

Short Communication

Freezing mortality of faucet snails, *Bithynia tentaculata*: a viable method for equipment decontamination to reduce aquatic invasive species spreadLynda R. LaFond^{1,*}, Jared E. House², Sabin J. Adams³, Debbie L. Guelda⁴ and Charlotte L. Roy⁵¹Biology, Bemidji State University, Bemidji, MN 56601, USA

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Abstract

Reliable, inexpensive, and publicly accessible decontamination techniques are needed for equipment used in waters infested with aquatic invasive species. One such aquatic invasive species is the faucet snail (*Bithynia tentaculata*) that hosts several parasitic trematodes which have caused mass waterfowl die-offs in the United States and Canada. We examined freezing as a potential decontamination method using a household chest freezer. Snails were frozen in time increments of 0, 5, 10, 30, 60, 120, 180, and 240 min in dry conditions (water removed) and in water. Snails in dry conditions reached 100% mortality after 30 min while snails in water reached 80% mortality after 240 min. Snail mortality was inversely related to size, with larger snails requiring longer times to achieve mortality. Snails in water could tolerate freezing temperatures longer than snails in dry conditions. We determined that freezing is a viable decontamination method for contaminated equipment, but longer freezing times will be necessary for larger snails, especially when equipment is wet.

Key words: cold tolerance, contaminated equipment, desiccation, invasive species removal, mollusk, trematodes, waterfowl

Introduction

Aquatic invasive species (AIS) cause an estimated \$97–137 billion in cumulative economic damage to agriculture, forestry, and fisheries within the United States (Lovell et al. 2006). Preventative methods to reduce AIS spread in the United States are heavily regulated through federal acts like the National Invasive Species Act of 1996 and Clean Boating Act of 2008. These acts were established to limit invasive species from entering the United States and prevent further spread by recreational boaters within the

country (Boothe 2007; Larson et al. 2011). Although many U.S. state agencies have codes, statutes, and/or rules that regulate the possession and transportation of AIS within their borders (Boothe 2007), AIS continue to be introduced into new waterbodies.

In Minnesota, it is unlawful to transport species classified as harmful to natural resources without a permit. If caught, intentionally or unintentionally, AIS transportation may result in fines and penalties for the offender. Recreational water users, professionals, and state agencies are encouraged to clean, drain, and dry their watercraft and equipment whenever exiting the water. However, drying equipment may not efficiently destroy all species (Ricciardi et al. 1995; Morse 2009; Comeau et al. 2011). One such species that has been shown to survive desiccation for 42 d (Havel et al. 2014) is the faucet snail, *Bithynia tentaculata*, due in part to an ability to close the aperture of the shell using a conical operculum (Burch 1989; Mitchell and Cole 2008).

The faucet snail is an AIS originally from Europe that made its way into the United States in the 1870's (Berry 1943; Mills et al. 1993; Mitchell and Cole 2008). Faucet snails are thought to have entered into the Great Lakes through marsh grasses used in packaging (Latchford 1914, 1925) and/or ballast in cargo ships used to haul timber (Baker 1928). Major pathways for introduction and spread into and within the United States has been primarily through cargo ship ballast water, exotic species trade, recreational boating and fishing, and intentional stocking (Mackie 1999; Padilla and Williams 2004).

Faucet snails act as primary and secondary intermediate hosts to the digenetic trematodes; *Sphaeridiotrema globulus*, *Sphaeridiotrema pseudoglobulus* (McLaughlin et al. 1993; Mattison et al. 1995), and *Cyathocotyle bushiensis* (Menard and Scott 1987; Sandland et al. 2013), and as the primary host to *Leyogonimus polyoon* (Cole and Friend 1999). These parasites may cause trematodiasis, an important cause of waterfowl die-offs in the United States and Canada (Roscoe and Huffman 1982; Hoeve and Scott 1988; McKindsey and McLaughlin 1993; Sauer et al. 2007; Bergmame et al. 2011). Since faucet snail detection in the Upper Mississippi River in 2002, more than 130,000 waterfowl have died in the Great Lakes Region due to trematodiasis (USGS National Wildlife Health Center, J. Chipault, unpubl. data cited in Roy et al. 2016). Numerous waterfowl species are affected, with lesser scaup (*Aythya affinis*) and American coots (*Fulica americana*) most commonly affected (Roy et al. 2016). Northern shoveler (*Anas clypeata*), mallard (*Anas platyrhynchos*), northern pintail (*Anas acuta*), ring-necked ducks (*Aythya collaris*), blue-winged teal (*Spatula discors*), redheads (*Aythya americana*), and herring gull (*Larus argentatus*) have also been affected by trematode infections during 1981–2012 in North America (Roy et al. 2016).

To minimize further impacts to wildlife and aquatic systems, limiting the spread of faucet snails is desired. Because of economic and ecological costs, including blockage of municipal water supplies (Baker 1902, cited in Mills et al. 1994), preventing new infestations is preferred to responding and managing after invasion (Leung et al. 2002). Understanding the mechanisms of invasive species transmission is necessary to prevent expansion, determine risks, and identify management options (Hulme 2009). Aquatic invasive species are known to spread by boats and associated equipment (Johnson et al. 2001; Rothlisberger et al. 2010) and *Bithynia tentaculata* is not an exception.

Effective decontamination treatments for these snails include chemical (Hydrothol 191) exposure for 24 hrs and heat treatments of greater than 50 °C for 1 min (Mitchell and Cole 2008). Obtaining these chemicals can be costly and hazardous and is not recommended as an effective treatment (Mitchell and Cole 2008). In addition, chemicals and heat treatments may be damaging to delicate equipment (i.e. nets, etc.). Alternative treatments are needed for equipment (watercraft, anchors, fishing nets, hunting equipment, scientific equipment, etc.) to reduce the risk of transportation and provide tools to reduce economic and ecological risks from unintentional transportation of AIS. This study evaluates freezing as a method of equipment decontamination. We used a residential chest freezer which is widely available to and commonly owned by private citizens, unlike the other available decontamination options that require specialized equipment or chemicals. Our goal was to determine the amount of time needed to achieve 100% mortality of faucet snails under typical freezer conditions in water and in dry conditions.

Materials and methods

Faucet snails were collected with D-nets (0.09 m²) from the Crow Wing River near Hubbard, Minnesota (47°49.394N; 094°52.299E) on 13 June 2014, and from Lake Winnibigoshish near Winnie Campground (47°42.744N; 094°31.315E) 14 June 2014, 24 May 2015, 20 May 2016, and 20 June 2016. Faucet snails were collected according to the Minnesota Department of Natural Resources Prohibited Invasive Species Permit (#298 issued to D. Guelda). Water temperature at the time of collection ranged between 20–22 °C. Snails were transported to Bemidji State University in screw-top containers within an insulated container to maintain collection temperatures.

Experiments began shortly after reaching the laboratory to reduce snail stress. Only live snails, as determined by active locomotion, were used in treatments (Mitchell and Brandt 2005). All water used during the trials was collected from the snail collection site. All snail shells were measured using calipers to the nearest 0.1 mm vertically from the apex to point on the basal lip linearly below the apex.

Snails were randomly assigned to 1 of 12 treatments for experiments that included 2 conditions (in water or dry) and 6 durations of freezing (0 [control], 5, 10, 30, 60, or 120 mins). We placed 4,096 snails in plastic trays with 16–8 × 4 cm wells. We randomly assigned 1,536 snails in 2014, 768 snails in 2015, and 1,792 snails in 2016 to treatments. Three additional treatments were added in 2016; 128 snails were assigned to wet conditions with a freeze duration of 180 min, 64 snails were assigned to dry conditions for 180 min, and 64 snails were assigned to water with a freeze duration of 240 min. For treatments in water, 10 mL of water from the snail collection site was added to each well while the dry treatment had no water added. Control snails remained at room temperature in water from the collection site for the full duration of treatment.

We moved snails from room temperature (19.2–20.1 °C) directly into a Kenmore® elite chest freezer (–17.3––19.9 °C). We placed control samples at room temperature (19.2–20.2 °C) for the duration of freezer treatments. Upon removal from the freezer, snails were acclimated to room temperature for 2 h before adding 10 mL of room temperature water collected at the same site as the snails to dry samples. Snails remained at room temperature (19.2–19.8 °C) for 2 d to allow for recovery and to aid in mortality determinations according to methods described in Mitchell and Brandt (2005). Snails were categorized as alive if any movement was observed after the 2 d waiting period. In order to assess mortality, mantles were agitated on inactive snails; if movement was observed, snails were deemed alive, if no movement was observed, snails were determined dead. Additionally, if the operculum of individuals had sloughed off, snails were considered dead. All snails were killed by exposure to 50 °C water for 1 min after the experiments concluded (Mitchell and Cole 2008).

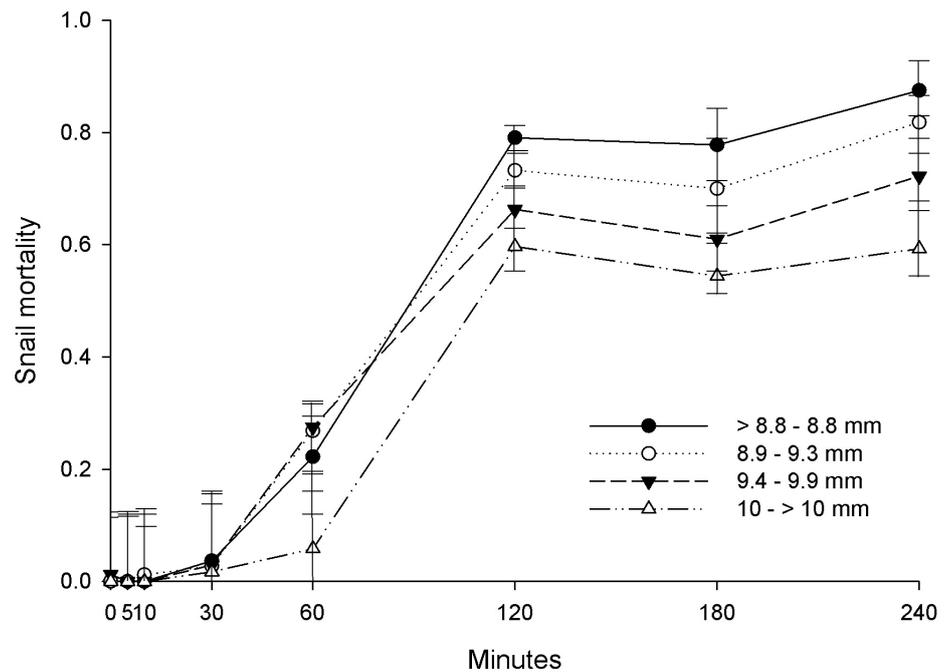
Statistical analysis

We used a generalized linear mixed model with mortality as the dependent variable and a Bernoulli distribution. We examined 3 categorical predictor variables including location, year collected, and treatment condition (in water or dry), as well as the continuous predictor variables, freezing duration and snail length. We standardized snail length to a mean of zero and a standard deviation of one. We examined all 2 variable interactions for significance and dropped them from consideration if they were not significant ($P < 0.05$). We considered all combinations of variables in models with additive effects, as well as models with significant interaction terms. We evaluated model performance with log-likelihood values and examined the significance of fixed effects. We also used Akaike's information criterion (ΔAIC) to compare models, where only models with $\Delta AIC < 4$ were considered competitive. Additionally, we used a generalized linear mixed model to further explore the relationship between shell length and mortality in each condition (in water or dry) as a function of time.

Table 1. Parameter estimates of fixed effects for the top model explaining snail mortality.

Fixed effect	Estimate	Standard Error	T	df	P
Intercept ¹	3.49	0.15	23.25	1,4090	< 0.0001
Freeze duration * Condition (dry)	-0.49	0.02	-21.18	1,4090	< 0.0001
Freeze duration * Condition (in water)	-0.04	0.002	-24.50	1,4090	< 0.0001
Year (2015)	2.31	0.21	11.22	1,4090	< 0.0001
Year (2016)	2.29	0.18	12.65	1,4090	< 0.0001
Snail length	0.27	0.07	3.89	1,4091	0.0001

¹ For conditions where Year is 2014.


Figure 1. Faucet snail mortality during freezing durations in water for various snail lengths.

Results

All faucet snails in the dry treatment died when exposed to $-20\text{ }^{\circ}\text{C}$ for 60 min. Longer exposure times were necessary to achieve the same level of mortality when snails were in water, with most snails dying within 180 min. However, some of the largest snails were able to survive 240 min when submersed in water.

The best model to predict snail mortality was condition \times freeze duration ($F_{2,4090} = 310.68$, $P < 0.001$) + year ($F_{2,4090} = 104.72$, $P < 0.001$) + snail length ($F_{1,4090} = 15.17$, $P < 0.001$). All parameter estimates in this model were significant ($P < 0.01$; Table 1). The next closest model, condition \times freeze duration + year \times length had a ΔAIC of 218 therefore we considered no other models to have support.

Snail length (mm) was divided into 4 categories based on distribution within quartiles: ≤ 8.8 , 8.9–9.3, 9.4–9.9, ≥ 10 mm. Mortality of snails in water gradually increased with time (Figure 1). For snails ≤ 8.8 mm in length, mortality increased from 0.0 ± 0.12 at 5 min to 0.88 ± 0.04 at 240 min. For snails 8.9–9.3 mm in length, mortality increased from 0.0 ± 0.1 at 5 min to

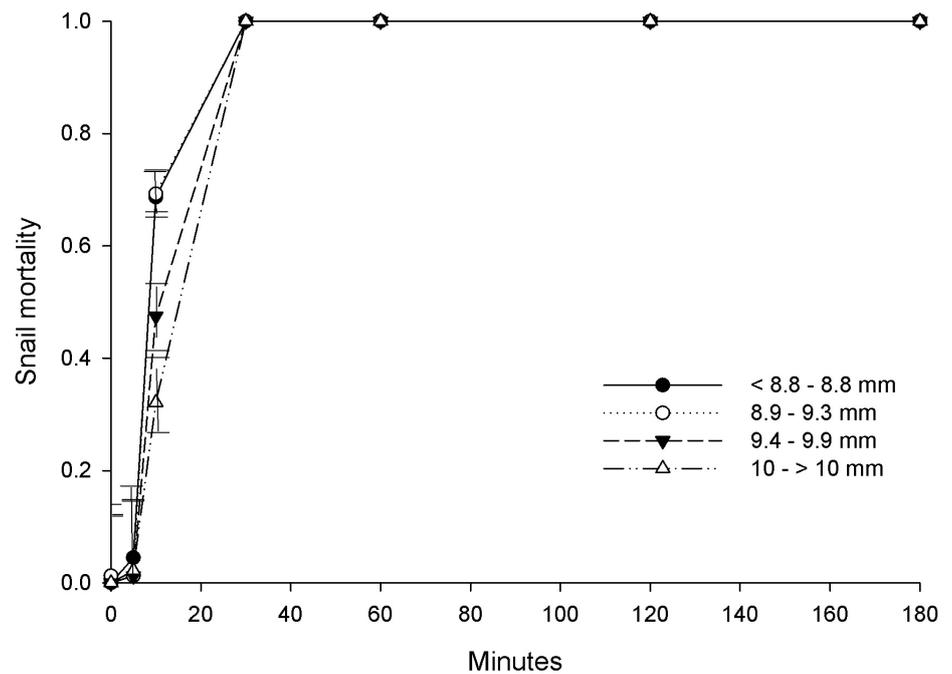


Figure 2. Snail mortality as a function of freezing duration in dry conditions for various faucet snail lengths.

0.82 ± 0.05 at 240 min. For snails 9.4–9.9 mm in length, mortality increased from 0.0 ± 0.1 at 5 min to 0.72 ± 0.07 at 240 min. For snails ≥ 10 mm in length, mortality increased from 0.0 ± 0.11 at 5 min to 0.6 ± 0.08 at 240 min. In dry conditions, snail mortality reached 100% mortality for all length categories by 60 min (Figure 2).

Discussion

Snail mortality was higher and occurred faster when water was removed before freezing. This is consistent with previous findings that rate of freezing can be an important factor in snail mortality, with faster freezing producing higher mortality (Murphy and Johnson 1980). These findings have significant implication for use of freezing as a decontamination method. Specifically, draining water from equipment should be attempted whenever possible. When equipment holds water, the water must be cooled and provides a temperature buffer to snails so that they do not immediately experience freezing temperatures and experience a slower freezing rate. Importantly, the volume of water in which snails are submersed will also be an important factor determining time to 100% mortality, because it takes more time for larger volumes of water to freeze at a given temperature. Furthermore, freezing times will vary depending on initial water temperature because more energy must be lost from water at a higher initial temperature.

Snail size was an important predictor of mortality of snails in water with larger snails having lower mortality than small ones for similar durations of time in the freezer. Larger body sizes require more time to drop

temperature and thus larger snails freeze more slowly (Murphy and Johnson 1980). Complete mortality (100%) was obtained for snails ≤ 8 mm in length in dry conditions after 30 min. In water, 100% mortality for snails ≤ 7 mm in length was reached at 120 min. Exposure times longer than those we used (> 240 min) will be necessary to achieve complete mortality of snails of all sizes in water beginning at room temperature and exposed to -20 °C. However, smaller faucet snails (e.g. pinhead size, ≤ 1 mm), which are much harder to find and more easily missed than large snails, have high mortality from exposure to freezing temperatures.

We suggest that freezing can offer another decontamination method, and one that is more accessible to private citizens than other options requiring access to chemicals or high temperature power washers. Use of freezing as a decontamination method with chest freezers will work best for equipment such as anchor lines, fishing nets, soft-soled waders, benthic sampling equipment for research, and other equipment where visual inspection may not detect the smallest snails and equipment is being moved among waterbodies. Use of a freezer for boat and trailer decontamination is not practical, but in much of the Great Lakes Region where faucet snail infestations occur (Sauer et al. 2007; Roy et al. 2016), outside temperatures dip below -20 °C during winter, effectively decontaminating equipment stored outside or in cold storage. In Walker, Minnesota, United States (near several of our collection sites) the average date where temperatures dropped below 0 °C was in October and last freeze occurred in May during 1981–2010 (Minnesota Department of Natural Resources, Leech Lake, Walker, MN). We did not determine if temperatures > -20 °C might also achieve 100% mortality in dry conditions. Thus, future work exploring mortality of snails subjected to temperatures 0 °C – -20 °C would help refine recommendations. Such information on temperature thresholds for freezing decontamination could be helpful to boaters that frequent lakes during late fall before ice up (e.g. waterfowl hunters) and early spring shortly after ice out (e.g. anglers).

Our research is specific to faucet snails. Previous research has shown that several intertidal and marine mollusks have the ability to withstand freezing temperatures or have partial freezing tolerances including *Lottia digitalis*, *Littorina littorea*, *Mytilus edulis*, and *Geukensia demissa* (Murphy and Johnson 1980; Hylleberg and Siegismund 1987; Ansart et al. 2001, 2002a). Mechanisms that allow ectotherm survival of freezing temperatures include 1) super-cooling which is a reduction in the temperature of the body fluid at which ice spontaneously forms (i.e. the temperature of crystallization, which is often below -10 °C), and 2) extracellular ice formation in tissues which allows avoidance of hyperosmotic stress originating from pure water ice crystals forming (Storey and Storey 1996). This cryoprotection often occurs through the production of polyols, sugars, ice nucleation proteins, or antifreeze proteins (Ansart et al. 2001). The land snail, *Cornu aspersum*,

can tolerate up to 40–60% of its body water freezing for short durations (Ansart et al. 2001) and can later reproduce (Ansart et al. 2002b). Some *Cornu aspersum* survived exposure to $-20\text{ }^{\circ}\text{C}$ air temperatures for up to 4 h, but longer times were lethal (Ansart et al. 2001). Likewise, *Littorina littorea* can prevent formation of tissue ice at temperatures in air above $-7\text{ }^{\circ}\text{C}$ and can survive up to 7 d at $-9.1\text{ }^{\circ}\text{C}$, 120 h at $-11.1\text{ }^{\circ}\text{C}$, 36 h at $-13.0\text{ }^{\circ}\text{C}$, and large snails (20.1–25.0 mm) were able to survive lower temperatures (-7°C – $-20\text{ }^{\circ}\text{C}$ examined; Murphy and Johnson 1980). We did not test temperatures other than $-20\text{ }^{\circ}\text{C}$ because our focus was on applications for decontamination with common household freezers, but complete mortality in dry conditions after 1 h suggests that faucet snails may lack survival mechanisms for cold-tolerance comparable to those of *Cornu aspersum* (a larger snail, 25–35 mm) and that the rate of freezing that occurs at $-20\text{ }^{\circ}\text{C}$ in dry conditions is too rapid to allow survival.

Freezing tolerance can also be influenced by the temperatures experienced before, during, or after freezing (Murphy and Pierce 1975; Murphy and Johnson 1980). When initial water temperatures are colder, snails might be more tolerant to freezing than snails collected from warmer initial temperatures during May and June for our experiments. However, in marine mollusks, 2–3 wks of exposure to $0\text{--}5\text{ }^{\circ}\text{C}$ was necessary to increase freezing tolerance and lethal freezing temperatures were only reduced by a few degrees in mussels acclimated to cold temperatures (Murphy and Pierce 1975). Therefore, we would not expect freezing mortality to vary much throughout the year with exposure to colder temperatures in waterbodies prior to decontamination.

Our research also contributes to the existing knowledge of faucet snail overwintering biology; experiments with freezing tolerance have parallels with natural winter mortality in other snail species (Hylleberg and Siegismund 1987). Faucet snail overwintering behavior likely varies depending on climate and the potential for freezing of waterbodies. Winter temperatures and/or the length of winter might influence overwinter mortality (Richter 2001). Some studies have reported that faucet snails migrate from shallow areas to hibernate in muddy sediments (Hahn 2005), but others have reported that these snails did not make deep to shallow migrations and preferred shallow depths (Strayer 1987; Vincent et al. 1991). Yet, in infested waterbodies in Minnesota, faucet snail abundance varied with depth seasonally (Roy et al. 2016). Given that hibernation in surface mud is unlikely in this region because of ground freeze and lakes developing $> 1\text{ m}$ of ice cover, faucet snails would be expected to have higher survival at depths where mud and water do not freeze. Not surprisingly, small snails have higher overwinter mortality than large snails (Richter 2001), which would suggest that they may be more vulnerable to cold temperatures, freezing, and other stressors than large snails, which is consistent with findings in our experiments.

We conclude that freezing to $-20\text{ }^{\circ}\text{C}$ is an effective method for decontamination of *Bithynia tentaculata* from aquatic field equipment when excess water is removed. Storage of equipment outside during such temperatures would achieve the same results. This work provides another method for prevention of faucet snail spread to uninfested waterbodies, and a method that is more accessible to private citizens than currently available methods.

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