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Research Article

Ingested toxicity of antimycin A to grass carp *Ctenopharyngodon idella* and black carp *Mylopharyngodon piceus* in two carriers

Patrick T. Kroboth*, Duane C. Chapman, Jeffery A. Steevens and Curtis G. Byrd

U.S. Geological Survey, Columbia Environmental Research Center, Columbia, MO 65201, USA

**Corresponding author*

E-mail: *pkroboth@usgs.gov*

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Abstract

Toxic baits are a potential control mechanism for nuisance carps, but rotenone-based baits for grass carp *Ctenopharyngodon idella* have been ineffective. Failures have been attributed to the palatability of rotenone because innocuous training pellets are readily consumed prior to provision of piscicide baits. Several studies suggest antimycin A, a common alternative piscicide, typically applied directly to water, may be suitable as an ingested bait. The oral toxicity of antimycin A is not well described. We evaluated the oral toxicity of antimycin A in two carriers (ethanol and corn oil) on grass carp and black carp *Mylopharyngodon piceus,* administered via gavage. Doses ranged from 1–16 mg/kg. Lethal dose estimates for 50% of treated fish (LD50) were calculated, and the observed treatment levels resulting in complete mortality are reported at 24- and 96-hours post-treatment. Ethanol was a more effective carrier than corn oil with lower LD50 estimates and observed treatment levels with complete mortality. Antimycin A in corn oil produced only partial mortality of black carp even 96 hours from treatment and at the highest dose administered. Results document ingested doses required for mortality of grass carp and black carp that may be used for future development of species-selective antimycin A baits.

Key words: invasive carp, invasive species, bait, piscicide, corn oil, ethanol

Introduction

Ingested piscicide baits have been tested to selectively target and control undesired fish species. Fajt (1996) explained the process required for the development and application of a piscicide bait that is swallowed, with piscicide absorption through the gastrointestinal tract. Rotenone baits were developed for control of common carp *Cyprinus carpio* Linnaeus, 1758 and grass carp *Ctenopharyngodon idella* Valenciennes, 1844 (Fajt and Grizzle 1993; Fajt 1996). Subsequent research with rotenone baits indicated that the product can kill both common carp and grass carp (Fajt and Grizzle 1993; Fajt 1996), but field results varied. Field methods generally consist of a feed training phase where innocuous similarly formulated floating food pellets are distributed by fish feeders to attract and congregate the target species prior to piscicide application. Both innocuous feed training pellets

and piscicide baits float to facilitate observations of ingestion. Piscicide baits have been tested in small lakes and reservoirs for control of common carp (Bonneau and Scarnecchia 2001; Gehrke 2003; Mangan 2003) and for removal of stocked triploid grass carp (Mallison et al. 1995; Fajt 1996). Stocking records for triploid grass carp were used to estimate the effect of baits on population density, and results ranged from 1 to 77% removal of stocked triploid grass carp (Fajt 1996). Others reported no or minimal mortality to common carp, attributing failure to the poor palatability of rotenone because feed attractants, which lack rotenone, were readily consumed throughout trials (Bonneau and Scarnecchia 2001; Gehrke 2003; Mangan 2003).

Antimycin A has been commonly used as a piscicide in fisheries management and is a rotenone alternative. The mechanism of antimycin A is decoupling of electron transport in the mitochondria during cellular respiration (Lennon and Vezina 1973). Rotenone acts similarly but on a different location in the same biochemical pathway (Horgan et al. 1968). Sensitivity of fish to aqueous antimycin A exposure is likely due to its rapid uptake across the gills into the circulatory system. Lethal concentrations (LC) and exposure times of antimycin A required to kill cyprinid species, such as grass carp, common carp, bighead carp *Hypophthalmichthys nobilis* Richardson, 1845, and silver carp *Hypophthalmichthys molitrix* Valenciennes, 1844 are much lower than for rotenone (Marking and Bills 1981). Antimycin A is reported to be undetected by fish, unlike rotenone (Bettoli and Maceina 1996), and once exposed, fish do not recover when moved to toxin-free water after receiving a lethal dose (Berger et al. 1969).

The aqueous toxicity of antimycin A for fish has been studied, but ingested toxicity has been infrequently examined, although common carp are one of the few species for which ingested toxicity has been reported. Rach et al. (1994) determined the 96-hour dose lethal to 100% of common carp was 0.8 mg antimycin A/kg body weight, measured using force-fed baits composed of extruded fish meal and antimycin A (Rach et al. 1994). In contrast, Poole et al. (2018) found the force-fed dose lethal to 50% of fish (LD50) to be 4 mg antimycin A/kg body weight for common carp, with complete mortality of treated common carp observed at 8 mg/kg; their baits consisted of wax-coated antimycin A microparticles mixed with corn meal and gelatin in extruded pellets. Kroon et al. (2005) tested the palatability of antimycin A baits to grass carp; baits consisted of fish meal pellets soaked in differing amounts of antimycin A (1.0 and 2.6% wet weight antimycin A) and coated with an algin stabilizing agent. Complete mortality of treated grass carp was observed, but the reported methods did not allow for estimation of the required lethal dose (Kroon et al. 2005).

Wild populations of grass carp and black carp *Mylopharyngodon piceus* Richardson, 1846 are considered invasive in North America. Both are anticipated to have deleterious effects on native ecosystems. Grass carp

consume aquatic vegetation, resulting in substantial habitat alteration (Krupska et al. 2012; Cudmore et al. 2017). Black carp consume benthic invertebrates, dominated by mollusks (Poulton et al. 2019), of which many North American taxa are listed as threatened or endangered with small or fragmented populations. The ecosystem-level effects of grass carp and black carp can be great and there are few options for control. Prior literature has emphasized the need for more palatable, effective piscicide baits (Bonneau and Scarnecchia 2001; Mangan 2003); however, there is a lack of ingested antimycin A toxicity data for grass carp and black carp. We present new data on the ingested toxicity of antimycin A for these carp species. Although antimycin A is not commercially available for fisheries use at this time, these data could be used in the future development of orally consumed antimycin A baits. Antimycin A is an antibiotic produced from the culture of *Streptomyces* sp. The purity of antimycin A among cultured batches varies with the cumulative proportion of the four main stereoisomer pairs ($A_{1\text{-}4}$); thus, we initially tested the toxicity of available antimycin A in aqueous exposures for comparison with previously reported aqueous results of fathead minnow *Pimephales promelas* Rafinesque, 1820 bioassays (Berger et al. 1969). We then observed the ingested toxicity of antimycin A via 24-hour and 96-hour bioassays with grass carp and black carp. Antimycin A was presented in two carriers: ethanol (a common solvent) and corn oil (a more palatable lipid anticipated to match the digestion rate of diet items).

Materials and methods

The purity of antimycin A is measured by the cumulative percent composition of the four stereoisomer pairs. For consistency among trials, we used antimycin A from batch number 096M4064V (Sigma-Aldrich, St. Louis, Missouri, USA) with reported stereoisomer pairs A_1 30.55%, A_2 18.94%, A_3 17.99%, and A_4 18.66%. The total of these percentages (86.14%) represents batch purity; the mass of antimycin A used with ethanol or corn oil carriers in stock solutions was adjusted to compensate for batch purity during mixing.

All experiments were conducted at the U.S. Geological Survey Columbia Environmental Research Center in Columbia, Missouri (CERC). Test animals were quarantined and observed to be in good health for a minimum of four weeks prior to treatment. Adult fathead minnow and juvenile diploid grass carp were propagated and cultured at CERC. Black carp were procured from Keo Fish Farms, Keo, Arkansas. Black carp were diploid, their status confirmed via Coulter counter method (Wattendorf 1986). Laboratory standard protocols for care, feeding, humane procedures for anaesthetization and handling of fish, acclimation to research waters and euthanasia of test organisms were followed throughout this project (ASTM International 2014). All project methods were reviewed under

CERC's Institutional Animal Care and Use Committee; permit numbers IACUC18-009 and IACUC18-030. The data set for this project has been archived by the U.S. Geological Survey (Kroboth et al. 2020).

Confirmation of batch toxicity

Prior to testing the toxicity of antimycin A to grass carp and black carp, we confirmed stock material met the aqueous toxicity standard similar to that established by Berger et al. (1969). Adult fathead minnows with an average total length 41 ± 2 mm (Mean ± SD) and an average weight 0.71 ± 0.15 g were used in our bioassay. A 96-hour static renewal bioassay with five treatment concentrations 0.025, 0.05, 0.10, 0.20, 0.40 ug/L, and a negative control were used. Five adult fathead minnows were stocked into each of four replicate 3.78-L glass test chambers per treatment concentration. Designated treatments were prepared by serial dilution of 10 mg antimycin A in 100 mL of acetone stock solution, as Berger et al. (1969) used both ethanol and acetone solvents. Berger et al. (1969) bioassays used reconstituted deionized water (hardness 40 mg/L), so similarly, the antimycin A stock solution was mixed with a similar reconstituted deionized water with hardness confirmed via titration at 42 mg/L (ASTM 2019) for aqueous tests.

Water temperature was maintained at 17 °C via partial immersion of test chambers in a water bath. Water bath temperature was recorded with a HOBO temperature logger (Onset Computer Corporation, Bourne, Massachusetts). Antimycin A is reported to degrade via oxidation; thus, no aeration was used in the 3.78-L test chambers (Chapman et al. 2003) to avoid oxidation of antimycin A in aqueous treatments. To increase dissolved oxygen, an 80% water exchange was completed at 48 hours, with treatment water from the initial stock that was stored within the water bath until exchange. Dissolved oxygen, water temperature, and pH were measured at the beginning of the experiment in one replicate of each treatment level and upon completion or mortality with a YSI Pro Plus meter (Yellow Springs Instruments, Yellow Springs, Ohio).

Survival was recorded daily, and deceased fish were removed from each container. Mortality was defined as a lack of operculum and general movement exceeding 5 minutes. Upon completion of the experiment, surviving fish were euthanized with tricaine methanesulfonate (MS-222; Syndel, Ferndale, Washington), weighed, and length was measured. Toxicity was estimated via median LC50 using the Trimmed Spearman-Karber Method (Hamilton et al. 1977) in the ecotoxicology package in R statistical software (Gama 2015). This method was chosen as it allows estimation of an LC50 in a bioassay with poor intermediate effects. The estimated value and confidence intervals were compared to the LC50 reported by Berger et al. (1969).

Ingested dose testing

Similar-size diploid juvenile grass carp and black carp were used in ingested dose testing, with grass carp average total length 209 ± 18 mm and average

weight 102.76 ± 29.76 g, and black carp average total length 237 ± 26 mm and average weight 126.42 ± 44.85 g. A sample size of 20 grass carp at each treatment level were administered antimycin A gavage doses during both ethanol and corn oil tests. Black carp aquaculture production has been restricted since listing under the Lacey Act (U.S. Fish and Wildlife Service 2007), thus a limited number of black carp were available for experiments. Ten black carp were tested at each treatment level.

Doses were administered from a standard 5 mg antimycin A/mL concentration stock solution. Storage and preparation of this stock varied by carrier. Pure non-denatured ethanol was mixed directly with the appropriate volume of antimycin A and stored overnight at 4 °C. Fresh stock solutions were prepared prior to experiments using ethanol carrier for both species. Corn oil is a viscous carrier, and we observed that corn oil possesses a lower solubility for antimycin A than ethanol. A sole stock was prepared the day prior to grass carp testing and stored at 24 °C throughout the 96-hour duration of grass carp observation until it was used in the black carp 96-hour test. For the duration of this storage period, temperature was recorded with a HOBO temperature logger. The corn oil stock was prepared by measuring the desired mass of antimycin A into a 50 mL glass conical bottom centrifuge tube. Antimycin A was mixed with an initial solution of acetone. Corn oil (Sigma-Aldrich, St. Louis, Missouri) was gradually added with nitrogen evaporation of the acetone until the desired concentration was reached and initial volume of acetone dissipated. Both ethanol and corn oil mixtures were sonicated (Branson Ultrasonics, Danbury, Connecticut) and agitated with a vortex mixer (Barnstead Thermolyne, Ramsey, Minnesota). All stored suspensions using corn oil were purged with argon gas after mixing to prohibit oxidation of corn oil. Upon completion of corn oil trials, an additional ten black carp were administered a freshly prepared stock at the highest dose and were observed at 24- and 96-hours post-treatment to assess the effect of storage methods on toxicity.

Treatment levels were 1, 2, 4, 8, and 16 mg antimycin A/kg of fish. Doses were administered from one 5 mg antimycin A/mL stock solution at the appropriate volume to achieve the desired dose. These volumes ranged from 0.01–1.08 mL of carrier (mean 0.18 mL). A negative control consisted of the highest treatment volume of innocuous carrier per kg of fish. Prior to treatment, individual fish were anaesthetized in 60–70 mg/L MS-222 until loss of equilibrium. Fish length and weight were recorded, and the appropriate volume of the 5 mg antimycin A/mL stock was administered by an 18 gage 76 mm-long reusable gavage needle (Pet Surgical, Phoenix, Arizona) attached to a 1 mL BD syringe (Becton, Dickinson and Company, Franklin Lakes, New Jersey) to meet the desired treatment level.

Following gavage, fish were placed into one of six 378 L tanks identified by dose. Flow-through well water resulted in a renewal rate equivalent to twice per hour. Tanks were fitted with a dual standpipe system to draw

water from the bottom of the tank and facilitate exchange of the complete tank volume. Aeration was maintained via an airstone fed from a compressed air supply. Dissolved oxygen, water temperature, and pH were measured daily in each tank with a YSI Pro Plus meter.

Mortality was monitored, and deceased fish were removed daily until the termination of each experiment at 96 hours. Mortality was defined as a lack of operculum and general movement exceeding 5 minutes. After 96 hours, surviving test animals were euthanized with MS-222.

We estimated the 24-hour and 96-hour LD50 by fitting a sigmoidal survival curve using the four-parameter log-logistic model in the drc package in R statistical software (Ritz and Strebig 2016). The log-logistic model is a common method for fitting the sigmoidal curve (Ritz et al. 2015). The estimated LD50 value represents the standard intermediate level of mortality reported and the inflection point in the sigmoidal curve. The lowest observed treatment levels resulting in complete mortality are commonly reported (Rach et al. 1994; Poole et al. 2018) as the desired endpoint in toxic bait studies. We report LD50 estimates and standard errors and the observed treatment levels resulting in complete mortality by species and carrier. Plots of the survival curves are presented for comparison among tests.

Results

Confirmation of batch toxicity

Within the fathead minnow bioassay water bath, average temperature was 16.64 ± 0.22 °C. Prior to stocking, mean water temperature was 18.68 ± 0.23 °C, mean dissolved oxygen was 7.45 ± 0.32 mg/L, and mean pH was 7.89 ± 0.11 among treatment levels. Dissolved oxygen declined during the test to an average of 2.44 ± 0.89 mg/L among test chambers; pH remained neutral 7.32 ± 0.32. The LD50 was observed at the 0.1 µg/L concentration, and no mortality occurred at the 0.05 or 0.025 µg/L concentrations or negative controls. Results produced an estimated median LC50 of 0.09 µg/L with 95% confidence interval 0.08–0.11 µg/L.

Ingested dose testing

Water temperature, dissolved oxygen, and pH were similar among gavage trials for grass carp and black carp (Table 1). Similar mortality occurred for grass carp with both ethanol and corn oil carriers (Table 2) at 24 hours and 96 hours post treatment. Complete mortality of grass carp occurred at the 4 mg antimycin A/kg in ethanol treatment and in the 8 mg antimycin A/kg in corn oil treatment, between 24 and 96 hours post treatment (Table 2). The dose response of black carp differed from grass carp. Complete mortality of black carp was observed 24 hours post treatment in the 8 mg antimycin A/kg ethanol treatment, declining to 4 mg antimycin A/kg at 96

Table 1. Average water temperature, dissolved oxygen, and pH and their respective standard deviation (SD) among the six-400 L ingested dose tanks.

Species	Carrier	Water temperature °C (SD)	Dissolved oxygen mg/L (SD)	pH (SD)
Grass carp	Ethanol	16.5 (0.1)	8.80 (0.52)	7.90 (0.04)
Black carp	Ethanol	17.6 (0.1)	8.58 (0.25)	7.87 (0.04)
Grass carp	Corn oil	17.5 (0.1)	8.83 (0.52)	7.89 (0.03)
Black carp	Corn oil	17.6 (0.1)	8.38 (0.25)	7.82 (0.07)

Table 2. The 24- and 96-hour response of grass carp and black carp to oral dosing of antimycin A in ethanol and corn oil carriers, and their respective standard errors (SE). Note, due to partial mortality of black carp at the highest doses of antimycin A in corn oil, these LD50s are not reported. Complete mortality represents the lowest observed treatment levels resulting in complete mortality.

Species	Carriers	LD50		Complete mortality	
		24-hr mg/kg (SE)	96-hr mg/kg (SE)	24-hr mg/kg	96-hr mg/kg
Grass carp	Ethanol	1.49 (0.08)	0.66 (0.02)	4	4
Black carp	Ethanol	2.16 (1.84)	0.83 (0.11)	8	4
Grass carp	Corn oil	1.68 (0.16)	0.99 (0.03)	8	8
Black carp	Corn oil	–	–	> 16	> 16

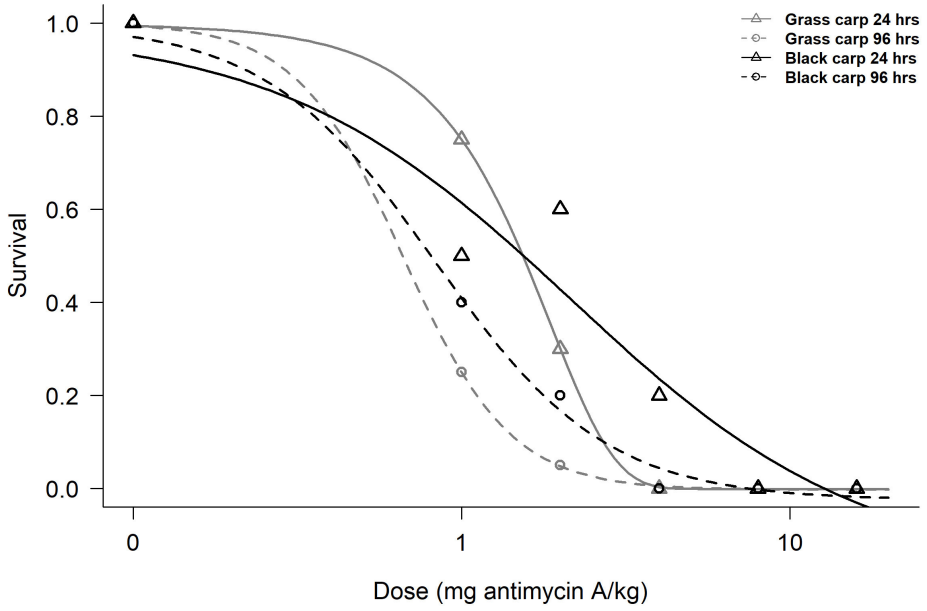

Figure 1. Grass carp and black carp proportional survival at 24- and 96-hour intervals post gavage treatment of antimycin A in ethanol.

hours. High standard error in the black carp 24-hour ethanol carrier LD50 estimate was the result of greater mortality at the lowest concentration (n = 5 fish) than the medium-low (n = 4 fish; Table 2). After 96 hours mortality approached a sigmoidal pattern (Figure 1), similar to that observed in grass carp for antimycin A in ethanol and a comparable complete mortality at the 4 mg antimycin A/kg treatment level. Black carp were less susceptible than grass carp to ingested antimycin A in the corn oil carrier (Figure 2). Insufficient mortality limited estimation of the dose response relationship. After 96 hours of observation, complete mortality had not occurred in the highest treatment level, indicating complete mortality occurs at >16 mg antimycin A/kg. The lowest observed effect occurred at 2 mg antimycin A/kg of fish at 24 hours after dosing. In the subsequent additional treatment of ten black carp with a freshly prepared antimycin A in corn oil stock, eight

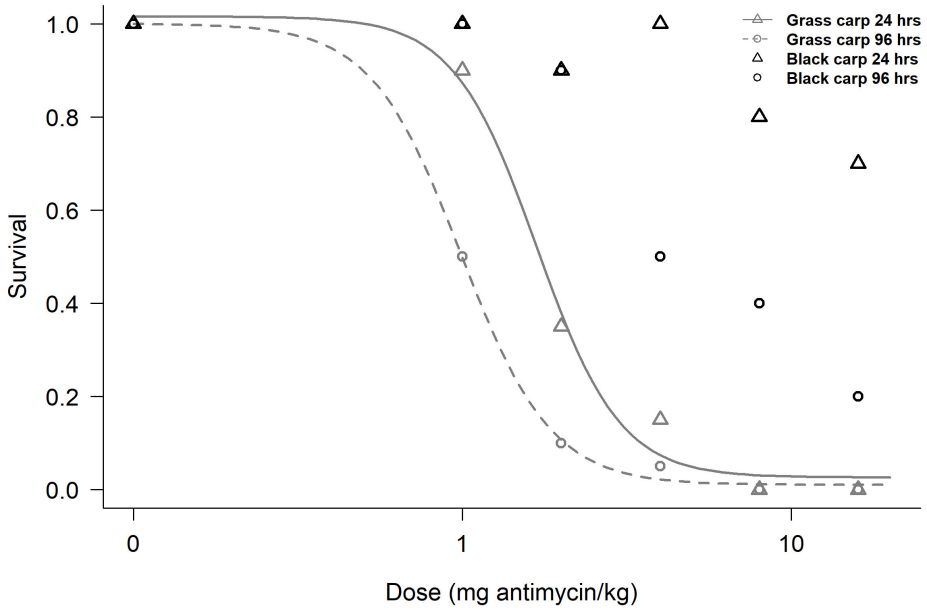

Figure 2. Grass carp and black carp proportional survival at 24- and 96-hour intervals post gavage treatment of antimycin A in corn oil.

fish survived 24 hours post treatment and four fish survived 96 hours post treatment. These results do not differ sufficiently from initial testing to suggest that the prior stock had degraded.

Discussion

This study sought to determine the ingested toxicity of antimycin A via 24- and 96-hour bioassays to grass carp and black carp presented in two carriers, ethanol and corn oil, via gavage methods. Antimycin A is a lethal piscicide when ingested by grass carp or black carp. We found that ethanol was a more efficient carrier than corn oil, but toxicity was observed within 24 hours for both species regardless of carrier. The complete survival of control individuals suggests that gavage methods such as treatment volume and handling did not affect survival. Our fathead minnow bioassay results confirm that the batch of antimycin A used had similar toxicity to batches used in prior published research. The resulting estimated LC50 for the aqueous fathead minnow bioassay was within the 0.08–0.10 ug/L 95% confidence interval reported by Berger et al. (1969).

Black carp survival was slightly higher than grass carp. This may be an artifact of different sample sizes, with ten black carp used at each treatment level compared to twenty grass carp. At a lower sample size, the effect of one individual has a greater influence on the overall model fit and error estimates. This effect is observed in the results of black carp treated with antimycin A in the ethanol carrier, where the 24-hour LD50 estimate has a high standard error caused by higher survival between two progressive low doses. Our plan to have equivalent sample sizes was limited by the availability of black carp from aquaculture sources.

Ethanol has been commonly used as a carrier of antimycin A, and it elicited response at lower doses than corn oil. This suggests antimycin A doses in the ethanol carrier were absorbed more efficiently. In a bait application, dosage rate may be restricted because ethanol is anticipated to challenge palatability to either black carp or grass carp. We chose to apply ethanol carriers because this standard is commonly used for preparation of antimycin A solutions and because dilution in water may facilitate degradation as described by hydrolysis of the antimycin A stereoisomer A_1 (Hussain 1969).

Degradation via hydrolysis is a limitation of antimycin A in bait applications independent of the carriers tested here. Fajt (1997) proposed a bait with a solid or liquid toxicant core suspended in a solid matrix, encapsulated in a water-resistant gel with an exterior fish-food attractant layer. Fajt (1997) did not identify the toxicant, but additional research led to the development of the rotenone based-fish management bait (Fajt 1996). This concept of encapsulating antimycin A is similarly applied by Poole et al. (2018) through use of wax-coated microparticles. Delivery methods favoring an encapsulated piscicide may be desirable to block hydrolysis or leaching of antimycin A, resulting in mortality of non-target species. Black carp possess specially adapted molariform pharyngeal teeth and muscle structure for the manipulation, breaking, and consumption of mollusk prey (Gidmark et al. 2015). We are currently investigating methods for delivery of piscicide encapsulated in a borosilicate glass ampule that may be broken by the specially adapted pharyngeal teeth of black carp and ingested, similar to shell material observed in diet samples (Poulton et al. 2019). The types and dimensions of ampules, attractants, and methods of delivery may be manipulated to target specific species and mitigate non-target effects. For example, black carp piscicide delivery may consist of an ampule with wall thickness sufficient to avoid breaking by non-target species being consumed and excreted without administering the piscicide. Similarly, the herbivorous diet of grass carp may be used to select attractants or delivery methods adapted to the digestive or pharyngeal adaptations of grass carp to avoid non-target effects through lack of consumption.

Mortality varied for antimycin A in corn oil by treated species (Figure 2). We considered the possibility that degradation may have occurred due to storage between grass carp and black carp testing. According to the manufacturer, antimycin A powder is stable at ambient temperature for up to two weeks. Additionally, Berger et al. (1969) comment that antimycin A solutions in acetone stored in cool, dark storage for two years produced equivalent 96-hour EC50s on rainbow trout *Oncorhynchus mykiss* Walbaum, 1792 at 12 °C during intermediate testing throughout the two-year period. Our corn-oil stock was stored in a climate-controlled room and not chilled to 4 °C, as was ethanol, to reduce the potential dissociation of the antimycin A from corn oil for the eight days from the time of mixing to the

time of black carp dosing following grass carp tests. Storage of the corn oil stock did not deviate substantially from the anticipated 24 °C; mean temperature was 23.77 °C (SD 1.33 °C). This temperature is not sufficient to degrade antimycin A (Berger et al. 1969). Additionally, when we reran the highest dose of antimycin A in corn oil from a fresh stock on ten black carp following initial experiments, the results were comparable, suggesting that the antimycin A did not degrade at stored temperatures.

Variation in toxicity among carriers and species is likely due to reduced bioaccessibility and slower absorption rate from the corn oil mixture in the gastrointestinal tract. Ethanol as a carrier increases the uptake of poorly soluble chemicals in the gastrointestinal system by increasing delivery at the membrane or by increased transmembrane absorption by destabilizing membrane structure (Gurtovenko and Anwar 2009; Fagerberg et al. 2015). The corn oil carrier takes longer to digest and does not cause destabilization across membranes. Because antimycin A is relatively lipophilic, it is most stable in the oil carrier. Variation in the anatomy of both species' gastrointestinal tracts and diet requirements may also affect absorption (i.e., differences in enzymatic or digestive microbiota) because grass carp are adapted for an herbivorous diet (Cudmore and Mandrak 2004), unlike black carp whose diet consists of aquatic insect and mollusk taxa (Poulton et al. 2019).

Additional knowledge is needed for development and deployment of toxic baits as a control method for invasive grass carp and black carp. Prior research selected rotenone-based baits as a control method in small closed lentic systems such as reservoirs and lakes (Mallison et al. 1995; Fajt 1996; Bonneau and Scarnecchia 2001; Mangan 2003). This approach is still suitable for the development and initial testing of antimycin A grass carp baits, although effects in large open systems are more difficult to measure. Little is known of the population, habitats, or distribution of black carp in North America, though commercial fishing data have facilitated development of some base knowledge of the population density and habitats where black carp may be encountered (Kroboth et al. 2019). Many of these reports are from lotic habitats where food-based attractants are anticipated to rapidly dissolve, maintaining only a brief timeframe of effectiveness. Black carp control methods will likely be implemented for specific protection of at-risk endangered mussel beds, thus a better understanding of black carp diet to identify potentially affected mussel taxa and their habitats will assist in toxic bait development. For both carp species, methods to identify target habitats, attraction or congregation of the target species, and facilitation of ingestion of the piscicide bait need further development.

Several authors have suggested that the prior failures of rotenone baits were due to the palatability of rotenone (Bonneau and Scarnecchia 2001; Gehrke 2003; Mangan 2003) and detection of the piscicide by target animals. Kroon et al. (2005) found that this palatability of rotenone for common

carp could not be overcome, even following an extended feed-training period. This extended feed-training period was not required for antimycin A baits, which were consumed immediately after the hard food-based products softened, allowing consumption, with complete mortality following (Kroon et al. 2005). Several authors have tested the toxicity of ingested antimycin A to nuisance carps (Rach et al. 1994; Kroon et al. 2005; Poole et al. 2018), but only the pellets soaked in antimycin A (1.0 and 2.6% wet weight antimycin A) used by Kroon et al. (2005) resulted in toxicity to grass carp, and no estimates of the required lethal doses were provided. No similar results are available for black carp. We present here initial data on the ingested doses required for mortality of grass carp and black carp that may be used for future development of species-specific antimycin A baits.

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Author's contributions

The authors contributed as follows to this research and the production of the manuscript: PK and DC – research conceptualization; PK, DC and CB – sample design and methodology; PK and CB – investigation and data collection; PK and JS – data analysis and interpretation; PK and CB – ethics approval; DC – funding provision; and PK produced the initial draft with editing and revision by DC, JS, and CB.

Ethics and permits

All methods followed established USGS standard operating procedures and conducted under the USGS Columbia Environmental Research Center's Institutional Animal Care and Use Committee; permit numbers IACUC18-009 and IACUC18-030.

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