

**New Zealand Mudsnaills: Effects on Native Hydrobiid Species, Reproduction after Digestion by Fish,
and Update on Range within Utah**

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Executive Summary

We worked on four tasks that were intended to: a) improve our understanding of the effects that invasive New Zealand mudsnails (NZMS) have on native snails, b) determine whether NZMS that survive passage through fish digestive tracts can reproduce, and c) update our understanding on the distribution of NZMS within Utah.

In the first task, we determined the effect that NZMS had on the behavior of two native hydrobiid snail species; the Toquerville springsnail *Pyrgulopsis kolobensis* and the mud amnicola *Amnicola limosus*. To accomplish this, we established test arena that contained various densities of the native snails and NZMS. We video recorded the movements of the snails and compared movements of the native snails both before and after the addition of NZMS to the arena. We found that the addition of NZMS did not affect the net distance that the native snails moved during the trials. The distance separating the native snails from conspecifics did not change when NZMS were added. The native snails, however, tended to spend more cumulative time closer to conspecifics than to NZMS and this tendency to be closer to a conspecific increased as the number of NZMS within the test arena increased.

In the second task, we evaluated the effects of NZMS on the survival and recruitment of the same two native hydrobiid species evaluated in the first task. We found that the presence of NZMS had no influence on the survival of the native species. New Zealand mud snails produced more recruits and a higher number of recruits/adult than the native species. This research demonstrates that the high invasion rates of NZMS can at least be partially explained by their high recruitment rates.

In the next task, we fed NZMS to rainbow trout from two size classes (average total length 141 and 207 mm, respectively). The surviving NZMS were harvested and the neonate production among these snails was evaluated. We found neonate production among NZMS that were digested by both size classes of fish. The results indicate that NZMS that survive digestion are not so compromised that they can reproduce. This shows that extreme caution should be taken when stocking fish from hatcheries that contain NZMS.

In the final task, we surveyed Posey, Wide Hollow, Upper Box Creek, and Lower Box Creek Reservoirs for NZMS and did not find the snail in any of these reservoirs. We present an updated map showing the distribution of NZMS within Utah.

Task 1: Effect of the Invasive New Zealand Mudsail on the Behavior of Two Native Snails

Background

New Zealand mudsnails (NZMS) *Potamopyrgus antipodarum* are an example of a successful invasive species (Alonso and Castro-Diez 2008). Once established, NZMS can become a dominant component of an invertebrate community and consequently, NZMS have the ability to negatively affect other taxa. For example, several studies have found that the growth rates of other snail taxa are suppressed in the presence of NZMS (Krist and Dybdahl 2005; Riley and Dybdahl 2015). Riley et al. (2008) found that NZMS limited the growth of the native snail *Pyrgulopsis robusta* and that the native snail facilitated the growth of NZMS. Studies have also found the abundance of other invertebrate taxa to covary with NZMS abundance (Kerans et al. 2005; Moore et al. 2012). Finally, NZMS have been shown to alter the behavior of other species. For example, Kerans et al. (2005) found that other species of macroinvertebrates were less abundant on tiles that were heavily colonized by NZMS. Kerans et al. (2010) found that NZMS interfered with the foraging of mayflies in the family Baetidae but that the caddisfly *Brachycentrus occidentalis* caused NZMS to abandon the surfaces of tiles. The presence of NZMS has also been associated with shifts in feeding preferences in the isopod *Asellus aquaticus* (Aberle et al. 2005) and baetid mayflies (Kerans et al. 2010).

New Zealand mud snails are members of the snail family Hydrobiidae, which is the largest freshwater mollusk group with more than 1,000 species (Hershler and Ponder 1998). Many species within the family have specific habitat requirements, limited ranges, and are sensitive to habitat alteration (Hershler and Ponder 1998). Consequently, many hydrobiid species are considered threatened or endangered (e.g., Bliss Rapids snail *Taylorconcha serpenticola*; Richards 2004). The effects that NZMS have on other hydrobiid taxa are poorly understood but similarities in body size, habitat, and life-history requirements would suggest that NZMS could have stronger competitive effects on other hydrobiid species than other invertebrate taxa.

The goals of our research were to assess how NZMS affect the behavior of two other hydrobiid species, the Toquerville springsnail *Pyrgulopsis kolobensis* and mud amnicola *Amnicola limosus*, both of which are native to the United States. To do this we placed *P. kolobensis* or *A. limosus* along with varying densities of NZMS within Petri dishes and videotaped the interactions among the snails. We determined how NZMS affected the movement of the native snails and whether the native snails preferred to congregate near conspecifics over NZMS. We hypothesize that the presence of NZMS would reduce the movement of native snails and force native snails to congregate with conspecifics.

Methods

Snail Collection and Maintenance

Pyrgulopsis kolobensis, *A. limosus*, and NZMS were collected in the field and transported to the Utah Division of Wildlife Resource's Fisheries Experiment Station (FES; Cache County, Utah). Snails were transported in 4.0 L volume plastic bags that were filled with 1-2 L of water and natural vegetation from

the collection sites. Bags were filled with oxygen and transported in coolers with ice. *P. kolobensis* were collected on July 28, 2015 from a small spring situated on National Forest land near Pine Valley, Utah (Washington County). Mud amnicola were collected on August 3, 2015 from the Right Hand Fork of the Logan River (Cache County, Utah). NZMS were collected on July 27, 2015 from the Loa State Fish Hatchery (Wayne County, Utah). Upon arrival to FES, snails, water, and collection site vegetation were transferred to plastic aquaria (35 x 21 x 12.5 cm, length x width x height) with one aquarium used per species. An airstone connected to an aquarium air pump was used to aerate the aquaria and were stored in a refrigerator (Frigidaire Electrolux FFHT2021QW10, Charlotte, North Carolina, USA) with a on/off cycle controlled by a temperature control switch (Johnson Controls model A419ABG-3, West Valley City, Utah, USA). The temperature in the refrigerator was set at 6°C and averaged $5.2 \pm 2.3^\circ\text{C}$ (mean \pm SD; measured every 15 min with a Hobo Onset temperature logger, Bourne, Massachusetts, USA). Water exchanges were performed twice weekly using FES well water (pH = 7.2, hardness and alkalinity = 200 mg/L). To provide food, watercress *Nasturtium officinale* was periodically collected from below the FES fish hatchery, rinsed 4-5 x with water to remove other invertebrates, and added to the aquaria.

Video Collection Apparatus

Four Logitech c525 web cameras (Newark, California, USA) were mounted on a shelf within the same refrigerator where the aquaria were stored and these cameras were aimed downward to capture images on a shelf that was 32 cm below. The web cameras were attached via four separate universal serial bus (USB) connectors to a Hewlett Packard Compaq 8510p laptop computer (Palo Alto, California, USA) that was situated outside the refrigerator. The web cameras were managed and videos were captured using iSpy video surveillance software (version 4.1.9.0; <http://ispyconnect.com>). The default iSpy settings were used, except the software settings were changed to capture one image every second. Videos were captured using iSpy in a MP4 format. They were then converted to a Windows Media video file (WMV format) using Microsoft Live Movie Maker (2011 Version; Redmond, Washington, USA). Decompile Video Master version 1.8 (<http://audane.com>) was used to separate the WMV files into separate JPG images with one image captured every 10 seconds. Finally, MakeAVI software (<http://makeavi.sourceforge.net/>) was used to compress/convert the JPG images into a single AVI format video. AVI files were processed using the "Manual Tracking" plugin in Image Processing and Analysis in Java (ImageJ) software (<http://imagej.nih.gov/ij/>).

Experimental Design

Experimental trials were designed to assess the effects that NZMS have on the two native species. Trials lasted 48 hrs with the first 24 hrs serving as a control period where three individuals, all from the same species were in a test arena. After the control period was complete, 1, 3, or 6 individuals from either the same species or NZMS were added to the arenas with five replicates of each combination tested. Thus, the treatments for the *P. kolobensis* were three *P. kolobensis* for the first 24 hrs followed by the addition of 1, 3, or 6 *P. kolobensis* for the second 24 hrs. Alternatively, 1, 3, or 6 NZMS were added. Similarly, 1, 3, or 6 *A. limosus* or 1, 3, or 6 NZMS were added for trials that began with *A. limosus*. Only NZMS were added after 24 hrs to arenas that had NZMS for the 24 hr control

period (1, 3, or 6 individuals added). Test arenas were 150 mm diameter Petri dishes filled with 25 mL of FES well water and a 1 cm² piece of fresh, organic, store purchased spinach *Spinacia oleracea* was added to the Petri dishes to provide the snails with forage. Snail densities during the first 24 hrs were 170 individuals/m² and were 226, 340, or 509 individuals/m² during the second 24 hrs depending on whether 1, 3, or 6 individuals were added. Each web camera recorded a single test arena. A total of 75 videos were recorded and recordings began on August 10, 2015 and were shot at four videos at a time until all videos were recorded. To prevent bias, the order that the videos were recorded was randomized among treatments and individual snails only participated in the recording of a single video.

Video Processing/Statistical Analysis

The processing of the videos followed techniques that are similar to those described in Myrick (2009). Even though 48 hrs of video was recorded for each replicate, only four hours was further processed. We decided to process the second and last hour of video from each 24 hr period (i.e., hours 2, 24, 26, and 48). These times were selected to represent behavior changes (e.g., tiring) across the entire observation period and we wanted to give the snails at least one hour to acclimate after addition to the test arenas. Regardless that we only observed four hours of video, individual snails were followed by watching the videos at accelerated speed so individuals that participated in the initial 24 hour control period could be separated from the additional snails that were added after 24 hrs.

The position of the snails was tracked using the "Manual tracking" plugin in ImageJ and coordinates were copied into a Microsoft Excel spreadsheet (Redmond, Washington, USA). Coordinate position was determined every 10 s (since images were captured off the original videos every 10 s). Pixel coordinates were converted to millimeter coordinates using the formula (Myrick 2009):

$$\text{coordinate (mm)} = \text{coordinate (pixels)} \times \frac{\text{measured width of arena (mm)}}{\text{measured width of arena (pixels)}}$$

Then, coordinates were converted to distances using the formula (Myrick 2009):

$$\text{distance}_{i+1} = \sqrt{(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2}$$

where x_{i+1} and x_i are the x-coordinates and y_{i+1} and y_i are the y-coordinates.

The distances traveled during each 10 s increment were summed to determine the cumulative distance traveled in an hour. In addition, the distance to the nearest conspecific snail and NZMS was determined for each 10 s increment and were averaged for each hour. Then values from hours 2 and 24 were averaged to assess behavior before additional snails were added and hours 26 and 48 were averaged to assess behavior after additional snails were added. For each snail, the averages from after the addition of snails were subtracted from the averages from before the snails were added to determine how the cumulative distance moved and the distance to the nearest conspecific differed between the first and second 24 h periods. Thus, positive values represented greater net distance traveled and conspecific separation before additional snails were added than after additional snails were added. Also, in the second 24 h period, for each 10 s increment, we subtracted the distance to the

nearest NZMS (in treatments that included NZMS) from the distance to the nearest conspecific and computed an average for hours 26 and 48. Positive values represent snails being nearer NZMS than a conspecific and negative values represent being nearer to a conspecific.

There were four primary questions that we wished to address with these data; 1) does the addition of NZMS reduce the distance native snails move, 2) do native snails tend to reduce separation among conspecifics after NZMS are added, 3) do native snails tend to be closer to a conspecific than NZMS, and 4) do native snails spend more cumulative time closer to a conspecific than a NZMS? Data for two of the response variables, net distance traveled and distance to nearest conspecific were analyzed as a 3-way ANOVA with initial species, species added after 24 hrs, and number of individuals added as main effects. The response of whether snails were closer to NZMS or conspecifics was analyzed as a two-way ANOVA with species during the initial 24 hrs and the number of additional snails added as main effects. Data were analyzed with the software program R (Hornik 2016) and values were considered significant at $P < 0.05$. Significant main effects and associated interactions were further explored using contrasts (Kuehl 2000). A log-linear analysis was performed to determine whether snails spent more cumulative time nearer a conspecific than a NZMS. For this analysis, the proportion of ten second time increments during hours 26 and 48 that each individual native snail was closer to a conspecific than to a NZMS was determined and we determined whether the cumulative time that snails were closer to a conspecific varied between the two native species and with the number of NZMS added. The analysis was performed using the "GLM" function in program R (Hornik 2016).

Results

The net distance the snails moved did not vary with species that was added after 24 hrs (Figure 1; $F_{2,63} = 1.61$, $P = 0.21$), indicating that the movement of native snails was not significantly reduced with the addition of NZMS. Also, the net distance that the snails moved did not vary with the number of individuals added after 24 hrs ($F_{2,63} = 1.38$, $P = 0.26$), indicating that the movement of the snails was not density dependant. On average, *P. kolobensis* moved 228 ± 146 mm/hr (mean \pm SD), *A. limosus* moved 275 ± 128 mm/hr, and NZMS moved 212 ± 168 mm/hr.

The separation among conspecific snails after 24 hrs varied with the species of snail added (Figure 2; $F_{2,63} = 4.08$, $P = 0.02$) but did not vary with the number of snails added ($F_{2,63} = 2.61$, $P = 0.08$). The only significant change in separation occurred among *P. kolobensis* (Tukey's HSD Test, $P = 0.02$), where distance to the nearest conspecific decreased by 15.8 ± 10.3 mm after more conspecifics were added. For both native species, the distance to the nearest conspecific did not change after NZMS were added (both $P \geq 0.06$).

Native snail preference for residing nearer to a conspecific than a NZMS varied with the number of NZMS added (Figure 3; $F_{2,25} = 14.69$, $P < 0.01$) but did not vary between the two native species ($F_{1,25} = 0.62$, $P = 0.44$). Contrasts indicated that when one NZMS is added that snails were on average 29.0 ± 5.5 mm closer to a conspecific than a NZMS than when either three or six NZMS were added ($t_{1,25} = 5.26$, $P < 0.01$). In contrast, when either three or six NZMS were added, the native snails showed no preference towards being nearer a conspecific or a NZMS ($t_{1,25} = 0.82$, $P = 0.42$). Regardless of the distance

separating native snails and NZMS, both native species spent more cumulative time closer to a conspecific than a NZMS and this varied with the number of NZMS added (Table 1; χ^2 , $P < 0.01$, $df = 50$). Odd ratios showed that *P. kolobensis* were 1.4 times more likely than *A. limosus* to be closer to a conspecific than a NZMS when six NZMS was added to a test arena. This odds ratio was 0.9 when one NZMS was added and 1.1 when three NZMS were added. *Mud amnicola* were 0.8 times as likely to be nearest to a conspecific than a NZMS when one NZMS was added and 1.2 and 1.4 times more likely to be nearer other *A. limosus* than NZMS when either three or six NZMS were added, respectively.

Finally, the distance among NZMS varied with the number of additional NZMS added (Figure 4; $F_{2,26} = 32.6$, $P < 0.01$). The results of a Tukey's multiple comparison test showed that the distance to the nearest NZMS was significantly closer when six NZMS were added than when three were added and the addition of three NZMS lead to snails being closer to one another than when a single NZMS was added (P for all pairwise comparisons ≤ 0.04).

Conclusions

Invasions by exotic species are becoming increasingly common and more research on the interactions between native and non-native species is required (Byers 2000). We observed snails in our behavior trial to determine whether interference occurs between NZMS and *P. kolobensis* or *A. limosus*. The results from these trials indicate that the addition of NZMS did not alter the willingness of the native snail species to move. The distances and hence velocities of the native snails did not vary by species added, nor did the distance moved change between the first and second 24 hr observation periods. Also, the distance to the nearest conspecific did not change when NZMS were added, indicating that NZMS did not have an antagonistic effect on the native snails. Both *P. kolobensis* and NZMS moved closer to one another when conspecifics were added, suggesting attraction among individuals within these species. Both *A. limosus* and *P. kolobensis* were equidistantly spaced between conspecifics and NZMS when either three or six NZMS were added, which again indicates a lack of antagonistic effect. Interestingly, however, spacing among species was similar when either three or six NZMS were added, which provides some indication that the native snails sought company of conspecifics at high NZMS densities. This is further supported by data showing that both native species spent more cumulative time nearer a conspecific than NZMS at higher NZMS densities than lower NZMS densities. Other studies have documented similar aggregation among snails including *Achatina fulica* (Chase et al. 1980) and *Biomphalaria glabrata* (Simpson et al. 1973).

The results from our research indicate that NZMS did not directly affect the behavior of *P. kolobensis* or *A. limosus*. There are few NZMS predators and parasites in North America and NZMS tolerate a wide range of physiochemical conditions (Alonso and Castro-Diez 2008) and thus NZMS may exert a stronger competitive effect on *P. kolobensis* or *A. limosus* under certain environmental conditions. Little is known about *P. kolobensis* and *A. limosus*, but the range of these species has not expanded like NZMS and it is likely that these species are less tolerant to extreme conditions as the NZMS. Thus, human alteration of ecosystems may create conditions that are more conducive for NZMS and less favorable for *P. kolobensis* and *A. limosus*. In general, there are many hydrobiid species in the Intermountain West (Hershler and Ponder 1998) and many of these species have restricted ranges. Due

to relatedness, competition between NZMS and native hydrobiids would be expected but little evidence of competition was observed in our studies. Future research should evaluate the effect of NZMS on additional species and should focus on NZMS effects in natural environments.

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Table 1: Percentage of observations where *P. kolobensis* and *A. limosus* were closer to a conspecific than to NZMS. The percentage of observations and standard deviation (SD; in parentheses) are based on 5 replicates of each treatment and 720 observations per replicate (120 min observation x 6 observation periods/min).

Native Species	Number of NZMS in Arena	% of Observations Closer to Conspecific
Toquerville Springsnail	1	45.6 (5.6)
	3	57.6 (23.4)
	6	72.6 (4.7)
Mud Amnicola	1	37.3 (9.3)
	3	61.0 (9.8)
	6	70.9 (13.7)

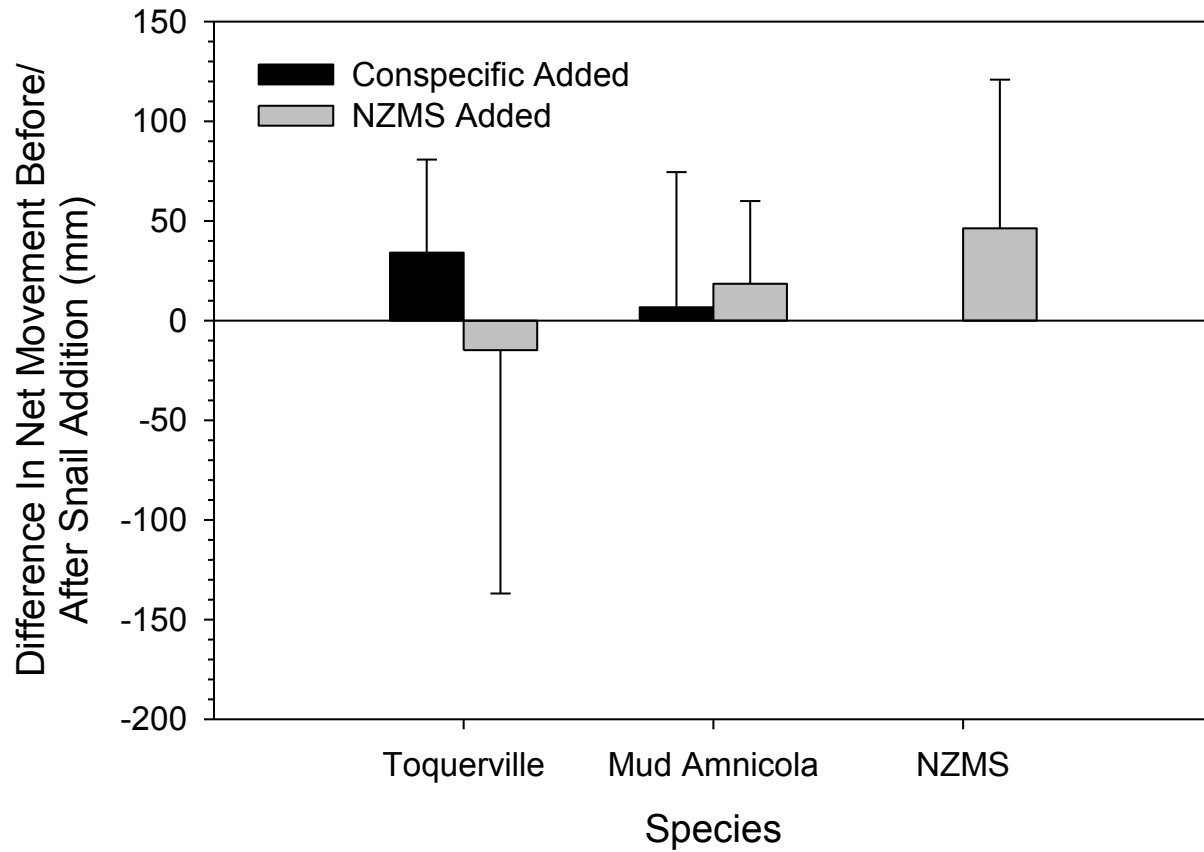


Figure 1: Difference in movement of *P. kolobensis* and *A. limosus* after the addition of conspecifics compared to the addition of NZMS. Data on the y-axis represent the average net distance that snails moved after additional snails were added subtracted from the average net distance moved before additional snails were added. Positive values represent greater distances traveled before additional snails were added. Error bars represent ± 1 SD.

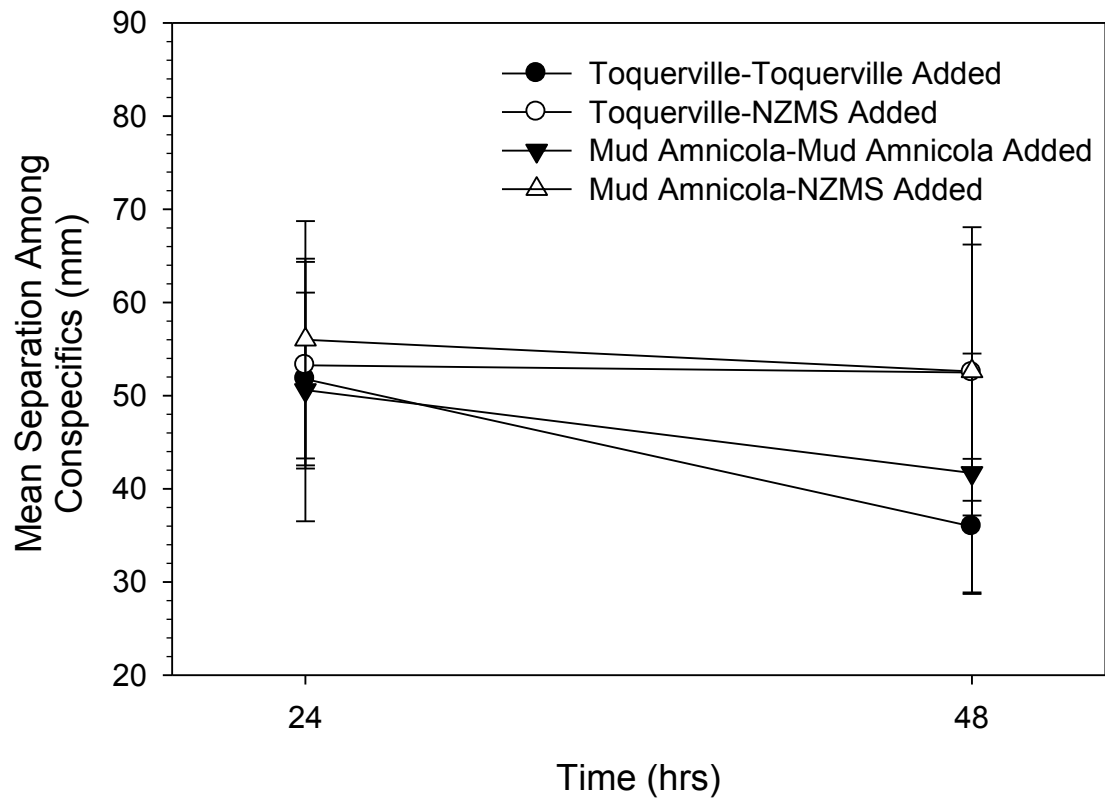


Figure 2: Average distance between *P. kolobensis* or *A. limosus* either after the addition of conspecifics or the addition of NZMS. Circles represent data from *P. kolobensis* and triangles represent *A. limosus*. Closed symbols represent the addition of conspecifics and open symbols represent the addition of NZMS. Error bars represent ± 1 SD.

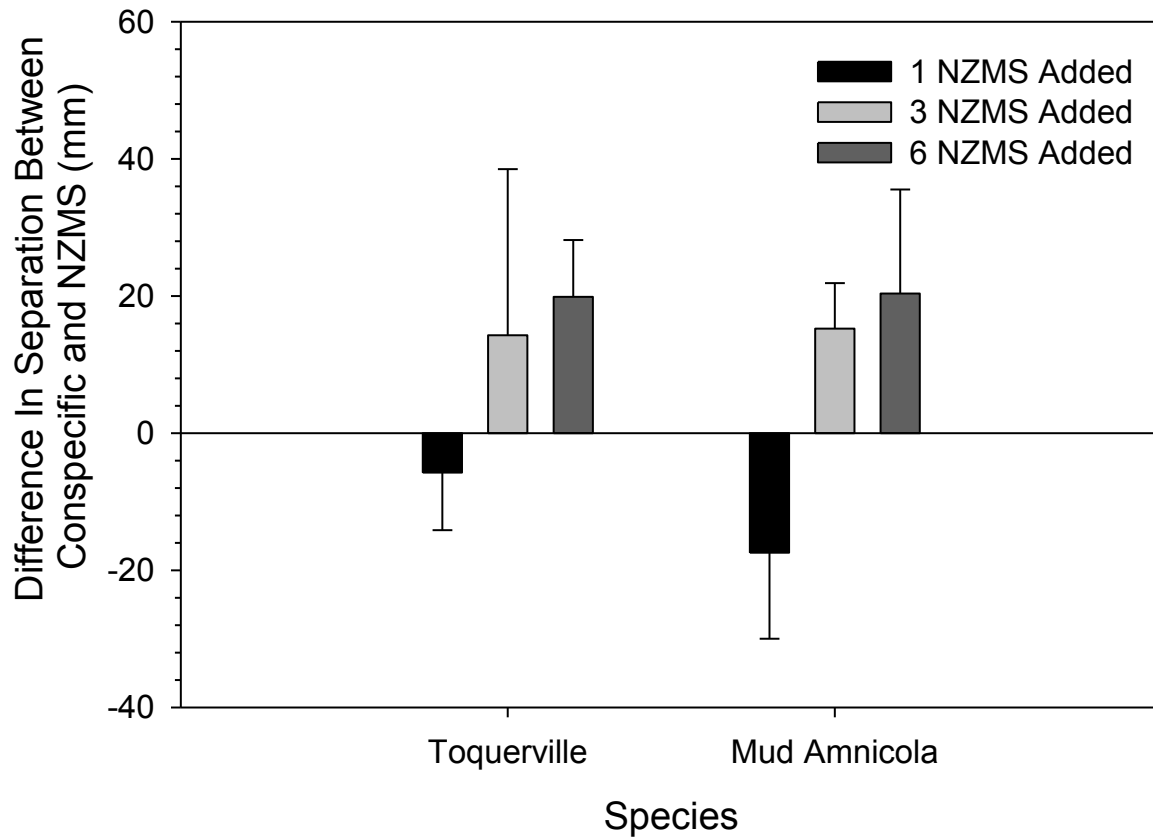


Figure 3: Average difference in distance between nearest conspecific and nearest NZMS after the addition of NZMS at three different densities. Data on the y-axis represent the average distance to the nearest conspecific minus the average distance to the nearest NZMS. Positive values represent snails being nearer to conspecifics and negative values represent snails being closer to NZMS. Error bars represent ± 1 SD.

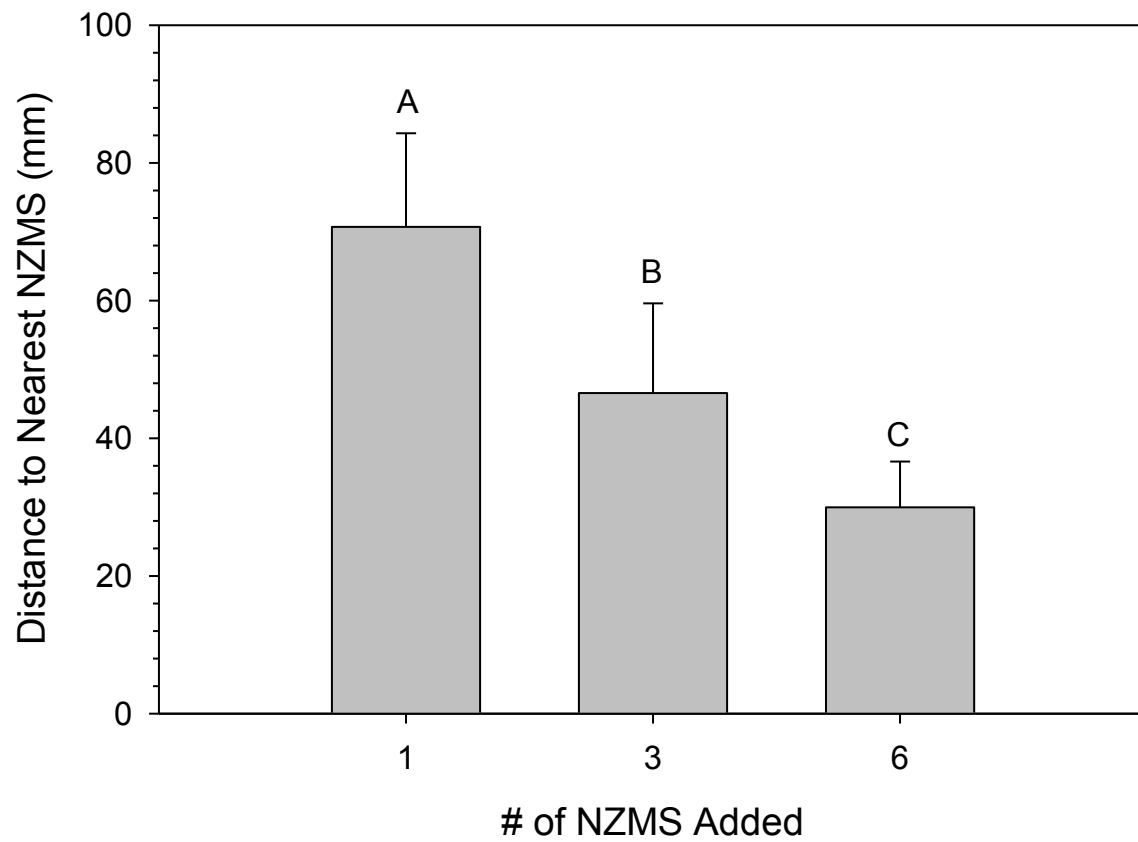


Figure 4: Average distance of NZMS to the nearest NZMS after the addition of either 1, 3, or 6 NZMS to the test arenas. Treatments with different letters above the bars are significantly different from one another at $P < 0.05$. Error bars represent ± 1 SD.

Task 2: Effect of the Invasive New Zealand Mudsnail on the Recruitment and Survival of Two Native Snails

Background

New Zealand mudsnails (NZMS) *Potamopyrgus antipodarum* are an example of a successful invasive species (Alonso and Castro-Diez 2008). Once established, NZMS can become a dominant component of an invertebrate community and consequently, NZMS have the ability to negatively affect other taxa. For example, several studies have found that the growth rates of other snail taxa are suppressed in the presence of NZMS (Krist and Dybdahl 2005; Riley and Dybdahl 2015). Riley et al. (2008) found that NZMS limited the growth of the native snail *Pyrgulopsis robusta* and that the native snail facilitated the growth of NZMS. Studies have also found the abundance of other invertebrate taxa to covary with NZMS abundance (Kerans et al. 2005; Moore et al. 2012). Finally, NZMS have been shown to alter the behavior of other species. For example, Kerans et al. (2005) found that other species of macroinvertebrates were less abundant on tiles that were heavily colonized by NZMS. Kerans et al. (2010) found that NZMS interfered with the foraging of mayflies in the family Baetidae but that the caddisfly *Brachycentrus occidentalis* caused NZMS to abandon the surfaces of tiles. The presence of NZMS has also been associated with shifts in feeding preferences in the isopod *Asellus aquaticus* (Aberle et al. 2005) and baetid mayflies (Kerans et al. 2010).

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The goals of our research were to assess how NZMS affect the behavior of two other hydrobiid species, the Toquerville springsnail *Pyrgulopsis kolobensis* and mud amnicola *Amnicola limosus*, both of which are native to the United States. To do this we placed *P. kolobensis* or *A. limosus* in experimental mesocosms with varying densities of NZMS and determined how NZMS affected the survival and recruitment of the native snails. We hypothesized that NZMS would reduce the survival and recruitment of the two native species.

Methods

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the collection sites. Bags were filled with oxygen and transported in coolers with ice. *P. kolobensis* were collected on July 28, 2015 from a small spring situated on National Forest land near Pine Valley, Utah (Washington County). Mud amnicola were collected on August 3, 2015 from the Right Hand Fork of the Logan River (Cache County, Utah). NZMS were collected on July 27, 2015 from the Loa State Fish Hatchery (Wayne County, Utah). Upon arrival to FES, snails, water, and collection site vegetation were transferred to plastic aquaria (35 x 21 x 12.5 cm, length x width x height) with one aquarium used per species. An airstone connected to an aquarium air pump was used to aerate the aquaria and were stored in a refrigerator (Frigidaire Electrolux FFHT2021QW10, Charlotte, North Carolina, USA) with a on/off cycle controlled by a temperature control switch (Johnson Controls model A419ABG-3, West Valley City, Utah, USA). The temperature in the refrigerator was set at 6°C and averaged $5.2 \pm 2.3^\circ\text{C}$ (mean \pm SD; measured every 15 min with a Hobo Onset temperature logger, Bourne, Massachusetts, USA). Water exchanges were performed twice weekly using FES well water (pH = 7.2, hardness and alkalinity = 200 mg/L). To provide food, watercress *Nasturtium officinale* was periodically collected from below the FES fish hatchery, rinsed 4-5 x with water to remove other invertebrates, and added to the aquaria.

Experimental Design

The effect of NZMS on the survival and recruitment of *A. limosus* and *P. kolobensis* was determined using seven different treatments with five replicates of each treatment. The seven treatments included ten individuals from each species (NZMS, *A. limosus*, and *P. kolobensis*) housed allopatrically, five individuals from the two native species held sympatrically with five NZMS, and five individuals from the two native species held sympatrically along with ten NZMS. Snails were added to plastic aquaria (35 x 21 x 12.5 cm, length x width x height; 136 snails/m² in treatments with 10 snails and 204 snails/m² in treatments with 15 snails) on December 9, 2015 and lasted 60 d. The aquaria were placed on two adjacent storage shelving units, each with three shelves. We were concerned about temperature differences among shelves and stratified the experiment by placing one replicate of each treatment on each shelf (one shelf on one storage unit not used). Treatment placement on individual shelves was randomized. Temperature data collected with Hobo Onset temperature loggers (Bourne, Massachusetts) revealed that treatment stratification was justified as there were significant temperature differences among shelves ($F_{2,17379} = 6738$, $P < 0.01$, top shelves: $13.9 \pm 2.9^\circ\text{C}$, middle shelves: $12.4 \pm 2.3^\circ\text{C}$, bottom shelves: $8.6 \pm 2.4^\circ\text{C}$, all mean \pm SD). To provide forage, 10.0 g of watercress was added to each tote and was replenished by adding 5.0 additional grams half-way through the experiment. The water in each tote was replaced every 3-4 d by pouring the contents of the tote through a 100 μm sieve and adding the contents and fresh FES well water back to the aquaria. Aeration was provided to each tote via an aquarium airpump and airstone.

At the end of the trial, adult snails were removed from the aquaria and survival of these adult snails was assessed via observation under a dissecting microscope and snails that reacted to touch with a needle were considered alive. Snails that did not respond or had empty shells were considered dead (Schisler et al. 2008). The remaining aquaria contents were frozen and later thawed and numbers of juveniles from each species were determined using a dissecting microscope under 15 X magnification. The survival status of the juveniles at the end of the trial was not assessed. To facilitate in separating

juveniles from detritus, samples were stained using a 0.1% Alizarin Red solution for 2 hrs followed by rinsing with deionized water (Howard and Smith 1983). Samples were then stained for 10-15 s using 1.0% Light Green SF Yellowish followed by additional rinsing (Howard and Smith 1983).

Data (survival of adults from each species and numbers of juveniles produced for each species) were analyzed using one-way randomized complete block design (Kuehl 2000). The main effect was treatment and each shelf was considered a block. Contrasts were used to determine NZMS effects by comparing control conditions (species held allopatric) against the two NZMS density treatments. Similarly, contrasts were used to compare survival and juvenile production between the two NZMS densities. All analyses were performed using program R (Hornik 2016) and values were considered significant at $P < 0.05$.

Results

The survival of the adult snails did not vary among treatments ($F_{6,24} = 1.97$, $P = 0.11$) indicating that NZMS presence and density did not affect the survival of the native snails. The survival of NZMS did not vary between the two densities (5 versus 10 NZMS within aquaria; $F_{3,12} = 2.65$, $P = 0.10$). The numbers of juvenile snails produced varied among treatments (Table 2; $F_{6,24} = 4.67$, $P < 0.01$). When held allopatrically, NZMS production exceeded production of both *A. limosus* and *P. kolobensis* (both $P < 0.01$). No reduction in juvenile production was observed when NZMS were added, regardless of NZMS density (all $P \geq 0.15$). Significant differences in juvenile production were also observed after standardization to control for differences in numbers of adult native snails among treatments (i.e., number of juveniles produced/number of adults added to aquaria; $F_{6,24} = 3.45$, $P = 0.01$). When analyzed in this manner, under allopatric situations, NZMS produced more juveniles/adult than both *A. limosus* or *P. kolobensis* (both $P \leq 0.01$) and there was no difference in juvenile production among the two native species ($t_{1,24} = 1.22$, $P = 0.23$). Under sympatric conditions, the addition of NZMS had no effect on the production of *P. kolobensis* ($t_{1,24} = 0.29$, $P = 0.77$) whereas the addition of NZMS increased juvenile production among *A. limosus* ($t_{1,24} = 2.15$, $P = 0.04$). Regardless, chi-square tests showed that the production of juveniles was not disproportionately skewed towards NZMS (all $P \geq 0.10$).

Conclusions

The results indicate that the presence of NZMS did not affect the survival or recruitment of the native snails. We observed, however, that NZMS recruitment rates (juveniles produced/adult) were higher than the native species. Other studies (e.g., Alonso and Castro-Diez 2008) have also noted a high reproductive rate among NZMS. The reproductive rate for NZMS in our study (0.02 ± 0.01 individuals/d) was much lower than has been reported in other studies (0.1-1.3 individuals/d; Hall et al. 2006). It is possible that reproductive rates in NZMS are reduced when snails are removed from natural conditions, but regardless, we in general have seen less reproduction among NZMS collected from the Loa Hatchery than has been reported by other researchers (Oplinger, personal observation). It appears that the combination of high fecundity and grazing success contributes to the effect that NZMS have on other taxa. For example, Moore et al. (2012) found that the abundance of native grazers declined as NZMS numbers increased and then recovered when the NZMS population crashed. Riley et al. (2008) and Riley

and Dybdahl (2015) studied NZMS interactions with *Pyrgulopsis robusta*, which is in the same genus as the *P. kolobensis* and found asymmetric competitive interactions; the presence of NZMS affected *P. robusta* but *P. robusta* did not affect NZMS. The effects of NZMS were assessed in these studies in terms of growth and although not assessed in our study, changes in growth may ultimately affect recruitment and should be evaluated in *P. kolobensis* and *A. limosus*.

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Table 2: Average number (with SD in parentheses) of native snail and NZMS recruits produced per adult added into aquaria. MA represents mud amnicola and Toq represents Toquerville springsnail .

Treatment	# of Natives	# of NZMS
10 MA	0.08 (0.1)	
10 Toq	0.2 (0.2)	
10 NZMS		1.0 (0.6)
5 MA + 5 NZMS	0.3 (0.4)	0.5 (0.4)
5 MA + 10 NZMS	0.5 (0.5)	1.2 (0.5)
5 Toq + 5 NZMS	0.1 (0.2)	0.5 (0.4)
5 Toq + 10 NZMS	0.4 (0.3)	0.6 (0.1)

Task 3: Recruitment Potential Among New Zealand Mud Snails that Survive Fish Digestion

Background

New Zealand mud snails (NZMS; *Potamopyrgus antipodarum*) have been discovered in many public and private fish hatcheries. Within Utah, the NZMS is found within the Loa State Fish Hatchery and has been known to occur within the hatchery since November 2007. NZMS appear to have no adverse effects on fish within hatcheries. The presence of NZMS has been associated with decreases in growth and condition of wild fish (Vinson and Baker 2008).

Hatchery reared fish can be distributed to many waters and there are concerns in hatcheries that have NZMS that snails can be inadvertently stocked at the same time that fish are stocked. There are many steps that can be taken to decrease this risk. For example, hatchery distribution trucks can be filled with filtered water or water from a NZMS-free source and the likelihood of accidentally loading snails into the distribution truck can be decreased by not touching the raceway walls and floor with a net while loading fish. One larger concern, however, is that the fish themselves could have NZMS in their digestive tracts and that the snails could be excreted alive after stocking. Oplinger et al. (2009) found that 4.5% of NZMS that are ingested by sub-catchable sized rainbow trout survive digestion. Staff at the Loa Hatchery dissected 1,539 fish in raceways and found a total of 120 NZMS in the digestive tracts of these fish (Oplinger et al. 2011). Based on these averages it is estimated that one live NZMS could be inadvertently added to the wild for every 285 fish stocked from the hatchery. The hatchery has a policy stating that fish are quarantined prior to stocking. A total of 2,100 fish in specially cleaned quarantine raceways were dissected and no NZMS were found in the digestive tracts of these fish (Oplinger et al. 2011). This data indicates that in principle that there is no risk associated with the stocking of fish from the quarantine raceways. In reality, however, NZMS are known to occur in every raceway at the facility and it is likely that some NZMS are not killed during the preparation of the quarantine raceways. Thus there is some risk that the stocking of fish from these raceways could lead to the establishment of new NZMS populations, albeit the quarantine process greatly reduces this risk. Regardless, even though it is known that 4.5% of ingested NZMS survive digestion, it is not known whether snails that pass through the digestive tract of a fish make a full recovery and eventually reproduce or if these snails are so compromised by the digestion process that they never reproduce or ultimately pass away. This detail is important because NZMS reproduce asexually and the introduction of a single snail may be sufficient to lead to the establishment of a new NZMS population.

The purpose of this study was to determine whether NZMS that survive digestion are capable of reproducing. We compared the reproduction ability of snails that were digested by two size classes of rainbow trout *Oncorhynchus mykiss*. This was done to determine whether the "thoroughness" of digestion varies with fish size.

Methods

This study was performed at the Loa State Fish Hatchery. Six aquaria, each 110 L in volume were placed on raceway floors. A siphon was used to deliver water into the aquaria and outflow was

directed through a standpipe. Boards were placed on top of the aquaria to prevent fish escape. Three aquaria were used for each fish size class with 7-8 fish placed into each aquaria. Fish were acquired from the Egan and Glenwood State Fish Hatcheries. Fish in the smaller size class ranged between 122 and 159 mm total length with an average of 141 mm. Fish in the larger size class ranged between 160 and 240 mm total length with an average of 207 mm. We had hoped to have a greater discrepancy in length between the larger and smaller fish. The smaller fish are of a size consistent with "sub-catchables" that are stocked within Utah. We hoped to find "catchable" fish but the larger size class were the largest fish available at the time of the study.

We found that the fish did not volitionally consume NZMS. Instead, NZMS were force-fed to the fish by anesthetizing the fish with MS-222 and inserting a tube into their stomachs (Oplinger et al. 2009). Ten NZMS were placed into the tube and a syringe filled with water was used to flush the snails from the tube into the stomachs. The fish were held for 15 minutes in freshwater to allow for anesthesia recovery and to provide the fish the opportunity to regurgitate snails. Afterwards, the fish were placed back into the aquaria.

Twice daily, the bottoms of the aquaria were siphoned and waste was removed. NZMS were separated from the other waste and placed into beakers of hatchery water. Snails were provided at least 6 hours to recover and after recovery, snails that moved were considered alive. Live snails were separated from dead snails and were placed into 5.7 L aquaria that were placed on the floor of the Loa Hatchery Shop. A single live snail was added to each aquaria and 10 g of watercress *Nasturtium officinale* was added to provide the snails with forage. A total of 66 aquaria were established; 22 contained snails that survived digestion by smaller fish, 22 contained snails that survived digestion by larger fish, and 22 contained snails that were not fed to fish and were thus controls. The snails remained in the aquaria for 74 d. The water and watercress in each aquaria was replaced three times during the holding period. After the holding period was complete, the survival of the snails was assessed and the contents of each aquaria were preserved by freezing.

Samples were transported to the Fisheries Experiment Station where they were thawed and filtered using sieves to collect particles that were 100-500 μm in size. These particles were stained for 1-4 hrs in a 0.1% alizarin red solution (Howard and Smith 1983). These particles were then rinsed and counterstained for 10 s using 1.0% light green, SF yellowish + 1% glacial acetic acid and afterwards the particles were thoroughly rinsed (Howard and Smith 1983). A dissecting microscope was used to scan all of the particles under 20x magnification and NZMS neonates were observed and enumerated. Not all 66 samples were processed. We enumerated neonates from 11 of the 22 snails that survived digestion by the smaller rainbow trout, 7 of the 22 snails that survived digestion by the larger rainbow trout, and 5 of the 22 control samples. An one-way ANOVA was used to compare neonate numbers across treatments. In addition, a t-test was used to determine whether the number of neonates observed was greater among snails that survived the entire 74 d holding period. All data were analyzed using R (Hornik 2016) and considered statistically significant at $P < 0.05$. Logarithmic transformations were used to ensure the data met the normality assumption of the analyses.

Results/Discussion

Neonates were found in all treatments (Figure 5) and the number of neonates produced did not vary among treatments ($F_{2,20} = 0.48, P = 0.63$). Only 5 of the 66 snails (7.6%) of the snails survived the entire 74 d holding period. Two of those snails were from the control treatment, two survived digestion by smaller fish, and one survived digestion by larger fish. The number of neonates produced did not vary among aquaria where the snails survived the entire 74 d holding period compared to aquaria where the snails did not survive the entire holding period (Figure 6; $F_{1,21} = 0.89, P = 0.36$; 3 of 23 samples processed had NZMS that survived the 74 d holding period). These data clearly indicate that NZMS that survive digestion have the ability to reproduce. Thus, if fish are stocked from a hatchery that has NZMS, any snails that survive digestion have the ability to reproduce and establish new NZMS populations. Also, reproductive rates among snails that survived digestion were not reduced compared to controls, indicating the surviving snails were in good condition. Finally, similar numbers of neonates were produced by snails that survived the entire 74 d holding period compared to snails that did not survive. This would suggest that most neonates were produced early in the holding period, before significant numbers of snails died.

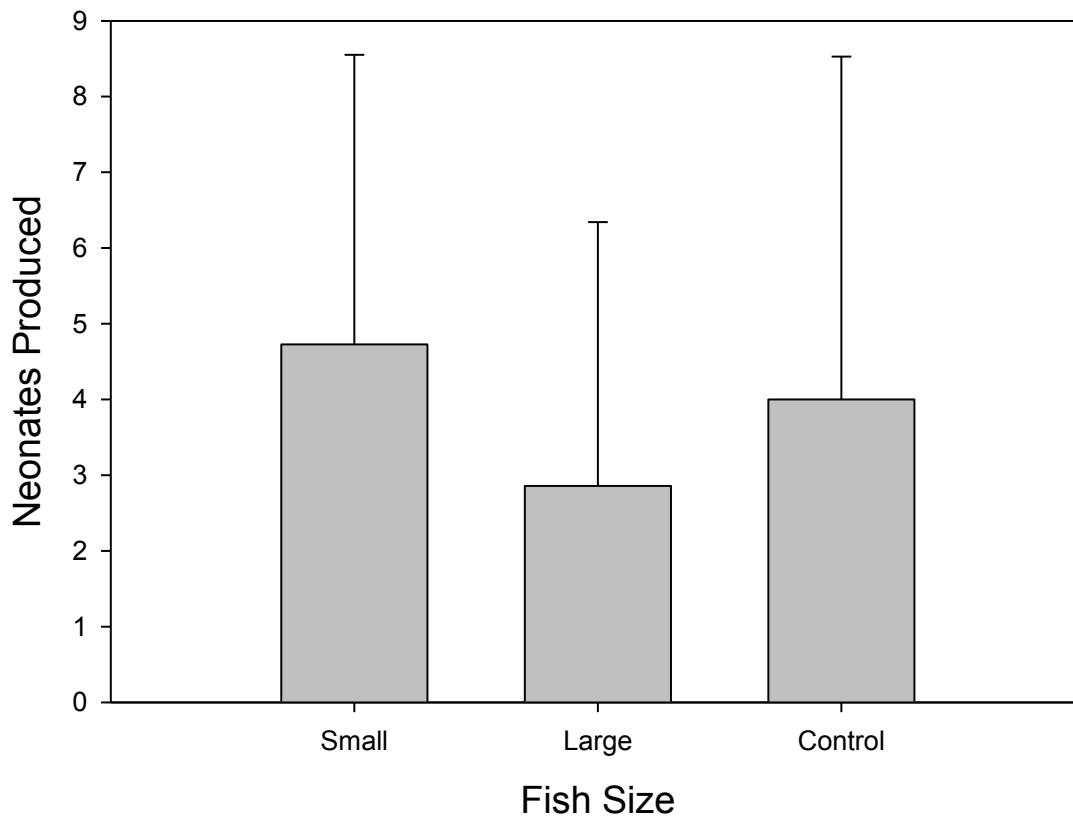


Figure 5: Average number of neonates produced by snails that survived digestion by either smaller or larger rainbow trout. The control treatment refers to snails that were not fed to fish. Error bars represent ± 1 standard deviation of the mean.

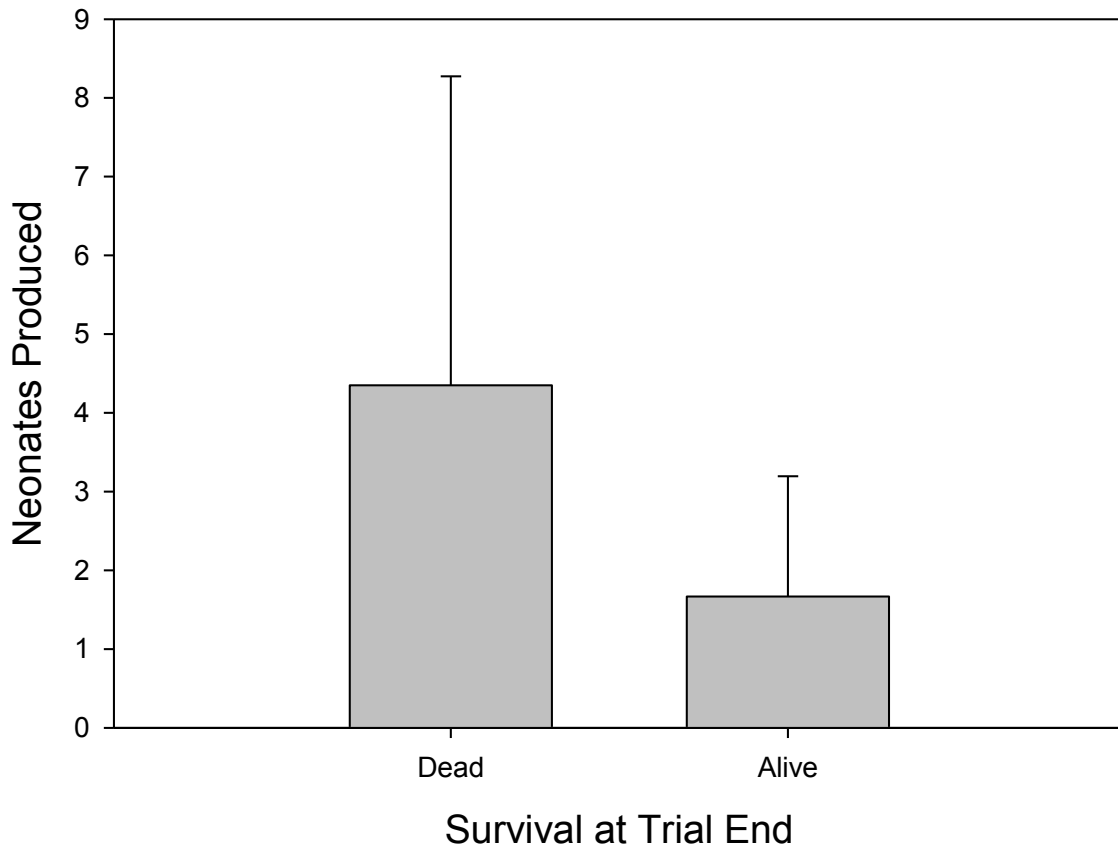


Figure 6: Average number of neonates produced by snails were either alive or dead 74 days after digestion by fish. Error bars represent ± 1 standard deviation of the mean.

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Task 4: Update of the Range of NZMS within Utah

New Zealand mudsnails (NZMS) were discovered in the Loa State Fish Hatchery in 2007. After their formal discovery, NZMS shells were found in piles of fish waste at the hatchery and these piles were produced prior to 2007 (likely 2004 or 2005). Thus, it is assumed that NZMS actually entered the hatchery earlier than 2007. The discovery of NZMS in 2007 prompted a quarantine of the Loa Hatchery and subsequent sampling found no NZMS in any waters that were stocked by Loa in 2007. Site sampling was performed by spending one man-hour of time assessing the presence/absence of NZMS in habitats that are suspected to harbor NZMS.

In light of the fact that NZMS were likely introduced into Loa prior to 2007, we reviewed stocking records and identified 22 sites (Table 3) that were stocked by Loa between 2004 and 2006 that had not previously been surveyed for NZMS. The goal is to visit 3-5 of these sites per year until all sites are sampled. In 2015, Wide Hollow Reservoir, Upper and Lower Box Creek Reservoirs, and Posey Lake were surveyed for NZMS. No NZMS were found at any of these sites. An updated map showing the distribution of NZMS within Utah is presented in Figure 7. To date, no NZMS have been found at any sites that were formerly stocked by Loa. Sampling from additional sites in Table 3 will be performed across the next several years.

Table 3: List of water bodies stocked by the Loa State Fish Hatchery that had not previously been assessed for the presence of NZMS.

Water Body	
Yeans Reservoir	Navajo Lake
Burraston Ponds	Enterprise Reservoir, Lower
Towne Reservoir	Enterprise Reservoir, Upper
Posey Lake	Wide Hollow Reservoir
Grantsville Reservoir	Palisade Reservoir
Gates Lake	Box Creek Reservoir, Lower
Beaver River	Box Creek Reservoir, Upper
Nine Mile Reservoir	Asay Creek
Coleman Reservoir	Panguitch Lake
Settlement Canyon Reservoir	Sevier River, East Fork
Minersville Reservoir	Mammoth Creek

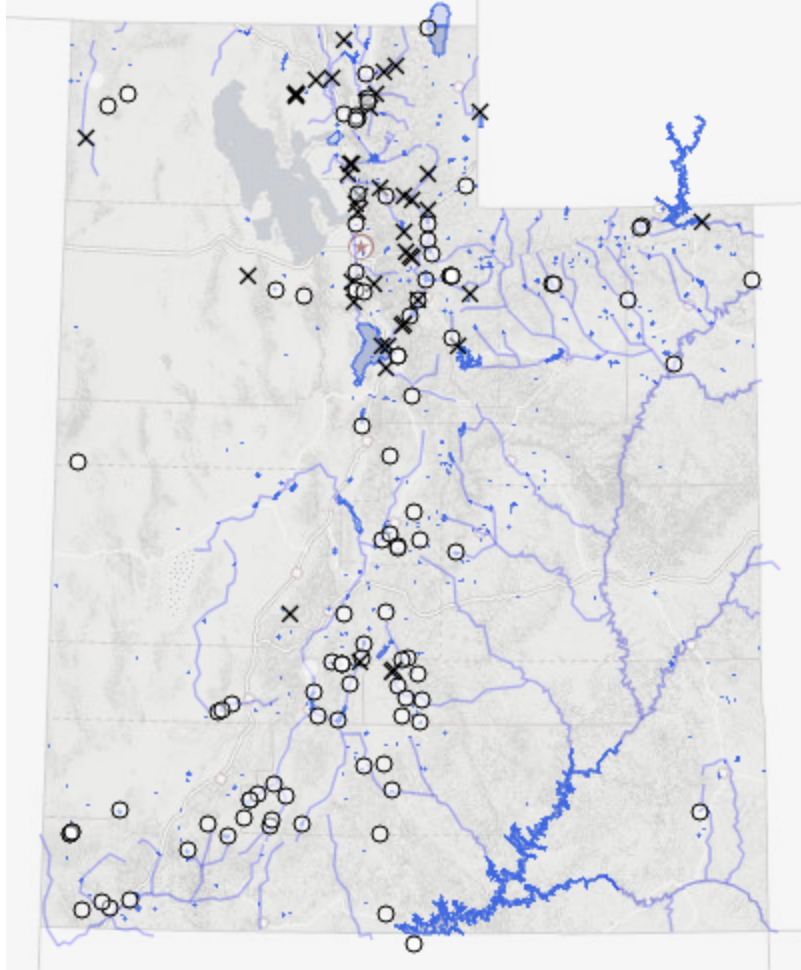


Figure 7: Map of the distribution of NZMS within Utah. X's represent sites containing NZMS and O's represent sites lacking NZMS.