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Research Article

Antimycin-A species sensitivity distribution: perspectives for non-indigenous fish control

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Abstract

The global transfer of aquatic biota outside their native geographical range has resulted in dramatic changes to biological communities. Many nonnative species introductions are facilitated by human activity and then spread intra-continentally through connected watersheds once established. Resource managers therefore utilize multiple control technologies, such as management chemicals, for fisheries management to remove non-indigenous fishes. Antimycin-A (ANT-A) is a management chemical, previously registered in the United States, that has been extensively studied and used to control non-indigenous fishes. The present study examines ANT-A species sensitivity among fish and aquatic invertebrates and summarizes factors that influence toxicity. ANT-A species sensitivity distributions 20th percentile hazard concentrations (HC20) for acute studies ≤ 24 h demonstrated fish (0.088 µg/L) are 174-fold more sensitive to ANT-A than invertebrates (15.35 µg/L). Similar to previous reports, toxicity was demonstrated to be influenced by water pH, temperature, and fish mass. Therefore, the present study and results characterize ANT-A toxicity for aquatic resource managers and future use in fisheries management.

Key words: fisheries, management, toxicity, aquatic biota, water chemistry, biotic factor

Introduction

The global transfer of aquatic biota outside their native geographical distribution has been indirectly and directly facilitated by humans for centuries (Kolar et al. 2010). Many foreign introductions of aquatic species and the intra-continental spread of biota among drainages and regions have resulted in dramatic changes to aquatic biological communities, which are becoming increasingly homogenized (Kolar et al. 2010). Fish and other aquatic biota have been introduced to new environments for additional food sources, to create new fisheries, restore depleted stocks, and for biological control of unwanted organisms (e.g., plants, invertebrates, fishes; Fuller et al. 1999). Although not every introduced species becomes established, several fishes such as non-indigenous carp (e.g., *Hypophthalmichthys nobilis* (bighead carp) Richardson, 1845, *Hypophthalmichthys molitrix* (silver carp) Valenciennes in Cuvier and Valenciennes, 1844, *Cyprinus carpio* (common carp) Linnaeus, 1758) pose substantial ecological and economic damages when

populations become dominant in waterways (Kolar et al. 2007, 2010). Therefore, management chemicals and biological agents (e.g., viruses) have been of great interest to resource managers over the past several decades for controlling nuisance fish species established in water bodies and in aquaculture.

In aquatic pest control, management chemicals are a key component of an integrated pest management plan (IPM). Although IPMs originated for insect pest management, the approach is widely recognized to be applicable for multiple environmental management practices (Sawyer 1980). Existing strategies to prevent the spread of non-indigenous species consist of physical barriers, deterrents (e.g., electric, carbon dioxide), and management chemicals. Management chemical control of non-indigenous species has been successfully demonstrated by the use of 4-nitro-3-(trifluoromethyl)phenol (TFM) to control *Petromyzon marinus* (sea lamprey) Linnaeus, 1758. TFM applications have reduced the sea lamprey population to ~ 90% of their previous population, in which they devasted *Salvelinus namaycush* (lake trout) Walbaum in Artedi, 1792, and other fish populations until the 1950s when lampricide use began (Siefkes 2017). Other management chemicals are of particular interest to control other aquatic nuisance species; however, none have proven as species specific and successful as the lampricide to date. Like the lampricide, other management chemicals have been used extensively for fisheries management practices, but they are non-selective, have a high degree of efficacy, are commonly available, and have low cost that make them attractive options for nuisance species control. Current registered pesticides for controlling non-indigenous fish include TFM and niclosamide, which are only registered for sea lamprey. In 2019, carbon dioxide (Carbon Dioxide — Carp) was registered with the U.S. Environmental Protection Agency (EPA) for use as a deterrent and for under-ice removal of aquatic nuisance species. The other registered pesticide is rotenone, a non-selective management chemical utilized for controlling a wide variety of nuisance fishes. Antimycin-A (ANT-A) is another non-selective pesticide used for fish removals, but its registration lapsed in 2017. Rotenone and ANT-A were first registered in 1947 and 1960, respectively, and used to remove nuisance fish species from streams, lakes, and aquaculture ponds to improve habitat for more desirable species (Gilderhus 1972; Fredricks et al. 2019). ANT-A is a bacteria toxin with antibiotic properties produced by *Streptomyces* sp. and consists of several stereoisomers with various potencies. Individual stereoisomer standards are available for purchase (e.g., A1–A4); however, stereoisomer-specific toxicity studies have not been conducted in fish although differences in potency have been reported with other organisms (Shiomi et al. 2005).

Gilderhus (1972) reported that water-based applications of ANT-A did not repel fish as had been observed for other fishery management chemicals (Gilderhus 1972). ANT-A is a potent, non-selective pesticide whose mode

of action is to irreversibly bind to the cytochrome B subunit Qi of complex III of the electron transport chain and disrupts adenosine triphosphate (ATP) production (Slater 1973; Moss and Bendall 1984). The effects of ANT-A on the electron transport chain are similar to rotenone, although rotenone binding is reversible. ANT-A has been demonstrated to be 100-fold (*Oncorhynchus mykiss* (rainbow trout) Walbaum, 1792) to 10-fold (*Esox lucius* (northern pike) Linnaeus, 1758, *Lepomis cyanellus* (green sunfish) Rafinesque, 1819) more toxic to fish than rotenone. Complete fish mortality can be achieved at application rates as low as 25 µg/L (liquid formulation; Gresswell 1991; Marking 1992; Moore et al. 2008); however, ANT-A induced lethality (e.g., 100%) to fish varies across family from < 1.0 µg/L for Salmonidae, 5–10 µg/L for Centrarchidae and Cyprinidae, and 25–200 µg/L for the tolerant Ictaluridae family (Finlayson et al. 2002). Marking and Bills (1981) reported ANT-A 96-hour lethal concentration to kill 50% of organism (LC_{50}) values for various carp species (e.g., common carp, *Ctenopharyngodon idella* (grass carp) Valenciennes in Cuvier and Valenciennes, 1844, bighead carp, silver carp) ranged from 0.6–1.0 µg/L. Fish sensitivity to ANT-A is size-dependent with fry, fingerling, and juvenile fish demonstrating higher sensitivity to ANT-A compared to adults (Berger et al. 1969; Brown et al. 2011).

ANT-A is moderately lipophilic (fat-loving) and an ionizable weak acid. The log octanol:water coefficient (log K_{OW}) and acid dissociation constant (pKa) are 4.7 and 7.51, respectively (Swain 2012). Due to the log K_{OW} and pKa of ANT-A, both water solubility and pH directly influence its bioavailability, which have been reported to influence the toxicological effects observed in fish. For example, the un-ionized or neutral speciation concentration of ANT-A has been directly associated to its temporal acute toxicity in fishes (Berger et al. 1969). Marking (1975) reported the toxicity of ANT-A decreased with increasing pH (from pH 6.5 to 8.5) and bioavailability severely diminished between pH 8.5 and 9.5 following acute exposures using common carp, green sunfish, and *Lepomis macrochirus* (bluegill) Rafinesque, 1819 (Marking 1975; Marking and Bills 1981). The half-life of ANT-A in water is influenced by pH and temperature. The degradation half-life of ANT-A in pH 6–6.5, 7.5, and 10 water was reported at 310, 120, and 1.5 hours, respectively (Marking and Dawson 1972).

ANT-A consists of four major compounds consisting of a pair of stereoisomers for a total of eight homologs plus additional minor homolog pairs (e.g., A6, A9; Abidi and Adams 1987; Abidi 1988). The concentration of each stereoisomer varies during streptomyces fermentation and purification/isolation of ANT-A, and because ANT-A degrades via hydrolysis, aqueous chemical solutions must be kept cold to minimize degradation during experiments and analytical concentration verification methods. Therefore, very few ANT-A toxicity studies analytically verified exposure concentrations (preferred; Klimisch et al. 1997) and most report toxicity values based on

nominal concentrations. Where studies verified ANT-A concentrations (e.g., degradation studies) the concentration in water was often monitored by means of biological activity using yeast or fish bioassays because the analytical instrumentation could not achieve the sensitivity required for analytical verification. Later, Bernardy et al. (2013) published an analytical method for detection of ANT-A in water by liquid chromatography/mass spectrometry over a limit of quantification range of 8–51,600 ng/L.

Despite the large amount of aquatic toxicity data available for ANT-A, very few reports characterize ANT-A species sensitivity and the factors that influence toxicity. This information is of interest to state resource managers that have specific species of interest and various protection goals based on their waterbody types and aquatic communities (e.g., vertebrate, invertebrate). Therefore, the current study aims to (1) examine the available ANT-A toxicity data for freshwater vertebrates (i.e., fish) and invertebrates; (2) use species sensitivity distributions to examine toxicity differences between fish and invertebrates; and (3) examine whether biotic and abiotic factors influence ANT-A toxicity.

Materials and methods

Antimycin-A acute toxicity data (lethal or effect concentration, LC_X or EC_X) and corresponding experimental conditions (e.g., temperature, pH) from each study were collected from the EPA ECOTOX Knowledgebase and the peer-reviewed literature (Google Scholar). Acute toxicity values derived from multiple study durations (e.g., 3–336 h) were considered. All studies where data quality consistency met and followed standard experimental designs, including reported common water quality parameters, were selected for use. For example, toxicity values were selected from studies reporting use of a standard toxicity guideline (e.g., American Society for Testing and Materials), sufficient water renewals, organismal conditions (e.g., species, biometrics, life stage, diet), water chemistry, and reported mortality observations across time (Saari et al. 2018). ANT-A endpoints reported as values greater or less than a concentration (e.g., inequality) were excluded from the analysis. Toxicity data used for species sensitivity distributions (SSDs) are listed in Supplementary material Table S1.

Species sensitivity distribution (SSD)

Compiled toxicity values (LC_{50}) were entered into the EPA SSD Toolbox (version 1.0) to summarize, visualize, and interpret SSDs according to previous published methods (Etterson 2020). The diversity of species and the abundance of toxicity values for ANT-A allowed datasets for specific classifications of animal groupings to be generated (e.g., all fish, salmonids, non-salmonids, invertebrates only) to examine whether taxonomic classification or experimental conditions (e.g., pH, temperature) influence ANT-A toxicity. Briefly, geometric means were calculated for each species with multiple

toxicity values within the same dataset. Datasets were imported into SSD Toolbox software and statistical distributions were fit to the data. Maximum likelihood was used for estimating the parameters of each SSD. Multiple distributions (normal, logistic, Weibull, gumbel, triangular, burr) were fit to individual data sets and a Goodness-of-Fit test was used to select distributions with the best fit, which were confirmed by Akaike's Information Criterion (AIC). Fitted distributions were used to calculate a susceptibility concentration, commonly referred to as hazard concentration (HC), that would affect a desired proportion of species. The proportion of species likely expected to be affected by ANT-A was recorded across multiple centiles (e.g., 5^{th}, 50^{th}, 95^{th}). Confidence limits (upper and lower 95% confidence intervals) for each HC value were reported according to methods previously described when using maximum likelihood method to fit distributions (Etterson 2020). To compare SSDs, HC at the 20^{th} percentile (i.e., HC20 or concentration protective of 20% of species) was calculated from each SSD (Saari et al. 2018). Lower percentile values were not compared because several SSDs contained less than 20 data values (minimum was 5) and would introduce excessive uncertainty in predictions (Grist et al. 2002; Wheeler et al. 2002; Saari et al. 2018). HC values calculated from each dataset were used to compute HC ratios to directly compare dataset differences (i.e., species sensitivity). When a ratio was greater than one, the dataset and/or species were considered more sensitive to ANT-A.

Water pH normalization

Studies reporting experimental water pH conditions and other water chemistry parameters were sorted and used for ionization modeling to pH-normalize all reported toxicity values. This was conducted to characterize apparent versus actual species sensitivities by determining the neutral bioavailable concentration of ANT-A in reported lethality endpoints (e.g., LC_{50}). The neutral ANT-A concentration of the reported toxicological endpoints was calculated from the acid dissociation constant or pKa (7.51; Swain 2012) and the pH for those studies that reported the water chemistry exposure conditions was calculated using the following equations:

$$Unionized = 10^{(pH-pKa)}$$

$$Ionized = \frac{1}{10^{(pH-pKa)}}$$

$$Fraction\ Unionized = \frac{Unionized}{Unionized + Ionized}$$

$$Unionized\ Concentration = Toxicity\ value \times Fraction\ Unionized,$$

where *unionized* is the neutral uncharged ANT-A speciation, *ionized* is the charged speciation, *fraction unionized* is the proportion of unionized speciation to total speciation, *toxicity value* is the species-specific value reported from the literature, and the *unionized concentration* is the

concentration of ANT-A in the neutral unionized form. Subsequently, toxicity values reported as the neutral unionized ANT-A concentration were entered into the EPA SSD Toolbox, like the above-mentioned categorical datasets, for derivation of HC values and confidence intervals.

To examine the influence of biotic and abiotic factors on ANT-A toxicity, a comprehensive meta-analysis was conducted to collate various acute lethality endpoints studied across multiple fish weights (mass), temperatures, and pH conditions. Datasets were generated from those fish species studied across greater than three different levels per biotic or abiotic factor (e.g., fish mass, temperature, pH). Datasets were plotted to determine whether significant correlations between fish size and water pH influenced ANT-A toxicity. Additionally, fish ANT-A toxicity endpoints studied across multiple temperatures (e.g., 4) were compiled. HC centiles were computed from each individual temperature dataset, plotted (Sigmaplot 14.0; San Jose, California, USA), and correlation coefficient (Pearson Correlation; $p < 0.05$) computed to examine whether ANT-A toxicity is temperature-dependent.

Results

Fish and invertebrate toxicity thresholds

Many more studies reported lethal thresholds for fish than for aquatic invertebrates. A large amount of ANT-A toxicity data published included fish species from both the Salmonidae family (salmonids; 6) and non-Salmonidae families (non-salmonids; 28). Acute toxicity data (LC_{50}) published over the last several decades were derived for 34 and 46 different fish and invertebrate species, respectively (Table S1). Several studies were performed across multiple pH conditions for both fish (6.0–10.0) and invertebrates (7.0–8.4) to investigate the effect of pH on ANT-A toxicity. Additionally, the influence of temperature on ANT-A toxicity has been examined and ranged from 7.0–27.0 °C and 2.0–24.0 °C for fish and invertebrates, respectively. *Polyodon spathula* (American paddlefish) Walbaum, 1792 were the most sensitive fish species within the ≤ 96h (0.0017 µg/L) and ≤ 24h LC_{50} datasets (0.0030 µg/L). The most sensitive invertebrate within the ≤ 96h and ≤ 24h LC_{50} datasets were *Gammarus fasciatus* (freshwater shrimp) Say, 1818 at 0.0089 µg/L and 0.01 µg/L, respectively. Results from different classified datasets for fish and invertebrates are described below.

ANT-A SSDs were created from datasets comprising only fish (Figure 1), salmonids and non-salmonids (Figure 2), and only invertebrates (Figure 3) to calculate HC values across multiple durations (e.g., ≤ 96 h, ≤ 24 h; Table S2). Fish and invertebrate SSDs were generated using acute (≤ 96 h) ANT-A toxicity values. Both fish and invertebrate datasets included both tolerant and intolerant species to ANT-A across both 96 h and 24 h durations. For example, minimum-maximum LC_{50} toxicity values for studies ≤ 96 h were 0.0017–17.82 µg/L and 0.0089–1000.0 µg/L for fish and invertebrates, respectively. Comparing HC ratios (20^{th} percentile) between invertebrate

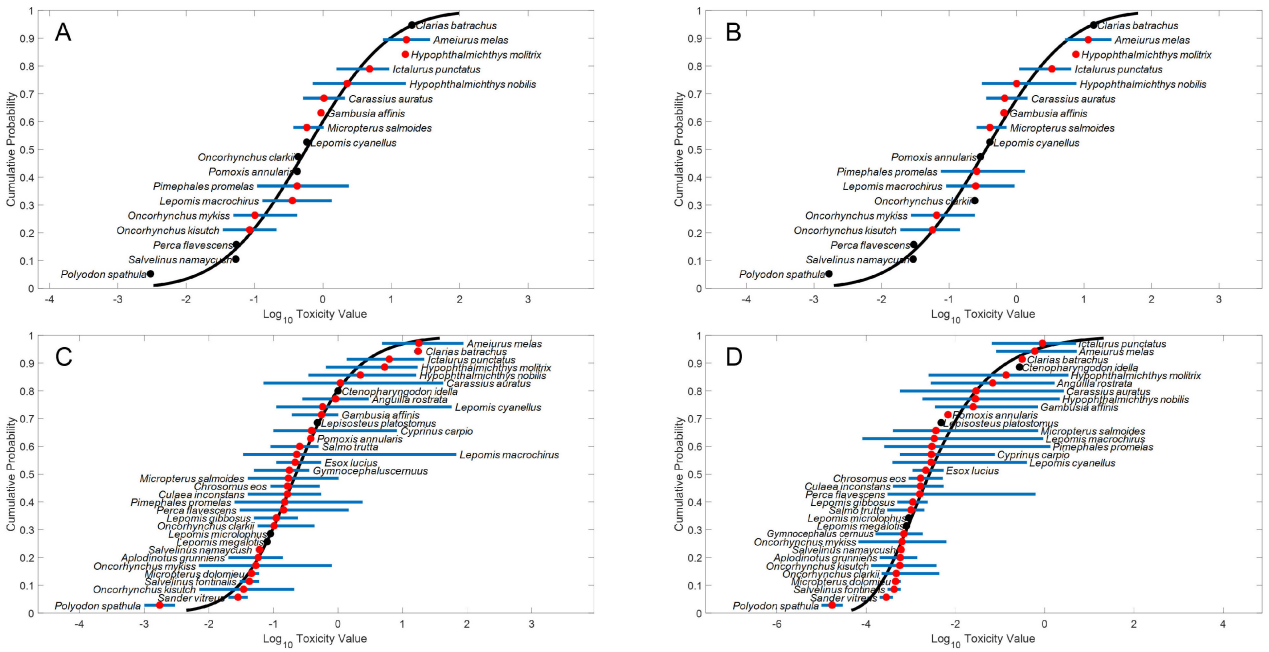

Figure 1. Species sensitivity distributions (SSDs) of all fish Antimycin-A toxicity values from both ≤ 24 h (A, B) and ≤ 96 h (C, D) duration studies. SSDs were produced using non-pH normalized (A, C) and pH-normalized (B, D) toxicity values for each duration. Black dots represent a species geometric mean consisting of a single toxicity value. Red dots represent a species geometric mean consisting of two or more toxicity values, and the blue horizontal bar represents the range of toxicity values per species.

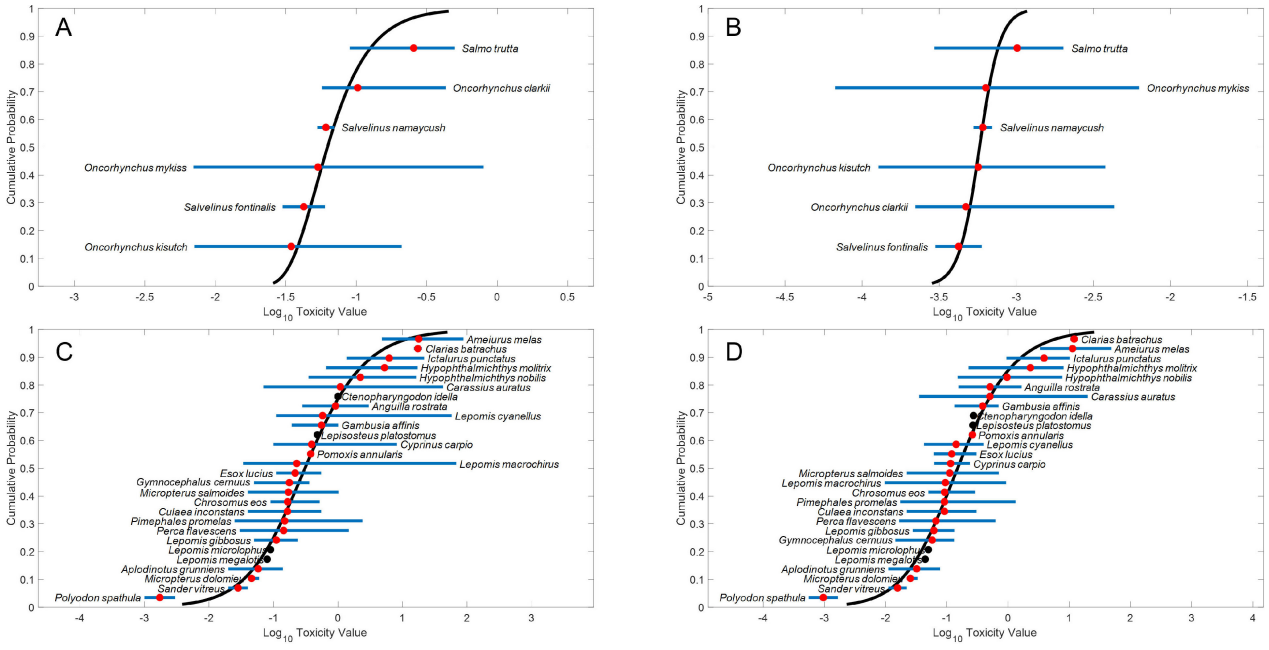

Figure 2. Species sensitivity distributions (SSDs) of salmonid (A, B) and non-salmonid (C, D) fish Antimycin-A toxicity values from ≤ 96 h duration studies. SSDs were produced using non-pH normalized (A, C) and pH-normalized (B, D) toxicity values for each duration. Black dots represent a species geometric mean consisting of a single toxicity value. Red dots represent a species geometric mean consisting of two or more toxicity values, and the blue horizontal bar represents the range of toxicity values per species.

and fish LC_{50} toxicity values from studies ≤ 24 h indicates fish (0.088 µg/L) are 174-fold more sensitive to ANT-A than invertebrates (15.35 µg/L). Similarly, datasets comparing ≤ 96 h toxicity values for fish and invertebrate indicates fish (0.059 µg/L) are 193-fold more sensitive to ANT-A than invertebrates (11.39 µg/L).

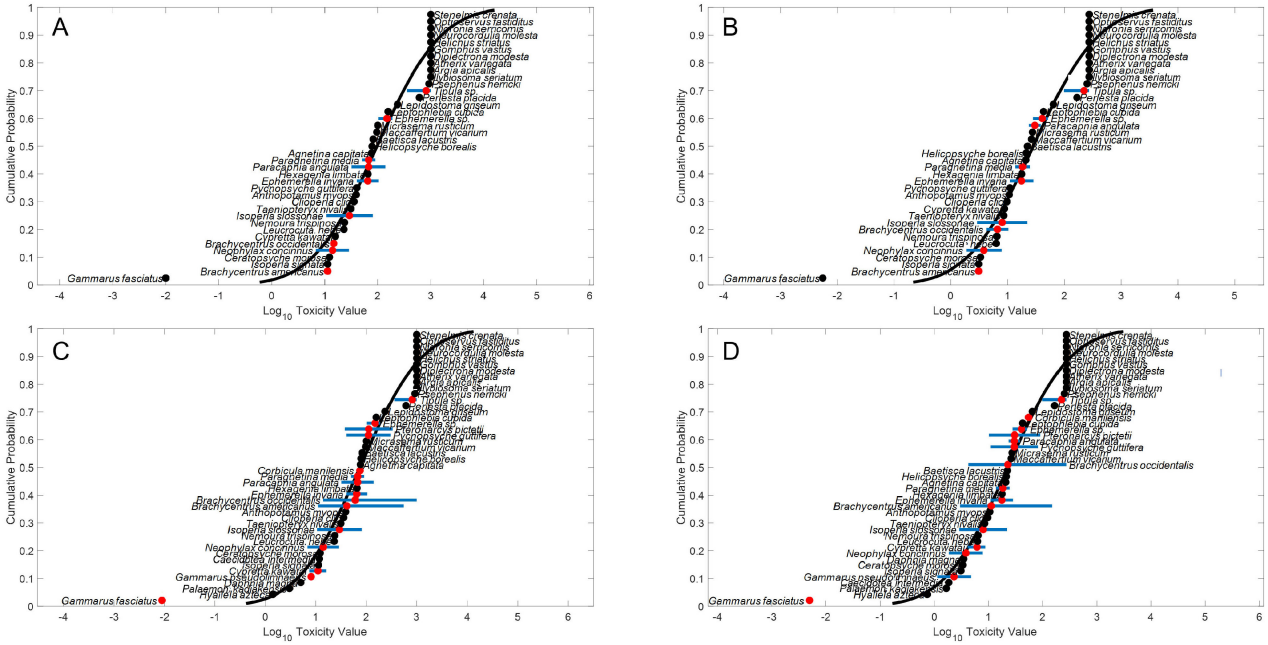

Figure 3. Species sensitivity distributions (SSDs) of invertebrates Antimycin-A toxicity values for ≤ 24 h (A, B) and ≤ 96 h (C, D) duration studies. SSDs were produced using non-pH normalized (A, C) and pH-normalized (B, D) toxicity values for each duration. Black dots represent a species geometric mean consisting of a single toxicity value. Red dots represent a species geometric mean consisting of two or more toxicity values, and the blue horizontal bar represents the range of toxicity values per species.

Fish were further classified into salmonid (cold water) and non-salmonid (warm water) datasets which included six salmonid species that have been acutely studied versus twenty-eight non-salmonid species (Table S2; Figure 2). Minimum-maximum LC_{50} toxicity values for ≤ 96h studies were 0.035–0.25 µg/L and 0.0017–17.82 µg/L for salmonid and non-salmonids (Table S2), respectively, and demonstrated overall that salmonids are extremely sensitive to ANT-A compared to non-salmonids. Non-pH normalized HC20 ratios computed from ≤ 96 h values between the two taxonomic groups indicate salmonids (0.042 µg/L) are approximately 1.8-fold more sensitive to ANT-A than non-salmonids (0.075 µg/L). The difference in sensitivity is similar when using pH-normalized HC20 values (1.7-fold) for salmonids (0.023 µg/L) versus non-salmonids (0.038 µg/L). Insufficient data were available to compare values derived at ≤ 24 h for fishes; however, the non-salmonid ≤ 24 h HC20 ratio for non-pH normalized (0.13 µg/L) to pH-normalized (0.080 µg/L) data indicated ANT-A toxicity increased 1.6-fold when considering toxicity study water pH conditions.

pH-dependent toxicity

The relationship between pH and ANT-A LC_{50} toxicity were similar to previous research demonstrating pH-dependent toxicity. The fish HC20 ratio between values derived from ≤ 24 h non-pH normalized and pH-normalized toxicity studies was 1.6, indicating fish ANT-A toxicity is almost 2-fold lower when considering study specific pH conditions (Table S2). The same HC20 ratio comparison for ≤ 96 h fish datasets indicated a 2.0-fold difference between non-pH normalized and pH-normalized values.

Subsequently, ≤ 24 h: ≤ 96h HC20 ratios for non-pH normalized indicated a minimal 1.5-fold difference between exposure durations when pH conditions are ignored. ANT-A fish sensitivity differed by 1.8-fold when comparing the pH-normalized HC20 values for ≤ 24 h: ≤ 96 h. Increasing the exposure duration of chemicals commonly increases an organism's sensitivity (i.e., decreases the LC_{50} value), which is similarly demonstrated here, and emphasizing the need to consider exposure water pH.

As mentioned above, invertebrates are less sensitive to ANT-A than are fish. pH-specific ANT-A toxicity relationships were examined using invertebrate data, and the results support previous reports indicating pH-dependent toxicity of ionizable weak acid chemicals. The HC20 ratio between those derived from ≤ 24 h non-pH normalized and pH-normalized toxicity studies was 3.2, indicating ANT-A invertebrate toxicity is ~ 3-fold lower when considering pH conditions (Table S2). The same HC20 ratio comparison for ≤ 96 h invertebrate toxicity data resulted in a similar 3.0-fold difference between non-pH normalized and pH-normalized values. Conversely, ≤ 24 h: ≤ 96 h HC20 ratios for non-pH normalized and pH-normalized indicated minimal (~ 1.3-fold) differences between exposure durations when pH conditions are both ignored and considered. Extending the exposure duration from 24h to 96h minimally decreased invertebrate LC_{50} values. These results complement the above-mentioned pH-dependent toxicity relationships observed for fish. Overall, normalizing for pH exposure conditions decreased the HC20 estimate, regardless of the study duration or taxonomic group, and the associated confidence interval, which clearly emphasizes the importance of water pH. Species specific pH-dependent ANT-A toxicity relationships were examined using fish representing both cold and warm water optimum temperatures. At 12 °C, both rainbow trout and *Ictalurus punctatus* (channel catfish) Rafinesque, 1818 had sufficient data to examine whether ANT-A acute lethality was pH-dependent. Significant ($p < 0.05$) positive correlations were observed for both species using non-pH normalized toxicity values and indicated increasing LC_{50} values with increasing pH conditions (Figure 4). When LC_{50} values were pH-normalized nonsignificant relationships were observed for both rainbow trout and channel catfish, indicating a defined range of neutral ANT-A concentration resulted in the same lethality regardless of ambient water pH.

Temperature-dependent trends

To examine whether fish ANT-A toxicity was temperature-dependent, those species with toxicity values studied across greater than three different temperatures were categorized into temperature-dependent datasets. Ten species (*Ameiurus melas* (black bullhead) Rafinesque, 1820, bluegill, *Culaea inconstans* (brook stickleback) Kirtland, 1840, channel catfish, common carp, *Pimephales promelas* (fathead minnow) Rafinesque, 1820, *Carassius auratus* (goldfish) Linnaeus, 1758, green sunfish, *Chrosomus eos*

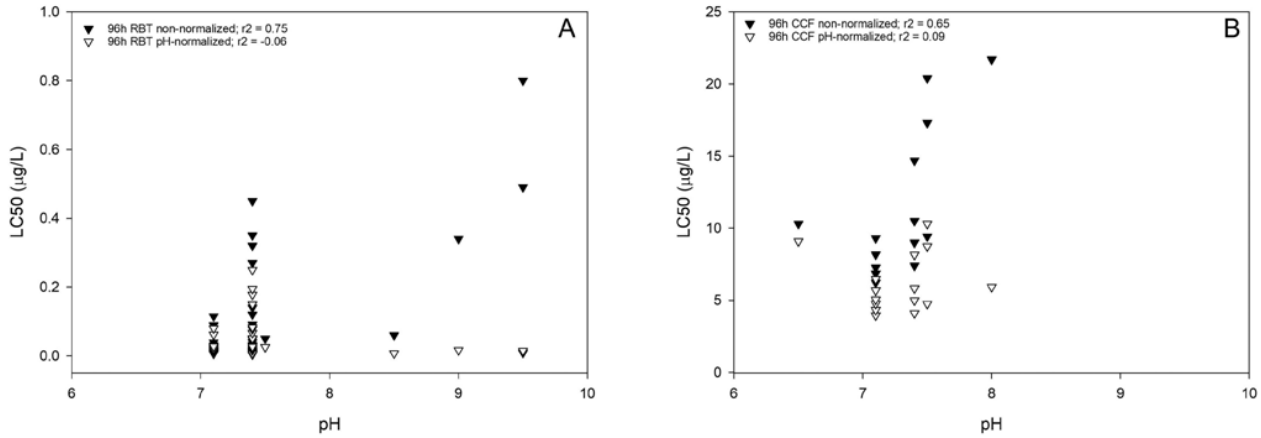

Figure 4. pH-dependent acute 96h Antimycin-A toxicity for (A) rainbow trout (RBT) and (B) channel catfish (CCF) correlations between pH condition (x-axis) and lethal concentration to kill 50% of species (LC50; y-axis). Both non-pH normalized and pH-normalized LC50 values were used for each relationship to demonstrate the influence of pH on Antimycin-A (weak acid) toxicity. Significant ($p < 0.05$) positive pH-dependent correlations were observed for both RBT and CCF non-pH normalized toxicity values; however, after normalizing the toxicity value for pH conditions (i.e., ionization) nonsignificant relationships were observed for both RBT and CCF pH-normalized toxicity values.

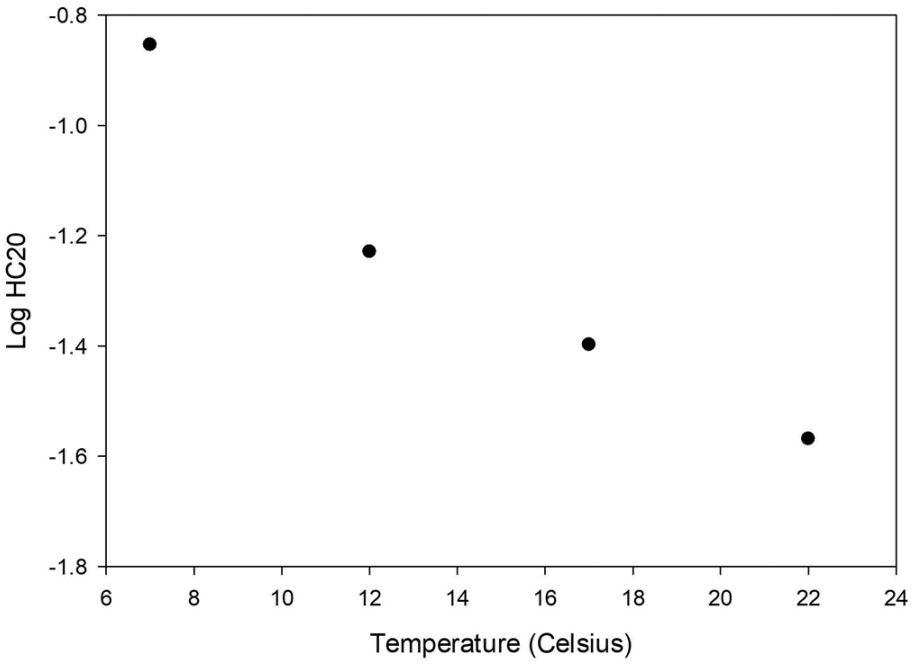

Figure 5. Temperature-dependent relationship for ten warm water fish species across four different temperatures. Hazard concentration values (HC20) were calculated from each separate LC50 toxicity distribution at temperatures 7, 12, 17, and 20 degrees Celsius. Each log HC20 value were plotted for each corresponding experimental temperature condition to demonstrate the significant negative temperature-dependent Antimycin-A toxicity relationship ($p = 0.023$; correlation coefficient = –0.98).

(northern redbelly dace) Cope, 1861, *Perca flavescens* (yellow perch) Mitchill, 1814) were studied across four different temperature conditions (7, 12, 17, 22 °C). From each classified temperature dataset, HC20 values were computed. By comparing HC20 values from the lowest temperature studied (7 °C), fish were 1.8-, 3.8-, and 5.2-fold more sensitive to ANT-A at 12, 17, and 22 °C, respectively (Figure 5). These results indicate a significant negative correlation between temperature-Log HC20 values ($p = 0.023$; correlation coefficient = –0.98; Figure 5).

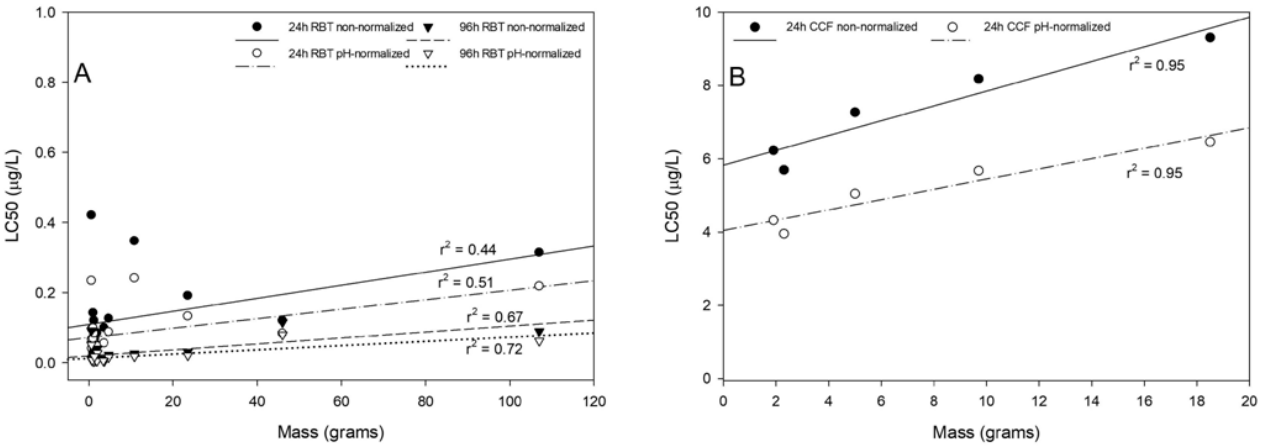

Figure 6. Size-dependent acute 24h and 96h (A) rainbow trout (RBT) and (B) channel catfish (CCF) correlations between fish mass (x-axis) and Antimycin-A lethal concentration to kill 50% of species (LC50; y-axis). Both non-pH normalized and pH-normalized LC50 values were used for each duration to demonstrate the influence of pH on Antimycin-A (weak acid) toxicity. 96h CCF relationships could not be determined due to insufficient data.

Influence of fish mass

In addition to temperature-dependent trends, relationships between fish mass and ANT-A toxicity in rainbow trout and channel catfish were examined. Both 24h and 96h ANT-A exposure durations were examined using non-pH normalized and pH-normalized toxicity values for rainbow trout. There was a significant positive correlation ($r^2 = 0.44$; $p = 0.035$) between rainbow trout mass (grams) and 24h non-pH normalized LC_{50} values, and a similar significant positive correlation ($r^2 = 0.51$; $p = 0.013$) was also observed for 24h pH-normalized LC_{50} values. The relationship between rainbow trout mass and 96h non-pH normalized and 96h pH-normalized LC_{50} values were also positive significant correlations ($r^2 = 0.67$; $p = 0.0002$ and $r^2 = 0.72$; $p = 0.0.00005$, respectively), indicating ANT-A toxicity is size-dependent for rainbow trout. Similarly, positive significant correlations ($r^2 = 0.95$; $p = 0.01$ and $r^2 = 0.95$; $p = 0.01$) between channel catfish mass and 24h ANT-A non-pH normalized and pH-normalized toxicity, respectively, were observed (Figure 6). The 96 h channel catfish mass-dependent relationships were not examined due to a lack of sufficient toxicity values. These results indicate regardless of water type, both cold and warm water fish acute 24h ANT-A toxicity is influenced by fish mass.

Discussion

Due to the increased distribution of species throughout the world, various types of management tools are necessary to prevent and control the spread of aquatic nuisance species. The spread of non-indigenous fishes (e.g., carp, lake trout, bluegill) pose substantial ecological and economic damages when populations become widespread in waterways (Kolar et al. 2007, 2010). Management control tools, such as ANT-A or rotenone, are important options for resource managers during aquatic non-indigenous species management efforts. When control tools are selected for management

applications, it is important to consider the aquatic species present in waterbodies and the biotic and abiotic factors influencing the effectiveness of the chemical. Therefore, the present study cohesively characterized the aquatic species sensitivity to ANT-A and the factors reported to influence its toxicity. The toxicity of ANT-A to fish across multiple exposure durations and water conditions has been studied extensively over the past 60+ years since its initial registration in 1960 as a pesticide for site-specific use to control nuisance fish species. Toxicity values for 34 fish and 46 invertebrate species were reported over the past seven decades. Among standard lethality endpoints reported for fish, 28 for non-salmonid and 6 for salmonid species were published. Regardless of the aquatic species studied, the present study supports previous research demonstrating ANT-A toxicity is influenced by pH, temperature, and biological factors such as fish size (e.g., mass). Therefore, the present results demonstrate the type of aquatic species (e.g., fish, invertebrate) with acute LC_{50} toxicity data and the biotic and abiotic factors within a waterbody that would need to be considered during fisheries management applications.

The acute (24–96 h) LC_{50} toxicity results indicate fish are more sensitive to ANT-A toxicity than invertebrates. This is consistent with the historical registered use of ANT-A and previous reports demonstrating how aquatic invertebrates are less sensitive to ANT-A than are fish (Fuller et al. 1999; Kolar et al. 2007). Differences in sensitivity between fish and invertebrates are similar regardless of exposure duration (e.g., 24–96 h). Among the fish species studied, ANT-A toxicity has been studied across more non-salmonid species compared to salmonids (Table S2). A summary of acute lethality endpoints for both cold and warm water species presented in the supplemental information can support regulatory and management decisions related to the use of ANT-A for nuisance and non-indigenous species control.

The pH-dependent ANT-A toxicity relationship results comparing non-pH normalized to pH-normalized HC20 values support previous data demonstrating decreasing toxicity with increasing pH (Berger et al. 1969). Rainbow trout and channel catfish non-pH normalized toxicity values across experimental pH conditions further support these inferences, which occurs in both cold (salmonid) and warm water (non-salmonid) species. This is consistent with the literature demonstrating other weak acid ionic organic chemical uptake is inversely correlated with bulk water pH (Armitage et al. 2017; Lee et al. 1971). The biological half-life of ANT-A was reported by Marking and Dawson (1972) to be pH-dependent from 310 hours to 1.5 hours at pH 6.0–6.5 to 10.0, respectively (Marking and Dawson 1972), and the effects of pH on ANT-A toxicity was similarly reported for common carp, green sunfish, and bluegill (Marking 1975). The present results complement these reports by providing evidence across tens of fish species to support resource management decisions during future removal applications.

Temperature-dependent toxicity relationships for rainbow trout and channel catfish emphasize how abiotic factors are important to consider when predicting ANT-A toxicity. Although pH greatly influences ANT-A toxicity, increasing water temperatures have been shown to increase toxicity (Figure 5; Marking and Dawson 1972; Berger et al. 1969). Oxygen-limited thermal tolerance models for ectotherms indicate fish have confined optimal temperature ranges where they function aerobically without physiological stress (Pörtner 2001, 2002). When fish experience increasing temperature conditions and those beyond optimum, a mismatch of energy demand and supply cause shifts to anaerobic respiration for enhanced energy supply to sustain cellular physiological functions (Pörtner 2010). Such change increases an organisms susceptibility to environmental stressors (e.g., ammonia, management chemicals; Marking and Dawson 1972; Lee et al. 1971). Subsequently, these same environmental conditions (e.g., pH, temperature) also increase ANT-A degradation in water. Marking and Dawson (1972) reported ANT-A deactivated rapidly with increasing temperature between 12 and 22 °C at pH 7.5. Increasing temperature conditions are likely to increase degradation rates accompanying an already high base hydrolysis rate. In pH studies conducted by the EPA, ANT-A degraded rapidly with half-life($t_{1/2}$) < 12 hours at pH 1 to 9. Strong oxidizing agents such as potassium permanganate are commonly used to detoxify ANT-A following field applications for fish removal (Marking and Bills 1975; Moore et al. 2008).

The increasing spread of non-indigenous fishes throughout the world warrants the evaluation of diverse approaches to manage populations of non-indigenous fishes, including the development and evaluation of new technological control tools to aid resource managers during removal applications. The long history of use of ANT-A in the control of non-indigenous fishes through waterborne applications was aided by the lack of chemosensory response by fish to ANT-A formulations and its irreversible mechanism of action. However, its non-selective toxicity has researchers studying ways to selectively deliver ANT-A and other management chemicals. Integrated delivery systems that use attractants together with management chemical-laden baits could allow practical and controlled removal of targeted fishes and reduce exposures (and hence, effects) to non-target species. ANT-A corn-based baits were previously demonstrated to to selectively target common carp, a member of the Cyprinidae family, compared to yellow perch and bluegill (Poole et al. 2018). The results by Poole et al. (2018) indicate bait formulations containing ANT-A can selectively target specific species. These initial results may benefit from further refinement of the corn-based baits and delivery processes to further inform management uses to selectively target common carp in waterbodies dominated by percids or centrarchids. Similarly, the Upper Midwest Environmental Science Center is currently developing oral delivery systems to exploit unique feeding behaviors for use with management chemicals during future fish removal efforts. The present results aim to inform

aquatic resource managers regarding ANT-A species sensitivity while emphasizing the influence of water chemistry on ANT-A toxicity. Management chemicals serve unique roles in fisheries management. Continued research with such chemicals can help to further inform the potential use of these chemical tools to enhance fish removals to meet management goals and control the spread of non-indigenous fishes.

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Authors' contribution

Gavin Saari conceived, performed the computations, developed, and wrote the present manuscript.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Fish and invertebrate toxicity values used for species sensitivity distributions (SSDs)

Table S2. Summary of species sensitivity distributions associated with Antimycin-A lethal concentrations to cause lethality in 50% (LC50) of fish and invertebrates across all temperature conditions and acute durations.