Short Communication

A pilot study examining the lethality of niclosamide monohydrate on the invasive mystery snails, *Callinina georgiana* and *Cipangopaludina japonica*

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Abstract

Callinina georgiana (banded mystery snail) and *Cipangopaludina japonica* (Japanese mystery snail) are two exotic viviparid snail species that have been introduced to North America. These snail species harbor parasites, predate on the eggs of native fish fauna and are prolific in their ability to spread across various freshwater environments. While several recent studies have explored the population structure and range expansion of the species in the United States, none have formally addressed control or eradication strategies. In this study, we attempted to do this by carrying out a series of assays to determine the efficacy of the popular molluscicide, niclosamide monohydrate against both species under both acute and chronic exposure. Our results show that the lethal dose required to achieve 50 % mortality (LD_{50}) for *C. georgiana* and *C. japonica* was 1.509 mg/L and 1.296 mg/L respectively. In acute exposure experiments we observed that at higher concentrations, all *C. georgiana* specimens in experimental treatment tanks were dead but *C. japonica* never reached 100% mortality even at the highest concentration. In chronic exposure experiments, *C. georgiana* did not survive more than a week while *C. japonica* did not survive more than 11 days in the experimental tanks. We conclude that niclosamide is an effective chemical control agent for eradication of invasive mystery snails in North America. However, further studies exploring the effect of the molluscide on non-target species, especially local native fauna, should be investigated prior to any scaled up field applications.

Key words: LD_{50}, molluscicide, freshwater, mortality, Viviparidae

Introduction

Aquatic invasions are the second leading cause of freshwater biodiversity loss, and are also responsible for incurring significant economic costs to the regions where their impacts are most felt (Reid et al. 2019; Essl et al. 2020; Haubrock et al. 2022). Invasive mollusks in particular have been implicated in some of the most high-profile invasion events in the last few decades. For example, the invasion of the zebra mussel (*Dreissena polymorpha*) into the Great Lakes of North America in the late 1980s have been responsible for completely reshaping the biogeochemistry of this critical region (Strayer 2009). In addition, the economic damage caused by zebra mussels hovers in the tens of millions of dollars (USD) (Adams and Lee 2012). More recently, two exotic freshwater gastropods, *Callinina georgiana* known colloquially as the banded mystery snail and the Japanese mystery snail, *Cipangopaludina* (=*Heterogen*) *japonica*, have been recorded as the dominant aquatic snails in several rivers and lakes across the United States (David et al. 2017; David and Cote 2019; Abeyrathna et al. 2023). *Callinina georgiana* is native to the southeastern United States, including parts of Florida, Mississippi, Alabama and Georgia (Clench 1962; Jokinen 1992). The species was introduced to the Hudson River through an intentional release by an amateur malacologist in 1870 (Jokinen 1992). Since then, this snail has invaded almost every major water body in the northeastern United States, upper midwestern states and most recently, have been recorded in high densities in parts of southern Canada. *Callinina georgiana* is believed to be problematic in areas where it has invaded. The snail is a voracious predator of largemouth bass embryos (Eckblad and Shealy Jr 1972; David et al. 2017), and may also harbor trematodes from its native range that could infect native fish and boreal birds when they consume the snail (David et al. 2017). *Cipangopaludina japonica*, another vivparid, is native to Japan and southeastern Asia (Clench and Fuller 1965; Bury et al. 2007; Fowler et al. 2022) and along with the closely related Chinese mystery snail (*Cipangopaludina chinensis*) was intentionally introduced to North America as a cheap source of protein for human consumption (Bury et al. 2007; Van Bocxlaer and Strong 2016). However, due to their unpopularity in the exotic food markets, many were released into the waterways of San Francisco, this region being the likely point source of their introduction. In the U.S., both of these so-called 'Asian mystery snails' now has a cosmopolitan distribution with self-sustaining populations recorded from every corner of the country including northern California, Texas and New York (Abeyrathna et al. 2023). This cosmopolitan distribution is reflective of *C. japonica's* broad physiological tolerance which will likely be a significant obstacle for control and eradication efforts. Unlike *C. georgiana*, evidence regarding its direct impact on biotic communities is currently lacking. However, the species is known to reach extremely high densities in lakes where it has become established, which may give it a competitive advantage over native snails for resources. Furthermore, *C. japonica* is known to harbor medically important trematodes and nematodes in its native range, which may pose a threat to human health (Ando and Ozaki 1923; Chung and Jung 1999).

Currently, there are no specific management strategies to control the spread of any of these mystery snails in North America. Considering the ecological threat that these snails pose in their non-native range, research into the various management options and their efficacy should be of paramount importance for state environmental agencies. Methods for controlling the spread of invasive species are generally classified into three types: mechanical, chemical and biological control. Mechanical control would involve the physical removal of snails either by hand or through dredging using heavy machinery. A previous removal project by Bernatis and Warren (2014) successfully used handpicking to eradicate invasive apple snails from an urban pond in Florida while dredging was used to eradicate the non-indigenous brown mussel from a soft sediment environment in New Zealand (Hopkins et al. 2011). Biological control would involve the use of a biological agent to regulate and or eradicate the target invader. For example, a laboratory trial by Ben-Ami and Heller (2001) found that the black carp would be an efficient biocontrol agent for two pest snails with different habitat requirements. Chemical control would involve the use of a chemical agent to eliminate the target species. In the case of pest snails, especially those which harbor medically important parasites (e.g., *Schistosoma*), specialized chemicals (molluscicides) have been specifically developed for this. Of these, the use of molluscicides is the only control method that has been formally sanctioned by the World Health Organization and remain arguably the most efficient option for elimination of invasive mollusks from large areas where they have achieved extremely high densities (see Crossland et al. 1963 for the usage of Bayulside in the field and Olivier et al. 2016 for the usage of nicolsamide monohydrate and an organic option (tea seed derivative – TSD) in laboratory trials).

The overarching objective of this study was to characterize the application and potential utility of the synthetic molluscicide niclosamide monohydrate in controlling *C. georgiana* and *C. japonica* in their invasive range. We wanted to determine through a series of trials, whether this highly popular molluscicide is effective at eliminating both *C. georgiana* and *C. japonica* We performed in-laboratory experiments by exposing *C. georgiana* and *C. japonica* to acute and chronic doses of niclosamide. The aim was to (1) discover the lethal dose (LD_{50}) of niclosamide for *C. georgiana* and *C. japonica* and (2) to determine survivorship range under chronic exposure. Our experiments are mainly focused on providing helpful insight on the potential effect of niclosamide on the selected two snail species rather than exclusively evaluating its efficacy. While our study concentrated on niclosamide we acknowledge other molluscicides like copper sulfate, gramoxone, calcium hydroxide, and N-tritylmorpholine (Frescon) could also play a vital role in managing pest snail species. However, our specific emphasis on niclosamide contributes to the understanding of its potential impacts on *C. georgiana* and *C. japonica* populations, thereby aiding in broader discussions surrounding molluscicide utilization. This study aims to provide valuable insights into the potential utility of niclosamide within a comprehensive context of molluscicide alternatives.

Materials and methods

Overview of niclosamide as a molluscicide

Niclosamide is a commercially available synthetic molluscicide which contains 2',5-dichloro-4'-nitrosalicylanilide as the active ingredient (Clearwater et al. 2008; Olivier et al. 2016). Common formulations of niclosamide include an emulsifiable concentrate, a wettable powder, an aqueous formulation and a granular formulation. The mode of action of niclosamide against the snails is not completely understood; however, it has been shown to affect vital enzyme activities in the body tissues which consequentially results in the death of the snails (Andrews et al. 1982; Cheng et al. 2007; Clearwater et al. 2008). Currently, niclosamide is the most effective chemical agent for use on pest aquatic snails (World Health Organization 2017). Niclosamide is non persistent in aquatic environments with a half-life of 1.2-3.9 days and is the only approved molluscicide to kill snails that harbor *Schistosoma* by the World Health Organization (WHO) (Oliveira-Filho and Paumgartten 2000; Olivier et al. 2016; World Health Organization 2017). Niclosamide has a very low toxicity to non-target species with no reported toxicity for humans. It was also recently deployed by the US Fish and Wildlife Services to eliminate the ram's horn snails (*Heliosoma sp.*) (Terhune et al. 2003; Olivier et al. 2016; World Health Organization 2017). Niclosamide has also been used in combination with other pesticide formulations in the Great Lakes region to control invasive species of sea lampreys (*Petromyzon marinus*) (McDonald and Kolar 2007).

Specimen collection

All snails used in this trial were collected from the Raquette River in the Adirondack Park of New York State. The Adirondack Park is the largest protected forested area in the contiguous United States with more than 103,000 hectares of lakes and streams (Tuttle and Heintzelman 2015). *Callinina georgiana* was collected from the town of Potsdam (44.666694, -74.989775) and *Cipangopaludina japonica* was collected from the town of Colton (44.545411, -74.936492). Collected specimens were morphologically identified in the field using the taxonomic key of Jokinen (1992), and the most recent redescriptions of the species (David et al. 2017; David and Cote 2019). Sampling, handling and processing of both snail species were approved by the New York State Department of Environmental Conservation (Permit numbers: 6-21-001 & 6-21-002). Snails were collected in August 2022 by hand picking each snail from the specified locations. Collected snails were kept in river water until they were safely transported to the Davinack Lab at Clarkson University. Only adult snails within the size range of 3-4 cm were chosen for *C. georgiana*, while those within the range of 5-6 cm were chosen for *C. japonica* for the experiments. Snails were then placed in 40 L aquariums at 25°C for acclimatization. The aquariums were supplied with aeration, along with water refills and drains on a daily basis. The snails were fed a micro mix which contains sinking blended diet pellets (see Appendix 1 for ingredients list) once a day until the experimental treatments commenced.

Experimental trials

To investigate the acute lethal dosage of niclosamide required to reach 50% mortality (LD_{50}) for each species, a concentration series was prepared as follows: 0 (control), 0.6, 0.9, 1.2, 1.5, 2.1, 2.4, 3 mg/L. The concentrations used in this study were selected based on a combination of factors based on a review of the literature, available guidelines and practical considerations such as the availability of niclosamide. World Health Organization (WHO) or Environmental Protection Agency (EPA) currently do not have specific concentrations listed for both snail species that we use in this experimental setup. Given the lack of universally recommended concentrations, we performed a series of preliminary experiments to understand the suitable concentration range for our LD_{50} assays. Each concentration consisted of three replicates with five snails per treatment. Thus, 120 adult snails from each species were used for the LD_{50} assays. We used analytical grade niclosamide monohydrate, Pestanal® (99.9% pure, Sigma-Aldrich) to prepare the desired concentration series of niclosamide. Following the protocol of Olivier et al. (2016), niclosamide was weighed and dissolved in 1 ml ethanol, and then diluted in 750 ml degassed tap water. To conduct the LD_{50} experiments, smaller 1L aquariums were used. Each treatment tank was filled with 750 ml of desired concentrations of niclosamide while control tanks were filled with a similar volume of degassed tap water. The acute exposure for niclosamide was set for 24 hrs. After 24 hrs., mortality was assessed and recorded by probing the snails with a blunt wooden probe to trigger withdrawal movements. To assess the mortality of the snails after probing we observed the snail for a continuous five minutes observation window to confirm immobility and death. Snails that were alive were placed in degassed tap water for 48 hrs with food and monitored for recoveries or mortalities (World Health Organization 2017). LD_{50} for both species were obtained using Probit Analysis in SPSS v. 28.0, and results were represented via survivorship line graphs. Differences among the replicates were verified using a chi-square test. In the Probit analysis we used a Probit link function which models the relationship between the predictor variable (concentration) and the probability of the response variable (mortality) on transformed data. A relative mean potency (RMP) analysis, was also used to assess the differences in among LD_{50} values of both snail species.

To investigate responses to chronic exposure of niclosamide in both snail species, we selected three concentrations of the molluscicides (one concentration below LD_{50} slope and two concentrations over LD_{50} slope) to conduct long term experiments. The selected experimental concentrations contained three replicates and a control with experimental and control tank containing five adult snails. Thus, we used 60 adult snails for each species to conduct chronic exposure experiments. Chronic exposure experiments were conducted in 1L smaller aquariums and each treatment tank was filled with 750 ml of desired concentrations of niclosamide while control tanks were filled with degassed tap water. Mortality was assessed and recorded each day until all snails in the experimental tanks were dead. Finally, a one-way ANOVA was performed to assess whether there were significant differences in mortalities across the different niclosamide concentration levels for both species.

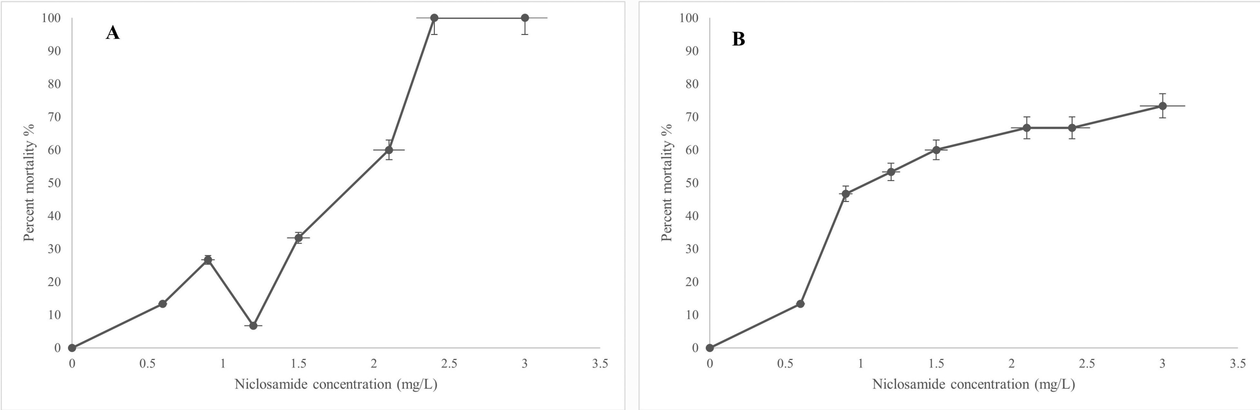

Figure 1. A) Survivorship of *Callinina georgiana* after 24-hour exposure to niclosamide monohydrate. B) Survivorship of *Cipangopaludina japonica* after 24-hour exposure to niclosamide monohydrate.

Results

After 24 hr acute exposure to niclosamide some *Callinina georgiana* individuals retracted into their shells with some snails floating at the surface with splayed appendages. *Callinina georgiana* in the control tanks were all alive and no mortalities were recorded for this treatment. At 2.5 mg/L concentration, all *C. georgiana* were dead after 24 hr exposure (Figure 1A). Some *Cipangopaludina japonica* specimens exhibited similar behavior, however this species did not approach 100% mortality in any of the experimental tanks, even at highest concentration (Figure 1B). No mortality was observed in the control tanks for *C. japonica*.

After 48 hrs in the recovery tanks, some *C. georgiana* snails which were exposed to lower concentrations were still surviving and no mortality was observed in the control tanks. *Cipangopaludina japonica* also behaved similarly, however, mortality did not reach 100% and fewer of the snails survived after 48 hrs in the recovery tanks. Using the 24 hr mortality percentages, the calculated LD_{50} values were 1.509 mg/L with a 95% confidence interval (0.841 mg/L, 2.846 mg/L), and 1.296 mg/L with a 95% confident interval (0.863 mg/L, 1.744 mg/L) for *C. georgiana* and *C. japonica* respectively, and there were no significant differences observed among the treatments. The RMP value between the two species was 1.164, which indicates a significant difference in their LD_{50} values. Additionally, the calculated 95% confident interval for RMP value is between 0.274 and 4.956, which suggests that the difference in sensitivity between the two species is statistically significant at the 5% significance level. The chronic exposure experiments to niclosamide in *C. georgiana* resulted in no surviving individuals after the seventh day of the experiment and in *C. japonica* no surviving individuals were found in the treatment tanks after the 11th day (Tables 1 and 2). No mortalities were recorded in the control tanks for both species. Statistical analyses confirmed that there was a significant difference in mortalities across concentrations for both *C. georgiana* ($F = 38.26$, $p = 0.0008$) and *C. japonica* ($F = 22.64$, $p = 0.0031$) (Table 3)

Table 1. Percentage of surviving *Callinina georgiana* individuals after chronic exposure of to niclosamide monohydrate.

Treatment	Percentage of individuals surviving at day 3	Percentage of individuals surviving at day 7	Percentage of individuals surviving at day 11
Control	100	100	100
1.2 mg/L (< LD_{50})	40	0	0
2.1 mg/L (> LD_{50})	13.3	0	0
2.4 mg/L (> LD_{50})	0	0	0

Table 2. Percentage of surviving *Cipangopapludina japonica* individuals after chronic exposure to niclosamide monohydrate.

Treatment	Percentage of individuals surviving at day 3	Percentage of individuals surviving at day 7	Percentage of individuals surviving at day 11
Control	100	100	100
0.9 mg/L (< LD_{50})	66.6	46.7	0
1.5 mg/L (> LD_{50})	46.7	26.7	0
2.1 mg/L (> LD_{50})	46.7	33.3	0

Table 3. 24-hr mortality data across different molluscicide concentration levels for *Callinina georgiana* and *Cipangopaludina japonica*

Niclosamide monohydrate concentration series (mg/L)	Mortality (%) for *C. georgiana*	Mortality (%) for *C. japonica*
0	0	0
0.6	13.33	13.33
0.9	26.67	46.67
1.2	6.67	53.33
1.5	33.33	60
2.1	60	66.67
2.4	100	66.67
3	100	73.33

Discussion

Despite the fact that both *Callinina georgiana* and *Cipangopaludina japonica* are listed as 'species of concern' in several states, no control methods have been proposed or studied to deal with them. The current study is the first attempt at exploring the potential for chemical eradication of these so-called mystery snails. In our study we tested whether niclosamide monohydrate (under the Pestanal trade name) is an effective molluscicide for the banded mystery snail *Callinina georgiana* and the Japanese mystery snail *C. japonica*. Under acute exposure, the LD_{50} of *C. georgiana* was 1.509 mg/L while the LD_{50} for *C. japonica* was 1.296 mg/. In acute exposure experiments for 24 hrs, we observed that, at higher concentrations (>2.5 mg/L), all *C. georgiana* in experimental treatment tanks died whereas *C. japonica* never reached 100% mortality in acute exposure levels even at higher concentrations. One reason for this could be difference in the body sizes of the two snails as *C. japonica* is relatively larger than *C. georgiana* (Jokinen 1992). Adult *C. georgiana* may grow up to 4.5 cm in shell height and *C. japonica* may get relatively larger in shell height (~ 6.5 cm) (Jokinen 1992). Historically there is limited information and mixed understandings about the relationship between snail body size and tolerance to molluscicides. Raheem et al. (1979) investigated the susceptibility of different body-sized *Bulinus truncatus* snails for two different molluscicides (Bayluscide and Mollutox) at two different temperatures and reported that at warmer temperatures (30°C) larger bodied snails are more susceptible to Bayluscide and smaller bodied snails are more susceptible at lower temperature (20°C). The dip in the survivorship in figure 1A is interesting and unexpected. This might have been due to several reasons such as the dosage responses of the study snails, specific physiological responses of the snails to niclosamide, variation in test conditions or any biological variations in tested snails (Hrovat et al. 2009). However, to test the underlying cause a detailed analysis of the experimental setup and physiological condition of the snails will be required.

We also observed that under chronic exposure, *C. georgiana* did not survive more than seven days while *C. japonica* did not survive more than 11 days. Both snail species possess an operculum which helps them to survive and thrive in their habitats. The operculum is a tight seal which gives protection to the soft body parts, helps to retain water while preventing dehydration and helps in locomotion as the snail pushes harder on the operculum when it moves (Paul 1991). However, in the previous literature there is no direct evidence that operculum of freshwater snails can act as a barrier/protectant to prevent the exposure to molluscicides. Therefore, our data suggests that niclosamide monohydrate would be an effective molluscicide against both invasive snails.

Previous studies have also investigated the use of niclosamide as a chemical agent for other pest mollusks. For e.g., Olivier et al. 2016, found that a niclosamide concentration of 1.3 mg/L resulted in 100% mortality of the apple snail *Pomacea maculata* while the lowest concentration (0.13 mg/L) resulted in a 17% mortality rate. Even though the concentrations they tested and the LD_{50} values were different from our study, they exposed the snails to molluscicides for a longer period (72 hrs) which might make a difference in the test results for the mortality percentages compared to our acute dosage results. Niclosamide has also been used in field trials to control aquatic snails that host medically important parasites. In a field study conducted by Crossland (1963), niclosamide under the trade name Bayluscide, was used to eliminate the planorbid snail *Biomphalaria pfeifferi* (host of the parasitic trematode *Schistosoma mansoni*) from an irrigation system in Tanzania. This study applied a dosage of 1 ppm (1 mg/L) which resulted in elimination of most *B. pfeifferi* snails two months after initial application. However, a few snails were able to recover and evade the chemical stressor after seeking refuge in pocket canals during a rainy season.

It should be noted that this was not a mesocosm study and so we are unable to determine how niclosamide affects non-target species such as fish which co-occur with the snail (e.g., largemouth bass and rainbow trout, in the Adirondacks). There are few studies that reported the effect of niclosamide on non-target species. Olivier et al. (2016) reported minimal effects of niclosamide non-target species like crayfish (*Procambarus clarkii*) and sunfish (*Lepomis microlophus*) and there were no significant effects on the growth, mortality and the behavior of these non-target species. It is noteworthy that in their study there were no crayfish mortalities for both the synthetic (niclosamide) and organic (tea seed derivative) molluscicides but the sunfish had experienced 21% mortality with niclosamide at 0.13 mg/L and 100% mortality with 0.03 g/L of TSD. In another study by Wilkie et al. (2019), the authors discussed the use of niclosamide as a potential chemical control agent to halt the spread of sea lamprey (*Petromyzon marinus*) along with the piscicide, TFM (3-trifluoromethyl-4-nitrophenol). They found that both chemicals are effective at controlling sea lamprey populations but found that niclosamide had lower risk of toxicity to fish and invertebrates when compared to TFM. Recent studies suggest that usage of niclosamide should be integrated with new formulations of molluscicides that are less harmful for non-target species and are selective for invasive snails to target areas with heavy infestation of pest snails (Coelho and Caldeira 2016). A study by Dai et al. (2008) used a novel formulation of niclosamide which contained a suspension concentrate of niclosamide (SCN) and compared its efficacy with the existing 50% wettable powder of niclosamide ethanolamine salt (WPN) both in laboratory and in the field. The new formulation of niclosamide, SCN was physically more stable, more effective and had better molluscicidal effect than WPN. New formulation SCN was also tested against zebrafish (*Brachydanio rerio*) as a non- target species and its acute toxicity was less than the WPN. While these synthetic molluscicides have had a successful track-record, there is now a growing trend towards adoption of plant based molluscicides ('organic') as potential eco-friendly alternatives. However, developing any effective treatment with plant-based products is always difficult due to the lack of availability of active ingredients, and the need to use large quantities to induce actual molluscicidal effects (Cheng et al. 2007). For the few studies which have used these alternatives, the results have been mixed. For example, Giovanelli et al. (2001) assessed the effects of latex (a fluid taken from the cuts in the trunk of the plant) of *Euphorbia splendens var. hislopii* on *Melaoides tuberculata* (a snail that harbors schistosomiasis-causing trematodes) and found that the latex is effective in killing *M. tuberculata* with a LD_{50} value of 3.57 mg/L. However, they had to use a very high concentration (10 mg/L) to reach 100% mortality at the end of the experimental trials. In another study, Olivier et al. (2016) also used a Tea Seed Derivative (TSD) and compared its effectiveness against niclosamide. The researchers had to use higher concentrations of TSD to see comparatively higher mortality rates as was observed for niclosamide at much lower concentrations.

It is likely that the most practical approach towards management and eradication of the mystery snails in the Adirondacks will be an integrated one which combines chemical and other methods. For example, in Cambodia, invasive apple snails (*Pomacea spp.*) have become the major pest species impacting rice cultivation in the region. Agricultural authorities there have implemented a management strategy that rotates among different approaches such as mechanical removal, installing screens, using different rice varieties which is less preferred by apple snails, along with the usage of niclosamide-based chemical molluscicides (Khay et al. 2018). In the northeast and midwestern states where both *C. georgiana* and *C. japonica* have reached high densities in several lakes, mechanical removal of the snails is only feasible during the summer months when they are more abundant in shallow areas. During winter, both species overwinter into deeper waters which are inaccessible since they are intermittently frozen from later winter to early spring (Jokinen 1992). In larger water bodies which are infested with these snails it will be difficult to implement mechanical control methods because it will be extremely labor intensive, possibly exceeding efforts provided by an all-volunteer force. Biological control of invasive snails is another popular control strategy which could be implemented. However, criticism of biocontrol methods including the potential negative impact to non-target species and difficulties in interpreting the complexities the control agent may cause in the ecosystem (since it takes a long time to detect such interactions) may (Ip et al. 2014).

In summary, this pilot study shows that the synthetic molluscicide, niclosamide monohydrate is an effective eradication method for the banded mystery snail (*C. georgiana*) and the Japanese mystery snail (*C. japonica*). From a practical point of view, the use of niclosamide may be cost prohibitive depending on the scale at which it is being used but new formulations are being developed that may reduce the cost of applying the chemical in the field (see Yang et al. 2012 as an example). However, prior to any open discussion regarding field trials, a larger mesocosm-based follow-up study will be needed to determine the effect of the molluscicide on non-target species in the region. This would provide a comprehensive dataset to environmental authorities and all relevant stakeholders to make an informed conservation decision regarding chemical control of these snails.

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Author Contribution

Both WANUA and AAD conceived of the study; WANUA carried out the experimental trials, raw data analysis and initial manuscript drafts and AAD verified data analyses, revised and wrote the final manuscript draft. Both WANUA and AAD were involved in final revisions.

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Supplementary material

The following supplementary material is available for this article:

Appendix 1. Ingredients of the micro mix.

This material is available as part of online article from:
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