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Malathion Fate in Water and Catfish

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Abstract

Several experiments were conducted in 1998 to test malathion degradation in water and to study residue accumulation in catfish. Laboratory and pond experiments were conducted at the Thad Cochran National Warmwater Aquaculture Center in Stoneville, MS, and chemical analyses were done at the Mississippi State Chemical Laboratory at Mississippi State University. Laboratory experiments using fiberglass tanks and glass vessels tested rate of malathion degradation in water and level of residues accumulated in catfish fillets and carcasses. Other laboratory experiments tested the effects of pond water, pond sediments, water pH, and the presence or absence of fish on degradation of malathion. Pond experiments also tested degradation of malathion in pond water and residue accumulation in catfish. Results of the research showed rapid degradation of malathion in well and pond water at higher pH levels. Essentially no malathion residues were detected in samples of catfish fillets or carcasses analyzed in these studies. A trace amount detected in one carcass sample was less than 0.01 part per million. This amount is less than the limit of quantification by the analytical methods used, which are four times more sensitive than the methods currently used by the U.S. Food and Drug Administration. Even when exposed to malathion doses exceeding levels that would be produced by direct application to a pond, catfish were not shown to accumulate detectable levels of malathion residue. These results suggest that detectable residues of malathion in catfish grown in commercial ponds are unlikely to occur due to ultra-low-volume applications of malathion in the Mississippi boll weevil eradication program.

Introduction

In early August 1994, the Mississippi Boll Weevil Management Corporation started a boll weevil eradication program in the south Delta, which contains a large concentration of commercial catfish ponds. The corporation and its primary contractee, the Southeastern Boll Weevil Eradication Foundation, along with area cotton and catfish producers, questioned the application of malathion near commercial catfish ponds. The main issues of concern were potential for pond contamination by spray drift and risk of malathion residues in catfish, where there is zero tolerance for residue. Research was conducted in 1998 to address these issues.

Malathion applied ultra-low-volume (ULV) to cotton is a component of the boll weevil eradication program. The product used in this program is Fyfanon ULV (Cheminova Agro A/S, Denmark), an ultra-low-volume concentrate insecticide that contains 96.5% malathion. Each gallon of Fyfanon ULV contains 9.9 pounds (4.5 kilograms) of malathion. The standard approach to boll weevil eradication is for all cotton acreage to receive multiple applications of malathion ULV during the first and second year of the 5-year eradication program and for a decreasing portion of acreage to be treated each subsequent year until eradication is achieved.

Since cotton grown near catfish ponds is treated with malathion ULV, these ponds could potentially be exposed to malathion contamination. Eradication program personnel, cotton producers, aerial applicators, and catfish producers have been concerned about the exposure of catfish ponds and the potential for malathion residue accumulation in catfish. Regulations established by the U.S. Environmental Protection Agency and the U.S. Food and Drug Administration set the level of tolerance for malathion residues in catfish to zero. Catfish producers and processors also demand that the product be free of malathion.

Malathion is used in boll weevil eradication for several reasons: (1) it is highly effective; (2) low application rates and the undiluted ULV method of application make it economical; and (3) it is safe to mammals and many other non-target organisms, including channel catfish. Malathion is widely used for mosquito control for similar

reasons.

Malathion ULV is applied at the rate of 296 milliliters (10 fluid ounces or 0.76 pounds) of active ingredient per acre. Direct application of this rate of malathion to the surface of a 1.2-meter-deep pond (4 feet deep) would result in a malathion concentration of about 70 parts per billion (ppb) in the pond water. The highest dose rates used in these studies exceeded the concentration that would result from direct application to a pond.

The studies reported in this technical bulletin were conducted to provide data on the fate of malathion in water and to study detection of malathion residues in catfish exposed to various single-application, discrete-dose treatments in water.

Methods

Laboratory Studies of Uptake and Clearance

Rate and Exposure Time

Uptake and clearance studies were conducted in circular fiberglass tanks containing 1,360 liters (360 gallons) of continuously flowing well water (9.5 liters per minute). Water pH was 8.9. Twelve tanks were each stocked with 25 channel catfish, each of which weighed approximately 454 grams (1 pound). Fish were obtained from an earthen experimental pond at the Thad Cochran National Warmwater Aquaculture Center. Two weeks after stocking, the water flow was stopped and malathion was introduced into each tank as an acetone solution to produce four final concentrations of the pesticide: 60, 12, 2.4, and 0.48 ppb. Each concentration was replicated three times. Malathion solutions were prepared by adding 2.125 grams of technical-grade malathion (96.5% active ingredient) to 25 milliliters of acetone to form a working stock solution. Water was not exchanged during the first 48 hours of the study. Water flow (fresh well water) was resumed after the initial period of static exposure and was maintained at a rate of 9.5 liters per minute for the duration of the study.

Water samples (1,000 milliliters) were taken from each replication 15 minutes after dosing to establish the exposure level for each tank. Other collections (fish and water) were composite samples taken after 1, 2, 3, 5, 7, and 14 days. Water composites were made by combining approximately 500 milliliters of tank water from each of the replicate tanks into 3-liter, pesticide-residue-quality glass containers (Teflon lined lids) containing 200 milliliters of methanol. Fish (fillet and carcass) composites were made by combining two fish from each tank at each dosing level (six fish total). Fillet samples consisted of one skinned shank fillet per fish, and carcass samples consisted of a portion of the fish body extending posterior from the operculum to the adipose fin. Each composite was placed in a labeled freezer bag. Four composite samples of water (one composite per dosing level) and one composite sample of fish (one fish from each tank) were collected before dosing to establish background malathion levels. The water samples taken 15 minutes after dosing were placed on ice and transported to the Mississippi State Chemical Laboratory for immediate extraction and analysis. All other samples were frozen (-20^{o}C) and later transported on ice to the Chemical Laboratory for extraction and analysis.

The test was statistically analyzed (water analyses data) as a two-way factorial (Dose x Time) with unequal replication, and differences were determined by LSD ($p = 0.05$). Data were subjected to regression analysis using log (malathion concentration in water) as the dependent variable and log (time) as the independent variable (i.e. log Y - log X trend).

Influence of Fish

Another study was conducted in two circular fiberglass tanks under conditions similar to those described in the section on Rate and Exposure Time. The purpose was to measure the influence of the presence of catfish on degradation of malathion in water. One tank was filled with 1,360 liters of well water and stocked with 25 channel catfish (approximately 454 grams per fish). The other tank was filled with 1,360 liters of well water but was not stocked with fish. Water pH in both tanks was 8.9. After stocking, the water flow to both tanks was stopped and malathion was added to each tank. The target dose was 60 ppb, but analysis of the

malathion/acetone stock solution indicated the actual dose level in each tank was at 46 ppb.

Water samples (1,000 milliliters) were collected from both tanks 15 minutes and 6, 24, and 48 hours after the malathion stock solution was introduced to each tank. Fish samples (two fish per tank) were collected after 24 and 48 hours. Before dosing, water and fish samples were collected from each tank to establish background levels of malathion. Fish and water samples were processed as previously described. The 15-minute and 6-hour water samples were extracted and immediately transported to the Chemical Laboratory for analysis. Remaining samples were placed in glass containers filled with 200 milliliters of methanol, stored at -20^{o}C, and later transported on ice to the Chemical Laboratory for extraction and analysis.

The test was statistically analyzed (water analyses data) as a two-way factorial (Fish Presence x Time) with the interaction used as an error term and mean differences determined by LSD ($p = 0.05$). Data were subjected to regression analysis with log (malathion concentration in water) as the dependent variable and log (time) as the independent variable.

Influence of pH

Effect of water pH on malathion degradation was evaluated in glass containers without fish. Two glass containers were filled with 3,500 milliliters of well water (pH 8.9); the pH of water in one beaker was adjusted to 7.0 using hydrochloric acid. Source of well water was a deep well at the Warmwater Aquaculture Center. A working stock solution of malathion and acetone was added to the water in both containers to create a final concentration of 42 ppb. The water was constantly stirred using a magnetic stir bar, and the water temperature was maintained at 24^{o}C.

Water samples (500 milliliters) were collected from each container after 15 minutes and 6, 24, and 48 hours. The 15-minute and 6-hour samples were extracted and analyzed immediately after collection. The remaining samples were placed in glass containers filled with 200 milliliters of methanol, stored at -20^{o}C, and later transported on ice to the Chemical Laboratory for extraction and analysis.

A second study of pH effect was conducted with deionized distilled water at the MSU Chemical Laboratory. Water pH was adjusted to 7.0 with a 1% hydrochloric acid solution and to 8.9 with a 1% ammonium hydroxide solution. The water was heavily buffered with a phosphate buffer to stabilize pH. Initial concentration of malathion in pH 8.9 water was 60 ppb. Two initial concentrations of malathion were used in pH 7.0 water: 60 ppb and 12 ppb. Water samples for analysis were taken within 15 minutes after dosing and at 1, 2, 4, 8, 24, and 48 hours after dosing.

Both of these studies of pH influence were statistically analyzed as two-way factorials (pH/Dose x Time) with the interaction used as an error term and mean differences determined by LSD ($p = 0.05$). Data from the first pH experiment were analyzed by regression analysis with log (malathion concentration in water) as the dependent variable and time as the independent variable (i.e., log Y - linear X trend). Data from the second pH experiment were analyzed by regression analysis with log (malathion concentration in water) as the dependent variable and log (time) as the independent variable.

Pond Studies of Uptake and Clearance

Pond Water

Influences of pond water, pond sediment, and water pH on malathion degradation were studied in the laboratory in glass containers. Pond water and sediment were obtained from Warmwater Aquaculture Center research ponds. Sediment was composed of the soil particles and incidental organic components from the bottom of the earthen ponds. Three treatments included (1) deionized distilled water at a pH of 7.0, (2) pond water at a pH of 7.85, and (3) pond water and sediment at a pH of 7.85. Water was not buffered in any of the treatments. The pH of deionized distilled water was adjusted with hydrochloric acid, but the pH of pond water was not adjusted. Pond water was collected around noon, which is before peak pH for the day. Malathion dosage in all treatments was 51 ppb. Water samples for analysis were taken within 15 minutes after dosing and at 6, 24, and 48 hours after dosing.

The experiment was statistically analyzed as a two-way factorial (Water x Time) with the interaction used as an error term, and mean differences were determined by LSD (p = 0.05). Data were subjected to regression analysis with log (malathion concentration in water) as the dependent variable and log (time) as the independent variable.

Fish in Ponds

Two experiments were conducted in earthen research ponds at the Warmwater Aquaculture Center. Pond A (0.1 hectare) and pond B (0.3 hectare) were stocked with production-run channel catfish. Each pond was dosed with 3.8 liters (1 gallon) of malathion acetone solution (containing 88.8 grams of technical-grade malathion) to create an estimated final concentration of 82 ppb for pond A and 35 ppb for pond B. Both ponds were dosed on June 2, 1998. Aliquots of the solution were distributed across the ponds. Calculated dose levels were based on the estimated volume of pond water. Pond water was continuously mixed with electrical aerators to help ensure even distribution of the malathion and prevent stratification of the water.

Water samples were collected from both ponds 1 hour after dosing. Water and fish samples from the pond dosed with 35 ppb were collected after 6, 24, 48, 72, and 168 hours. Water and fish samples from the pond water dosed with 82 ppb were collected after 17, 48, 72, and 168 hours.

Water samples (1,000 milliliters) were collected approximately 15.2 centimeters (6 inches) below the pond surface. Samples came from the middle of each pond and from the four corners of each pond approximately 1.2 to 1.5 meters (4 to 5 feet) from the pond bank. Each collection was given a designation: site 1, northwest corner; site 2, northeast corner; site 3, southeast corner; site 4, southwest corner; and site 5, center of pond. Each water sample was placed in separate glass containers.

Water samples collected at 1 and 6 hours after dosing pond B (35 ppb dose) and at 1 hour after dosing pond A (82 ppb dose) were extracted onsite. Subsequent water samples were placed in glass containers filled with 200 milliliters of methanol and frozen for transport to the MSU Chemical Laboratory. Fish (fillet and carcass) composites were made by combining six fish collected from each pond. Fillet and carcass samples were processed as previously described and stored at -20^{o}C. Fish and water composites from pond A were collected before dosing to establish background levels of malathion.

The two experiments were statistically analyzed as randomized complete blocks with sample sites treated as replicates. Data were subjected to regression analysis with log (malathion concentration in water) as the dependent variable and log (time) as the independent variable.

Analytical Methods, Sample Handling, and Preparation

Instrumentation and Materials

Gas chromatographic analyses were performed with a Varian 3600 gas chromatograph equipped with dual electron capture detectors, a Varian 8100 autosampler, and a Varian Star workstation using Star 4.02 software. The injection was split onto two columns for simultaneous analysis and confirmation. The primary column was a J&W DB-5 megabore (30-meter x 0.53-millimeter inside diameter, 1.5-micron film thickness), and the confirmatory column was a DB-608 megabore (30-meter x 0.53-millimeter inside diameter, 0.83-micron film thickness). The carrier was hydrogen, and the makeup gas was nitrogen.

Temperature was maintained at 230^{o}C in the injector and at 300^{o}C in the detector. Column temperature was programmed from 150^{o}C (5 minutes hold), to 170^{o}C at five per minute (10 minutes hold at 170^{o}C), then to 220^{o}C at 10 per minute (held at 220^{o}C for 15 minutes).

Water Sample Handling and Preparation

Water samples were usually extracted immediately or packed in ice and transported to the laboratory within a few hours. Samples that could not be handled in this manner were added to 3.8-liter glass containers along

with 200 milliliters of methanol and placed in a freezer for storage until transportation to the lab was possible. A 23-day storage study was performed, and it was found that recoveries were quantitative over this time. The frozen samples were held no more than 9 days before analysis. Water samples were usually 1,000 milliliters. In some cases, composites of 1,500-milliliter total volume (500 milliliters per sample) were analyzed. Single samples of 500 milliliters were analyzed on occasion. In these latter cases, the reagents used in the extraction were scaled proportionally.

Typically, 1 liter of water was measured into a separatory funnel. Sodium chloride (100 grams) and 50 milliliters of phosphate buffer (0.1M K_2HPO_4, pH 7) were added and the pH adjusted to 7.0 with 6N H_2SO_4 or 6N NaOH before addition of the surrogate standard. The sample was then extracted with three 60-milliliter portions of methylene chloride and shaken each time for 2 minutes. The extracts were dried by passing each through anhydrous sodium sulfate, combined, and exchanged into hexane for analysis by GC/ECD. Quality control consisting of at least one blank water and one malathion-spiked water was run with each set.

Fish Sample Handling and Preparation

Fish were divided into two portions: fillets and carcasses. The fillets were from muscle posterior to the body cavity (skinned shank fillet), and the carcass samples were obtained by taking the entire section of each fish body from a point just posterior of the operculum to the adipose fin. These samples were packaged separately and frozen until they were ground for analysis in the laboratory. All cutting and cleaning utensils and surfaces were rinsed with acetone and petroleum ether before use.

A 5-gram sample of the ground tissue was thoroughly mixed with 65 grams of sodium sulfate and continuously extracted with hexane in a Soxhlet extractor for at least 7 hours. The extract was concentrated by rotary evaporation and transferred to a test tube. The lipid obtained in this process was dissolved in petroleum ether (5 milliliters), and this solution was partitioned four times with acetonitrile saturated with petroleum ether (30 milliliters). The extracted petroleum ether solution was washed twice with 100 milliliters of water, dried by passing the petroleum ether extracts through a bed of anhydrous sodium sulfate, and concentrated. Then, the entire sample was transferred to a glass chromatographic containing 20 grams of activated Florisil. The column was eluted with 200 milliliters of 15% diethyl ether/85% petroleum ether followed by 200 milliliters of 50% diethyl ether/50% petroleum ether. Malathion elutes in the second fraction. Both fractions were concentrated separately to 10 milliliters and analyzed by gas chromatography. Quality control consisting of at least one blank tissue and one malathion-spiked tissue was performed with each set. Fillets and carcasses were treated alike.

Results

Results of Laboratory Studies of Uptake and Clearance

Rate and Exposure Time

Data indicating the influence of dosage rate on residual malathion in water are summarized in Table 1. Negative regression slopes for the 60-ppb and 12-ppb doses were significant (Ho: slope = 0). Slope for the 2.4-ppb dose was non-significant ($p = 0.087$), and slope for the 0.48-ppb dose was non-significant ($p = 0.53$). Malathion concentrations were predicted to reach the quantification limit of 0.02 ppb at 2.42 days after dosing for the 60-ppb dose; 2.21 days, 12-ppb; 2.10 days, 2.4-ppb; and 1.91 days, 0.48-ppb. These data indicate rapid degradation of malathion applied to water of pH 8.9 in fiberglass tanks. Water samples taken 0, 1, and 2 days after dosing were from dosed water held static. Samples taken on days 3, 5, 7, and 14 were after well water flow (9.5 liters per minute) was restarted. When the analyses showed no detection of malathion in water for a sample date for all doses, no further analyses were done on samples taken at later dates.

Carcasses of the fish analyzed after 1, 2, and 3 days at all four dose levels had no detectable levels of malathion. Chemical analyses of the fish from 5, 7, and 14 days after dosing were not performed because after the second day there was no further potential for exposure, and the earlier samples had no residues of malathion. The quantification limit in fish was 0.02 ppm. Fillets analyzed after 1 day at all four levels had no detectable residues of malathion.

Influence of Fish

Table 2 summarizes data showing disappearance of malathion from fiberglass tanks with and without channel catfish. There were no statistically significant differences in the slopes for the treatment with fish and the treatment without fish. Negative regression slope of the no-fish treatment was not different from 0. Slope of the fish-present treatment was significantly different from 0. Concentrations averaged across both treatments for each sample time showed significant differences in mean concentrations ($p = 0.05$). The average concentration of 36.5 ppb at the 0-hour sample time was significantly higher than for samples at 6, 24, and 48 hours. The average concentration of 15 ppb at the 6-hour sample time was significantly higher than for samples at 48 hours. The predicted time after dosing for malathion concentrations to reach 0.02 ppb (quantification limit) was 416.7 hours for the treatment without fish and 60.2 hours for the treatment with fish. The 416.7-hour time to reach 0.02 ppb for the no-fish treatment was unrealistically beyond the range of data. This suggests that sample times after dosing should have been extended beyond 48 hours to improve model prediction.

Influence of pH

Data showing degradation of malathion in well water of pH 7.0 and pH 8.5 (in glass containers) are summarized in Table 3. Difference in the negative regression slopes for the two treatments was significant ($p = 0.03$). Negative regression slopes for both treatments were highly significant (Ho: slope = 0). The predicted time after dosing for malathion concentrations to reach 0.02 ppb (quantification limit) was 98.3 hours for pH 7.0 and 59.9 hours for pH 8.5. These data show rapid degradation of malathion applied to water of both pH 7.0 and pH 8.5. Data also show a significant increase in degradation rate in water at the higher pH.

Data showing influence of deionized distilled water and water pH are summarized in Table 4. There were few significant differences when malathion concentrations for different sample times after dosing were averaged across treatments. There was no statistically significant difference in average concentration (41.1 ppb) at 0 hour after dosing and average concentration (34.3 ppb) at 48 hours after dosing (LSD, $p = 0.05$). These findings indicate a much slower rate of malathion degradation than occurred in the other tests. Regression slopes for the three treatments were essentially flat and not significantly different from 0. Buffers used in the experiment increased ionic strength in the water and may have enhanced stability of malathion.

Results of Pond Studies of Uptake and Clearance

Pond Water

Table 5 summarizes results of tests concerning the influences of pond water and pond sediments on malathion degradation. Slopes for the three treatments were not significantly different. Comparison of concentrations averaged for sample times across the three treatments showed significant differences ($p = 0.05$). The average concentration of 44 ppb at 0-hour after dosing was significantly different from the average concentrations at 6, 24, and 48 hours after dosing. Average concentrations of 31.7 ppb at the 6-hour sample time and 28.3 ppb at the 24-hour sample time were significantly higher than the average concentration (21.3 ppb) for the 48-hour sample time. The negative regression slopes for the three treatments were all significantly different from 0 ($p < 0.05$). However, the regression equations for the three treatments all predicted 0 ppb at very long times after dosing. A linear regression model predicted much shorter times to reach 0.02 ppb: 137.9 hours for laboratory water; 86.9 hours, pond water; and 84.7 hours, pond water with sediment. Although the linear model predicted shorter time to reach 0.02 ppb, the model fit was not as good as the log-log model. Additional sample times beyond 48 hours will be required to model this system adequately.

Fish in Ponds

Table 6 summarizes data showing the degradation of malathion in water in pond B, which was dosed initially with approximately 35 ppb of malathion. Table 7 summarizes data for pond A, which was dosed initially with approximately 82 ppb of malathion. These data show rapid significant ($p = 0.05$) reduction in concentrations of malathion in pond water. In both treatments, the average concentration detected 1 hour after dosing was significantly higher than concentrations detected 22 hours or longer after dosing. Malathion was not detected in water samples taken from pond B at 48 hours after dosing or in samples taken from pond A at 168 hours (7

days) after dosing. Using equations for the log-log trend, the predicted time after dosing for concentrations to reach the quantification limit of 0.02 ppb was 100.23 hours for the 35-ppb dose and 218.24 hours for the 82-ppb dose. These data show that malathion degraded rapidly in the earthen ponds under typical environmental and production conditions. Pond water temperature was not measured in this study, but a nearby pond of similar dimensions had a water temperature of 32^{o}C on June 2, 1998 (date of dosing). Water in most laboratory studies was allowed to adjust to an ambient laboratory temperature (about 21^{o}C).

Tables 8 and 9 summarize data from analyses of channel catfish fillets and carcasses of fish grown in ponds A and B. These data show essentially no detectable level of malathion in catfish fillets or carcasses at any sample time after dosing. One composite carcass sample from the high-dose pond showed a trace of malathion 17 hours after dosing, but it was less than 0.01 PPM, which is below the limit of quantification.

Discussion

Probability of Malathion Residue Problems in Catfish

The results of these studies show that when catfish ponds were exposed to relatively high levels of malathion, detectable levels of malathion did not accumulate in the tissues of channel catfish. Malathion was found to degrade rapidly in water in most experiments conducted during this study. Certain studies suggested the need for additional sample times to predict degradation to the limit of quantification adequately. Malathion was probably stabilized by buffers used in one study in which malathion degradation was slow. Water pH influenced malathion degradation in that there was more rapid degradation at higher pH levels. Factors, unmeasured in this study, that may contribute to increasing malathion degradation in pond water are exposure to sunlight and warm water temperatures. Detectable residues in catfish are unlikely to occur due to application of malathion ULV in the Mississippi boll weevil eradication program. In fact, our studies found only one fish sample that showed a trace of malathion residue. This sample was detected in fish from a high-dose exposure using analytical methods four times more sensitive than the methods currently employed by the U.S. Food and Drug Administration. The probable level of accidental drift contamination from aerial application of malathion ULV is much lower than most of the levels tested in these studies. Careful application techniques to manage drift should eliminate problems with malathion residues in catfish.

Drift Potential of Malathion ULV Applications

Spray drift from aerial application of malathion ULV would be the most probable source of inadvertent contamination in catfish ponds. Runoff from rain or irrigation from treated fields does not flow into commercial catfish ponds.

Research conducted in June 1995 by the USDA Agricultural Research Service Application and Production Technology Research Unit at Stoneville, MS, compared the drift of malathion ULV when applied with different application parameters and with different aircraft (Mulrooney, unpublished data). Drift from application with a turbine Air Tractor 402 and a Cessna Ag Truck were compared. Three different application setups on the Ag Truck were evaluated: F&W-60, F&W-75, and normal. Drift models were developed from data obtained in the studies. These models predicted the total concentration of malathion expected in a 4-foot-deep pond adjacent to and directly downwind from the treated area. Predicted concentration from 19 swaths with the Air Tractor 402 was 14.3 ppb. Predicted concentrations from 19 swaths with the Ag Truck were 1.4 ppb for the normal application setup, 2.2 ppb for the F&W-75 setup, and 2.4 ppb for the F&W-60 setup. Smaller size of the Cessna aircraft and slower flight speed probably contributed to reduced drift compared with the larger and faster Air Tractor 402.

Such low dose concentrations from an application of malathion ULV would dissipate rapidly in catfish pond water and would be unlikely to produce detectable malathion residues in catfish.

Conclusions

Malathion appears to degrade rapidly in water. No malathion residues above the limit of quantification (0.02 PPM) were detected in catfish carcasses and fillet samples in this study. Therefore, aerial application of malathion ULV in the Mississippi boll weevil eradication program is unlikely to cause detectable malathion residues in catfish grown in adjacent commercial ponds.

Acknowledgements

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