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An Examination of the Sensitivity of Bighead Carp and Silver Carp to Antimycin *A* and Rotenone

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Abstract

An electrical barrier has been constructed in the Chicago Ship and Sanitary Canal (CSSC) to prevent movement of invasive species between the Great Lakes and the Mississippi River system. Bighead and silver carp which are present downstream of the barrier may be able to eventually penetrate this barrier. If the fish near the barrier, it may be necessary to remove the fish from immediate vicinity. The only reasonable method to affect the complete removal of the fish is the use of a piscicide. We tested the sensitivity of bighead and silver carp to two piscicides, rotenone and antimycin *a*.

The fish were sensitive to rotenone, falling near the middle of the range of sensitivities reported for various species. A concentration of 250 μg/L, the highest concentration permitted for control of fishes, killed all bighead and silver carp within 4 hours. Both large and small fish were sensitive to rotenone. Rotenone should be a suitable toxicant for the removal of bighead and silver carp from the CSSC.

Bighead and silver carp were fairly insensitive to antimycin *a*. Compared to values in the literature, they were more sensitive than black bullheads, but less sensitive than most other species. A concentration of 20 μg/L, the highest concentration permitted for control of fish at the pH and temperatures likely in the CSSC, required 32 hours to kill all bighead and silver carp. Because the canal is important for commercial transportation, the duration of exposure required to affect the removal of bighead and silver carp with antimycin would be unacceptably long.

Introduction

Bighead carp and silver carp are Asian cyprinids imported to the United States in the 1970's for use in aquaculture and improvement of water quality in municipal effluents. These species have escaped from confinement and now have self-sustaining populations in the Mississippi River basin. The fish have been expanding their range northward up the Missouri, Mississippi, Illinois, and Ohio River systems. Because bighead and silver carp are known to reach high biomasses and because they feed low on the food chain, negative effects on desirable native species are anticipated.

Lake Michigan is connected to the Illinois River system by means of the Chicago Ship and Sanitary Canal (CSSC). This canal is a potential avenue for Asian carp to invade the Great Lakes system. Invasion of the Great Lakes by Asian carp would have unknown and potentially highly deleterious effects on this important fishery, worth billions of dollars annually.

An electric barrier has been constructed in the CSSC to impede invasive species from moving through the canal in either direction. It is still unknown if this barrier will be adequate to keep Asian carp from entering the Great Lakes. Added levels of security in the form of another electrical barrier and other potential barrier types are planned. Until these additional barriers are in place, other control methods may be necessary. If Asian carp invade the section of the CSSC between the barrier and the lock and dam below it, it may be necessary to remove the fish from this section before they have an opportunity to cross the barrier. The use of a piscicide to effect the complete mortality of Asian carp within that section is the only realistic method available for removal of all bighead and silver carp.

Before piscicides can be used, it is necessary to know the sensitivity of the target fish. In addition, the label requirements for antimycin *a*, one of the two piscicides available for this use, require that a toxicity test be performed with the target fish in site water prior to deployment. The following study was performed to fill those requirements.

Methods

We tested the sensitivity of Asian carp to rotenone and antimycin in three testing periods. The first period tested the sensitivity of bighead carp and silver carp to a range of concentrations of rotenone. The second period tested the sensitivity of bighead and silver carp to a range of concentrations of antimycin *a*. During the second period, we duplicated one treatment in full sunlight in an attempt to test the effect of photodegradation. In the third period, we performed a confirmatory test to determine if the piscicide concentrations that caused mortality in the first and second periods would be suitable for Asian carp of different sizes and to determine if those concentrations would be suitable in water collected from the Chicago Ship and Sanitary Canal. Also during the third period we performed another test with antimycin *a*, similar to that performed in the second period, but with only three concentrations, to determine if a different method of mixing resulted in different toxicity. Table 1 lists the treatments that were performed in the three periods. All treatments in all tests were "static"; all toxicants were added at the beginning of the test and there was no renewal of water during the test. Both rotenone and antimycin are known to oxidize, therefore no aeration was provided during the tests.

Small bighead carp and goldfish were purchased from the Ozark Catfisheries, Osage Beach, Missouri. Small silver carp were captured from a backwater of the Illinois River near Havana, Illinois, with the aid of Mark Pegg, Illinois Natural History Survey. Large silver carp and bighead carp were captured from the Missouri River near Hartsburg, Missouri. Fish were transported to Columbia Missouri in a live-car equipped with oxygen injection, and released into 700 m^2 ponds, where they were allowed to acclimate for at least two days prior to the test. Table 2 gives the sizes of the fish used in the various periods.

Test containers were 1000L high-density polyethylene cattle tanks. Except for one treatment in the second period specified above, the tanks were shaded from the sun. Temperature in the tanks was measured every 30 minutes by means of a temperature logging device. Volume of water was 1000 L in all treatments except treatments 1, 2, and 3 in the third period, (small fish in CSSC water) when the volume of water was 850 L. The smaller volume of water was used because of a limited supply of CSSC water.

The water used in periods 1 and 2 and in some treatments in period 3 was Columbia Environmental Research Center (CERC) well water diluted with deionized water to mimic the alkalinity and conductivity of CSSC water. The pH of the reconstituted water was adjusted to between 7 and 7.5 by addition of hydrochloric acid or sodium hydroxide. Reconstituted water was made up 24 hours in advance and final adjustments were then made on the day of the test. No effort to readjust pH was made once the tests had begun. The concentration of dissolved iron in the reconstituted water was determined by atomic absorption spectrophotometry.

CSSC water was collected from the canal and transported to Columbia Missouri in a 2000 gallon water hauling truck. The truck was thoroughly rinsed on the inside using CERC well water prior to the leaving Columbia. The truck was filled with CSSC water at the electric barrier site in Romeoville Illinois on 10/24/03. Dissolved oxygen concentration in the CSSC water at the time of collection was 2.5 mg/L. The dissolved oxygen concentration increased during the trip to Columbia Missouri, presumably because of agitation during transportation. Dissolved oxygen concentration in the CSSC water was 7.4 mg/L after offloading into the test containers on the evening of 10/24/03. The inside of the hauling container was treated with undiluted bleach after offloading the CSSC water to eliminate the potential for translocation of any exotic species, including zebra mussels. Likewise, after completion of the toxicity test, bleach was added to all tanks containing CSSC water or any tank containing piscicides prior to dumping. This served not only to destroy potential exotic species, but also to detoxify the piscicides. The tanks were left to stand with bleach for at least four hours prior to dumping.

Rotenone concentrations in test chambers were prepared by first preparing a stock dilution of Prenfish® 5% rotenone technical grade solution (Prentiss Incorporated, C.B. 2000, Floral Park, New York) mixed in deionized water. A predetermined amount of this stock solution was added to each tank to arrive at the correct final concentration.

The technical grade of antimycin *a* used in this study is a 23% w/w (20% w/v) solution (Fintrol®concentrate, Aquabiotics Incorporated, Bainbridge Island, Washington). Other ingredients in this solution are acetone and soy lipids. Fintrol is packaged with a "Fintrol Diluent" consisting of acetone, a detergent, and a surfactant. This diluent is an aid to dissolving the concentrate in water. We did not use the Fintrol diluent in this study. Instead, we followed the method of Burress (1975) and made a stock dilution

of Fintrol in acetone without the detergent carrier. In period 2, we made a stock dilution of Fintrol by drawing an aliquot from the amber bottle of technical grade solution and injecting the aliquot into a volumetric flask. The flask was then filled with unadulterated acetone. At that time, we noted that a droplet of oil, apparently incompletely dissolved in the technical grade solution, was clinging to the pipette tip used to draw the aliquot. Toxicity to Asian carp in the period 2 test was somewhat lower than we expected, so we investigated the possibility that antimycin *a* might have partitioned unequally into undissolved lipids in the technical solution, resulting in lowered toxicity. In period 3, we emptied the entire contents of the technical grade solution into a graduated cylinder to measure the remaining volume. About 10 mL of undissolved lipids (in 222 mL total volume of solution) were noted. This entire volume was then rinsed into a volumetric flask with acetone and all lipids were thoroughly dissolved by mixing. This solution was then used to make up the stock solution, as in period 2.

Temperature in the tanks was recorded at half-hour intervals during the test by the use of temperature logging devices (Tidbit® loggers, Onset Computer, Inc). Dissolved oxygen concentrations were measured at 24-hour intervals with a Yellow Springs Instruments multi-parameter probe. The pH of the tanks was recorded at 24-hour intervals with an Orion hand-held pH meter. The dissolved oxygen probe and the pH meter were calibrated each day prior to measurement.

Mortality was recorded at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 24 hours, 32 hours, 40 hours, 48 hours, 72 hours, and 96 hours. Criteria for determination of mortality were absence of any respiratory movement and lack of a response to gentle prodding. Fish that were alive but incapable of swimming away when prodded were termed "morbid". Dead fish were removed and weighed and measured immediately.

Analytical confirmation of rotenone concentrations was performed by the high performance liquid chromatography (HPLC) after the method of Dawson et al. (1983). Grab samples were taken from the test chambers by filling an amber glass bottle directly from the test chamber and capping with a Teflon® closure. In period 1, samples were taken from all treatments one half hour after mixing the rotenone into the tanks, just before addition of the fish. Grab samples were also taken from two treatments at 24, 48, 72, and 96 hours. On two occasions, duplicate grab samples were taken. In period 3, grab samples were taken from two treatments at the start of the test. Grab samples were stored refrigerated (2°C) until analysis. In addition to the samples taken from the test containers, the concentration of rotenone in the technical grade solution used to make the test concentrations was confirmed by analysis.

There are no established methods for the analysis of antimycin *a* at the low concentrations used for fish control. Aquabiotics Corporation, P.O Box 10576 Bainbridge Island, Washington, performed the confirmation of the antimycin *a* concentration in the technical solution. Two blind dilutions (in acetone) of the technical samples were provided to Aquabiotics Corporation. The flourometric method of Sehgal and Vezina (1967) was used for the analysis. One of the blind samples was prepared similarly to the stock solution in period 2 (prior to dissolving any remaining lipids in acetone) and the second blind sample was prepared similarly to the stock solution in period 3 (after dissolving remaining lipids in acetone). In lieu of chemical analysis of antimycin concentrations in the test chambers, we used goldfish as a positive control organism. Goldfish toxicity was then compared to established data in the literature. Caged goldfish, introduced at 24 and 48 hours post-dosing, were used to empirically test the degradation rates of antimycin. Mortality of these fish was then compared to mortality in fish placed in the chambers at the start of the test. At the end of the antimycin test (period 2), cages containing live goldfish were moved to a tank containing water with no toxicant. Surviving fish (all species) from both controls and from treatment 9 were also placed in cages and moved to a tank with no toxicant. These caged fish were observed for an additional 24 hours to determine any delayed toxicity.

The concentration of toxicant causing 50% mortality (LC50) at the various time periods was calculated using the Spearman – Karber method. A 10% trim was used if possible, and for data sets in which the 10% trim would not work, we increased the trim up to 30%. (Trimming refers to a statistical manipulation and does not entail discarding data.) For data sets which did not fit the Spearman-Karber model, we used the probit method with Abbot's correction. These methods of developing an LC50 have been in use for many years and are preferred by the Environmental Protection Agency (West, Inc. 1996). We used the program Toxstat (Western EcoSystems Technology, Inc., 2003 Central Avenue, Cheyenne, WY 82001) for LC50 calculations.

Results and Discussion

Analytical confirmation of nominal concentrations

Measured rotenone concentration in Prenfish technical grade solution was very close to the nominal value, but measured rotenone concentrations in samples taken from the test chambers were lower than nominal concentrations (Table 3). Measured concentrations in samples taken at 0.5 hours after dosing the chambers were strongly correlated with nominal values (Fig. 1), but averaged 28% of nominal values. We think this reflects degradation. Goldfish mortality occurred at nominal concentrations that were close to values in the literature. Spike recovery ranged from 84 to 99.5%, with most recoveries over 95%, so poor recovery of rotenone from samples is also not expected to be a reason for these low values.

Chromatograms from the analyses of rotenone in this test (Fig. 2) show peaks at the same loci as two of the rotenone breakdown products reported in Draper et al (1999). They were often higher than the measured rotenone concentrations and likely account for the missing toxicant. Standards for the breakdown products are not commercially available. Because the breakdown products have a structure very similar to rotenone, they should have a similar response on the UV detector. This should provide a close estimate of degradation product concentrations. Measured concentrations of rotenone plus estimated concentrations of breakdown products averaged 70.1% of nominal (Table 3). Measured concentrations taken over time from the same chamber to test for degradation showed no real pattern and were highly variable (Fig. 3), but if degradation was continuing in the stored bottles (which were held in the dark at 2°C) one would not expect such a pattern to be detectable.

The single treatment of rotenone in CSSC water was the lowest in recoverable rotenone. This could be due to sorption to solids and organics present in CSSC water. Rotenone and antimycin are easily adsorbed to silts and suspended solids (Illinois Natural History Survey 1975). CSSC water contained significant amounts of suspended solids. This had settled to the bottom of the chambers by the time toxicants were added, but it was resuspended temporarily during mixing of the toxicants into the test chambers.

The measured antimycin concentrations in blind samples made from the Fintrol technical solution were very close to the nominal concentration, both in the sample with dissolved and undissolved residual lipids (Table 4). The confidence interval encompassed the nominal concentration on Blind B, in which the lipids were dissolved. The confidence interval of the measured concentration fell just above the nominal concentration in Blind A. Thus, there is no evidence that test concentrations were lower than nominal concentrations due to partitioning into the undissolved lipid fraction. The Merck Index (Budavari et al, 1989) describes antimycin *a* as “freely soluble in acetone”, but gives no lipid solubility.

Water Quality

Temperature, pH, dissolved oxygen and dissolved iron are water quality variables that are important in understanding the effects of these toxicants. Antimycin degrades rapidly at pH > 8 (Marking and Dawson, 1972), so tracking pH throughout the test was important. Antimycin and rotenone degrade faster in higher temperature water, but they are more effective, probably because higher temperatures increase the metabolism and respiration rate of the fish, thereby increasing the rate of toxicant uptake (Berry and Larkin 1954). Dissolved iron strongly binds free antimycin, reducing the effectiveness of the compound (Schnick, 1974). Dissolved oxygen concentrations are important in any test with fish, because fish cannot survive low dissolved oxygen concentrations. In addition, both the toxicants degraded to non-toxic or less toxic byproducts by oxidation.

The pH of the reconstituted water (but not CSSC water) was adjusted to between 7 and 7.5 prior to the test, and pH values varied only slightly through the tests. CSSC water generally varies between these pH values, (data from Metropolitan Water Reclamation District of Greater Chicago) but the pH values of CSSC water brought from the CSSC to Columbia were slightly higher before the start of the test (between 7.54 and 7.66). In period 1 (rotenone test with small fish) pH values varied between 7.13 and 7.65 during the test. In period 2 (antimycin test with small fish) pH values varied between 7.34 and 7.79. In period 3, (confirmatory tests with large and small fish) pH values in reconstituted water ranged from 7.11 to 7.46, and in CSSC water ranged from 7.11 to 7.69. Tanks with large fish had the lowest pH values, undoubtedly due to respiration.

Temperature varied during the test according to the outside temperature, as it would in a field application of toxicant. In periods 1 and 2, all tests were performed under a canopy with shade-cloth walling. Because conditions were similar between tanks, temperature in the tanks varied together over time. In period 1, mean temperature was 15.4 °C over the 96 hours of the test, but gradually decreased from a high of 18 °C at the beginning of the test (Fig. 4). Because of the need to remove bighead and silver carp quickly to avoid a long duration of field application of chemical, managers will be most concerned about the temperature at the beginning of the test. Temperature during the first 8 hours of the test decreased from 18 to 17C. Temperature in period 2 was much lower than in period 1, and there was a warming trend during the 96 hours (Fig. 4). Temperature during the first 8 hours ranged from 9.8 to 11.6 °C. In period 3, CSSC water treatments were performed in an uninsulated building (Fig 4) whereas reconstituted water treatments were performed in the tent (Fig 4). Mean water temperature in the CSSC water was 12.5 °C over the duration of the test, ranging from 11.17 – 13.99°C, and ranging from 12.2 to 12.0 °C over the first eight hours. Mean temperature in the reconstituted water test during period 3 was also 12.5 °C , but the range was slightly larger (Fig. 4).

Concentrations of dissolved iron in CSSC water are ranged from 0.01 to 0.1 mg/L in 2002 and 2003 (data from Metropolitan Water Reclamation District of Greater Chicago). Concentration of dissolved iron in the reconstituted water was less than 0.01, and was not adjusted to match CSSC water. The CSSC is often very low in dissolved oxygen, which may account for elevated iron concentrations. Iron is not highly soluble in water high in oxygen. It would not have been feasible to lower the dissolved oxygen in the test in order to achieve higher dissolved iron concentrations, because the test organisms require oxygen.

Dissolved oxygen was more than adequate in all treatments with small fish, remaining above 7.5 mg/L at all times. Dissolved oxygen in the CSSC water was low (2.5 mg/L) at the time of collection at the canal, but it was aerated by sloshing during transportation and was at 7.6 mg/L when transferred to the test chambers. Dissolved oxygen in treatments with large fish in reconstituted water was adequate (above 5 mg/L) for the 40-hour duration of that test. Dissolved oxygen concentrations in treatments with large fish in CSSC water dropped as low as 0.6 mg/L by 24 hours, resulting in mortality of test fish in both the control and antimycin chambers. CSSC water is largely composed of wastewater from Chicago, therefore it is very high in biological oxygen demand. This, coupled with the oxygen demand of the large fish and the long time to death when treated with antimycin, resulted in oxygen concentrations that were insufficient to support the fish. However, all large fish subjected to antimycin were alive at the 20 hour period, indicating that antimycin at the test concentrations would not be a suitable chemical for rapid removal of bighead and silver carp.

Behavior of fishes in the toxicity tests

Regardless of toxicant or test concentration, fish of all three species schooled together and there was no obvious intra- or inter-species aggression. Fish were observed coughing after they were introduced into the 1000 and 500 μg rotenone/L test chambers. Otherwise, there were no obvious immediate reactions to either toxicant. Fish did not swim rapidly or try to jump from the test chambers when introduced to higher concentrations of either toxicant. As fish succumbed to the toxicants, they became more lethargic and eventually moribund, but rarely or never showed any sign of struggling or thrashing. Some goldfish floated at the surface when they were moribund or dead, but bighead and silver carp, with few exceptions, remained on the bottom or sank when they died. Assuming that this behavior is similar during field applications, most bighead and silver carp that die or become moribund will not be visible to field personnel until gas buildup in the peritoneal cavity resulting from decomposition raises the fish to the surface.

Sensitivity of test organisms to rotenone

Bighead carp and silver carp were sensitive to rotenone. At nominal concentrations of 15 μg/L and above both bighead and silver carp were either dead or moribund at 4 hours, and all were dead at 8 hours. Control survival of bighead carp was 100%. One of ten silver carp in the control died at the last measurement period, apparently due to fin rot. No other individuals in any concentration showed evidence of fin rot. Figure 5 and Table 5 show the LC50s and the concentrations that caused complete mortality of bighead and silver carp at the various time periods. Marking and Bills (1981) reported 96 hour LC50s for

bighead and silver carp in static tests (2.2μg/L , 95% CI 1.7 - 2.7 and 2.8 μg/L, CI 1.9 – 4.0, respectively). We could not calculate a 96 hour LC50 for bighead carp because of high mortality in our lowest concentrations, but the silver carp 96 hour LC50 in this study was very similar (3.6 μg/L, CI 0.1 – 7.5) to the Marking and Bills (1981) study. Marking and Bills (1981) did not report water quality information, the size of the fish in the tests, mortality at earlier time periods, complete mortality at any time period, or the test temperature, which makes further comparisons difficult. In an unpublished report, Henderson (1975) reported complete mortality of bighead and silver carp in static tests in 6 hours at 30 ug/L and at 10 μg/L, and at 24 hours at 5 μg/L, which indicates toxicity slightly higher than we report here. Henderson did not report the number or size of the fish used, but the fish were undoubtedly small because the test chambers were 20-gallon aquaria. Henderson also did not report the temperature or water quality in the test, but he did mention difficulty in measurement of tiny quantities of the powdered rotenone used.

Marking and Bills (1976) tested the toxicity of rotenone on a variety of fishes under varying conditions. Comparing the 24 hr LC50s of bighead and silver carp in this test to the data of Marking and Bills (1976), performed under similar conditions, bighead and silver carp were less sensitive than bowfin, coho or chinook salmon, rainbow, brook, or lake trout, northern pike, longnose or white sucker, or walleye. They were more sensitive than fathead minnows, goldfish, channel catfish, black bullhead, green sunfish, or largemouth bass. Yellow perch, smallmouth bass, bluegill, and common carp LC50s fell between the LC50s of bighead and silver carp.

Goldfish were much less sensitive to rotenone than bighead and silver carp. Goldfish at nominal concentrations of 250 ug/L and above were dead or moribund at 4 hours and were dead at 8 hours. Some goldfish were alive in the 15ug/L treatment at the 72 hour measurement, but were finally dead by 96 hours (the end of the test). LC50s for the various time periods are given in Table 5. Three of ten goldfish died in the control by the end of the test; none of these fish showed signs of external lesions, but there may have been some incidental mortality of goldfish. The 96-hour LC50 for goldfish in this test was 6.8μg/L, compared to 24 μg/L in Marking and Bills (1976).

Large bighead and silver carp subjected to 50 μg rotenone/L in reconstituted water were reasonably similar in sensitivity to the small fish. All large fish subjected to rotenone were dead or moribund by 4 hours and dead by 8 hours. Control survival was 100% to 40 hours, when the test with large fish with rotenone was terminated.

Small fish subjected to 50 μg rotenone/L in CSSC water (period 3) were moribund at 4 hours but no deaths occurred until the 8-hour observation, by which time all bighead and silver carp were dead. All goldfish were dead by 48 hours. There was no apparent difference in toxicity between the tests conducted in reconstituted water and those conducted in CSSC water, despite the cooler temperatures under which the CSSC test was conducted. Control survival of small fish in CSSC water was 100%, all species.

Sensitivity of test organisms to antimycin *a*

Antimycin *a* is often more toxic than rotenone to fish, but in this study antimycin (as active ingredient) was not as effective at killing Asian carp as rotenone. In general, effective contact time for rotenone is longer than the contact time for antimycin, but fishes exposed to rotenone exhibit physiological responses earlier. In a study by Gilderhus (1972), fish that had become lethargic due to exposure to rotenone recovered when returned to water without toxicant, but fishes exposed to antimycin that had begun to show signs of distress did not recover when released to toxicant-free water. In our study, fairly high dosages of antimycin failed to kill bighead and silver carp within a reasonable period of time. The highest dose tested (20 μg/L) did not kill all silver carp until the 24-hr observation, and did not kill all bighead carp until the 32-hr observation. 96-hr LC50s for bighead and silver carp in this study were 4.6 and 6.3 μg/L antimycin. Figure 6 and Table 7 give the LC50 and 100% mortality data for antimycin from this study. Control survival was 100%, all species, in both the reconstituted water control and the reconstituted water with acetone control.

Goldfish sensitivity to antimycin was similar to that reported in some other studies and differed somewhat from others. Berger et al (1969) reported that 10 μg/L of antimycin was required to kill goldfish at 96 hours. Ten μg/L was also the 96-hour 100% mortality concentration in this study. Temperature and pH strongly affect the toxicity of antimycin to fishes. Antimycin and rotenone degrade slower in lower temperature water, but they are less effective, probably because at lower temperatures the metabolism and respiration rate of the fish decrease, thereby decreasing the rate of toxicant uptake (Berry and Larkin 1954).

Marking and Dawson (1972) reported 96-hour LC50s for goldfish ranging from 0.42 μg/L at pH 6 to 29.25 at pH 9.5. In that study, the 96-hour LC50 was 1.22 μg/L at pHs similar to those in this test (pH ~7.25), which is about 20% of the 96-hour LC50 reported in this study (5.7 μg/L), but they did not report the temperature. Marking and Dawson (1972) also reported 96-hour LC50s to goldfish ranging from 0.07 to 0.34 at a temperature of 22C, but they did not report pH values in that portion of the study. Marking and Dawson did not report LC50s for exposures of less than 96 hours or complete kills at any time period. Walker et al (1964) reported 24-hour complete mortality of goldfish at 40 μg/L, higher than any concentration tested in this study.

Dissolving lipids in the technical grade product prior to making the test dilutions had no apparent affect on toxicity. Silver and bighead carp exposed to 10 μg antimycin/L were all dead at 96 hours regardless of dilution method. The 5μg/L concentration caused partial mortality to silver carp in both periods (33% with undissolved lipids and 30% with dissolved lipids) and caused partial mortality of bighead carp in the test with undissolved lipids, but did not kill bighead carp in the test with dissolved lipids, although some fish were moribund. Control survival was 100%, all species, in both tests. This corresponds to the chemical analyses comparing the two methods of mixing the antimycin. If antimycin had partitioned strongly into the undissolved lipids in the technical solution container, the data would have shown erroneously low sensitivity of fishes to antimycin. It does not appear that this was the case.

The range of sensitivity of fishes to antimycin is known to be quite wide, a fact which has been useful in selective control of fish (Berger et al., 1969). Finlayson et al. (2002) compared values of antimycin and rotenone in the literature and found antimycin to be 50 times as toxic as rotenone to rainbow trout, but half as toxic to black bullhead as rotenone.

A study by Marking and Bills (1981) found the 96-hr LC50s of antimycin to bighead carp and silver carp to be 0.600 (95% CI 0.54 – 0.67) μg/L, and to silver carp to be 0.83 (95%CI 0.71 – 0.97). The LC50 values in our study are more than 5 times higher. The conditions of that test (water quality, temperature, test chambers, lighting conditions, whether technical or reagent-grade chemical was used, whether or not dispersants or detergents were used to dissolve the antimycin, and the size of the fish) were not reported, nor were analytical or bioassay support of nominal concentrations performed in that test. Thus it is difficult to determine the reason for the difference between their test and ours. Further testing would be required to determine if Asian carp might be more sensitive to antimycin under other conditions.

Caged goldfish were added to some chambers at the 24- and 48-hr intervals in the antimycin test with small fish, to biologically determine degradation of the activity of antimycin. In the 20μg/L treatment, all goldfish added at 24 hours were dead at 96 hours, a time-to-death of 72 hours, which was exactly equivalent to goldfish stocked at zero hour. In the 5 μg/L treatment, one of ten goldfish added at 24 hours was dead at 96 hours (time-to-death 72 hours) compared to three goldfish stocked at the beginning of the test that were dead at the 72 hour observation. Survival in other cages was 100% at the end of the test. Marking and Dawson (1972) developed half-life curves for antimycin at pH 7.5. According to those data, half-life of antimycin in these tests would have been between 93 and 120 hours.

Survival of bighead and silver carp in the 2.5 μg/L antimycin was 100% in 96 hours, in the shaded as well as in the full sunlight treatment. Thus, it is impossible to tell from these data if sunlight degraded the antimycin rapidly enough to reduce toxicity to fishes.

At the end of period 2, remaining fish from the 2.5 and 5 μg/L concentrations were placed in cages and transferred to clean water to observe any delayed mortality. Remaining fish in cages stocked at 24 hours were also retained for 24 hours in clean water. No further mortality occurred except for one of six fish in the cage containing goldfish from the 5μg/L treatment. Thus, there was no significant delayed mortality of fish exposed to antimycin.

Toxicity of antimycin (7.5 μg/L) to small fish in CSSC water (period 3) was not different from the toxicity we observed in reconstituted water at that concentration. There was no mortality of any fish prior to the 96-hour observation. At 96 hours, 60% of the bighead carp and 20% of the silver carp were dead. No goldfish died in the test. Survival of small fish in the CSSC water control was 100%, all species.

The confirmatory test of antimycin (7.5 μg/L) to large fish in CSSC water failed due to low dissolved oxygen, a result of high fish biomass combined with the biological oxygen demand of CSSC water. No fish had died in any concentration prior to the 24-hour observation, but by that time dissolved oxygen concentrations in those tanks had decreased to values ranging from 0.56 to 3.46 mg/L. Most fish had died by 48 hours, but it was impossible to separate the effects of low dissolved oxygen from the effects

of antimycin. Generally, large fish take longer to die from antimycin exposure than small fish (Nick Romeo, Aquabiotics, Inc, personal communication).

The unexpectedly low sensitivity of silver and bighead carp to antimycin in this study may be in part due to the low temperatures during both test periods 2 and 3. Fish must bioaccumulate antimycin from the environment, and reduced metabolism due to low temperatures will delay bioaccumulation and the onset of mortality. However, temperatures were somewhat higher during period 3 than in period 2, and no significant increase in mortality was observed.

Summary

Young-of-the-year and adult silver carp and bighead carp were sensitive to rotenone in both of the waters and at both of the temperature regimes in this study. They were less sensitive than salmonids and more sensitive than catfishes and other insensitive species. The sensitivity of silver and bighead carp to rotenone in this test was similar to that reported in the limited available literature. The Prenfish label allows application concentrations of up to 250 μg/L, active ingredient. In our study, that concentration killed all bighead and silver carp in 4 hours. A concentration of 15 μg/L killed all the bighead carp and 90% of this silver carp in that time period, and the remaining fish were moribund. However, in field applications, mixing will be less complete and fish may avoid the highest concentrations of rotenone. Because of the importance of keeping bighead and silver carp from invading the Great Lakes, and because of the high value of commercial transportation in the CSSC, it is imperative that a complete kill be achieved in the lowest possible time period. Therefore it is our recommendation that the highest allowable concentration of rotenone (250 μg/L) be used, and that this concentration be maintained for longer than 4 hours, to allow for a margin of security and ensuring a complete kill.

Silver carp and bighead carp were relatively insensitive to antimycin in our study. They were more sensitive than bullhead catfish, but less sensitive than most other species. In both of the two tests in our study, bighead and silver carp were less sensitive than in the single test reported in the literature for those species. The reasons for this difference are not clear. Based on the results of this study, only the highest label recommendations would be advisable for the removal of bighead and silver carp, and those concentrations might require a contact period of more than 24 hours.

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Table 1. Treatments performed. Unless otherwise specified, each treatment consisted of a round, high-density polyethylene tank containing 1000 L of water. Tanks with "small" fish had ten fish of each species (bighead carp, silver carp, and goldfish). Tanks with "large" fish had two bighead carp and two silver carp. All fish in periods 1 and 2 were "small" fish. "Recon" water was CERC well water mixed with deionized water to approximate the conductivity and alkalinity of Chicago Ship and Sanitary Canal (CSSC) water, and treated with acid or base to a pH between 7 and 7.5, to imitate the normal pH of CSSC water.

Treatment number	**Period 1**		**Period 2**		**Period 3**			
	Water	Rotenone concentration (ug/L)	Water	Antimycin *a* concentration (ug/L)	Water	Fish size	Piscicide	Piscicide Concentration (ug/L)
1	**Recon**	0	**Recon**	0	CSSC*	Small	Control	0
2	Recon	3.25	Recon	0.155	CSSC*	Small	Rotenone	50
3	Recon	7.5	Recon	0.310	CSSC*	Small	Antimycin	7.5
4	Recon	15	Recon	0.625	CSSC	Large	Control	0
5	Recon	31	Recon	1.25	CSSC	Large	Antimycin	7.5
6	Recon	62.5	Recon	2.5	CSSC	Large	Antimycin	7.5
7	Recon	125	Recon	5	CSSC	Large	Antimycin	7.5
8	Recon	250	Recon	10	Recon	Large	Control	0
9	Recon	500	Recon	20	Recon	Large	Rotenone	50
10	Recon	1000	Recon	0 (acetone control)	Recon	Large	Rotenone	50
11			Recon	2.5 (full sunlight)	Recon	Large	Rotenone	50
12					Recon	Large	Rotenone	50
13					Recon	Small	NA	0
14					Recon	Small	Antimycin	10
15					Recon	Small	Antimycin	5
16					Recon	Small	Antimycin	2.5

*Volume of water in these tanks was 850 L. A smaller volume was used with the small fish in CSSC water because with limited availability of CSSC water; this allowed for an additional treatment.

Table 2. Size of fish used in toxicity tests with rotenone and antimycin.

Testing period	"Large" or "small"	Species	Mean Total Length (mm)	Length range (mm)	Length standard deviation	Mean Weight (g)	Weight Range (g)	Weight standard deviation
1	Small	Bighead carp	104	79 - 130	8.29	10.8	4.8 – 20.3	2.80
1	Small	Silver carp	123	91 - 163	11.2	15.8	6.1 – 40.5	4.54
1	Small	Goldfish	105	82 - 126	8.47	14.4	4.4 – 22.3	2.93
2	Small	Bighead carp	104	89 - 126	8.97	11.6	7.2 – 20.4	2.85
2	Small	Silver carp	123	106 - 159	9.53	16.5	9.6 – 39.0	4.76
2	Small	Goldfish	102	80 - 120	9.09	13.8	7.9 – 20.8	3.27
3	Small	Bighead carp	103	86 - 117	7.36	10.8	6.5 – 15.3	2.12
3	Small	Silver carp	122	100 - 156	12.8	16.8	9.8 – 36.4	6.06
3	Small	Goldfish	98.3	58 - 117	15.1	14.1	3.0 – 22.3	4.80
3	Large	Bighead carp	676	605 - 771	58.0	3378	2500 - 5500	900
3	Large	Silver carp	743	706 - 884	72.9	4714	3200 - 6250	1210

Table 3. Measured versus nomimal concentrations of rotenone in test chambers. Samples were from period 1 except for the two 50 µg/L samples, which were taken from period 3. All samples were in reconstituted water made by diluting CERC well water with deionized water and pH adjusting, except for the technical grade product (50,000,000 µg/L) and one of the period 3 samples, which was in Chicago Ship and Sanitary Canal water. Rotenolone and Tephrosin are breakdown products of rotenone. All concentrations are in µg/L (as active ingredient). Solutions were made using Prenfish 5% rotenone technical grade product.

Rotenone Nominal Concentration (µg /L)	**Sampling Time (hr. after addition of toxicant to test chamber)**	**Rotenone Measured Concentration (µg /L)**	**Measured to Nomimal Ratio**	**Rotenone Measured Concentration + Rotenolone and Tephrosin Estimated Concentration (µg /L)**	**Measured to Nomimal Ratio Including Degradation Products**
50,000,000	NA	50,000,000	1	NA	NA
0	0.5	0.0	NA	0.0	NA
3.25	0.5	1.1	0.338	2.9	0.892
7.5	0.5	2.3	0.307	5.2	0.693
15	0.5	3.2	0.213	11	0.753
31	0.5	8.1	0.261	20	0.642
62.5	0.5	22	0.355	45	0.722
125	0.5	52	0.417	90	0.722
250	0.5	42	0.168	163	0.652
250[1]	0.5	47	0.190	190	0.760
500	0.5	88	0.175	329	0.658
500[1]	0.5	62	0.124	434	0.868
1000	0.5	410	0.410	754	0.754
1000[1]	0.5	610	0.610	1210	1.210
1000[2]	0.5	320	0.320	684	0.684
1000[1,2]	0.5	555	0.555	972	0.972
31	24	7.5	0.242	20	0.655
250	24	43	0.172	162	0.649
250[1]	24	52	0.210	164	0.656
250[2]	24	82	0.328	178	0.713
62.5	48	16	0.258	37	0.586
250	48	87	0.348	163	0.653
62.5	72	11	0.173	35	0.563
250	72	54	0.216	132	0.526
62.5	96	10	0.165	31	0.494
250	96	71	0.285	149	0.594
50[3]	2	12	0.232	37	0.744
50[3,4]	2	5.5	0.110	27	0.538

[1]duplicate analysis
[2]Duplicate sample
[3]Sample from period 3
[4]Dilution water was from the Chicago Ship and Sanitary Canal

Table 4. Concentrations of antimycin *a* in blind samples made from Fintrol® technical solution. Blind A was made by taking an aliquot of the liquid in the original bottle without dissolving residual lipids, then dilution to the "blind" nominal concentration with acetone. Blind B was made by dissolving all lipids by the addition of additional acetone, then diluting the solution to the blind concentration.

	Nominal concentration (% active ingredient, w/v)	**Measured concentration (% active ingredient, w/v)**
Blind A (lipids not dissolved – compares to period 2 dilution)	1.8	2.04 ± 0.10
Blind B (lipids dissolved – compares to period 3 dilution)	1.02	0.98 ± 0.05

Table 5. Rotenone LC50s (calculated lethal concentrations to 50% of the organisms and confidence intervals) and the concentration lethal to 100% of the organisms for bighead and silver carp and goldfish. All values are reported as rotenone active ingredient, with mg/L of the technical grade solution below in parenthesis. Toxicant used for these exposures was Prenfish, a 5% rotenone technical grade product. Unless otherwise indicated, LC50s were calculated using the Spearman-Karber method with a 10% trim. LC50s were not calculable for some time periods.

Time period (hours)	Bighead carp				Silver carp				Goldfish			
	LC50	CI Low[1]	CI High[2]	100%[3]	LC50	CI Low[1]	CI High[2]	100%[3]	LC50	CI Low[1]	CI High[2]	100%[3]
1	707 (14.1)	250 (5)	1000 (20)	NA[4]	311 (6.2)	125 (2.5)	500 (10)	NA	NC[5]	NC	NC	NA
2	328 (6.6)	250 (5)	500 (10)	500 (10)	188 (3.8)	125 (2.5)	250 (5)	500 (10)	NC	NC	NC	NA
4	27.2 (0.5)	15 (0.3)	31 (0.6)	62.5 (1.3)	9.9 (0.2)	7.5 (0.2)	15 (0.3)	250[6] (5)	237[8] (4.7)	178 (1.6)	298 (6.0)	500 (10)
8	10.6 (0.2)	7.5 (0.2)	15 (0.3)	62.5[7] (1.3)	8.8 (0.2)	3.25 (0.1)	15 (0.3)	15 (0.3)	178[8] (3.6)	134 (2.6)	224 (4.5)	500 (10)
12	5.8 (0.1)	3.25 (0.1)	7.5 (0.2)	15 (0.3)	7.5 (0.2)	3.25 (0.1)	15 (0.3)	15 (0.3)	99.2[8] (1.9)	63.9 (1.3)	135 (2.7)	250 (5)
16	4.5 (0.1)	3.25 (0.1)	7.5 (0.2)	7.5 (0.2)	7.5 (0.2)	3.25 (0.1)	15 (0.3)	15 (0.3)	112[8] (2.2)	76.9 (1.5)	148 (3.0)	250 (5)
20	4.5 (0.1)	NC	NC	7.5 (0.2)	7.5 (0.2)	NC	NC	15 (0.3)	125[8] (2.5)	89.8 (1.8)	159 (3.2)	250 (5)
24	4.2 (0.1)	NC	NC	7.5 (0.2)	7.5 (0.2)	3.25 (0.1)	15 (0.3)	15 (0.3)	104[8] (2.1)	68.9 (1.4)	140 (2.8)	250 (5)
32	4.2 (0.1)	0.1 (0.002)	7.5 (0.2)	7.5 (0.2)	5.4 (0.1)	0.1 (0.002)	7.5 (0.2)	15 (0.3)	28.4[8] (0.6)	20.0 (0.4)	36.7 (0.7)	62.5 (1.3)
40	NC	NC	NC	7.5 (0.2)	5.4 (0.1)	NC	NC	15 (0.3)	24.6[8] (0.5)	15.5 (0.3)	33.6 (0.6)	62.5 (0.7)
48	NC	NC	NC	7.5 (0.2)	4.6 (0.1)	0.1 (0.002)	7.5 (0.2)	15 (0.3)	18.3[8] (0.4)	11.2 (0.2)	25.4 (0.5)	62.5 (1.3)
72	NC	NC	NC	7.5 (0.2)	3.9 (0.1)	0.1 (0.002)	7.5 (0.2)	7.5 (0.2)	11.1[8] (0.2)	7.31 (0.15)	15.0 (0.30)	31 (0.6)
96	NC	NC	NC	7.5 (0.2)	3.6 (0.1)	0.1 (0.002)	7.5 (0.2)	7.5 (0.2)	6.82[8] (0.14)	4.18 (0.08)	9.46 (0.19)	15 (0.3)

[1]Lower boundary of the 95% confidence interval for the LC50
[2]Upper boundary of the 95% confidence interval for the LC50
[3]Test concentration causing 100% mortality.
[4]NA = Not applicable
[5]NC = Not calculable
[6]At 4 hours all silver carp at concentrations 15 and above were dead, except one fish in 62.5ug/L and one fish at 125 ug/L. These fish were moribund at 4 hours.
[7]One moribund bighead carp alive in each of the 31 and 15 ug/L concentrations
[8]Calculated using Probit analysis with Abbot's correction

Table 6. Antimycin LC50s (calculated lethal concentrations to 50% of the organisms) and confidence intervals for bighead and silver carp and goldfish. All values are reported as antimycin, active ingredient. Toxicant used for these exposures was Fintrol,, a 20% antimycin (w/v) technical grade product. To arrive at the concentration as technical grade solution, these data should be multiplied by a factor of 5. Unless otherwise indicated, LC50s were calculated using the Spearman-Karber method with a 10% trim. LC50s were not calculable for some time periods.

Time period (hours)	Bighead carp				Silver carp				Goldfish			
	LC50 (µg/L)	CI Low[1]	CI High[2]	100%[3]	LC50 (µg/L)	CI Low[1]	CI High[2]	100%[3]	LC50 (µg/L)	CI Low[1]	CI High[2]	100%[3]
20	NC[4]	NC	NC	NA[5]	17.1[6]	14.2	20.04	NA	NC	NC	NC	NA
24	16.3	14.3	18.2	NA	15	NC	NC	20	NC	NC	NC	NA
32	14.3	12.6	16.0	20	9.2	6.8	11.67	20	42.5[7]	-8.5637	93.5954	NA
40	8.8	6.3	11.3	20	7.5	NC	NC	10	13.2[7]	-6.8359	33.25	NA
48	7.5	NC	NC	10	7.2	6.5	7.88	10	29.4[7]	12.0134	46.8746	NA
72	7.5	NC	NC	10	7.2	6.5	7.88	10	9.7	6.61	12.88	20
96	4.6	3.4	5.8	10	6.3	4.9	7.63	10	5.7	4.38	7.03	10

[1]Lower boundary of the 95% confidence interval for the LC50
[2]Upper boundary of the 95% confidence interval for the LC50
[3]Lowest test concentration causing 100% mortality.
[4]NC = not calculable
[5]NA = not applicable; no test concentration killed all fish of this species at this time period
[6] a 30% trim was used to calculate this LC50; 10 and 20% trims would not allow calculation of an LC50
[7]Probit analysis was used to calculate these LC50s and associated confidence intervals because the data did not fit the Spearman-Karber model.

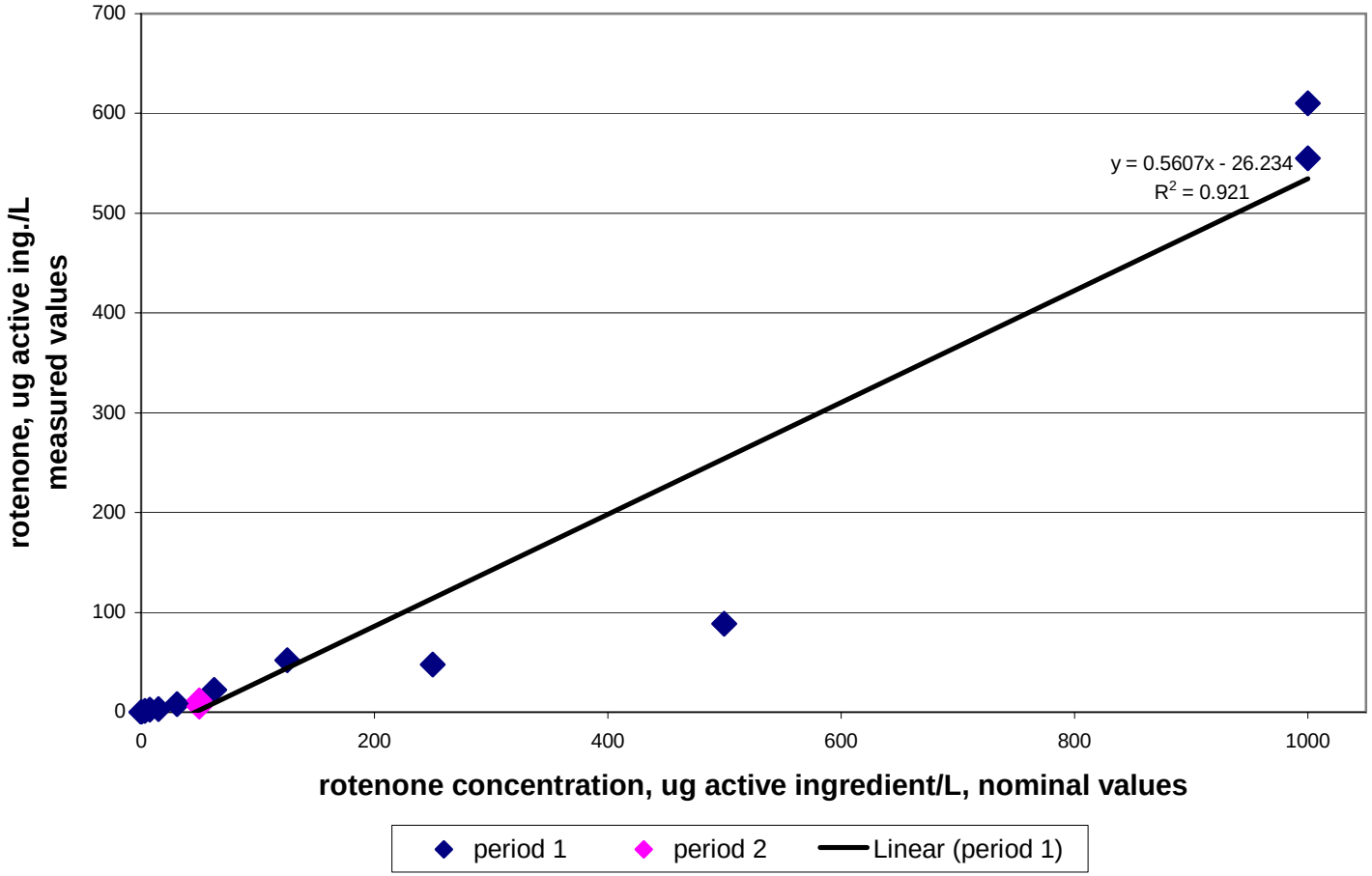


Figure 1. Measured versus nominal concentrations of rotenone in a toxicity test with Asian carp.

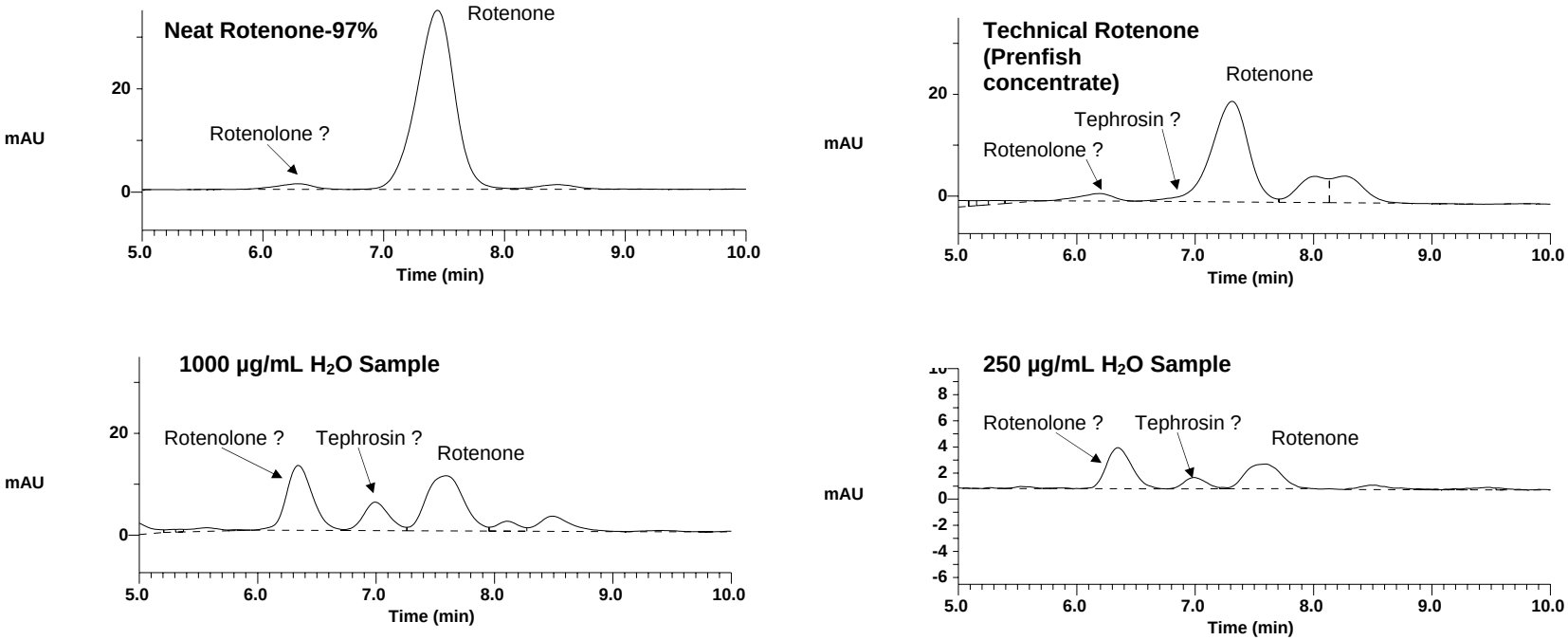


Figure 2. Chromatograms from analysis of rotenone. Peaks to the left of the rotenone peak are situated similarly on the chromatogram to peaks identified by Draper et al. (1999) to be rotenone's breakdown products rotenolone and tephrosin. 1000 and 250 µg/L chromatograms were from samples collected at the start of period 1.

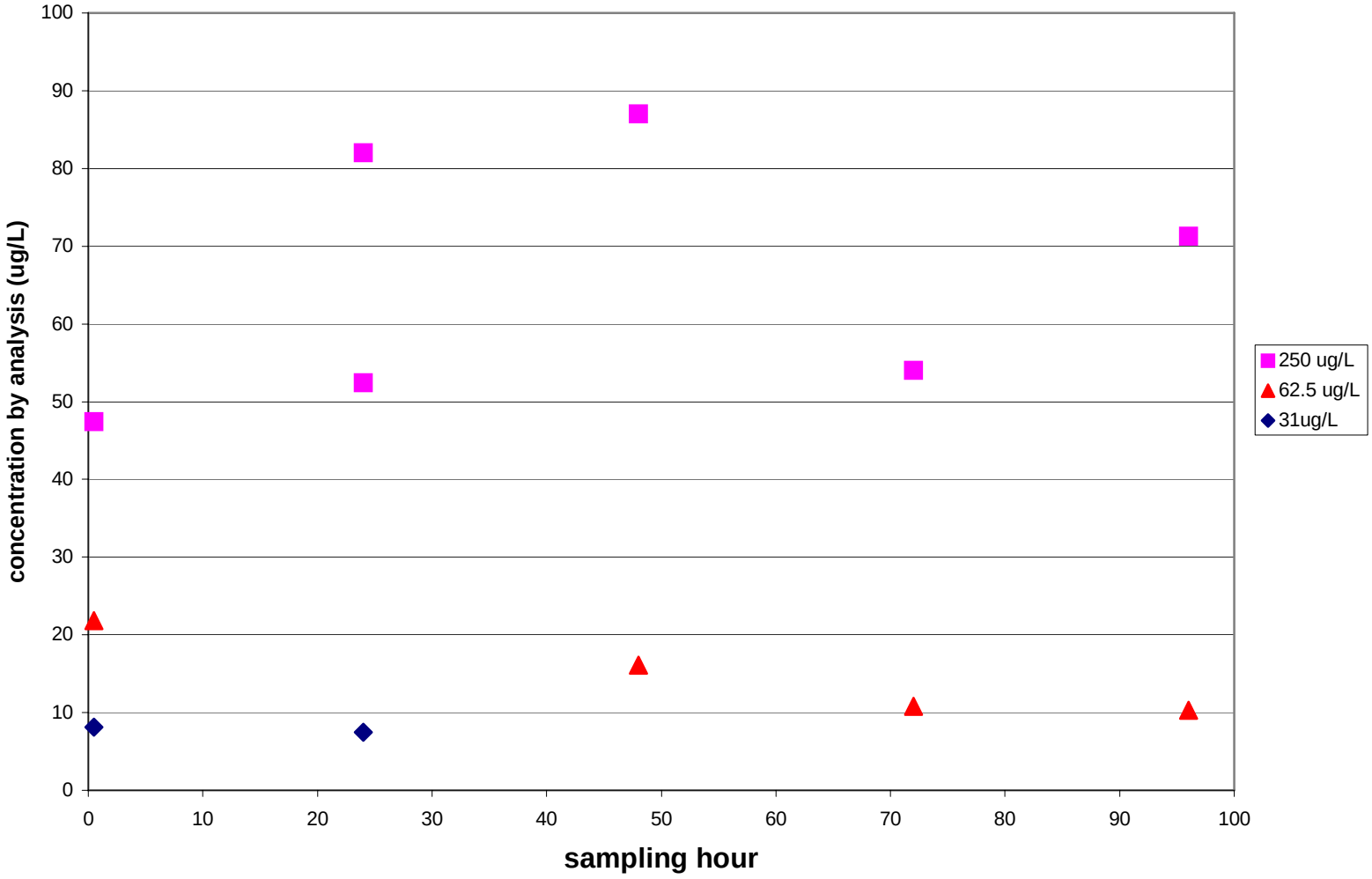
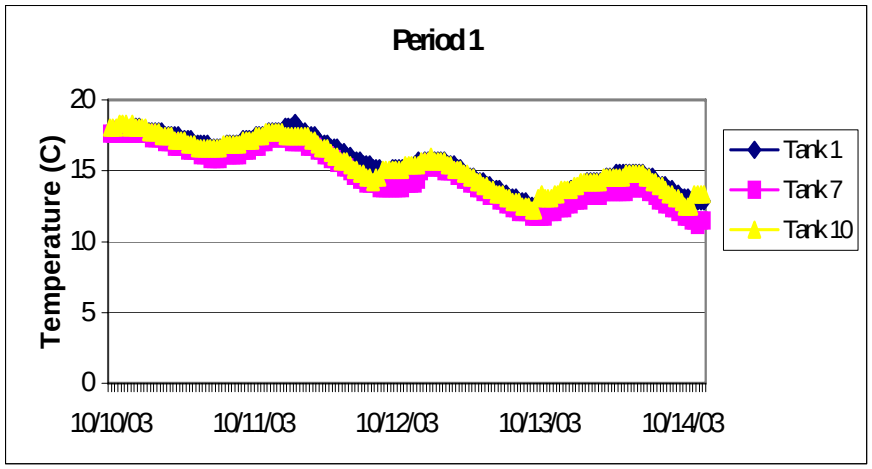
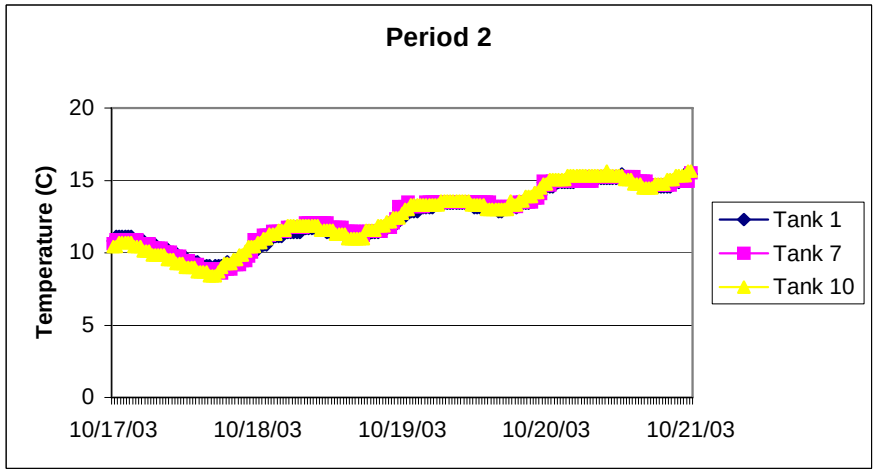
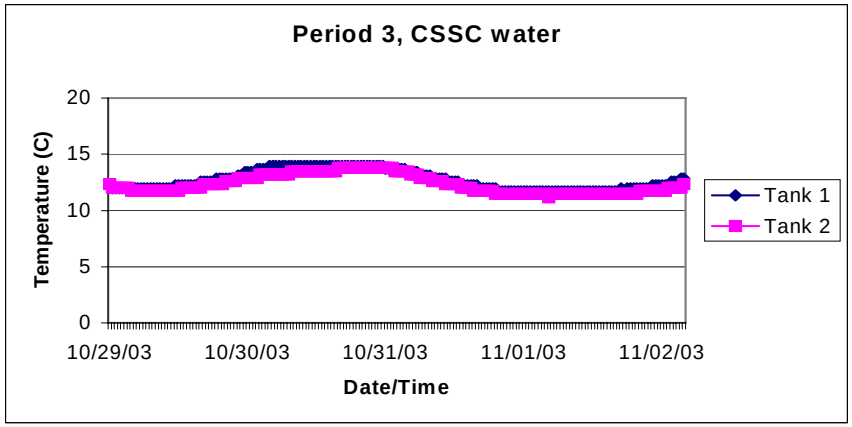


Figure 3. Concentrations of rotenone from samples taken during a toxicity test with Asian carp. All concentrations are as active ingredient.







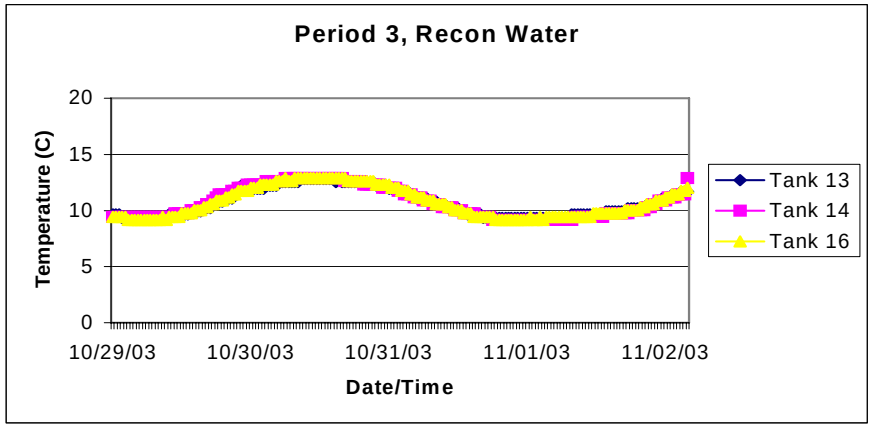


Figure 4. Temperatures of representative test chambers during toxicity tests with Asian carp. All tests were conducted outdoors under at tent, except for the period 3 test with CSSC water, which was conducted in an uninsulated building.

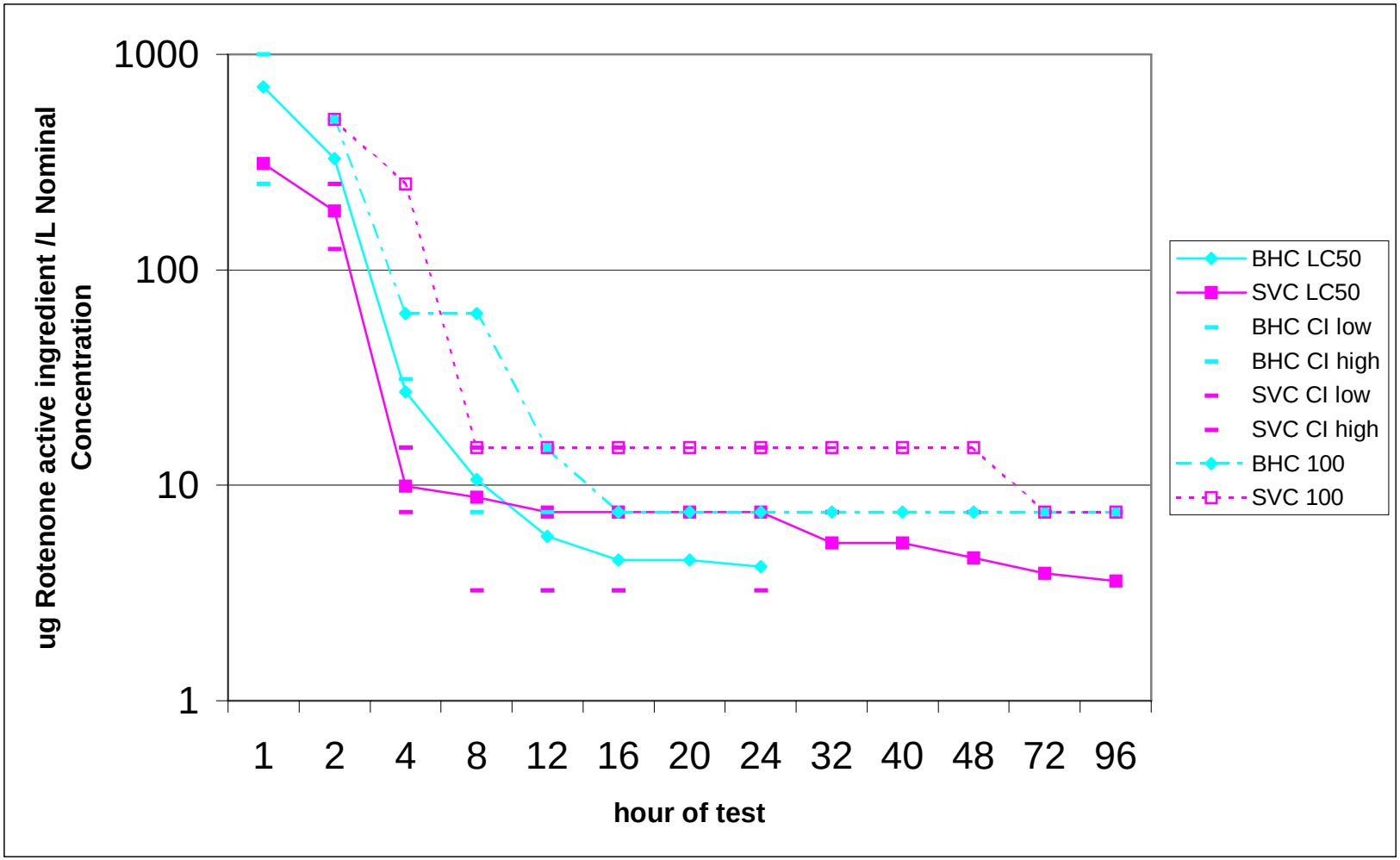


Figure 5. LC50s and confidence intervals and concentrations that caused complete mortality in bighead and silver carp exposed to rotenone. BHC = bighead carp, SVC = silver carp, CI low = low 95% confidence interval value, CI high = high 95% confidence interval value, BHC 100 lowest concentration that killed 100% of bighead carp, and SVC 100 is the lowest concentration that killed 100% of silver carp.

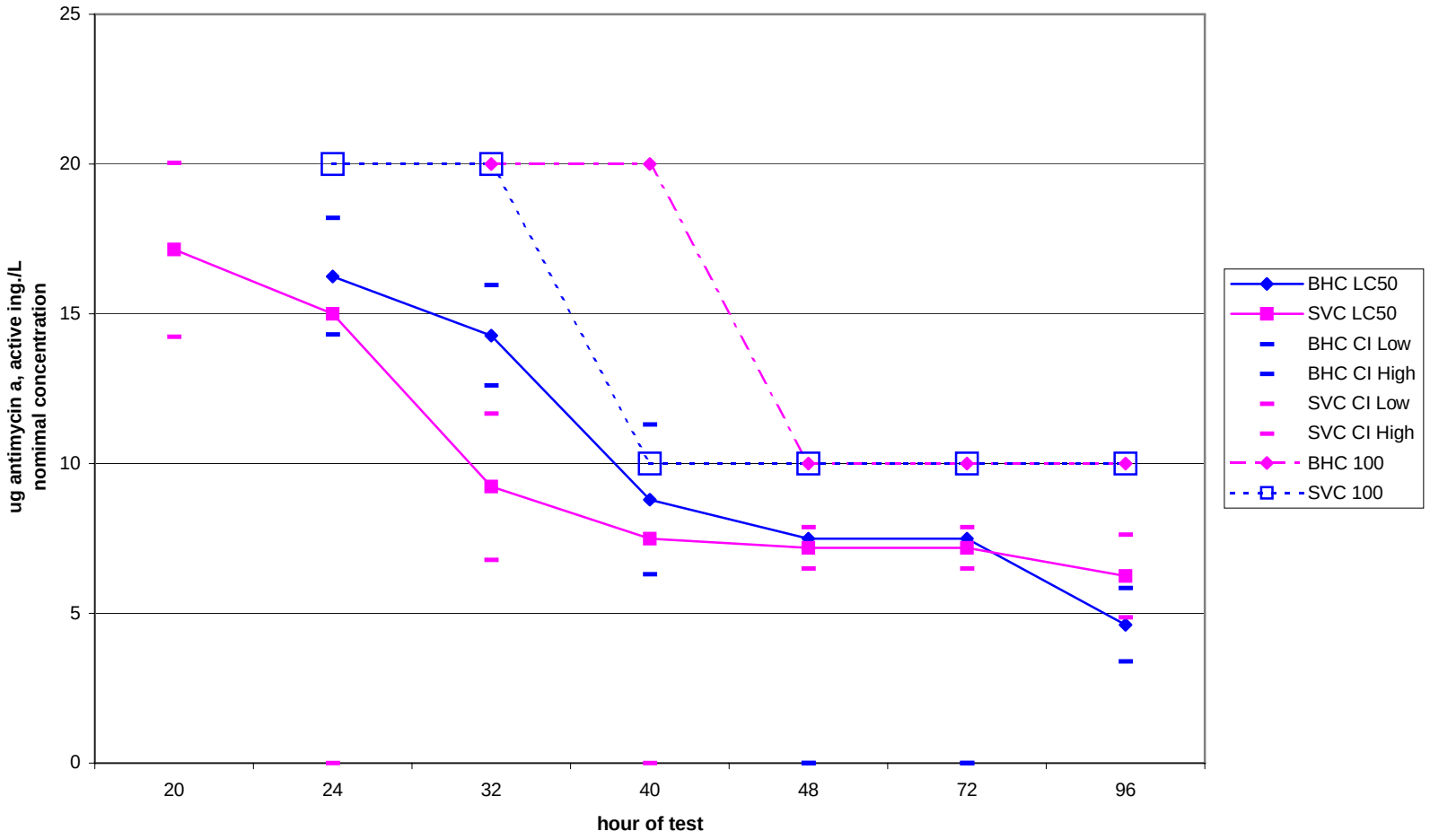


Figure 6. Antimycin LC50s (lethal concentration to 50% of the organisms) and concentrations which killed all organisms at different time periods. BHC = bighead carp, SVC = silver carp, CI low = low 95% confidence interval value, CI high = high 95% confidence interval value, BHC 100 lowest concentration that killed 100% of bighead carp, and SVC 100 is the lowest concentration that killed 100% of silver carp