during which fish were fed with corn (cracked corn) and corn pellets without pesticide. During this phase, 390 g of corn and 30 g of corn pellets (non-toxic) were placed on the pond bottom, in the center of the PIT antenna. The amount of corn and corn pellets provided (420 g) was based on 3% of the Carp biomass in each pond. Feeding occurred at 15:00 every day. Presence of fish of each species that visited the baited site was recorded using the PIT antennas and reader. Phase 3 consisted of a 4-d toxic bait treatment (TBT) that included two parts, a 2-d treatment with 390 g of corn and 30 g of corn pellets containing ANT-A followed by a 2-d observation without feeding. In the TBT phase Ponds 2, 3, and 5 were randomly selected to receive pellets containing ANT-A, while Ponds 1, 4, and 6 served as controls by only being fed corn and corn pellets without ANT-A. TBT experiments were conducted in three ponds at a time (first group included Ponds 1, 2, 3 and second group included Ponds 4, 5, 6). During the 4-d TBT period the ponds were observed at least twice daily to recover dead fish. At the end of the 4-d TBT period, ponds were drained to recover remaining live and dead fish. Remaining live fish were euthanized by tricane methanesulfonate overdose. Fish were frozen and retained until assessments of digestive tracts were completed to determine if corn or corn pellets were consumed.

Data from the pond experiment were analyzed in three ways. First, we analyzed the detections at the PIT antennas to determine what percentage of fish of each species visited the PIT antennas during Phase 1 and Phase 2 of the experiment. We hypothesized that while the number of Carp would increase rapidly in Phase 2 once baiting with corn started, the numbers of other species would decline or remain unchanged. This analysis was restricted to fish that acclimated to the ponds and were assumed to be in good health; i.e., we excluded fish that perished in Phase 1 or 2 (see regression analysis below).

Second, we performed a regression analysis to examine mortalities during TBT (Phase 3). We hypothesized that only Carp would show increased mortality during TBT. Due to the length of this study and potential for handling or tagging-related mortality prior to TBT, it was important to determine the number of fish that perished in each pond prior to TBT (Phase 1 and 2) because high mortality prior to TBT (e.g., due to disease or handling) could have been correlated with mortality during TBT regardless of the application of toxic pellets. By taking mortalities prior to TBT into account, we were able to estimate fish mortality during TBT in an unbiased way. The number of fish of each species that perished prior to TBT was estimated as: 40 (number initially stocked) minus fish that were recovered alive when the ponds were drained minus individuals that were recovered dead during TBT. All fish that were detected by PIT antenna 24 hrs prior to TBT were recovered alive when the ponds were drained, showing that we were able to account for all the fish. Due to a faulty PIT tag antenna and HDX PIT reader, all PIT data collected in Pond 3 were deemed unusable for analyses reliant on detection data (absent from Figure 1). A generalized mixed linear model in R ver. 3.4.0 (R Core Team 2017) was used to test if the pesticide had a significant effect on the number of fish of each species that died during TBT: Death during TBT ~ species + death before TBT + pond + pesticide + pesticide:species. In the regression model, the response variable was the number of mortalities after the TBT began. The predictors in the regression model were species (factors: Carp, Yellow Perch, White Sucker, and Bluegill), number of dead before TBT period (numerical variable), pond (factor: pond numbers 1–6), pesticide (factor: yes or no), and the interaction term between pesticide and species.

Third, frozen fish were transported to the University of Minnesota for analysis of digestive tract contents. Fish were thawed, digestive tracts were removed and the contents were extruded onto a plate and sorted. Corn bait was noticeably hard when manipulated and easily recognizable, when viewed through a binocular microscope, as it was mostly undigested and retained a dull yellow color.

Results

Laboratory experiment

Nine out of the 17 Carp assigned to the test group consumed the corn pellet containing ANT-A, and eight out of the 17 Carp assigned to the control

Table 1. Number of Carp tested in each group, number of Carp that consumed the pellet, percent of Carp that consumed the pellet after they approached it, number of Carp that never approached the pellet or showed any interest in it, total number of times the pellets were ingested (sometimes the pellets were ingested and spat out before being ingested again), total number of times the pellets were rejected (all of those were eventually ingested again and consumed), and the mean amount of time the pellet remained in the mouth before it was swallowed.

Pellet type	Carp tested	Consumed pellet	Approached-consumed	No approach	Times ingested	Times rejected	Time to swallow (s)
Toxic	17	9	100%	8	10	1	41.2
Control	17	8	100%	9	12	4	57.5

group consumed the corn pellet within the 7-minute experimental window. Other Carp showed no interest in the corn pellet (i.e., did not approach or ingest the pellet). The Carp showed no significant difference in their willingness to consume toxic versus control corn pellets, χ^2 (1, N = 34) = 0.118, P = 0.73. All Carp that approached and ingested a pellet into the oral cavity consumed it, both among the test and control groups (Table 1). The Ind_{pal} was 5.88 showing slightly positive selection for ANT-A pellets over control pellets. For Carp that ingested the pellet into their oral cavity, there was not a significant difference in the time the corn pellet was retained in the mouth before it was swallowed between the two groups (Table 1; t = 1.18, df = 13.67, P = 0.26). Carp rarely rejected the pellets (one test fish once, one control fish once, one control fish three times), and all corn pellets that were initially rejected were eventually consumed (Table 1).

Pond experiment

The percent of each species that were detected by the PIT antennas during Phase 2 of the experiment varied between 10% and 100% (Figure 1). After feeding with corn and corn pellets without pesticide was initiated, the number of detected Carp increased to ~ 90% and the number of White Suckers, Perch, and Bluegill tended to decline or remain the same (Figure 1). The pattern indicates the other species were being detected at the antenna, some at high percentages, but did not increase with the addition of corn, while Carp visited the antenna more in all but one pond in response to addition of corn (Figure 1).

Most pre-TBT deaths mortalities occurred within the first day of stocking and unforeseen mortalities ceased before TBT began. Although pre-TBT mortality of carp was low (< 25%), pre-TBT mortality was considerably higher and ranged from 37.5% to 92.5% for other species and ponds (Table 2). A total of 22 of 120 Carp died (18.3%), i.e. were recovered dead during visual inspections during TBT, all in ponds where toxic pellets were used (Table 2). In Ponds 2 and 5, most Carp died on the 4th day of TBT (Table 2). All Carp that died were detected by PIT antennas at the baited site during TBT, and they had evidence of corn in their digestive tracts. Two White Suckers (4.0 % of all White Suckers in treatment ponds during TBT) also died (recovered dead during visual pond checks) during TBT, all in ponds where toxic pellets were applied (Table 2, Supplementary material Table S1).

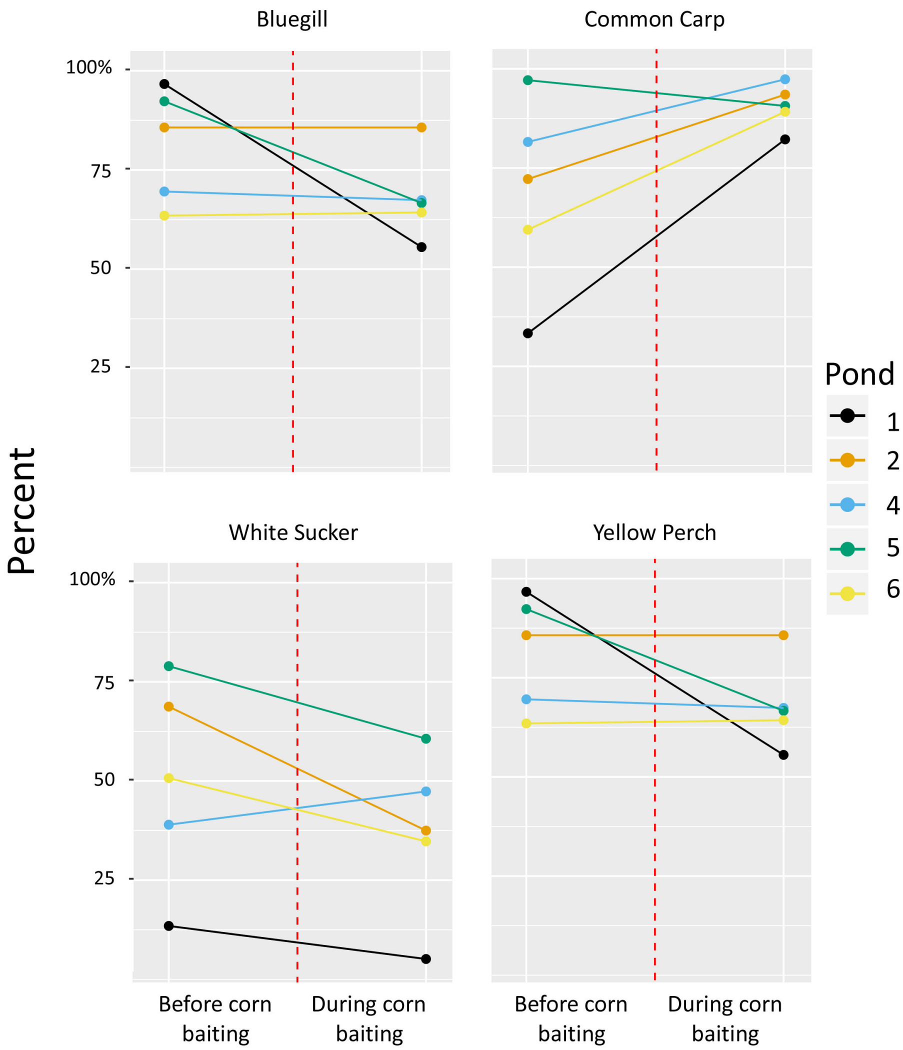

Figure 1. Average percent of fish, by species, detected at feeding station/antenna before corn baiting (Phase 1) and during corn baiting (Phase 2 of the experiment, 5 days each). Phase 1: fish were acclimating to ponds and no corn baiting occurred. Phase 2: cracked corn and corn pellets (without pesticide) were placed daily at the antenna. Pond 3 is not included due to malfunctioning PIT antenna and reader. For specific number of each species in pond see Table 2.

However, none were found to have corn in the digestive tract and none had been detected by the PIT antenna during TBT (see discussion). No other fish were found dead during TBT in either treatment or control ponds. The only significant factors in the regression model (F-statistic: 5.988 on 12 DF, p-value = 0.003) were the increased mortality in Pond 3 (Table S1, p-value = 0.049) and the interaction between the Carp and pesticide (Table S1, p-value = 0.001) showing that the toxic corn pellets significantly increased Carp mortality.

A total of 270 (30 Bluegill, 106 Carp, 66 White Sucker, and 59 Yellow Perch) and 203 fish (34 Bluegill, 97 Carp, 46 White Sucker, and 26 Yellow Perch) were recovered alive from control and treatment ponds, respectively, when the ponds were drained (Table 2). Observation of the pond substrate after the ponds were drained revealed corn pellets and corn at the feeding stations in ponds receiving toxic corn pellets. No corn or corn pellets were found at the feeding stations in control ponds (Figure S1).

Table 2. Number of fish detected at PIT antennas 24 hrs prior to TBT, found dead during TBT, or found alive after TBT; 40 individuals of each species were initially stocked in each pond. Ponds receiving the toxic pellets with Antimycin-A (ANT-A) are marked with *.

	Pond					
	1	2*	3*	4	5*	6
Bluegill						
Detected 24 hrs prior to TBT	8	15	n.a.	12	3	12
Died during treatment	0	0	0	0	0	0
Alive after treatment	8	16	13	14	3	12
Common Carp						
Detected 24 hrs prior to TBT	20	28	n.a.	37	35	33
Died during treatment	0	8	11	0	3	0
Alive after treatment	30	27	29	38	28	37
White sucker						
Detected 24 hrs prior to TBT	2	8	n.a.	3	4	3
Died during treatment	0	1	1	0	0	0
Alive after treatment	25	14	14	19	18	22
Yellow Perch						
Detected 24 hrs prior to TBT	5	5	n.a.	11	7	9
Died during treatment	0	0	0	0	0	0
Alive after treatment	15	7	6	23	13	21

Corn was found in digestive tracts of all Carp recovered from the ponds (either those that died during TBT or were recovered alive at the end), but in no other species.

Discussion

Our results provide further evidence that corn pellets containing ANT-A could be used to target Common Carp with a relatively high degree of selectivity in systems containing centrarchids, percids, and suckers. Our results expand upon recent work by Poole et al. (2018) by showing that in natural earthen ponds, ~ 90% of the Carp visited baited sites daily, Carp were the only species whose visits at the baited site increased once baiting with corn commenced, Carp were the only species with corn in their digestive tract, and were the only species that showed a significant mortality during TBT. Our laboratory experiment showed that presence of lethal doses of ANT-A did not deter Carp from consuming toxic corn pellets. Carp handled the toxic corn pellets in the same way they handled control corn pellets and we saw no indication that ANT-A containing pellets were rejected more than control pellets. However, this study also signaled potential limitations of the “bait and switch” approach. Even though nearly all Carp visited baited sites daily and the amounts of toxic pellets were designed to inflict 100% mortality among Carp in a single application, only 22.5% of Carp perished as a result of the application of pellets containing ANT-A during two consecutive days. This might be attributed to several processes that would need to be addressed in future studies. Relatively low mortality rates in our study might be potentially attributed to uneven consumption of toxic pellets among individual Carp and/or insufficient length of observation. It is possible that a small number of Carp consumed the majority of toxic pellets making them unavailable to other Carp. This could be addressed in future studies by dosing the toxic pellets more gradually over time or for a longer period to allow more individuals to consume toxic pellets. It is also plausible that some Carp experienced sub-lethal doses of pellets with ANT-A and ceased consumption altogether during the TBT. This might explain large amounts of uneaten corn and corn pellets in treatment ponds once the ponds were drained (all corn was consumed in control ponds) (Figure S1). These aspects would need to be addressed in controlled experiments, most likely in laboratory settings, where consumption and behavior of individual fish is closely monitored.

A critical aspect of the "bait and switch" method is to minimize non-target mortality. Our results indicated that percids (Yellow Perch), centrarchids (Bluegill), and catostomids (White Sucker) might not be strongly affected by the application of corn pellets containing ANT-A to control Carp. We found no evidence of corn in the digestive tracts of Bluegill, Perch, and White Sucker. Three White Suckers died during TBT period whereas no Bluegill or Yellow Perch died during TBT. We speculate that these mortalities were not related to the application of pellets with ANT-A because none of the three White Sucker that died were detected by the antennas placed at the bait. Further, White Suckers are known for consuming invertebrates (e.g., Diptera, chironomid larvae, and Cladocera), detritus, and some aquatic plants, but are not known to consume large seeds (Ahlgren 1990; Saint-Jacques et al. 2000). Poole et al. (2018) reported that Fathead Minnows readily consumed corn pellets with ANT-A and perished. Other minnows might also show similar responses, which would limit the potential application of this technique in regions dominated by large-bodied minnows, such as Europe (Rapp et al. 2008; Monk and Arlinghaus 2017). Direct in-situ observations in natural systems, for example using PIT systems and underwater cameras, are needed to determine potential effects of the "bait and switch" strategy described in this paper on fish other than Carp in specific ecosystems and regions. Utilization of corn pellets not containing ANT-A would be beneficial in such evaluations. Such studies would be especially important in regions dominated by fishes known to consume grains or seeds, like many cypriniform fishes from Europe and Asia (e.g. goldfish *Carassius auratus* Linnaeus, 1758).

The development of toxic corn pellets containing ANT-A for use in Common Carp management appears to be promising. However, before corn pellets containing ANT-A could be used in a natural setting, proper ethical, regulatory, and safety concerns warrant consideration (see discussion in Poole et al. 2018). Additionally, researchers need to further explore the complexities of Carp feeding aggregations induced by corn baiting to make the use of toxic pellets as targeted as possible. To minimize undesired effects and the number of pellets required to achieve desired mortality levels, it would be prudent to condition Carp to aggregate at corn baited sites in large numbers and at highly predictable times. Image-recognition technologies in combination with underwater cameras and PIT technologies could be developed to ensure a high-precision dosing of pellets containing ANT-A to minimize non-target effects.

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Ethics and Permits

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. Research was conducted under animal care and use protocol 1601-33424A approved by the University of Minnesota Animal Care and Use Committee.

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Supplementary material

The following supplementary material is available for this article:

Figure S1. Photos taken after six earthen experimental ponds were drained at outdoor complex in La Crosse, Wisconsin. Corn remained in only the ponds (2, 3, and 5) with treated corn pellets containing a piscicide Antimycin-A (*).

Table S1. Regression model results from data collected during pond experiments. The predictors in the regression model are: species (factor: Carp, Yellow Perch, White Sucker, and Bluegill), number of dead before the experiment (numerical variable), pond (factor: pond numbers 1–6), toxin (factor, yes or no), and the interaction effect between toxin and species. * Results were considered significant at $P < 0.05$.

This material is available as part of online article from:
http://www.reabic.net/journals/mbi/2020/Supplements/MBI_2020_Hundt_etal_SupplementaryMaterials.pdf

Other data associated with this study are available at http://hdl.handle.net/11299/214094