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

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Executive Summary

- Virile or northern crayfish *Orconectes virilis,* is an invasive species throughout much of the United States. Therefore identification of effective methods for their eradication from these areas is important.
- We studied effectiveness of two commercial formulations of rotenone, Chemfish Regular™ and CFT Legumine™, for virile crayfish control. Earlier observations suggested relative effectiveness of the two formulations differed.
- The only noteworthy difference between the formulations was that the former contained a synergist. Therefore, we also administered Chemfish Regular™ with potassium permanganate to see if we could neutralize the rotenone and isolate the underlying synergist, potentially identifying a treatment effective against crayfish but not against fish.
- In our first experiment, we tested each toxicant at the maximum labeled dosage (5ppm) and found CFT Legumine™ to be 100% ineffective (0% mortality), while the Chemfish Regular™ and Chemfish Regular™ + potassium permanganate treatments resulted in 12.5% and 15.0% mortality, respectively.
- We then tested Chemfish Regular™ administered with various concentrations of potassium permanganate in containers with both goldfish and crayfish. We found it was not possible to isolate the synergist as all fish died, while no crayfish were affected.
- After we deemed Chemfish Regular™ to be the only toxicant with any effectiveness against virile crayfish we tested concentrations from 5 to 50 ppm and found it took 10x the maximum labeled dosage (50 ppm rotenone) to kill all virile crayfish (LD_{100})

- Because crayfish burrow, are able to leave the water, and 100% eradication is usually desired, rotenone applied at labeled rates will not be effective for crayfish control.
- However, treating a water body with CFT Legumine™ to eradicate invasive fish while leaving desirable crayfish unharmed is possible.
- Other chemicals such as bifenthrin, liquid ammonia, and the cypermethrin-based compound BETAMAX VET® show better promise for crayfish control.

Introduction

The range of virile crayfish *Orconectes virilis* has expanded into many environments, especially across western North America (Hobbs et al. 1989; Phillips et al. 2009; Larson et al. 2010). When virile crayfish *Orconectes virilis* are introduced to new environments, they often become invasive. These invasive populations can adversely affect abundance of aquatic plants in ponds and streams (Chambers et al. 1990; Lorman and Magnuson, 1998). 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 detrimental effects caused by virile crayfish, controlling and/or eradicating crayfish from undesired locations is needed. Resource managers are interested in possibly using pesticides to eradicate virile crayfish, but unfortunately, rotenone is not labeled for use against crayfish. However, if effective, a label change by the manufacturer may be sought.

Much literature is available describing 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 manufacture for fish eradication (5 ppm) resulted in 100% mortality of rusty crayfish *Orconectes rusticus* in laboratory tests. Farringer (1972) tested effects of Noxfish on the calico crayfish *Orconectes immunis* and found LC_{50} values for 24 and 96 hours to be 34.50 and 1.02 mg/l respectively for soft water and 47.20 and 1.18 mg/l respectively for soft water. Fish species differ considerably in their susceptibility to rotenone. More information is needed about the efficacy of different formulations of rotenone for control of various crayfish species. Two types of rotenone solutions currently in common use are

CFT Legumine™ Fish Toxicant and Chem Fish Regular™ Fish Toxicant; however, neither formulation is labeled specifically for use against crayfish. Both are effective for fish removal; however, preliminary observations suggested that Chemfish Regular™ may show promise for crayfish removal, while CFT Legumine™ would not (J. Sorensen, Arizona Game and Fish Department, Unpublished Data). Both fish toxicants are commercial brands containing 5% rotenone as the active ingredient. CFT Legumine™ contains the solvents N-methylpyrrolidone (NMP) and di(ethylene glycol) ethyl ether (DGEE) which help reduce petroleum hydrocarbons and increase the water solubility of rotenone. N-methylpyrrolidone is currently used as a pharmaceutical solvent for oral ingestion and neither NMP nor DGEE have been found to bioaccumulate (Ott 2010). Chem Fish Regular™ is listed as a 5% emulsifiable concentrate of rotenone that contains emulsifiers, which it labels only as "other associated resins", and also a synergist, piperonyl butoxide (EPA 2012; PMRA 2006). Therefore, the underlying difference between the two rotenone formulations is the presence of a synergist (piperonyl butoxide) in Chemfish Regular^{TM} and not in CFT Legumine^{TM}. 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 if either rotenone formulation was effective for eliminating virile crayfish, and if so, at what concentrations. Furthermore, we wanted to combine potassium permanganate, a common agent used to neutralize rotenone, with rotenone solutions and apply it to crayfish treatments. This was to test whether a chemical in the Chemfish Regular^{TM} rotenone formulation - besides the rotenone and unaffected by the potassium permanganate - would have an underlying toxic effect on virile crayfish without affecting fish. These results could help managers evaluate effectiveness of different rotenone formulations and concentrations on virile crayfish. If we could isolate the synergist or other chemical in the formulation and show it is effective at crayfish removal, then perhaps it could be considered for crayfish eradication in habitats where desired fish are still 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. Over 180 crayfish were caught and transported back to holding tanks at the University of Arizona's Environmental Research Laboratory (ERL). The tanks were filled to 75 L and held at approximately 25°C.

Once required numbers of crayfish were attained, 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 water in each tank. Crayfish were randomly selected and placed in each of 32 tanks until all tanks contained 5 crayfish. Rotenone can be applied at any temperature; however, it is most toxic at higher temperatures and persists a shorter time in the environment (Meadows 1972; Finlayson et al. 2010). Therefore, each tank was filled to 75 L and 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 Company, 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 bio balls and filter screens, through a UV sterilizer (COM6390-UL, Emperor Aquatics, Pottstown, Pennsylvania) and 2 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. The crayfish were placed on a 12 hrs light: 12 hrs dark light cycle. Recirculation of system water was halted just prior to rotenone treatment.

Four treatments were randomly assigned to the tanks so that there were 8 replicates for each treatment, thereby utilizing 32 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). In the last treatment we administered Chem Fish Regular™ simultaneously with a 2:1 ratio of potassium permanganate, a chemical commonly used to neutralize rotenone. According to studies and field applications, a 2:1 or greater ratio is needed to neutralize rotenone unless being used in soft water with less than 20 ppm hardness (Engstrom-Heg 1972). In effect, this should have allowed us to isolate the synergist or at least neutralize the rotenone. A goldfish *Carassius auratus auratus* was also placed in each tank, on the opposite side of a stainless steel screen, to insure 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. Then appropriate concentrations of rotenone were administered. Guilderhus (1972) found that contact times of 0.5 – 8.0 hrs of 100 ppb rotenone active ingredient were required to kill various fish species tested. The half-life of rotenone ranges from 14-32 h at temperatures ranging from 22-24°C (Guilderhus et al. 1986; Dawson et al. 1991). Therefore our experiments were monitored over a 120 hr period without any feeding or water changes taking place to ensure all effects were observed. Mortality and any sublethal effects on the crayfish were observed in each tank. Sublethal effects included laying on the back or side with legs, swimmerets or antennae still moving or dragging of the claws (unable to use). We compared all treatments using one-way analysis of variance (ANOVA). Post hoc comparisons were done using the Tukey HSD ($\alpha = 0.05$).

Experiment 2: Effects of different rotenone / potassium permanganate ratios on virile crayfish and goldfish

To test different combinations of potassium permanganate and rotenone, a single crayfish and goldfish were placed in a 1000 mL beaker with an air stone, separated by a stainless steel grate. A piece of 1-cm square plastic grating was placed over the top of each beaker to prevent crayfish from climbing out. After a 24-hr acclimation period, the most effective rotenone formulation, identified from the first experiment, was administered to each beaker at 10 ppm (twice the maximum labeled dosage of 5 ppm) simultaneously with 0, 10, 20, 40, and 80 ppm potassium permanganate. This would insure that the rotenone should be completely neutralized in at least one of the treatments. There was also a control set up with no rotenone or potassium permanganate administered.

Experiment 3: 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 the same protocols as the first experiment. All conditions were kept the same as the first experiment, except the treatments this time only utilized the most effective rotenone formulation identified in experiment 1. Immediately prior to administering the treatments, hoses and sponge filters were also 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 also 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 hrs. 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.

Experiment 4: 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, rotenone was not administered directly to the tanks but for each tank, rotenone was first added to a 1000 mL beaker filled with 300 mL of tank water, vigorously stirred with a glass stirring rod and then applied. This was to better emulsify the rotenone. 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 7x (35 ppm) and 10x (50 ppm) maximum labeled dosage treatments. The experiment was monitored over 96 hr 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 hours of treatment with the maximum labeled dosage (5ppm) of CFT Legumine™ there was 0% mortality and crayfish appeared unaffected by exposure to the chemical. The Chemfish 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%). Similarly in the treatment where Chemfish Regular™ and potassium permangenate were applied simultaneously, there was a mean mortality of 15.0% (SE, 3.27%). Including subjects observed displaying sublethal effects mean effectiveness was 25.0% (SE, 4.05%) after the 5-d treatment. No physical effects developed after 72-h exposure. In one tank treated with Chemfish Regular™ + potassium permanganate treatment, three of the subjects seen on their sides/backs after 24 h appeared functional again by the next day. All goldfish succumbed to death within 1 h of all treatments. No crayfish or goldfish died in the control tanks. Mean mortality in the Chemfish Regular™ treatments and the Chemfish Regular™ + potassium permanganate were not significantly different from each other ($P = 0.537$). Mean mortality in the Chemfish Regular™ treatments and the Chemfish Regular™ + potassium permanganate were both significantly higher ($P < 0.05$) than CFT Legumine™ and the control.

Experiment 2: Effects of Different Rotenone / Potassium Permanganate Ratios on Virile Crayfish and Goldfish

Upon completion of the first experiment, CFT Legumine™ was found to be completely ineffective against crayfish at the maximum labeled dosage. However, Chemfish Regular™ and Chemfish Regular™ + Potassium Permangenate were found to be about 15% effective at the same labeled dosage. Every goldfish died within the first 8 hours of all treatments except in the control, in which they all survived. Therefore in the treatment with the thought to be 'isolated synergist', either the synergist was responsible for the 15% mortality, the potassium permanganate was itself responsible, or the potassium permanganate was ineffective at neutralizing the rotenone in the Chemfish Regular™.

No treatment in the rotenone/potassium permanganate ratio experiment was effective at killing crayfish. However, each rotenone and potassium permanganate combination tested was lethal to fish. After 1 hour, all goldfish, except for the control were dead, so a 2nd goldfish was added to each to account for a possible lag time in rotenone neutralization (Engstrom-Heg 1972). The first fish to die, almost immediately, was that in the rotenone-only treatment followed by the 80 ppm potassium permanganate treatment, then the 40 ppm potassium permanganate treatment, the 20 ppm potassium permanganate treatment and after 7 h, the 10 ppm potassium permanganate treatment. The finding of no deaths of crayfish and complete mortality of goldfish suggested our treatments for eradicaing crayfish without killing fish was not effective.

Experiment 3: Identifying effects of different concentrations of the most effective rotenone formulation: Rotenone administered directly to tanks without dilution.

Chemfish 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 Chemfish Regular™ rotenone treatment. There was 24% (SE, 6.57%), 4% (SE, 1.79%), 20% (SE, 3.58%) and 24% (SE, 4.38%) mortality in the 10, 15, 20 and 25 ppm Chemfish Regular™ treatments respectively. In the 25 ppm treatment 4% of subjects experienced sublethal effects making it 28% (SE, 4.56%) effective when combined with mortality data. None of 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 effectivenss was not consistent within treatments. We were unable to establish an LD100 from the data but there did appear to be a greater effectiveness as rotenone concentration was increased.

Experiment 4: 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 Chemfish Regular™ rotenone treatment (Figures 1,2). We found 8% (SE, 2.19%), 12% (SE, 3.58%), 76% (SE, 5.22%), 68% (SE, 6.07%) and 76% (SE, 5.22%) mortality in the 10, 15, 20, 25 and 35 ppm Chemfish 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 hr of treatment (Figures 1,2). Mortality differed significantly among treatments (F= 25.024, df = 5, 32; P = 0.00).

Discussion

In the first experiment where we compared different chemical treatments on crayfish, we saw that a maximum dosage of CFT Legumine™ had no effect on crayfish survival, while Chemfish Regular™ administered at maximum dosage had a slight effect. We also had found a slight effectiveness when potassium permanganate was added simultaneously with the rotenone. Possibly, the potassium permanganate did not react fast enough to neutralize the rotenone in the Chemfish Regular™ when added in a 2:1 ratio. Potassium permanganate is known to oxidize a wide range of organic substances (USEPA 1999) and therefore may have reacted with dissolved organics within the tanks thus limiting its effectiveness for neutralizing rotenone. Our effort to isolate the chemical synergist in the Chemfish Regular™, by performing a pilot experiment that mixed potassium permanganate at different concentrations to see if there was a specific concentration that could be used to effectively neutralize the rotenone and allow fish to survive, was unsuccessful. All potassium permanganate/rotenone combinations were lethal to the goldfish at all concentrations within a few hours and ineffective against crayfish at all dosages. Possibly, when potassium permanganate was added at higher ratios to rotenone, the strong oxidizing effect of the potassium permanganate affected fish as well. The goldfish in the 1:1 ratio (10 mg/L) treatment survived for almost 6 h, those in the 2:1 potassium permanganate/rotenone treatment survived just under 4 h; those in the 4:1 ratio treatment survived almost 1 h and those in the 8:1 treatment died within 30 min. Numerous studies have shown potassium permanganate to be toxic to fish at a range of concentrations over 3 mg/L and that its toxicity increases in harder water (Marking and Bills 1975; Golow and Godzi 1996; Straus 2004; Kori-Siakpere 2008). In addition to the toxic effects of potassium permanganate on fish, that such a strong oxidizer could neutralize rotenone while leaving piperonyl butoxide in the water to affect the crayfish seems unlikely. Piperonyl butoxide is an organic compound and would likely react with potassium permanganate as well. Therefore using potassium permanganate/rotenone combinations to affect crayfish but not fish does not seem conceivable.

The most effective rotenone formulation, Chemfish 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 excessive and unreasonable. 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 management 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, in crayfish, which has an open circulatory system, we might expect death by tissue anoxia to take longer and probably require higher concentrations of rotenone. All of these factors considered in combination suggest that using either of the two rotenone formulations we tested would be completely ineffective for removal of virile crayfish.

Our study suggests managers can remove rotenone from consideration for controlling virile crayfish and concentrate on other control techniques. Trapping is a proven and effective way to catch and eliminate many crayfish from an unwanted location (Bills and Marking 1988; Hein et al. 2006; Rogowski et al. 2013); however, few argue it can provide 100% eradication. However, crayfish suppression is possible 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 2012; Kelly and Anup 2012; Ward et al. 2013). 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. 2012) 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. 2012). 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 ppb range (most chemicals are effective in the ppm range) while not harming larval goldfish, fathead minnows or golden shiners (Kelly and Anup 2012). Although not used specifically to target crayfish, its apparent effectiveness against other aquatic crustaceans suggest it may have promise 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 nuisance signal crayfish *Pasifastacus leniusculus* populations 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).

In summary, we conclude that the two rotenone formulations we tested are ineffective for removal of virile crayfish. Much better methods exist for controlling these crustaceans. Efforts should be spent in other areas of crayfish control such as further testing of the more promising toxicants, identification of new toxicants or improvement of mechanical suppression techniques. However, use of CFT Legumine™ to eradicate invasive fish while leaving desirable crayfish unharmed is possible where this is the goal.

References

Archer, D.L, 2001. Rotenone Neutralization Methods. American Fisheries Society Publication. http://www.fisheriessociety.org/rotenone/rewards/01archer.pdf

Bills. T. D., and L. L. Marking. 1988. Control of nuisance populations of crayfish with traps and toxicants, The Progressive Fish-Culturist, 50:103-106.

Byron, C. J., and K. A. Wilson. 2001. Rusty crayfish (*Orconectes rusticus*) movement within and between habitats in Trout Lake, Vilas County, Wisconsin. Journal of the North American Benthological Society 20: 606-614.

Carpenter, J. 2005. Competition for food between an introduced crayfish and two fishes endemic to the Colorado River basin. Environmental Biology of Fishes 72:335-342.

Chambers, P. A., J. M. Hanson, J. M. Burke and E. E. Prepas. 1990. The impact of the crayfish *Orconectes virilis* on aquatic macrophytes Freshwater Biology (1990) 24, 81-91.

Dawson, V. K., W. H. Gingerich, R. A. Davis and P. A. Gilderhus. 1991. Rotenone persistence in freshwater ponds: Effects of temperature and sediment adsorption. North American Journal of Fisheries Management, 11:226-231.

Dorn, N. J., and J. M. Wojdak. 2004. The role of omnivorous crayfish in littoral communities. Oecologia 140: 150–159

Engstrom-Heg, R. 1972. Kinetics of rotenone-potassium permanganate reactions as applied to the protection of trout streams. New York Fish & Game Journal 19(1):47–58.

Farringer, J. E. 1972. The determination of the acute toxicity of rotenone and Bayer 73 to selected aquatic organisms. Master's thesis. University of Wisconsin – La Crosse.

Finlayson, B., R. Schnick, D. Skaar, J. Anderson, L. Demong, D. Duffield, W. Horton, and J. Steinkjer. 2010. Planning and standard operating procedures for the use of rotenone in fish management—rotenone SOP manual. American Fisheries Society, Bethesda, Maryland.

Gilderhus, P. A. 1972, Exposure times necessary for antimycin and rotenone to eliminate certain freshwater fish. Journal of the Fisheries Research Board of Canada 29: 199-202

Gilderhus, P. A., J. L. Allen, and V. K. Dawson. 1986. Persistance of rotenone in ponds at different temperatures. North American Journal of Fisheries Management 6:129-130

Hanson, J. M., P. A. Chambers and E. E. Prepas. 1990. Selective foraging by the crayfish *Orconectes virilus* and its impact on macroinvertebrates Freshwater Biology 24:69-80.

Hazlett, B., D. Rittschof and D. Rubenstein. 1974. Behavioral Biology of the Crayfish *Orconectes virilis* I. Home Range. American Midland Naturalist 92:301-319.

Hein, C. L., B. M. Roth, A. R. Ives, and M. J. Vander Zanden. 2006. Fish predation and trapping for rusty crayfish (*Orconectes rusticus*) control: a whole-lake experiment. Canadian Journal of Fisheries and Aquatic Sciences 63:383–393.

Hobbs, H. H. III, J. P. Jass and J. V. Huner. 1989. A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). Crustaceana 56: 299-316.

Hyatt, M.W. 2004. Investigation of crayfish control technology. Final Report, Cooperative Agreement Number 1448-20181-02-J850. Arizona Game and Fish Department, Phoenix.

Kelly, A. M., and K. C. Anup. 2012. Laboratory, mesocosm and pond acute toxicity tests of several agricultural chemicals to commonly used chemicals to copepods, larval baitfish and larval hybrid striped bass. Meeting Abstract. Aquaculture America 2012, World Aquaculture Society Annual Meeting, February 29-March 2, Las Vegas, Nevada.

Miller, T.A. and V.L. Salgado. 1985. The mode of action of pyrethroids on insects, in The Pyrethroid Insecticides. Leahy, J.P., Ed., Taylor & Francis, London. 43-97.

Larson, E. R., C. A. Busack, J. D. Anderson, J. D. Olden. 2010. Widespread distribution of the non-native virile crayfish (*Orconectes virilis*) in the Columbia River Basin. Northwest Science 84:108-111.

Ling, N. 2003. Rotenone—a Review of Its Toxicity and Use for Fisheries Management. Wellington (New Zealand): Department of Conservation. Science for Conversation Number 211.

Meadows, B. S. 1972. Toxicity of rotenone to some species of coarse fish and invertebrates. Journal of Fish Biology 5:155-163.

Phillips, I. D., R. D. Vinebrooke, M. A. Turner. 2009. Ecosystem consequences of potential range expansions of *Orconectes virilis* and *Orconectes rusticus* crayfish in Canada — a review. Environmental Reviews 17: 235-248.

Reynolds, J. and C. Souty-Grosset. 2011. Management of freshwater biodiversity. Crayfish as bioindicators. Cambridge University Press.

Reynolds, J., C. Souty-Grosset and F. Gherardi. 2011. Control and management of non-indigenous crayfish. Pages 197-218 *in* Reynolds, J. and C. Souty-Grosset, authors. Management of freshwater biodiversity. Crayfish as bioindicators. Cambridge University Press.

Rogers, W.D., D.M. Holdich, and E. Carter. 1997. Crayfish Eradication. Report for English Nature, Peterborough.

Rogowski, D. L., S. Sitko and S. A. Bonar. 2013. Optimizing control of invasive crayfish using life-history information. Freshwater Biology. http://onlinelibrary.wiley.com/doi/10.1111/fwb.12126/abstract

Sandodden, R. and S. I. Johnsen. 2010. Eradication of introduced signal crayfish *Pasifastacus leniusculus* using the pharmaceutical BETAMAX VET.®. Aquatic Invasions 5:75-81.

USEPA. 1999. Alternative disinfectants and oxidants guidance manual. EPA 815-R-99-014. U.S. Environmental Protection Agency, Cincinnati, Ohio.

Ward, D. L., R. Morton-Starner, and S. J. Hedwall. 2013. An evaluation of liquid ammonia (Ammonium hydroxide) as a candidate piscicide. North American Journal of Fisheries Management 33:400-405.

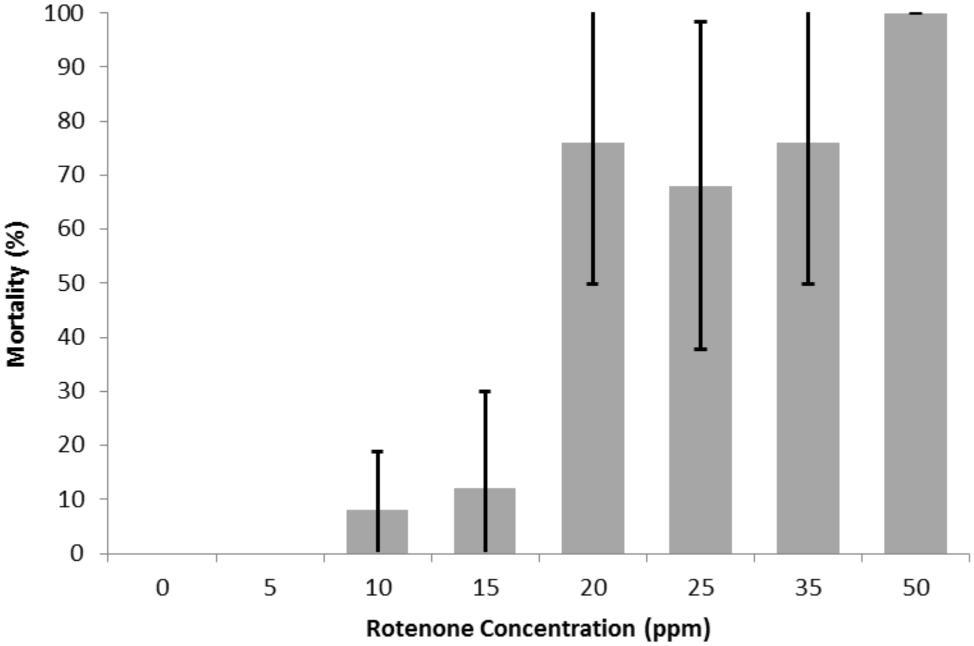

Figure 1—Mortality (Mean % and SD) of virile crayfish after 120-h treatment with varying concentrations of Chemfish Regular™

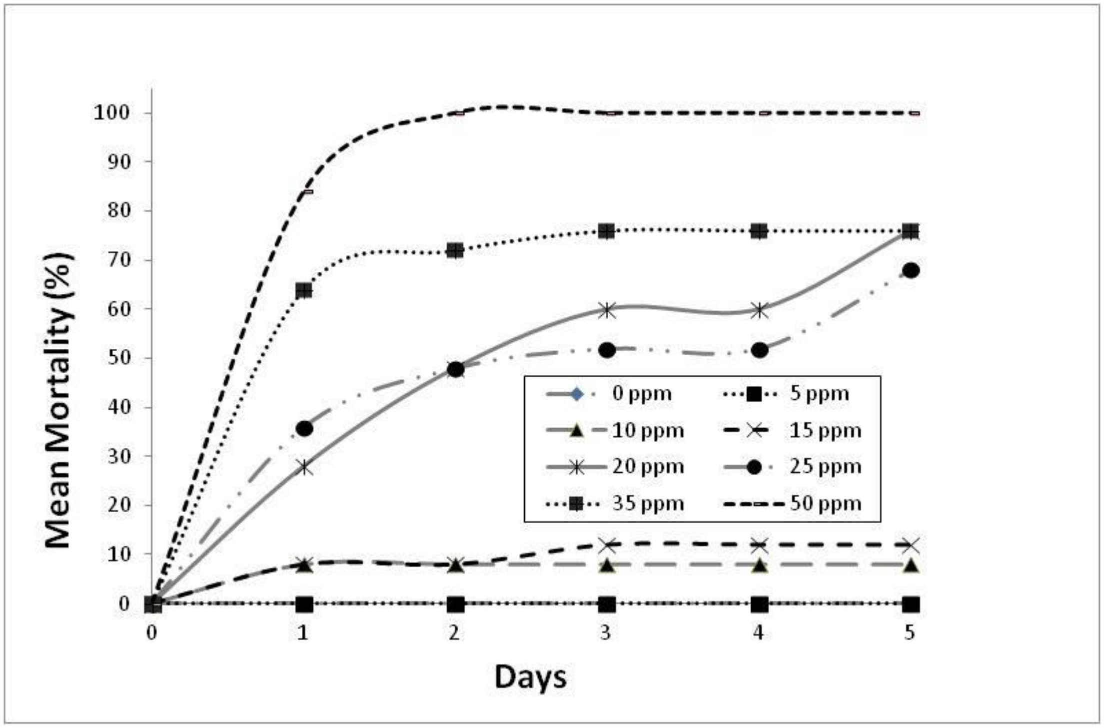

Figure 2—Mean Mortality (%) of virile crayfish over 5-d treatment with Chemfish Regular™ at different concentrations.