North American Journal of Fisheries Management
Publication details, including instructions for authors and subscription information:
http://afs.tandfonline.com/loi/ujfm20

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Published online: 27 May 2015.

North American Journal of Fisheries Management 35:616–620, 2015

ISSN: 0275-5947 print / 1548-8675 online
DOI: 10.1080/02755947.2015.1017127

MANAGEMENT BRIEF

Effectiveness of Two Commercial Rotenone Formulations in the Eradication of Virile Crayfish *Orconectes virilis*

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Abstract
The virile or northern crayfish *Orconectes virilis* is an invasive species throughout much of the USA, damaging aquatic communities where it is introduced. Therefore, identification of effective methods for its eradication from areas in which it is unwanted is important. We studied the effectiveness of two commercial formulations of rotenone, Chem Fish Regular and CFT Legumine, for virile crayfish control. Although both formulations were effective for fish eradication, earlier observations by fisheries managers suggested that the relative effectiveness of the two formulations differs for crayfish. The only noteworthy difference between the formulations is that the former contains a synergist. In our first experiment, we tested each toxicant at the maximum labeled dosage (5 ppm) and found CFT Legumine to be 100% ineffective (0% mortality), while the Chem Fish Regular treatment resulted in 12.5% mortality. After we deemed Chem Fish Regular to be the only toxicant with any effectiveness against virile crayfish, we tested concentrations from 5 to 50 ppm and found 10 times the maximum labeled dosage (50 ppm rotenone) was needed to kill all virile crayfish. Because crayfish burrow and can leave water, and because 100% eradication is usually desired, rotenone applied at the labeled rates will not be effective for crayfish control. However, treating a body of water with CFT Legumine to eradicate invasive fish while leaving desirable crayfish unharmed is possible.

Aquatic managers are consistently interested in finding new tools to control nuisance species. One such species that has become a substantial nuisance to those managing North American lakes and rivers is the virile or northern crayfish *Orconectes virilis*. The virile crayfish has expanded into many environments, especially across western North America, where it has major management implications (Hobbs et al. 1989; Phillips et al. 2009; Larson et al. 2010). When virile crayfish are introduced to new environments, they often become invasive. These invasive populations can adversely affect the abundance and diversity of aquatic plants (Lorman and Magnuson 1978; Chambers et al. 1990) and benthic macroinvertebrates (Moody and Sabo 2013) in ponds and streams. In addition, competition for food with introduced virile crayfish has been shown to lead to reduced growth in endemic species (Carpenter 2005). Furthermore, virile crayfish have been known to eat fish eggs (Dorn and Wojdak 2004), other macroinvertebrates (Hanson et al. 1990), and juvenile reptiles and amphibians (Fernandez and Rosen 1996). To mitigate the detrimental effects of virile crayfish, the ability to control or eradicate them from undesired locations is needed. Rotenone treatment of water bodies has been considered as one method for their eradication.

Much literature is available describing the effects of different rotenone formulations on fish (e.g., Finlayson et al. 2010), but much less is known about the effects of different rotenone formulations on crayfish. Bills and Marking (1988) found that twice the concentration (i.e., 10 ppm) of Noxfish (5% rotenone) recommended by the manufacturer for fish eradication (5 ppm) resulted in 100% mortality of rusty crayfish *O. rusticus* in laboratory tests. Farringer (1972) tested Noxfish on the calico crayfish *O. immunis* and found the concentration at which 50% died (LC50) at 24 and 96 h to be 34.50 and 1.02 mg/L, respectively, for soft water (40–48 mg/L $CaCO_3$)

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Received May 16, 2014; accepted February 4, 2015

and 47.20 and 1.18 mg/L, respectively, for hard water (160–180 mg/L $CaCO_3$). Fish species differ considerably in their susceptibility to rotenone. More information is needed about the efficacy of different formulations of rotenone for the control of various crayfish species. Two rotenone solutions currently in common use are CFT Legumine Fish Toxicant and Chem Fish Regular Fish Toxicant. Both are effective for fish removal; however, preliminary observations suggested that Chem Fish Regular would be effective for crayfish removal while CFT Legumine would not (J. Sorenson, Arizona Game and Fish Department, personal communication). Both fish toxicants are commercial brands containing 5% rotenone. Contained within CFT Legumine are the solvents *N*-methylpyrrolidone (NMP) and diethylene glycol ethyl ether (DGEE), which help reduce petroleum hydrocarbons and increase the water solubility of rotenone. Currently, *N*-methylpyrrolidone is used as a pharmaceutical solvent for oral ingestion, and neither NMP nor DGEE have been found to bioaccumulate according to the Toxicology Data Network (http://toxnet.nlm.nih.gov). Chem Fish Regular is listed as a 5% emulsifiable concentrate of rotenone that contains emulsifiers, which it labels only as "other associated resins," and a synergist, piperonyl butoxide according to the Canadian Pest Management Regulatory Agency (http://www.hc-sc.gc.ca/ahc-asc/branch-dirgen/pmra-arla/index-eng.php) and the U.S. Environmental Protection Agency (http://www.epa.gov/pesticides/chem_search/ppls/001439-00157-19940217.pdf). Therefore, the underlying difference between the two rotenone formulations is the presence of a synergist (piperonyl butoxide) in Chemfish Regular and not in CFT Legumine. The synergist by itself does not have pesticidal properties, but it increases the potency of pesticides by inhibiting cytochrome P450, allowing increased metabolism of these chemicals (Porte and Escartin 1998; Moores et al. 2009).

Our goal was to test whether either rotenone formulation was effective for eliminating virile crayfish and, if so, at what concentrations. These results could help managers evaluate the effectiveness of different rotenone formulations and concentrations on virile crayfish. If we could show one formulation to be completely ineffective at crayfish removal, then perhaps it could be considered for fish eradication in habitats where desired crayfish (or other invertebrates) are also present.

METHODS

Experiment 1: effectiveness of different fish toxicants.—Virile crayfish were collected using baited minnow traps from Patagonia Lake and Rose Canyon Lake, both located in southern Arizona. One hundred-sixty crayfish were needed for experiments, but to account for any unexpected mortality, 180 crayfish were caught and transported back to holding tanks at the University of Arizona's Environmental Research Laboratory (ERL). The holding tanks were filled to 75 L with filtered municipal water and held at approximately 25°C.

Treatments were initiated in a 36-tank recirculating system described by Widmer et al. (2006) and Recsetar and Bonar (2013). Each tank was equipped with an air stone, a biological sponge filter, and equal amounts of cracked clay pots to provide cover for the crayfish. Air to the sponge filter was supplied by flexible vinyl aquarium tubing, which allowed the filter to denitrify the water in each tank. Crayfish were randomly placed into each of 32 tanks until each contained five. The remaining four tanks served as holding tanks for the additional crayfish.

Rotenone can be applied at any temperature; however, it is most toxic at higher temperatures and persists a shorter time in the environment than at lower temperatures (Meadows 1972; Finlayson et al. 2010). Therefore, tank water temperatures were maintained at approximately 25°C during the acclimation period. Water from hot and cold head tanks was mixed to 25°C via Hass K-series intellifaucets (Hass Manufacturing, Averill Park, New York) and pumped into each tank for 3 min every half hour to help eliminate wastes and maintain the desired acclimation temperature. Overflow from each tank traveled to a homemade sump tank filled with plastic biofilter media and filter screens, through a UV sterilizer (COM6390-UL; Emperor Aquatics, Pottstown, Pennsylvania) and two Pentair cartridge filters (CC75; Moonpark, California) before being pumped back to the head tanks equipped with heaters and chillers. Crayfish were acclimated for 14 d and fed to satiation with Hikari brand sinking wafers once per day. Crayfish were also placed on a 12 h light:12 h dark light cycle. Recirculation of system water was halted just prior to rotenone treatment.

Three treatments were randomly assigned to the tanks so that there were eight replicates for each treatment, thereby utilizing 24 of the 36 tanks. The first treatment was a control and no rotenone was used. In the second treatment, we used CFT Legumine administered at the maximum labeled dose (5 ppm). In the third treatment, we used Chem Fish Regular measured to the maximum labeled dose (5 ppm). A Goldfish *Carassius auratus* was also placed in each tank, on the opposite side of a stainless steel screen, to ensure that rotenone was active in each tank. Immediately prior to the treatment period, wastes were removed from each tank using an aquarium siphon hose system, and then appropriate concentrations of rotenone were administered. Gilderhus (1972) found that contact times of 0.5–8.0 h of 100 ppb rotenone active ingredient were required to kill various fish species tested. The half-life of rotenone ranges from 14 to 32 h at temperatures ranging from 22°C to 24°C (Gilderhus et al. 1986; Dawson et al. 1991). Therefore, our experiments were monitored over a 120-h period without any feeding or water changes taking place to ensure that all effects were observed. Mortality and any sublethal effects on the crayfish were observed in each tank. Sublethal effects included lying on their backs or sides with legs, having swimmerets or antennae that were still moving, or dragging their claws (i.e., being unable to use them). Sublethal effects would leave a crayfish vulnerable to predation or unable to compete for food or shelter. We compared all treatments using one-way ANOVA. Post hoc comparisons were done using Tukey's honestly significantly different test ($\alpha = 0.05$).

Experiment 2: identifying effects of different concentrations of the most effective rotenone formulation—rotenone administered directly to tanks without dilution.—Crayfish were again collected from Rose Canyon Lake in southern Arizona using baited minnow traps. All conditions were kept the same as the first experiment, except these treatments only utilized the most effective rotenone formulation identified in experiment 1. Immediately prior to administering the treatments, hoses and sponge filters were removed from the tanks to prevent possible confounding factors. Five different concentrations of rotenone were tested, with five replicates of each treatment randomly assigned to tanks. The rotenone was administered using a pipette so that the five treatments would test rotenone effectiveness at 5, 10, 15, 20, and 25 ppm. The rotenone was vigorously mixed in the tanks using a glass stirring rod. Five randomly selected tanks served as a control and were also mixed using the glass stirring rod. Mortality was again observed over a 120-h test period and recorded after 8, 24, 48, 72, 96, and 120 h. We also noted sublethal physical effects from the rotenone. After the test period, all crayfish were measured for carapace length and sexed (male or female) to make sure size and sex were not confounding results.

Experiment 3: identifying effects of different concentrations of the most effective rotenone formulation—rotenone administered to tanks with prior dilution.—For a third time, crayfish were collected from Rose Canyon Lake in southern Arizona. All conditions were kept the same as in the first experiment except for two changes. First, sponge filters were taken out of the tanks just prior to rotenone administration. Second, instead of administering rotenone directly to the tanks, it was added to a 1,000-mL beaker filled with 300 mL of tank water and vigorously stirred before being applied. This was done to better emulsify the rotenone. Engstrom-Heg (1972) theorized that, at concentrations above 3.4 ppm, rotenone goes into a colloidal state, suggesting that it is wasteful to apply rotenone at any higher concentrations. Therefore, better mixing of the rotenone containing the synergist may make it more effective. The previously emulsified rotenone was evenly distributed across the water's surface in each tank by pouring. The concentrations of rotenone in this experiment were the same as those in experiment 2 except they also included seven times (35 ppm) and 10 times (50 ppm) the maximum labeled dosage treatments. The experiment was monitored over 96 h in the same manner as the previous experiment, and all crayfish were sexed and measured upon completion. The concentration at which total mortality occurred was recorded.

RESULTS

Experiment 1: Effectiveness of Different Fish Toxicants

After 72 h of treatment with the maximum labeled dosage (5 ppm) of CFT Legumine, there was 0% mortality and crayfish appeared to be unaffected by exposure to the chemical. The Chem Fish Regular treatment had a mean mortality of 12.5% (SE, 2.35%) after 120 h. Including sublethal effects, mean effectiveness was 15.0% (SE, 3.27%). No physical effects developed after a 72-h exposure. All Goldfish died within 1 h of all treatments. No crayfish or Goldfish died in the control tanks. Mean mortality in the Chem Fish Regular treatment was significantly higher ($P < 0.05$) than that in CFT Legumine and the control.

Experiment 2: Identifying Effects of Different Concentrations of the Most Effective Rotenone Formulation—Rotenone Administered Directly to Tanks without Dilution

Chem Fish Regular was used in all further treatments, as it was identified as the more effective rotenone formulation. There was 0% mortality in the control and in the 5-ppm Chem Fish Regular rotenone treatment. There was 24% (SE, 6.57%), 4% (1.79%), 20% (3.58%), and 24% (4.38%) mortality in the 10-, 15-, 20-, and 25-ppm Chem Fish Regular treatments, respectively. In the 25-ppm treatment, 4% of subjects experienced sublethal effects, making it 28% (SE, 4.56%) effective when this is combined with the mortality data. None of the fish in the other treatments experienced any sublethal effects. Goldfish died in every tank except the control tanks. We found no significant difference between treatments ($F = 2.242$; df = 5, 24; $P = 0.083$). Although we found that treatments with 10, 15, 20, and 25 ppm rotenone were at least partially effective, their effectiveness was not consistent within treatments. We were unable to establish a lethal dose that resulted in 100% mortality, but there did appear to be a greater effectiveness as the rotenone concentration was increased.

Experiment 3: Identifying Effects of Different Concentrations of the Most Effective Rotenone Formulation—Rotenone Administered to Tanks with Prior Dilution

No mortality occurred in the control or the 5-ppm Chem Fish Regular rotenone treatment (Figures 1, 2). We found 8% (SE, 2.19%), 12% (3.58%), 76% (5.22%), 68% (6.07%), and 76% (5.22%) mortality in the 10-, 15-, 20-, 25-, and 35-ppm Chem Fish Regular 5-d treatments, respectively. There was an additional 12% (SE, 5.37%) of crayfish exhibiting sublethal effects in the 35-ppm treatment. No crayfish died after the 5-d period that did not show sublethal effects within the first 24 h of treatment. In the 50-ppm rotenone treatment, we observed 100% mortality, all of which occurred within the initial 48 h

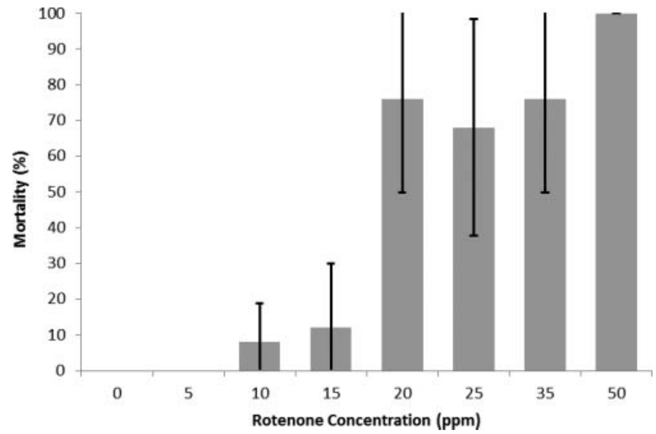

FIGURE 1. Mortality of virile crayfish after 5-d (120-h) treatments with varying concentrations of Chem Fish Regular; the error bars = SEs.

of treatment (Figures 1, 2). Mortality differed significantly among treatments ($F = 25.024$; df = 5, 32; $P < 0.0001$).

DISCUSSION

In the first experiment, in which we compared the effects of different chemical treatments on virile crayfish, we saw that a maximum dosage of CFT Legumine had no effect on crayfish survival while Chem Fish Regular administered at the maximum dosage was 12.5% effective. The most effective rotenone formulation, Chem Fish Regular, required 10 times the maximum labeled dosage for it to be 100% effective against crayfish. As total eradication of invasive virile crayfish is almost always the goal, this method of treatment appears to be impractical. Furthermore, our experiments were conducted in bare tanks. Sediment in ponds reduces the effectiveness of rotenone (see Gilderhus et al. 1986), so effective concentrations in natural settings would be higher still. Also, crayfish can burrow into the sediment (Hazlett et al. 1974) and move out of water bodies (Byron and Wilson 2001), so all individuals may not be vulnerable to chemical treatment. In addition, rotenone did not have an instantaneous effect, as it does with fish. Our experiments showed that even at 10 times the maximum dosage (50 ppm), 72 h passed before all subjects died (Figure 1). Rotenone causes death at the cellular level and not at the water–blood interface (Ling 2003). Therefore, we might expect death by tissue anoxia to take longer and probably require higher concentrations of rotenone in crayfish, which have an open circulatory system. Taken together, these factors suggest that using either of the two rotenone formulations that we tested would be completely ineffective for the removal of virile crayfish. However, the use of CFT Legumine to eradicate invasive fish while leaving desirable crayfish unharmed is possible where this is the goal.

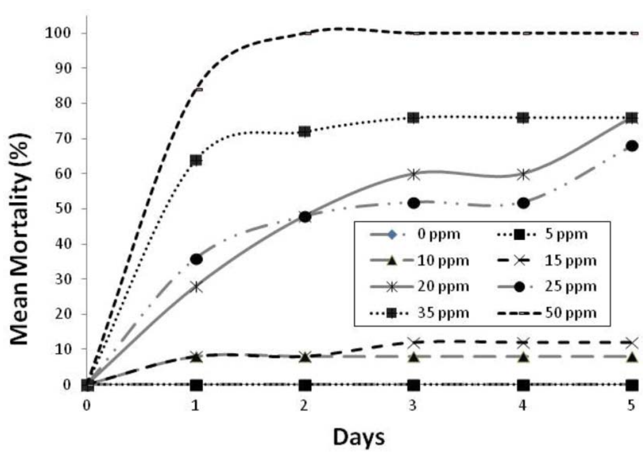

FIGURE 2. Mean mortality of virile crayfish over 5-d treatments with Chem Fish Regular at different concentrations.

Our study suggests that managers can remove rotenone from consideration for controlling virile crayfish and concentrate on other control techniques. Trapping has proven to be an effective way to catch and eliminate many crayfish from an unwanted location (Bills and Marking 1988; Hein et al. 2006; Rogowski et al. 2013), but few argue that it can provide 100% eradication. However, crayfish suppression is possible with trapping if conducted with considerable sustained effort or integrated with other control methods such as fish predation (Rogers et al. 1997).

A variety of other chemicals have been evaluated for crayfish control (e.g., Farringer 1972; Bills and Marking 1988; Hyatt 2004; Reynolds and Souty-Grosset 2011; Ward et al. 2013; A. M. Kelly and K. C. Anup, paper presented at the annual meeting of the World Aquaculture Society, 2012). Compared with other methods, biocides currently show the best promise for control of invasive crayfish (Hyatt 2004). A few compounds have emerged as most cost effective and easy to use. Derivatives of natural pyrethrum, such as Pyblast (Reynolds et al. 2011), have successfully been used to remove crayfish. Natural pyrethrum was first used to clear aquatic crustaceans water hoglouse *Asellus aquaticus* from public water mains and is still used for this purpose (Reynolds et al. 2011). Recent examples of crayfish–aquatic crustacean control chemicals tested include bifenthrin, liquid ammonia, and the cypermethrin-based compound BETAMAX VET. Bifenthrin is a pyrethroid insecticide that affects the central and peripheral nervous system of insects, leading to paralysis (Miller and Salgado 1985). Bifenthrin was effective against copepods in small ponds in the parts-per-billion range (most chemicals are effective in the parts-per-million range) while not harming larval Goldfish, Fathead Minnows *Pimephales promelas*, or Golden Shiners *Notemigonus crysoleucas* (Kelly and Anup, unpublished). Although not used specifically to target crayfish, its apparent effectiveness against other aquatic crustaceans suggests that it would be effective for crayfish control as well. The pharmaceutical BETAMAX VET, which is based on the synthetic pyrethroid cypermethrin, was originally used in European ponds to control salmon louse *Lepeophtherius salmonis*. The compound is widely licensed throughout Europe and was recently successful, in combination with pond draining, for controlling populations of nuisance signal crayfish *Pacifastacus leniusculus* in Scandinavian ponds (Sandodden and Johnsen 2010). Liquid ammonia (Ward et al. 2013) was successfully used to remove fish, crayfish, and tadpoles from two Arizona ponds. Because ammonia is a natural product of fish metabolism and is naturally present in the environment at low levels, it also shows promise as a biocide (Ward et al. 2013).

ACKNOWLEDGMENTS

This project was conducted with aquatic animal care and use in accordance with procedures at the University of Arizona. Funding and support for this study were provided by the Arizona Department of Game and Fish. We especially thank Jeff Sorensen, Arizona Game and Fish Department, for providing inspiration for this study and supplying us with crayfish traps and chemicals to make this study possible. We thank the staff at University of Arizona Environmental Research Laboratory, including Galen Bennett and Jeff Bliznick, for helping with maintenance of the laboratory facilities. We thank Olin Feurbacher for his assistance at the laboratory as well as Anita Kelly, University of Arkansas at Pine Bluff, for her contribution to the discussion. Reference to trade names does not imply endorsement by the U.S. Government.

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