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Research Article

Toxicity of copper sulfate and rotenone to Chinese mystery snail (*Bellamya chinensis*)

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Abstract

The Chinese mystery snail (*Bellamya chinensis*) is a freshwater snail native to Southeast Asia, Japan, and Russia and is currently classified as an invasive species in at least 27 states in the USA. The species tolerates a wide range of environmental conditions, making management of established populations difficult. We tested the efficacy of two traditional chemical treatments, rotenone and copper sulfate, on the elimination of adult Chinese mystery snails in laboratory experiments. All snails (N=50) survived 72-hour exposure to rotenone-treated lake water, and 96% (N=25) survived 72-hour exposure to pre-determined rotenone concentrations of 0.25, 2.5, and 25.0 mg/L. All snails (N=10) survived exposure to 1.25 mg/L copper sulfate solution, 90% (N=10) survived exposure to 2.50 mg/L copper sulfate solution, and 80% (N=5) survived exposure to 5.0 mg/L copper sulfate solution. Neither rotenone nor copper sulfate effectively killed adult Chinese mystery snails in laboratory experiments, most likely due to their relatively large size, thick shell, and operculum. Therefore, it appears that populations will be very difficult to control once established, and management should focus on preventing additional spread or introductions of this species.

Key words: *Bellamya chinensis*, aquatic invasive species, chemical treatment, invasive gastropod

Introduction

The Chinese mystery snail (*Bellamya* [=*Cipangopaludina*] *chinensis* (Reeve, 1863)) is a freshwater snail native to Southeast Asia, Japan, and Russia (Jokinen 1982). Transported for both food and the aquarium trade, they first appeared in the USA in the late 1800s and were reported on the East Coast as early as 1914 (Jokinen 1982). Currently, there are known populations in at least 27 states as well as southern portions of Canada (Solomon et al. 2010). The species thrives in cool-temperate to warm-temperate climates in permanent ponds, lakes, and slow-moving portions of rivers (Jokinen 1982) and is capable of producing high-density populations (Chaine et al. 2012). After reaching a critical size, individuals of this species are capable of switching between grazing and filter feeding, allowing them to thrive in a variety of environmental conditions (Olden et al. 2013). Community-level effects vary when the Chinese mystery snail is present with other invasive species, such as the rusty crayfish (*Orconectes rusticus* (Girard, 1852)). When present alone, the Chinese mystery snail does not appear to harm native snail abundance (Solomon et al. 2010), but when present with the rusty crayfish, native snail biomass decreases (Johnson et al. 2009). Furthermore, the Chinese mystery snail may increase the water column N:P ratio in invaded lakes (Johnson et al. 2009).

There are no uniform methods for the successful management of aquatic invasive species. If prevention efforts are unsuccessful, then controlling spread is critical (Vander Zanden and Olden 2008). Manipulation of aquatic ecosystems is difficult, especially when trying to target a single species. Efforts to manage a single invasive species may require mechanical or manual removal (Hewitt et al. 2005), biological control (Secord 2003), and/or chemical treatment (Bax et al. 2003; Mitchell and Hobbs 2003). Two traditional chemical treatment options are rotenone (McClay 2000; Britton and Brazier 2006) and copper sulfate (Waller et al. 1993; de Oliveira-Filho et al. 2004; Wise et al. 2006).

Rotenone is derived from the roots of *Derris* and *Longchocarpus* plants, typically grown in tropical and subtropical regions (Irvine and Freyre 1959), and is applied as an insecticide as well as a piscicide (Ling 2003). In some situations, it has also been used as a molluscicide (Mozley 1952). Rotenone works by blocking cellular aerobic respiration, and death results from tissue anoxia (McClay 2000, 2005; Ling 2003). Toxicity is greatest at water temperatures <25°C (Ling 2003), and the chemical has varying lethal effects on aquatic invertebrate species. Typically, rotenone is applied for fish elimination, though after treatment, most aquatic invertebrates are also eliminated (Ling 2003).

Copper sulfate is used as a molluscicide (Hoffman and Zakhary 1953), algicide (Fitzgerald and Faust 1963), and fungicide (Michaud and Grant 2003). High concentrations are toxic, disrupting the structure and function of DNA and proteins, resulting in death (de Oliveira-Filho et al. 2004). Marsh rams-horn snails (*Planorbella trivolvis* (Say, 1817)) have an average survival rate of 3% after a copper sulfate application of approximately 1.0 mg/L at water temperatures ≥21°C (Mitchell and Hobbs 2003).

We tested the efficacies of rotenone and copper sulfate for eliminating the invasive freshwater Chinese mystery snail. The Chinese mystery snail has a thick shell as well as an operculum, allowing individuals to seal up when environmental conditions are undesirable (Jokinen 1982). Although rotenone is rarely used to eradicate invertebrates, it is commonly used to eradicate fish (McClay 2000) and has the potential to eliminate non-target species during treatment (Mangum and Madrigal 1999). In contrast, copper sulfate is primarily marketed as a molluscicide.

Methods

Rotenone

Snail collection

Chinese mystery snails were collected in mid-August 2012 from Wild Plum Lake (40.614 N, 96.902 W), Lancaster County, Nebraska, approximately 27 km southwest of Lincoln. Snails were transported in a 19-L bucket of lake water back to the laboratory at the University of Nebraska – Lincoln and held in aerated tanks filled with de-chlorinated tap water maintained at room temperature (20ºC ± 1.0ºC). Snails were fed algal wafers three times per week while housed in the laboratory and were allowed eight weeks for acclimation before experiments began. Only adult snails were used in both rotenone and copper sulfate experiments because juveniles have exhibited high mortality rates in a laboratory setting and are extremely difficult to monitor for survival due to their small size.

Exposure experiment

During the first experiment, rotenone-treated lake water was collected immediately after application (the target rotenone concentration was 3.0 mg/L) to a sandpit within the Fremont State Recreation Area (41.439N, 96.490W). Water was collected in 19-L buckets and transported to the laboratory, where it was maintained at room temperature (approximately 20ºC, well within the identified range for maximum rotenone toxicity (Ling 2003)). Ten adult snails were randomly assigned to each of five buckets (N=50) containing rotenone-treated lake water. Rotenone degrades in sunlight, so buckets were kept covered, and snails were checked daily during the 72-hour experiment. After 72 hours, snails were removed and placed in recovery tanks filled with aerated and de-chlorinated tap water for 24 hours. Recovery tanks were used to confirm suspected deaths; if a snail did not open its operculum, move, or respond to gentle prodding of the operculum within 24 hours, it was considered dead.

We proceeded with a second experiment using one control aquarium (no rotenone) and three aquaria with pre-determined rotenone concentrations made with de-chlorinated tap water and 5% rotenone stock solution. The rotenone concentrations in the three treatment aquaria were 0.25, 2.5, and 25.0 mg/L, respectively. This concentration range encompasses the general rotenone concentration target of 3.0 mg/L used to eliminate fish (Bettoli and Maceina 1996) and includes a value almost one order of magnitude higher. Ten adult snails were randomly assigned to each tank (N=40) and checked daily during the 72-hour experiment. As previously mentioned, rotenone degrades rapidly, so 72 hours was an appropriate period to determine if death would occur after an application. After 72 hours, snails were removed and placed in recovery tanks filled with aerated and de-chlorinated tap water for 24 hours.

Copper sulfate

Snail collection

Chinese mystery snails were collected in mid-August 2013 from Eugene T. Mahoney State Park (41.026N, 96.314W), Cass County, Nebraska, approximately 43 km northeast of Lincoln. Snails were transported in a 19-L bucket of lake water back to the laboratory at the University of Nebraska – Lincoln and held in aerated tanks filled with de-chlorinated tap water maintained at room temperature (20ºC ± 1.0ºC). Snails were fed algal wafers three times per week while housed in the laboratory and were allowed 12 weeks of acclimation before the experiment began.

Exposure experiment

A stock solution of 50 mg/L copper sulfate pentahydrate ($CuSO_4$ $5H_2O$) was prepared and used to create three concentrations of copper sulfate solution: 1.25, 2.50, and 5.0 mg/L. These concentrations were chosen because they encompass a range of concentrations commonly used to kill mollusks as well as concentrations higher than usually used in management application. Snails were fed *ad libitum* during the 24 hours directly preceding the experiment. During the first trial, five adult snails (N=15) between 40–55 mm total shell length were randomly assigned to each of the treatment aquaria with copper sulfate concentrations of 1.25 and 2.50 mg/L as well as one control aquarium (no copper sulfate). During a second trial, five additional snails (N=20) in the same length range were randomly assigned to each of the treatment aquaria with copper sulfate concentrations of 1.25, 2.50, and 5.0 mg/L as well as one control aquarium. Because of high survival during the first trial, we introduced the 5.0 mg/L aquarium in the second trial. In both trials, after introduction, snails were observed every 12 hours for 72 hours, and dead individuals were removed once being declared dead after 2 consecutive observation periods. A snail was considered dead if did not open its operculum, move, or respond to gentle prodding of the operculum or if the operculum was open and the snail's body was limply hanging out. These individuals were placed in recovery tanks with de-chlorinated tap water for an additional 24 hours to confirm death. After this recovery period, the number of dead individuals from each aquarium was recorded. Water temperature was approximately 20ºC throughout the experiment.

Results and discussion

Rotenone

From an experimental population of 50 adult Chinese mystery snails used in rotenone-treated lake water, 72-hour survival was 100%. In the experiment using known rotenone concentrations, all snails survived in the control aquarium, the 0.25 mg/L aquarium, and the 2.5 mg/L aquarium. Nine of the ten snails in the 25.0 mg/L aquarium survived. As a result, we concluded that rotenone does not effectively eliminate adult Chinese mystery snails at concentrations used to eliminate fish or at concentrations nearly an order of magnitude higher.

Previous experiments testing rotenone toxicity of freshwater snails provide a range of lethal concentrations. Freshwater snails in the genus *Helisoma* (Family: Planorbidae) had an LC_{50} of 33.5 mg/L after three to six hours of rotenone exposure and an LC_{50} of 8.0 mg/L after 96 hours of rotenone exposure (Chandler and Marking 1982). However, snails in the genus *Lymnaea* (Family: Lymnaeidae) had 30–70% survival at rotenone concentrations of 0.5 mg/L and 1.0 mg/L, respectively, after 84 hours of exposure (Hamilton 1941). Finally, species in the genus *Physa* (Family: Physidae) had LC_{50} values of 1.0–4.0 mg/L after 96 hours of rotenone exposure (Leonard 1939; Chandler and Marking 1982). However, all of these examples are air-breathing snails that do not possess an operculum. Based on this, we would assume higher concentrations would be necessary to eliminate species with an operculum, such as the Chinese mystery snail, and this would most likely only occur if the Chinese mystery snail was the target species for removal. At concentrations that could potentially eliminate the Chinese mystery snail, many native mollusk species would be negatively affected, and all fish would be killed, making the practice undesirable unless the management objective was an entire biotic renovation.

Copper sulfate

All snails (N=10) survived 96 hours at 1.25 mg/L $CuSO_4$ solution, nine snails (N=10) survived 96 hours at 2.50 mg/L $CuSO_4$ solution, and four snails (N=5, only added in second trial of copper sulfate experiment) survived 96 hours at 5.0 mg/L $CuSO_4$. In comparison, when treating a similarly-sized reservoir (compared to the reservoir our Chinese mystery snails were collected from) for zebra mussels (*Dreissena polymorpha* (Pallas, 1971)), the target treatment concentration was 1.0 mg/L $CuSO_4$ (URS Group, Inc. 2009), Subsequent reports cited no reported zebra mussels immediately following application; however, adult zebra mussels were identified in the treated reservoir the following year (Holm et al. 2012). Based on this unsuccessful attempt and our experimental findings, it does not appear that copper sulfate is an effective treatment for eradicating Chinese mystery snails. As with rotenone, this is most likely due to the Chinese mystery snail's ability to close its operculum when presented with deleterious environmental conditions. During the experiment, snails did not emerge from their shells. This species can survive up to nine weeks out of water (Unstad et al. 2013), easily surpassing treatment lengths (approximately seven days) in lakes (URS Group, Inc. 2009). Additionally, we would expect the copper to have a longer residence time in our experiment because we did not include substrate in the aquaria; copper tends to bind to organic matter, reducing its bioavailability (Flores-Velez et al. 1996).

Freshwater snails in the genus *Biomphalaria* (Family: Planorbidae) do not possess an operculum, and they exhibited the highest tolerance to copper toxicity in a comparative study with a planktonic algae (*Raphidocelis subcapitata* (Korshikov, 1987)), a water flea (*Daphnia similis* (Claus, 1876)), and the zebrafish (*Danio rerio* (Hamilton, 1822)) (de Oliveira-Filho et al. 2004). The 24-hour LC_{50} of *Biomphalaria* was 1.868 mg/L (de Oliveira-Filho et al. 2004), falling within the range of $CuSO_4$ concentrations used in our experiments. Comparatively, in a field study investigating the toxicity of $CuSO_4$ on another rams-horn snail in the genus *Planorbella* (Family: Planorbidae) only 2.2% of individuals survived a shoreline application of $CuSO_4$ and citric acid with an initial copper concentration of approximately 4 mg/L (Mitchell 2002), also well within the range included in our experiments. Typically, higher concentrations are not used in field studies due to the fatal effects on non-target species (Post 1987).

Despite the ineffectiveness of rotenone and copper sulfate in eliminating the Chinese mystery snail, there are other chemicals that have been used as molluscicides for aquatic snails. The compound Bayer 73 (referred to as niclosamide (Clearwater et al. 2008) and bayluscide (Crossland 1963)) has been used in Asia, Africa, and New Zealand to eradicate snails. Sodium pentachlorophenate has been used in the Caribbean, West Africa, and South America to eliminate snails that act as hosts of human bilharziasis (Wright et al. 1958). Future research investigating the efficacy of these chemicals on the Chinese mystery snail may yield different results.

Conclusion

Neither rotenone nor copper sulfate effectively killed adult Chinese mystery snails in our experiments. Therefore, adult Chinese mystery snail populations will not be reduced by application of rotenone or copper sulfate at standard concentrations used by natural resource managers (though future research on the efficacy of these chemicals on the elimination of juvenile Chinese mystery snail snails may yield different results). The relatively large size, thick shell, and operculum present a challenge to managers looking for efficient ways to eliminate the species. Rotenone and copper sulfate are unlikely to be successful unless they are able to penetrate the operculum or the shell at higher concentrations than were tested. Such concentrations would likely have adverse effects on non-target species, complicating the issue of eliminating the Chinese mystery snail. These results indicate Chinese mystery snail populations will be very difficult to control once established, and management should focus on preventing additional spread or introductions of this species.

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