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Toxicity of a management bait for grass carp (*Ctenopharyngodon idella*) incorporated with Antimycin A

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Abstract

No current technology can specifically target grass carp (*Ctenopharyngodon idella*) for control within aquatic ecosystems. Rotenone and Carbon Dioxide-Carp are currently the only available registered pesticides for grass carp; they are nonselective and typically applied throughout the water, equally exposing target and native species. A more selective control tool or pesticide application could be used by resource managers to support mitigation efforts. Development of delivery systems that exploit carp feeding strategies could increase selectivity of pesticides and minimize effects on native fishes. A pesticide with selective delivery could be less labor intensive and used within an integrative pest management strategy. The present study examined Antimycin A toxicity in juvenile and sub-adult grass carp and rainbow trout (*Oncorhynchus mykiss*) across two routes of exposure. Water-based toxicity studies were used to calculate the concentration to cause lethality in 50% of treated fish (LC_{50}) at 24-h, while oral gavage toxicity studies were used to calculate the dose to cause lethality in 50% of treated grass carp and rainbow trout (LD_{50}) 24- to 96-h. Although rainbow trout were more sensitive than grass carp to Antimycin A through water-based exposure, oral toxicity was similar between species, even with inherent gastrointestinal morphological differences. Successful delivery of a lethal dose of Antimycin A to grass carp was achieved through an oral route of exposure using the rapeseed bait and shows promise for registration as a control tool and eventual use in pest management plans. Although a lethal dose of Antimycin A could be incorporated into a single bait pellet, more bait was required to achieve desired mortality when fed to fish under laboratory conditions.

Introduction

Concern over recruitment of grass carp (*Ctenopharyngodon idella*) in the Laurentian Great Lakes tributaries (Chapman et al. 2013; Embke et al. 2016) has prompted increased removal programs in an effort to prevent further population expansion throughout the watershed (Herbst et al. 2021). Although recent years of targeted response efforts by multiple management agencies and research groups have been successful at removing more grass carp from the Lake Erie Basin through traditional harvest methods, the catch per unit effort (CPUE) has remained low (Herbst et al. 2021). Whether low CPUE of grass carp in the region is indicative of a fish population in low abundance (Maunder et al. 2006), seasonal changes in local abundance of this migratory species (Bain et al. 1990; Sullivan et al. 2020), or overall low gear efficiency (Bonar et al. 1993; Herbst et al. 2021), CPUE will continue to diminish as management objectives are met and the population declines. Localized pesticide use at targeted time points is a potential management option that might be less labor intensive than traditional methods. Additionally, this strategy could supplement removal efforts as populations decline and more effort is required or at specific sites where traditional gear is less effective.

Rotenone and Carbon Dioxide-Carp are currently the only available registered pesticides for grass carp; they are nonselective and typically applied throughout the water, equally exposing target and native species. In an effort to

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reduce nontarget species effects from nonselective pesticide water-based applications, selectivity has been possible through pesticide incorporation into baits designed to exploit food preferences of target organisms such as grass carp and common carp (*Cyprinus carpio*; Fajt and Grizzle 1993; Rach et al. 1994; Mallison et al. 1995; Poole et al. 2018). Limited success of field applications with rotenone laden baits has been attributed to the ability of fish to detect and avoid rotenone (Bettoli and Maceina, 1996; Bonneau and Scarnecchia 2001; Gehrke 2001). Because of inadequate control of grass carp and common carp through oral delivery systems of rotenone, along with concerns of the negative human health effects (McClay 2005; Finlayson et al. 2012), additional management chemicals that can be selectively administered would be useful for resource managers. Antimycin A is an alternative to rotenone and is currently in the process of being reregistered for control of nonnative fishes. Antimycin A is a good candidate as a management chemical because it is substantially more toxic to fish than rotenone (Finlayson et al. 2002; Rach et al. 2009), is undetectable to fish (Bettoli and Maceina, 1996; Finlayson et al. 2002), and quickly degrades in water (Marking and Dawson 1972). The eutrophic conditions where grass carp management and removal is currently underway would cause Antimycin A to degrade more rapidly than other systems, where it was historically used as a fish pesticide, because of higher pH and water temperature (Marking and Dawson 1972; Marking 1975).

Although Antimycin A is nonselective, acute toxicity varies among fish species, with Salmonidae being more sensitive than members of the Cyprinid family (Finlayson et al. 2002). The 96-h water-based toxicity of Antimycin A to common carp has been reported to be lethal to 50% of fish at a concentration (LC_{50}) of 0.57 µg/L (Marking and Bills 1981), but only 0.04 µg/L for rainbow trout (*Oncorhynchus mykiss*; Berger et al. 1969; Finlayson et al. 2002). Similarly, the 96-h LC_{50} of Antimycin A for grass carp has been reported to be 1.00 µg/L (Marking and Bills 1981). Rach et al. (1994) observed 100% mortality of common carp when they were gavage fed a dose greater than 0.81 mg Antimycin A/kg fish. Successful oral delivery of Antimycin A to common carp using a corn-based bait has also been demonstrated to be selective based on the delivery mechanism (Rach et al. 1994; Poole et al. 2018). Poole et al. (2018) reported a 24-h oral dose lethal to 50% of fish (LD_{50}) to be 4 mg/kg and 100% mortality at 8 mg/kg, using encapsulated Antimycin A in beeswax. However, the 96-h Antimycin A LD_{50} gavage fed to grass carp was recently reported at 0.66–0.99 mg/kg depending on the carrier solvent (ethanol and corn oil respectively; Kroboth et al. 2022). In the same study, the 24-h LD_{50} was reported to be between 1.49 and 1.68 mg/kg (ethanol and corn oil carriers, respectively). Water-based and oral toxicity of Antimycin A has been reported for multiple species, however, a comparison using analytically verified concentrations and an orally deliverable formulation has not been made.

Effectiveness of a selective control tool, such as an Antimycin A laden bait, will rely on production of a palatable and attractive bait formulation that is selective to target organisms relative to non-targets, as well as protection of the control agent from rapid degradation when exposed to water. A nontoxic bait produced from rapeseed and corn has proven palatable and attractive to grass carp in the laboratory (Wamboldt et al. 2022) and is currently being evaluated as a management tool in the Lake Erie Basin. Although Antimycin A is relatively insoluble in water (0.2 µg/L; U.S. Environmental Protection Agency's Estimation Program Interface software), it does degrade rapidly through hydrolysis, and its half-life would be substantially shorter in waters typically inhabited by grass carp given an expected higher pH in eutrophic systems (Berger et al. 1969; Marking and Dawson, 1972). Furthermore, controlling the leach of Antimycin A from the bait will be vital for selectivity of the treatment and will reduce nontarget mortality through water-based exposure to the chemical.

From lack of access to proper analytical instrumentation, previous Antimycin A toxicity studies were based on nominal water concentrations. Biological activity (lethality) and Antimycin A degradation are most commonly verified using rainbow trout, fathead minnow (*Pimephales promelas*), or yeast bioassays (Berger et al. 1969; Kroboth et al. 2022) in lieu of analytical verification of Antimycin A concentration. Use of nominal concentration and different rates of Antimycin A degradation, dependent on water quality, likely affect LC_{50} values previously reported. Bernardy et al. (2013) published an analytical method for verifying Antimycin A water concentrations by liquid chromatography–mass spectrometry (LC–MS). This LC–MS method served as the basis for analytically verifying the present toxicity study exposure concentrations, which is essential in toxicological studies (Klimisch et al. 1997).

The goal of this study was to develop and examine a management bait that can be used for selective control of grass carp. Our objectives were to (1) quantify the water-based 24-h LC_{50} of Antimycin A for grass carp and rainbow trout, (2) quantify the 96-h LD_{50} of orally administered Antimycin A laden bait for grass carp and rainbow trout, (3) quantify the leaching rate of Antimycin A from the bait in water, and (4) determine if a management bait laden with Antimycin A will be consumed by grass carp and cause lethality in the laboratory. To meet our objectives, Antimycin A was encapsulated in a wax microparticle similar to Poole et al. (2018) and incorporated into a rapeseed bait for oral gavage feeding and consumption trials to demonstrate if Antimycin A can be orally delivered, protected from degradation, and readily consumed by grass carp.

Materials and methods

Animal husbandry

All experiments were completed at the U.S. Geological Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, Wisconsin. Fish were maintained in indoor flow-through systems and held at optimal temperatures for growth and feeding, specific for each species (12–15 °C and 17–25 °C for rainbow trout and grass carp, respectively). Water quality (temperature, dissolved oxygen, and pH) was measured daily within experimental tanks. Ammonia (NH_3-N), hardness as calcium carbonate ($CaCO_3$), and total alkalinity ($CaCO_3$) were measured at the beginning and end of each trail. Fish were taken off feed 24 h before being transferred to experimental tanks and were allowed to acclimate for a minimum of 48 h. Rainbow trout were tested as a model species along with grass carp because of the availability of Antimycin A toxicity data available through the U.S. Environmental Protection Agency ECOTOX Knowledgebase (cfpub.epa.gov) and their acceptance of gavage procedures (Wamboldt et al. 2022).

Water-based toxicity trials for rainbow trout and grass carp were conducted at 12 ± 1.0 °C in aerated 40-L stainless steel tanks partially submerged in a flow-through raceway to maintain temperature. Oral gavage toxicity trials were conducted in aerated community flow-through raceways (735-L) with flow rates adjusted for >1 tank exchange/h to maintain proper water quality. Fish were tagged for gavage toxicity trials to allow for dosing of fish based on individual mass. When transferred, fish were anesthetized in accordance with UMESC animal care and use standard operating procedures and usage label in an immersion bath of an aerated buffered solution (50–85 mg/L) of Syncaine® (tricaine methanesulfonate; Syndel, Ferndale, Washington, USA). While fish were anesthetized, individual fish weight was measured, and a uniquely coded T-bar whisker tag (Floy Tag, Seattle, Washington, USA) was injected into the dorsal musculature approximately 1 cm lateral and posterior to the midline of the dorsal fin. Bait consumption trials were conducted in 150-L cylindrical flow-through tanks with flow rates adjusted for >1 exchange/h to maintain proper water quality. Warmer temperatures (20 ± 1.0 °C) were maintained for grass carp consumption trials to facilitate active feeding behavior (Osborne and Riddle, 1999). Remaining fish were euthanized with a lethal dose of Syncaine® at the end of each trial.

Analytical

Water samples from experimental chambers were measured to 250 mL in glass volumetric flasks and extracted using solid phase extraction (Oasis MAX 60 mg, Waters Corp., Milford, Massachusetts, USA) to concentrate Antimycin A. Antimycin A was extracted from bait and microparticle samples to give empirically derived total Antimycin A concentration maximums for each trial. Bait samples were processed by addition of sodium sulfate (anhydrous sodium sulfate; CAS No. 7757-2-6, 99.0% purity), dried acetone, and one #7 steel shot bead, homogenized via SPEX Geno Grinder 2010 (SPEX Sample Prep, Metuchen, New Jersey, USA), and then centrifuged using an Avanti 30 Centrifuge (Beckman Coulter Life Sciences, Indianapolis, Indiana, USA). Supernatant was removed and diluted in dried acetonitrile containing 1% formic acid (ACNFA-1%) to a target Antimycin A concentration of 200 µg/L. Samples were passed through a 0.45-µm syringe filter (Gelman PTFE Acrodisc®, Gelman Sciences, Ann Arbor, Michigan, USA) directly into a liquid chromatography—mass spectrometry vial for analytical verification.

Analysis of water, bait, and microparticle samples was completed using an Agilent G6460A QQQ and 1290 Infinity II HPLC and an Agilent G6530A QTOF and 1290 Infinity I HPLC (Agilent Technologies, Santa Clara, California, USA). The ion source used was AJS ESI. The column on the HPLC was a Phenomenex Kinetex 1.7-µm particle size, XB-C18 100 Å, 100 × 2.10 mm (Phenomenex, Torrance, California, USA). Mobile phase A for the HPLC was 5-mM ammonium acetate in water/methanol (80:20 w/w), and mobile phase B was 5-mM ammonium acetate in methanol/isopropanol (70:30 w/w). A flow rate of 0.300 mL/min was used. The mobile phase gradient began with 50% mobile phase A and 50% mobile phase B for 2.15 min, followed by 100% mobile phase B for 2.15 min to 2.20 min, and then back to 50:50 until 3.30 min. Antimycin A (Sigma-Aldrich, St. Louis, Missouri, USA) was dissolved in acetonitrile with 1% formic acid to create analytical standards. Antimycin A concentrations were determined through the average response of combined integrated peaks corresponding to 4 targeted major Antimycin A structural analog pairs (A_1, A_2, A_3, A_4).

Static exposure

Antimycin A 24-h static water-based exposure trials were conducted to determine toxicity for rainbow trout and grass carp in hard water. Stable water quality conditions similar to expected environmental conditions are important because Antimycin A is an ionizable weak acid, and its toxicity is pH dependent. Historically, most fish toxicity data for Antimycin A were collected in soft water conditions. Trials conducted at UMESC were carried out in hard water (target range:160–180 mg/L $CaCO_3$) to mimic environmental conditions expected where a toxic bait for grass carp would likely be used by resources managers. Reconstituted hard

Table 1 Mean (±standard deviation) temperature (°C), pH, dissolved oxygen (DO; mg/L), alkalinity (mg/L $CaCO_3$), hardness (mg/L $CaCO_3$), and ammonia (mg/L NH_4) of experimental tanks containing rainbow trout (RBT; *Oncorhynchus mykiss*) and grass carp (GRC; *Ctenopharyngodon idella*) during multiple experiments at 0 h (a) and 24 h (b)

Experiment	Species	Temperature	pH	DO	Alkalinity	Hardness	NH_4
a.							
Static bath	RBT	13.1 (0.4)	8.26 (0.05)	9.89 (0.11)	111 (12)	121 (2)	0.01 (0.01)
Static bath	GRC	19.8 (0.2)	8.41 (0.03)	8.58 (0.15)	141 (12)	130 (3)	0.00 (0.00)
Gavage	RBT	13.6 (0.1)	7.78 (0.11)	8.54 (0.24)	138 (1)	190 (7)	0.05 (0.06)
Gavage	GRC	13.6 (0.2)	7.82 (0.16)	9.04 (0.50)	135 (7)	189 (9)	0.14 (0.14)
Leaching	-	-	8.36	9.30	118	197	0.0
Consumption	GRC	20.5 (0.3)	8.09 (0.08)	8.78 (0.09)	116 (5)	174 (7)	0.04 (0.03)
b.							
Static bath	RBT	12.1 (0.4)	8.23 (0.11)	10.18 (0.26)	107 (11)	125 (3)	0.25 (0.45)
Static bath	GRC	20.1 (0.2)	8.25 (0.09)	8.28 (0.14)	123 (12)	121 (1)	0.09 (0.06)
Gavage	RBT	13.9 (0.5)	7.90 (0.13)	9.25 (0.19)	134 (18)	188 (3)	0.10 (0.08)
Gavage	GRC	13.9 (0.2)	7.94 (0.03)	9.51 (0.01)	136 (5)	187 (0)	0.21 (0.17)
Leaching	-	-	-	-	-	-	-
Consumption	GRC	20.1 (0.1)	8.02 (0.06)	8.82 (0.05)	142 (8)	183 (7)	0.00 (0.00)

water was prepared from deionized water as described in ASTM International (2014). Water quality was measured at 0 and 24 h (Table 1) in tanks containing fish.

Static exposure trials were repeated twice per species with six treatment concentrations, a negative control, and a solvent (acetone) control. Antimycin A stock solutions (~800,000 µg/L) were prepared in acetone (manufacturer grade) and spiked into individual experimental tanks via pipette at various volumes to obtain desired concentrations. Tanks were mixed thoroughly using a glass stir rod before addition of fish. After tanks were mixed, a 300-mL water sample was collected from each tank (0 h) for analytical verification of Antimycin A concentration as described previously. Measured concentrations of Antimycin A were compared between trials using a two-way analysis of variance (ANOVA) with significance set at $\alpha = 0.05$. Differences between trials were determined with a Tukey's honest significance test. Ten fish were stocked into each of three replicate, stainless steel tanks (40-L) per treatment concentration. Treatments were randomly assigned to each tank. Mean ± standard deviation (±SD) weights of rainbow trout and grass carp were 3.1 (1.4) g and 4.4 (1.7) g, respectively. Nominal Antimycin A experimental concentrations were 0.31, 0.61, 1.25, 2.50, 5.00, and 10.00 µg/L for rainbow trout and 0.80, 1.46, 2.66, 4.84, 8.80, and 16.00 µg/L for grass carp. Toxicity values were calculated as described below for each trial using mean measured Antimycin A concentrations collected from experimental tanks at 0 h. Lethal concentration values were derived using a two-parameter log-logistic model in the drc package in R statistical software (Ritz et al. 2016). Sigmoidal survival curves were fitted using a two-parameter model that fixes the lower limit to zero and upper limit to one because survival data have a binary response bound by zero and one.

Bait formulation and leaching

Antimycin A laden microparticles were produced using methods similar to the methods described in Poole et al. (2018). Antimycin A (Sigma-Aldrich, St. Louis, Missouri, USA) was combined into a melted mixture of waxes and encapsulated using a ¼ inch JBCJ atomizing sprayer (Spraying Systems Co., Wheaton, Illinois, USA) with a 35100 spraying nozzle fitted to a 120-SS air cup. The nominal Antimycin A amount in the microparticle was 20% w/w. Microparticles were stored at −20 °C for later use and analytically verified before each study. The Antimycin A concentration was verified via solvent extraction as described previously.

Bulk quantities of nontoxic bait were produced by Prairie AquaTech (Brookings, South Dakota, USA) using the corn and rapeseed formulation described in Wamboldt et al. (2022). Small batch bait formulations produced in the laboratory were compared with bulk manufactured batches using proximate analyses, fatty acid profiles, and free amino acid profiles completed by the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, Missouri, USA). The manufactured bait was used as a base formulation for all experiments in the present study. Nontoxic manufactured bait was finely ground in a coffee mill and mixed with Antimycin A laden microparticles and other ingredients to produce the toxic formulation used to gavage feed fish and feed fish in consumption trials as described below.

The toxic bait was designed to be pelletized and slowly sink, mimicking the pelleted aquafeed that grass carp were acclimated to before experimentation. Stability of Antimycin A and wax microparticle within the bait was achieved through the use of a low temperature process for making floating bait (Orire and Sadiku 2014).

Manufacturing procedures were kept less than the mean (±SD) melting point of the microparticle formulation ingredients (42.4 ± 0.60 °C). The milled corn and rapeseed bait consisted of 64% dry weight (w/w) of the final toxic bait. Active dry yeast (7%), baking powder (3%), and microparticle (20%) were mixed with two binders, carboxymethyl cellulose powder (3%) and cellulose (10%). Once dry ingredients were thoroughly mixed, canola oil (4%) was incorporated into the mixture along with deionized water to form a doughball. Water was slowly added to the mixture to produce a malleable doughball as described in Orire and Sadiku (2014). After water was fully incorporated, the doughball was extruded through a garlic press to produce a uniform pellet. Yeast was activated to produce air bubbles within the dough, and thus increase its buoyancy, by placing bait pellets in an incubator at 38 °C for 45 min before being dried an additional 3 h at 30 °C. Once dry, toxic bait was stored at −20 °C for later use and analytically verified before each study. The mean (±SD) dry weight of an individual pellet was 0.014 (0.002) g.

Bait samples were analytically verified in triplicate throughout the production process to compare the amount of Antimycin A within the microparticle and final bait product. The mass of Antimycin A and percent recovery were compared between the original microparticle, dry mixture of bait ingredients, wet dough mixture, air dried bait, and oven dried bait using a one-way ANOVA with significance set at 0.05. Differences between samples were determined with a Tukey's honest significance test. To determine how much Antimycin A would leach from the final product in water, a 24-h leaching study was completed in well water at room temperature (18–20 °C) in complete darkness. Well water was collected from the UMESC fish culture source, and water quality was assessed as a bulk sample (Table 1). Approximately 50 mg of bait was added to 300 mL of well water in glass beakers and temporally sampled at 0, 1, 3, 6, and 24 h for Antimycin A analytical verification. Each time point consisted of four replicates each. Water samples were filtered through a 5-µm Whatman™ filter (PTFE Membrane Filters, GE Healthcare, Chicago, Illinois, USA), and Antimycin A was analytically verified in the water and filtered to determine bait-microparticle retention. Therefore, a mass balance of Antimycin A was possible, under the assumption that the degraded amount was not accounted for.

Oral gavage

Oral toxicity of the Antimycin A laden bait was determined for rainbow trout and grass carp through a set of oral gavage trials. Unlike the 24-h static water-based exposure, gavage fed fish were monitored for 96 hours to observe prolonged toxic effects because water quality could be maintained with flow-through test systems. It was also assumed that oral exposure to Antimycin A could be latent because of reduced bioavailability when incorporated into the bait/microparticle. Aquafeed was withheld from fish for four days before gavage feeding to ensure empty gastrointestinal (GI) tracts. Dose concentrations (mg Antimycin A/kg fish; hereinafter referred to as "mg/kg") were specific to individual fish and were preloaded in 1-mL Luer-lock syringes with the tip cut off. The total weight of the gavage fed bait and microparticle equaled 0.10% and 0.05% body weight (BW) for rainbow trout and grass carp, respectively. Although the amount of microparticle and Antimycin A dose remained similar between species, blank bait inclusion for gavage feeding grass carp was reduced because of their lack of a true stomach and fragile GI tract. Fish were anesthetized for gavage in small batches of ≤4 organisms with tricaine methanesulfonate as described previously. After gavage procedures, individual fish were monitored for regurgitation in separate 50-L tanks for 15 min before being placed in a 69 × 292 × 30 cm (735 L) temperature controlled, flow-through, community tank for 96 h. Fish that were injured or regurgitated microparticle were removed from the trial.

Nominal gavage doses were 0, 1.0, 2.0, 5.0, 10.0, and 15.0 mg/kg for rainbow trout and 0, 1.0, 2.5, 5.0, 7.5, 15.0, and 25 mg/kg for grass carp and were based on range-find testing of each species. Higher gavage doses were avoided to keep the microparticle to bait inclusion rate less than 50%. The LD_{50} of each species was calculated using the mean measured Antimycin A concentration encapsulated in the microparticle as described previously. Five fish per dose were gavaged per trial, with three replicate trials per species, for a total of 15 fish/treatment. The mean (±SD) weights of rainbow trout and grass carp were 130.6 (34.9) g and 138.4 (54.7) g, respectively. Lethal dose values were derived using a two-parameter log-logistic model in the *drc* package in R statistical software (Ritz et al. 2016). Sigmoidal survival curves were fitted using a two-parameter model that fixes the lower limit to zero and upper limit to one because survival data have a binary response bound by zero and one. Percent inclusion of microparticle in the bait ranged from 0.22 to 9.61% (w/w), representing a range of 0.18 mg to 4.42 mg of Antimycin A used for rainbow trout. Percent inclusion of microparticle in the bait ranged from 0.20% to 35.54% (w/w), representing a range of 0.10 mg to 5.17 mg of Antimycin A used for grass carp. The mean (±SD) measured inclusion rate (w/w) of Antimycin A in the microparticle was 19.96 ± 0.13%.

Consumption

Antimycin A laden bait was offered to fish to evaluate if grass carp would readily consume the toxic bait at a lethal

Table 2 The 24-h LC_{50} (μg/L) values of static water-based exposure to Antimycin A for rainbow trout (RBT; *Oncorhynchus mykiss*) and grass carp (GRC; *Ctenopharyngodon idella*) in reconstituted hard water (a) and 24–96-h LD_{50} (mg/kg) and standard error (SE) values for RBT and GRC gavage fed Antimycin A laden rapeseed bait (b) with upper and lower 95% confidence intervals (CI)

Species	Trial	LC_{50} (SE)	95% CI
a.			
RBT	1	0.61	0.60–0.62
RBT	2	0.77	0.67–0.87
GRC	1	3.91	3.10–4.71
GRC[a]	2	2.74	2.15–3.32
b.			
Species	Hour	LD_{50} (SE)	95% CI
RBT	24	5.35	5.24–5.46
RBT	48	5.10	4.99–5.21
RBT	72	5.10	4.99–5.21
RBT	96	5.10	4.99–5.21
GRC	24	6.74	4.52–8.96
GRC	48	4.56	3.00–6.12
GRC	72	3.76	2.45–5.06
GRC	96	3.76	2.45–5.06

[a]Nominal values used rather than measured

Treatment concentrations were derived from measured values in water (a) and bait (b) as reported by Bernardy et al. (2013)

dose. Sixteen cylindrical 150-L, flow-through tanks at 20 ± 1.0 °C were each stocked with 10 grass carp. The mean (±SD) weight of grass carp was 189.5 (54.3) g. Water flow was adjusted to allow for >2 tank exchanges per hour. Water quality (temperature, dissolved oxygen, and pH) was monitored daily (Table 1). Grass carp were monitored daily for feeding activity. Once normal feeding was observed for two consecutive days, toxic bait was offered the following day. Tanks were randomly assigned to three feeding regimes (0.05%, 0.3%, and 0.7% biomass) of toxic bait or a control (0.7% biomass) of their standard feed, with four replicates per treatment. Fish mortality was monitored at 6-, 24-, 48-, 72-, and 96- h posttreatment.

Waterflow was turned off 10 min before treatment application to minimize bait loss in effluent. One hour after the bait was offered, water samples (300 mL) were collected to measure Antimycin A in the water. Before turning the waterflow back on, any remaining bait was siphoned out, dried, and deducted from the amount offered to calculate nominal dose (mg/kg) for each tank. One hour after waterflow was restored, Antimycin A was measured again in pooled water samples from each experimental treatment to verify Antimycin A retention. To remove any particulate, water samples were filtered through a 5-μm Whatman™ filter before analysis. The mean (±SD) measured inclusion rate (w/w) of Antimycin A in the microparticle within the bait fed to grass carp was 20.2 ± 0.7%. The microparticle was incorporated into the toxic bait formulation at a nominal rate of 10% (w/w) with a mean (±SD) measured concentration of 20.27 (0.65) g Antimycin A/kg bait.

Results

Static exposure

Mean measured Antimycin A concentrations were significantly different between rainbow trout static exposure trials ($F = 19.0$; df = 1; $p < 0.005$). However, post-hoc analysis determined that the measured concentrations in the two highest treatments (5 and 10 mg/L nominal) were not statistically different between trials. Nominal Antimycin A concentrations were used (represented with an asterisk in Table 2) to calculate LC_{50} values from the second static exposure trial for grass carp due to supply chain shortages of analytical reagents. For clarity, trials for both species were also compared separately and LC_{50} values were recorded for each trial. Control survival was 100% for both species. Rainbow trout were more sensitive to acute exposure of Antimycin A than grass carp (Fig. 1). Complete mortality of rainbow trout occurred between 1.34 and 2.21 μg/L Antimycin A, with a 24-h LC_{50} almost 5 times less than grass carp (Table 2). Complete mortality of grass carp occurred between 7.72 and 12.93 μg/L Antimycin A, and only one treatment tank ($n = 5$ fish) had fish survive 24-h postexposure to 6.54 μg/L. The lowest concentration mortality was observed for rainbow trout and grass carp between 0.51–0.61 and 2.33–3.20 μg/L Antimycin A, respectively. Measured concentrations of Antimycin A from experimental tanks, after static exposure, had a calculated accuracy of 47.10–97.06% for grass carp, with greater accuracy at higher experimental concentrations. Rainbow trout static exposure to Antimycin A resulted in an accuracy range of 26.47–116.25% when comparing measured to nominal Antimycin A concentration in the bulk tank water.

Bait formulation and leaching

No degradation of Antimycin A was detected during bait production when air dried or oven dried at 30 °C. The mean (±SD) percent recovery of Antimycin A from the raw microparticle, air dried pellets, and oven dried pellets was 90.9 (4.6) %, 81.1 (2.3) %, and 84.3 (1.2) % of nominal, respectively. A significant difference was detected between the Antimycin A recovery rate and bait production stage ($F = 25.8$; df = 4; $p < 0.05$). Post-hoc analysis indicated mean Antimycin A recovery from the wet mixture of bait ingredients (60.2 ± 1.0%) was lower than other stages of bait production,

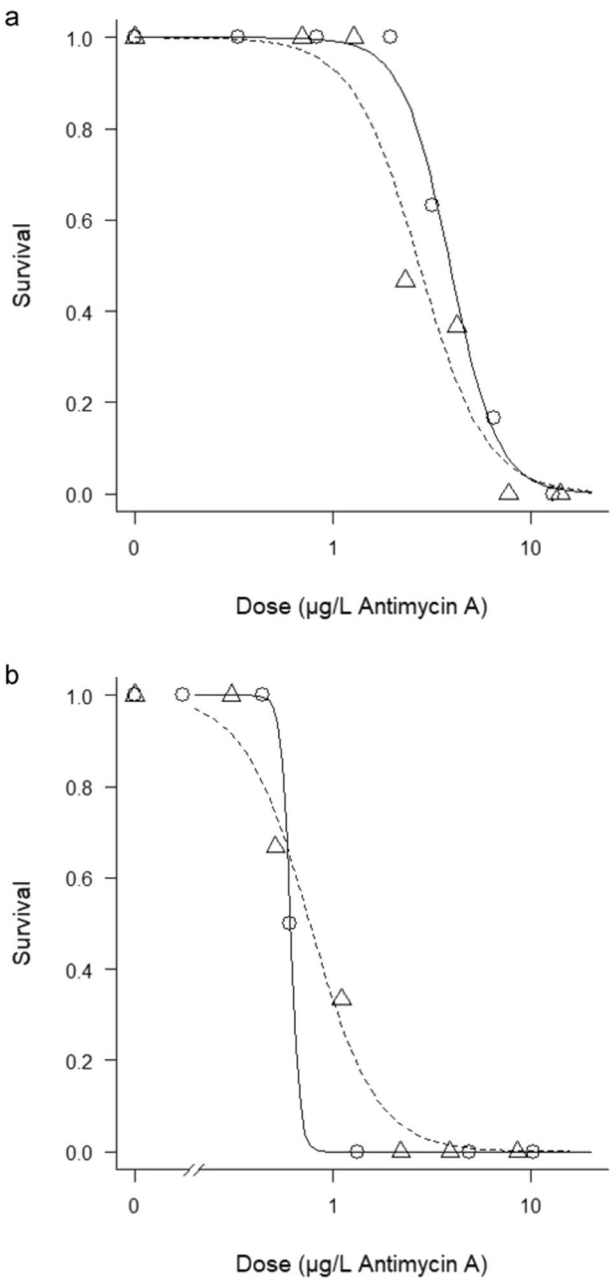

Fig. 1 Grass carp (*Ctenopharyngodon idella*) (**a**) and rainbow trout (*Oncorhynchus mykiss*) (**b**) 24-h survival curves from static exposure of Antimycin A in hard water in trial one (∘) and trial two (Δ)

indicating the time it takes to dry the doughball mixture is important to Antimycin A stability.

The mean (±SD) measured quantity of Antimycin A in the bait used for the leaching trial was 0.73 (0.05) mg, or 14.5 ± 0.01% w/w. At 1 h, 0.04 (0.03) mg of Antimycin A leached from the bait into the water (5.6 ± 3.9%). The majority of Antimycin A remained in the bait through the 6-h time point (89.6 ± 4.4%), with a mean (±SD) reduction of 44.9 (21.8) % by 24 h (Fig. 2). Mass balance of Antimycin A at 24 h in both the water and bait indicated a loss

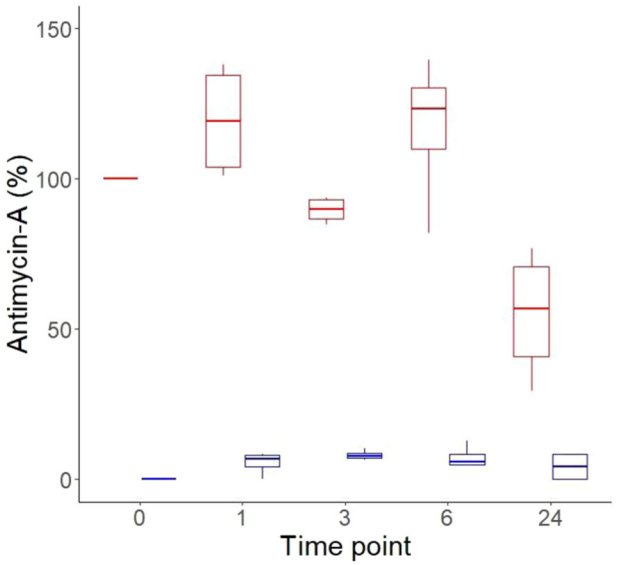

Fig. 2 Mass balanced percent of Antimycin A in water (blue) and microparticle (red) after 24 h. Box extents correspond to the median (centerline), lower quartile (25th percentile), and upper quartile (75th percentile), with whiskers extending to minimum and maximum datapoints. Mean (±standard deviation) measured quantity of Antimycin A in the bait used was 0.73 (0.05) mg, or 14.5 ± 0.01% w/w

or degradation of 0.30 mg (41%) with a mean (±SD) measured quantity of 0.03 (0.04) mg in the water and 0.40 (0.16) mg remaining in the bait. The maximum amount of leached Antimycin A into water from a single sample was 0.09 mg at the 6 h time point, with the majority of leach occurring between 6 and 24 hours.

Oral gavage

Control survival was 100% throughout gavage studies for both species. No rainbow trout were removed from the study because of injury during the gavage procedure or regurgitation thereafter. Five grass carp (5%) were removed from the study because of injury ($N = 2$) or regurgitation ($N = 3$). Complete mortality of rainbow trout occurred at 12.69 mg/kg with no mortality observed less than 1.62 mg/kg (Fig. 3). Rainbow trout mortality stabilized after 48 h with no observed mortality between 48 and 96 hours in any treatment groups. Complete mortality of grass carp was not achieved at any dose ≤22.76 mg/kg with two fish in the highest treatment level surviving until the 96-h time point. Grass carp mortality stabilized after 72 h; however, remaining fish euthanized at 96 h were observed to be lethargic with a loss of dermal pigmentation. Grass carp mortality was observed in every treatment level ≥1.08 mg/kg.

Consumption

Grass carp consumed bait in every tank with only one replicate in the highest treatment group (0.7% BW) having

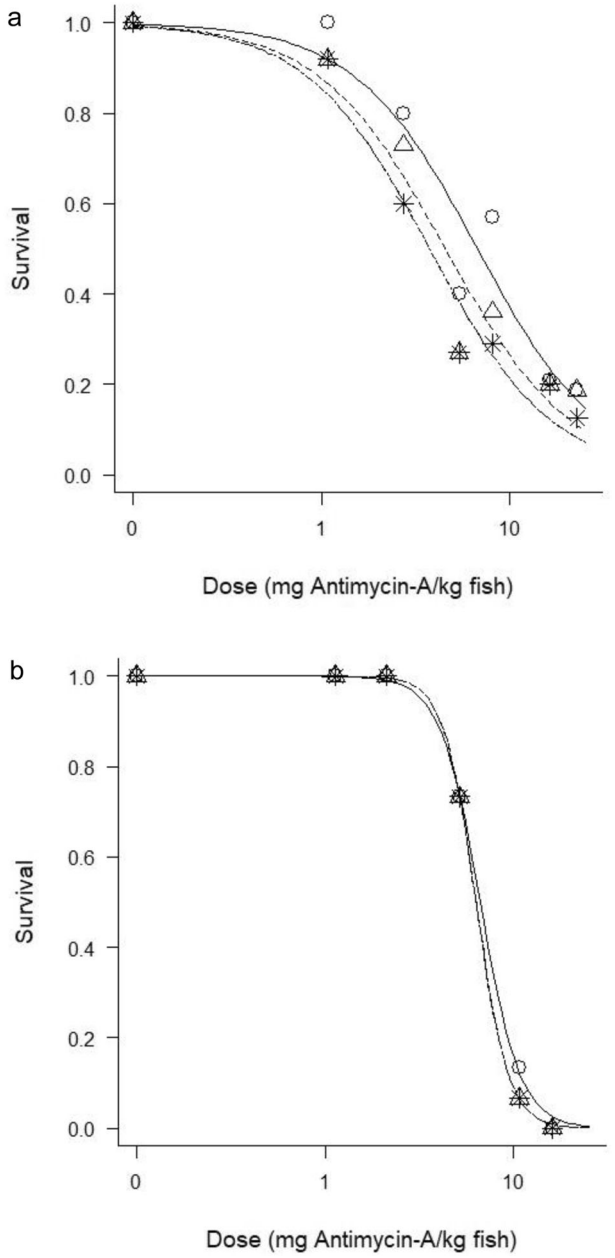

Fig. 3 Grass carp (*Ctenopharyngodon idella*) (**a**) and rainbow trout (*Oncorhynchus mykiss*) (**b**) survival curves at 24 (∘), 48 (Δ), and 72 h (+) post oral gavage of rapeseed bait incorporated with Antimycin A microparticles

visible signs of cohesive bait pellets remaining in the bottom of the tank after 1 h. Seven tanks had particulate matter at the bottom of the tank that was filtered out and dried for subtraction from nominal consumption estimates and dosing calculations. However, it was unclear how much of the material consisted of degraded bait, feces, or precipitate manganese from well water. This became evident by a greater amount of material siphoned out than what was originally offered in the lowest treatment group (0.05% BW), resulting in two replicates having an estimated consumption ≤0 g of bait. For transparency, the offered dose is reported in Table 3 while the adjusted mean (±SD) doses (siphoned material dry weight adjusted) were 8.4 (9.7), 57.3 (8.9), and 97.7 (19.2) mg/kg for the low, medium, and high treatments, respectively. No mortality was observed in replicate tanks with adjusted dosing estimates of 0 mg/kg.

One hour after offering bait to grass carp, the mean (±SD) Antimycin A concentrations measured in experimental tanks were 3.11 (0.77), 12.06 (7.12), and 9.93 (2.97) µg/L in the low, medium, and high treatment groups, respectively. After flushing experimental tanks with fresh well water for an additional hour (roughly 3 tank exchanges), Antimycin A concentrations ranged from 0.39 to 5.57 µg/L. Compared to the leaching trail conducted in beakers, the measured concentration in experimental tanks was 0.6–4.7 times greater than what was predicted from an expected mean (±SD) leach rate of 5.6 (3.9) % at 1 h. Control survival was 100% in all tanks. Mortality was observed in all treatment groups (Table 3). Complete mortality was observed by 24-h post bait application in the highest treatment group (0.7% feeding rate) with all but one fish dead by the 6-h time point. Partial mortality was observed at the 6-h time point in the medium treatment group with high variability between replicate tanks. Only one fish died in the low treatment group.

Discussion

The present study used standard acute toxicity studies to examine Antimycin A toxicity across multiple routes of exposure to rainbow trout and grass carp. Although multiple studies have published toxicity values from water-based exposures, only a few studies have characterized the effects of Antimycin A administered orally to fish. The route of exposure greatly affects the toxicity of Antimycin A, which was observed in the present study. A lethal dose of Antimycin A can be successfully delivered to grass carp through an oral route of exposure using the rapeseed bait. Mortality can be achieved during a single feeding event without apparent detection of the control chemical. Although the ingested toxicity of Antimycin A for grass carp has been demonstrated using multiple liquid carriers (Kroboth et al. 2022), encapsulation in a microparticle allows for a concentrated lethal dose to be incorporated in an oral formulation that was demonstrated to be accepted by laboratory fish. Poole et al. (2018) estimated a 24-h LD_{50} for common carp to be approximately 4.0 mg/kg for Antimycin A encapsulated in a microparticle. It is unclear if the 24-h LD_{50} observed for grass carp (6.74 ± 1.04 mg/kg) in the present study indicates a difference between species sensitivity, microparticle formulation, or Antimycin A potency between studies. However, common carp have

Table 3 Grass carp (*Ctenopharyngodon idella*) survival 96 h after consumption of Antimycin A laden bait at three treatment levels: low (0.05%), medium (0.3%), and high (0.7%) feeding rates (% fish body weight)

Time point	Negative				Low				Medium				High			
	r_1	r_2	r_3	r_4	r_1	r_2	r_3	r_4	r_1	r_2	r_3	r_4	r_1	r_2	r_3	r_4
6	10	10	10	10	10	10	10	10	3	8	0	5	0	0	1	0
24	10	10	10	10	10	10	10	9	0	8	0	2	0	0	0	0
48	10	10	10	10	10	10	10	9	0	8	0	2	0	0	0	0
72	10	10	10	10	10	10	10	9	0	8	0	2	0	0	0	0
96	10	10	10	10	10	10	10	9	0	8	0	2	0	0	0	0

Each replicate (r_x) tank contained 10 grass carp. Antimycin A laden bait contained 10% chemical (w/w) with negative controls containing 0% (w/w). Mean (±standard deviation) experimental Antimycin A concentrations were 17.2 (1.2), 61.8 (1.0), and 141.9 (0.7) mg/kg for the low, medium, and high treatments, respectively

been shown to be almost twice as sensitive to Antimycin A as grass carp through water-based exposure (Marking and Bills 1981).

Acute toxicity at 24 h observed for rainbow trout in the present study indicate they were less sensitive to Antimycin A when compared to previous studies (Mayer and Ellersieck 1986). However, this is likely the effect of using reconstituted water with a mean (±SD) pH of 8.26 (0.05) compared to the toxicity values from water with a pH between 7.1 and 7.5 and supports the known relationship between decreased toxicity of Antimycin A at higher a pH (Berger et al. 1969; Saari, 2023). Acute toxicity was not directly compared between species because different water temperatures maintained to facilitate feeding likely deactivated Antimycin A more rapidly at higher temperatures maintained in grass carp test systems (Marking and Dawson, 1972). Antimycin A is a weak acid and toxicity is dependent on water pH when there is a higher percentage of the neutral speciation of the chemical (Valenti et al. 2009; Armitage et al. 2017). Marking and Bills (1981) reported a 96-h LC_{50} for grass carp to be 1.0 µg/L at a pH of 8.0. In the present study, the 24-h LC_{50} for grass carp was 2.7 and 3.9 µg/L at a mean (±SD) pH of 8.41 (0.03) between the two replicate trials. Although most results in the published literature report LC and LD values from nominal concentrations of Antimycin A, the present study toxicity values were calculated using analytically verified concentrations using a modified method described in (Bernardy et al. 2013) to increase the certainty in our toxicity results between different species and water quality conditions. Although rainbow trout were observed to be roughly five times more sensitive to a water-based exposure of Antimycin A than grass carp, this does not seem to be the same for an oral route of exposure.

With the markedly different morphology and function of grass carp GI tracts (Mokhtar et al. 2021), we hypothesized a notable difference in the oral toxicity of the Antimycin A laden bait when compared to a piscivore like rainbow trout. Kroboth et al. (2022) observed a slight difference in LD_{50} values between grass carp and black carp (*Mylopharyngodon piceus*) and suggested possible differences in GI anatomy as an explanation but noted small sample size limitations. Although sample size in the present study was sufficient, complete mortality in the highest gavage treatment group was not achieved, affecting model fit and confidence intervals around estimated LD_{50} values. This limited our ability to observe notable differences between the two species. As demonstrated in trials where fish were offered the toxic bait, complete mortality can be achieved through consumption, but fragility of the grass carp GI tract and lack of true stomach (Mokhtar et al. 2021) limited the quantity of solid matter that could be orally gavage fed without internal damage. Successful production and application of this management bait require a balance between protecting Antimycin A from degradation without reducing its bioavailability and while not reducing consumption from poor palatability because of microparticle ingredients incorporated into the bait. Had the inclusion rate of Antimycin A in the microparticle or microparticle in the bait been increased, complete mortality could have been possible through gavage feeding but may have affected overall success of the bait from reduced palatability. Further formulation refinement and research may be warranted to optimize bait, microparticle, and Antimycin A inclusion rates.

Protecting Antimycin A from degradation in water is necessary and would need to be incorporated into any management bait. Antimycin A not only degrades quickly in water (Hussain 1969) but has reduced toxicity under high pH conditions (Marking 1975). Given the eutrophic conditions in which grass carp management would likely occur, high water pH conditions would be expected (Chislock et al. 2013). The original beeswax microparticle had a leach rate of 0.1% at 8 h (Poole et al. 2018). With the change in waxes used for microparticle encapsulation, we observed a mean (±SD) Antimycin A leach rate of 7.3 (3.8) % at 6 h. Unlike Poole et al. (2018), we assessed leach of Antimycin A from the bait in 300 mL of water, under controlled

conditions, noting that quantifying low concentrations in a large volume can be difficult. Greater leach from the microparticle was intended to increase the concentration of Antimycin A in the gut, increase oral toxicity, and reduce the amount of bait consumption necessary for lethality. Obtaining the correct microparticle formulation is complex because of oral routes of exposure requiring more Antimycin A for lethality while minimizing the amount of Antimycin A leaching out of the microparticle.

The concentration of Antimycin A detected in water 1 h after feeding was at, or greater than, the 24-h LC_{50} in all treatments. Given that the Antimycin A concentration was well above what was predicted from the leaching trial ($5.6 \pm 3.9\%$ of what was measured in the bait), additional factors from experimental conditions contributed to higher measured concentrations in the water. Antimycin A detected in water at the 2-h time point, after flushing experimental tanks approximately three times and removing any remaining bait particulate, indicates fish may have been excreting Antimycin A post consumption. A substantial percentage of the grass carp GI tract would have been expected to be evacuated within 2 h (Nekoubin and Sudagar 2013), with additional excretion possible across the gills (Fitzsimmons et al. 2001; Chang et al. 2021). Leaching from bait, excretion through the body, and mastication and emesis of bait during consumption make LD_{50} values calculated from feeding trials alone imprecise but useful for testing effectiveness under real-world conditions. These factors, along with the LD_{50} values from gavage fed grass carp, indicate that more toxic bait would need to be offered than is necessary based on the baits' efficacy and further formulation optimization may be necessary. Knowledge of the LD_{50} of Antimycin A encapsulated microparticles for grass carp sets a target inclusion rate necessary for lethality that can better guide bait optimization of inert ingredients to help with pellet stability and palatability.

Conclusion

Development of targeted pesticides as part of resource management agencies' integrated pest management strategies for invasive carp control would be beneficial (Cupp et al. 2021; Fredricks et al. 2021; Chapman et al. 2023). Apart from sea lamprey (*Petromyzon marinus*) controls, the majority of chemicals historically used in fisheries management, including Antimycin A, are nonselective and lethal to most fish species (Cupp et al. 2018; Fredricks et al. 2021). Invasive carps, including grass carp, are some of the least sensitive fishes to Antimycin A and would require a water-based exposure concentration greater than the lethal dose to many nontarget fishes for effective control (Saari 2023). However, reduction of nontarget mortality would be possible in targeted locations where invasive carps are concentrated through the combination of other strategies such as herding (Chapman 2020; Ridgway et al. 2023), use of attractants (Claus and Sorensen 2017; Sorensen et al. 2019), or use of selective baits (Ghosal et al. 2018; Poole et al. 2018; Wamboldt et al. 2022). Targeted use of toxic bait may also be of great use for grass carp control in locations where traditional removal techniques are ineffective or become too expensive when CPUE and abundance are low. Incorporation of a control chemical into a bait designed to exploit unique feeding strategies of target organisms is one method among many techniques that may enhance grass carp control and benefit from integration with other emerging control technologies (Cupp et al. 2021).

Data availability

All data are included in a USGS data release https://doi.org/10.5066/P9PI72Y6.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

References

Armitage JM, Erickson RJ, Luckenbach T, Ng CA, Prosser RS, Arnot JA, Schirmer K, Nichols JW (2017) Assessing the bioaccumulation potential of ionizable organic compounds: current knowledge and research priorities. Environ Toxicol Chem 36(4):882–897. https://doi.org/10.1002/etc.3680

ASTM International (2014) ASTM E729–96: standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. ASTM International, West Conshohocken, PA, p 22. https://doi.org/10.1520/E0729-96R14

Bain MB, Webb DH, Tangedal MD, Magnum LN (1990) Movements and habitat use by grass carp in a large mainstream reservoir. Trans Am Fish Soc 119(3):553–561. https://doi.org/10.1577/15488659(1990)119<0553:MAHUBG>2.3.CO;2

Berger BL, Lennon RE, Hogan JW (1969) Laboratory studies on Antimycin A as a fish toxicant. Investigations in Fish Control. Bureau of Sport Fisheries and Wildlife, Washington D.C

Bernardy JA, Hubert TD, Ogorek JM, Schmidt LJ (2013) Determination of Antimycin A in water by liquid chromatographic/mass spectrometry: single-laboratory validation. J AOAC Int 96(2):413–421. https://doi.org/10.5740/jaoacint.12-286

Bettoli PW, Maceina MJ (1996) Sampling with toxicants. Fisheries techniques, 2nd edn. American Fisheries Society, Bethesda, Maryland, p 303–333

Bonar SA, Vecht S, Bennett C, Pauley G, Thomas G (1993) Capture of grass carp from vegetated lakes. J Aquat Plant Manag 31:168–168

Bonneau JL, Scarnecchia DL (2001) Tests of a rotenone-impregnated bait for controlling common carp. J Iowa Acad Sci 108(1):6–7

Chang ED, Owen SF, Hogstrand C, Bury NR (2021) Developing in vitro models to assess fish gill excretion of emerging contaminants. Anal Methods 13(12):1470–1478. https://doi.org/10.1039/D0AY02282A

Chapman D, Amberg J, Calfee R, Hlavacek E, Hortness J, Jackson PR, Kazyak DC, Knights B, Roberts J (2023) U.S. Geological Survey invasive carp strategic framework 2023–27, vol 1504. U.S. Geological Survey Circular, p 30. https://doi.org/10.3133/cir1504

Chapman DC (2020) "Modified Unified Method" of carp capture. US Geological Survey Fact Sheet 20202327–69323005. https://doi.org/10.3133/fs20203005

Chapman DC, Davis JJ, Jenkins JA, Kocovsky PM, Miner JG, Farver J, Jackson PR (2013) First evidence of grass carp recruitment in the Great Lakes Basin. J Great Lakes Res 39(4):547–554. https://doi.org/10.1016/j.jglr.2013.09.019

Chislock MF, Doster E, Zitomer RA, Wilson AE (2013) Eutrophication: causes, consequences, and controls in aquatic ecosystems. Nat Educ Knowl 4(4):10–18

Claus AW, Sorensen PW (2017) Chemical cues which include amino acids mediate species-specific feeding behavior in invasive filter-feeding bigheaded carps. J Chem Ecol 43:374–384. https://doi.org/10.1007/s10886-017-0833-0

Cupp AR, Brey MK, Calfee RD, Chapman DC, Erickson R, Fischer J, Fritts AK, George AE, Jackson PR, Knights BC (2021) Emerging control strategies for integrated pest management of invasive carps. J Vertebr Biol 70(4):21057.1–21. https://doi.org/10.25225/jvb.21057

Cupp AR, Smerud JR, Tix JA, Schleis SM, Fredricks KT, Erickson RA, Amberg JJ, Morrow WS, Koebel C, Murphy EA (2018) Field evaluation of carbon dioxide as a fish deterrent at a water management structure along the Illinois River. Manag Biol Invasion 9(3):299–308. https://doi.org/10.3391/mbi.2018.9.3.12

Embke HS, Kocovsky PM, Richter CA, Pritt JJ, Mayer CM, Qian SS (2016) First direct confirmation of grass carp spawning in a Great Lakes tributary. J Great Lakes Res 42(4):899–903. https://doi.org/10.1016/j.jglr.2016.05.002

Fajt JR, Grizzle JM (1993) Oral toxicity of rotenone for common carp. Trans Am Fish Soc 122(2):302–304. https://doi.org/10.1577/1548-659(1993)122<0302:OTORFC>2.3.CO;2

Finlayson BJ, Schnick RA, Cailteux RL, DeMong L, Horton WD, McClay W, Thompson CW (2002) Assessment of Antimycin A use in fisheries and its potential for reregistration. Fisheries 27(6):10–18. https://doi.org/10.1577/1548-8446(2002)027<0010:AOAAUI>2.0.CO;2

Finlayson B, Schnick R, Skaar D, Anderson J, Demong L, Duffield D, Horton W, Steinkjer J, VanMaaren C (2012) Rotenone use in fish management and Parkinson's disease: another look. Fisheries 37(10):471–474. https://doi.org/10.1080/03632415.2012.723963

Fitzsimmons PN, Fernandez JD, Hoffman AD, Butterworth BC, Nichols JW (2001) Branchial elimination of superhydrophobic organic compounds by rainbow trout (*Oncorhynchus mykiss*). Aquat Toxicol 55(1–2):23–34. https://doi.org/10.1016/S0166-445X(01)00174-6

Fredricks KT, Hubert TD, Amberg JJ, Cupp AR, Dawson VK (2021) Chemical controls for an integrated pest management program. N Am J Fish Manag 41(2):289–300. https://doi.org/10.1002/nafm.10339

Gehrke PC (2001) Preliminary assessment of oral rotenone baits for carp control in New South Wales. Management Invasive Freshwater Fish in New Zealand, Proceedings of a Workshop Hosted by Department of Conservation, pp. 143–154

Ghosal R, Eichmiller JJ, Witthuhn BA, Sorensen PW (2018) Attracting Common Carp to a bait site with food reveals strong positive relationships between fish density, feeding activity, environmental DNA, and sex pheromone release that could be used in invasive fish management. Ecol Evol 8(13):6714–6727. https://doi.org/10.1002/ece3.4169

Herbst SJ, Nathan LR, Newcomb TJ, DuFour MR, Tyson J, Weimer E, Buszkiewicz J, Dettmers JM (2021) An adaptive management approach for implementing multi-jurisdictional response to grass carp in Lake Erie. J Great Lakes Res 47(1):96–107. https://doi.org/10.1016/j.jglr.2020.07.006

Hussain A (1969) kinetics and mechanism of hydrolysis of antimycin A1 in solution. J Pharm Sci 58(3):316–320

Klimisch HJ, Andreae M, Tillmann U (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul Toxicol Pharmacol 25(1):1–5. https://doi.org/10.1006/rtph.1996.1076

Kroboth P, Chapman D, Steevens J, Byrd C (2022) Ingested toxicity of Antimycin A to grass carp *Ctenopharyngodon idella* and black carp *Mylopharyngodon piceus* in two carriers. Manag Biol Invasion 13(4). https://doi.org/10.3391/mbi.2022.13.4.10

Mallison CT, Hestand III RS, Thompson BZ (1995) Removal of triploid grass carp with an oral rotenone bait in two central Florida lakes. Lake Reserv Manag 11(4):337–342. https://doi.org/10.1080/07438149509354215

Marking LL (1975) Effects of pH on toxicity of Antimycin to fish. J Fish Board Can 32(6):769–773. https://doi.org/10.1139/f75-100

Marking LL, Bills TD (1981) Sensitivity of four species of carp to selected fish toxicants. N Am J Fish Manag 1(1):51–54. https://doi.org/10.1577/1548-8659(1981)1<51:SOFSOC>2.0.CO;2

Marking LL, Dawson VK (1972) The half-life of biological activity of Antimycin determined by fish bioassay. Trans Am Fish Soc 101(1):100–105. https://doi.org/10.1577/1548-8659(1972)101<100:THOBAO>2.0.CO;2

Maunder MN, Sibert JR, Fonteneau A, Hampton J, Kleiber P, Harley SJ (2006) Interpreting catch per unit effort data to assess the status of individual stocks and communities. ICES J Mar Sci 63(8):1373–1385. https://doi.org/10.1016/j.icesjms.2006.05.008

Mayer FL, Ellersieck MR (1986) Manual of acute toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals. US Department of the Interior, Fish and Wildlife Service

McClay W (2005) Rotenone use in North America (1988–2002). Fisheries 30(4):29–31. https://doi.org/10.1577/1548-8446(2005)30[29:RUINA]2.0.CO;2

Mokhtar DM, Abd-Elhafez EA, Hassan AH (2021) Microanalysis of the intestinal bulb of grass carp (*Ctenopharyngodon idella*): Histological, histochemical, immunohistochemical, and scanning electron microscopical studies. Microsc Microanal 27(6):1564–1572. https://doi.org/10.1017/S1431927621012873

Nekoubin H, Sudagar M (2013) Effect of different types of plants (Lemna Sp., Azolla filiculoides and Alfalfa) and artificial diet (with two protein levels) on growth performance, survival rate, biochemical parameters and body composition of grass carp (Ctenopharyngodon idella). J Aquac Res Dev 4(2). https://doi.org/10.4172/2155-9546.1000167

Orire A, Sadiku S (2014) Development of farm made floating feed for aquaculture species. J Int Sci Pub Agric Food 2:521–523

Osborne JA, Riddle RD (1999) Feeding and growth rates for triploid grass carp as influenced by size and water temperature. J Freshw Ecol 14(1):41–45. https://doi.org/10.1080/02705060.1999.9663653

Poole JR, Sauey BW, Amberg JJ, Bajer PG (2018) Assessing the efficacy of corn-based bait containing Antimycin A to control common carp populations using laboratory and pond experiments. Biol Invasions 20(7):1809–1820. https://doi.org/10.1007/s10530-018-1662-y

Rach JJ, Boogaard M, Kolar C (2009) Toxicity of rotenone and Antimycin to silver carp and bighead carp. N Am J Fish Manag 29(2):388–395. https://doi.org/10.1577/M08-081.1

Rach JJ, Luoma JA, Marking LL (1994) Development of an Antimycin-impregnated bait for controlling common carp. N Am J Fish Manag 14(2):442–446. https://doi.org/10.1577/1548-8675(1994)014<0442:DOAAIB>2.3.CO;2

Ridgway JL, Lawson KM, Shier SA, Calfee RD, Chapman DC (2023) An assessment of fish herding techniques: management implications for mass removal and control of silver carp. N Am J Fish Manag 43(1):176–188. https://doi.org/10.1002/nafm.10685

Ritz C, Strebig JC, Ritz MC (2016) Package 'drc.' Creative Commons: Mountain View, CA, USA

Saari GN (2023) Antimycin-A speciessensitivity distribution: perspectives for non-indigenous fish control. Manag Biol Invasion 14(3):503–517. https://doi.org/10.3391/mbi.2023.14.3.09

Sorensen PW, Rue MC, Leese JM, Ghosal R, Lim H (2019) A blend of F prostaglandins functions as an attractive sex pheromone in silver carp. Fishes 4(2):27. https://doi.org/10.3390/fishes4020027

Sullivan CJ, Weber MJ, Pierce CL, Camacho CA (2020) A comparison of Grass Carp population characteristics upstream and downstream of Lock and Dam 19 of the Upper Mississippi River. J Fish Wildl Manag 11(1):99–111. https://doi.org/10.3996/062019-JFWM-046

Valenti Jr TW, Perez-Hurtado P, Chambliss CK, Brooks BW (2009) Aquatic toxicity of sertraline to *Pimephales promelas* at environmentally relevant surface water pH. Environ Toxicol Chem 28(12):2685–2694. https://doi.org/10.1897/08-546.1

Wamboldt JJ, Nelson JE, Thomas LM, Steiner JN, Hebert JL, Erickson RA, Putnam JG (2022) Quantitative method development to determine feed consumption using a dye. N Am J Aquac 84(3):354–369. https://doi.org/10.1002/naaq.10246

Wamboldt JJ, Steiner JN, Sauey BW, Lada BM, Putnam JG, Korducki BM, Saari GN (2024) Data release for toxicity of Antimycin A incorporated management bait for grass carp. U.S. Geological Survey, data release. https://doi.org/10.5066/P9PI72Y6