Control of New Zealand Mudsnails in Fish Hatcheries

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ABSTRACT

New Zealand mudsnails (NZMS) have infested several fish hatcheries in the western United States. Because of risks of transporting NZMS to naïve locations hatchery managers or regulators have denied permission for shipments of fish from infested hatcheries to non-infested waters. We tested the efficacy of a hydrocyclonic separator to remove New Zealand mudsnails from infested fish hatchery inflow waters. We found the system was fully successful in removing all sizes of NZMS from inflow waters to the underflow of the filter. We modeled transit patterns for water to verify that collection intervals for snails were reasonable and then modeled movement of three different snail sizes through the hydrocyclone at two flow rates to determine if flow affected filtration efficiency. We found that the smallest size of snail, neonate, had the fastest transit rate at both flows and adult and juvenile sizes had similar transit rates to each other. Snail transit was faster at the higher flow rate and remained efficient. As a treatment for snails filtered from hatchery flow by hydrocyclone we conducted laboratory studies of the toxicity of carbon dioxide to NZMS. Three different sizes of NZMS based on life history characteristics were exposed to CO_2 under 100 kPa pressure at several time intervals and three different temperatures to determine survival based on temperature units. Probit models were used to compare the different sizes of NZMS ability to survive pressurized CO_2 treatment. Snails exhibited a dose response to pressurized CO_2 treatment. Models indicated that adult and juvenile sized snails exhibited similar response to the treatment and neonate snails had a reduced ability to withstand treatment. A combination of filtration followed by a toxic treatment of CO_2 is an effective method for removing New Zealand mudsnails from infested hatcheries.

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CHAPTER I - HYDROCYCLONIC SEPARATION OF NEW ZEALAND MUDSNAILS FROM INFESTED FISH HATCHERY INFLOWS

Abstract

New Zealand mudsnails (NZMS) have infested several fish hatcheries in the western United States. Because of risks of transporting NZMS to naïve locations hatchery managers or regulators have denied permission for shipments of fish from infested hatcheries to non-infested waters. We tested the efficacy of a hydrocyclonic separator to remove New Zealand mudsnails from infested fish hatchery inflow waters. We found the system was fully successful in removing all sizes of NZMS from inflow waters to the underflow of the filter. The patterns of transit of water and adult, juvenile, and neonate NZMS through the test hydrocyclone at 367 L/min and 257 L/min were modeled to determine filtration efficiency at various flows. Residence time distribution analysis was completed on water to determine if the filtration system was reacting as a plug flow or mixed flow reactor and to determine appropriate collection intervals for determining NZMS hydrocyclone transit. NZMS were removed more rapidly at the higher flow rate. A four parameter logistic model was fit to the cumulative percent recovery by time for all sizes of NZMS at both flows. Parameter estimates from the model were compared using MANOVA to determine differences in transit pattern among three snail sizes and between two flows. Analysis of logistic curve parameter estimates showed no difference between transport time of adult and juvenile sized snails within flows. Parameter estimates for neonate logistic curves were different from the adult and juvenile sizes. More water can be filtered at the higher flow rate without reducing filtration efficiency and would be recommended for implementation in a hatchery setting.

Introduction

New Zealand mudsnails (*Potamopyrgus antipodarum,* NZMS) are native to both fresh and brackish water of New Zealand and its surrounding islands (Gerard and Lannic 2003). NZMS were discovered in the United States in 1987 in benthic samples collected in the Nature Conservancy's Thousand Springs Preserve near Hagerman, Idaho by D.W. Taylor (Bowler 1991). Since their introduction, NZMS have spread to every state in the Western U.S. with the exception of New Mexico (Gustafson et al. 2002). The snails have been introduced into the Great Lakes (Zaranko et al. 1997; Grigorovitch et al.2003), and Wisconsin and Minnesota (Minnesota Department of Natural Resources 2008).

New Zealand mudsnails reproduce both sexually and asexually via parthenogenesis (Winterbourn 1970). Unites States populations are primarily composed of asexually reproducing females (Wallace 1992) that can produce up to 120 embryos per snail (Winterbourn 1970b, Schreiber et al. 1998, Richards and Lester 2000, Richards 2004). This reproductive strategy requires only one female snail to found a new population in a naïve water body. Snails in the U.S. have escaped the suite of trematode parasites from their native range that leave them unable to reproduce (Jokela and Lively 1995, Levri and Lively 1996, Levri 1998, Jokela et al. 1999). Some populations in the Snake River and Yellowstone National Park have been reported at densities of over 500,000 NZMS per square meter (Richards 2002, Hall et al. 2003).

At densities such as those found in the Snake River and rivers in Yellowstone National Park, NZMS can disrupt natural ecosystem function by outcompeting native macroinvertebrates for consumption of primary production and thereby dominate carbon and nitrogen cycling (Hall et al. 2003; Hall et al. 2006).

Infestations of NZMS have posed dilemmas at infested hatcheries that provide fish for mitigation, conservation, recreation, and/or supplementation. Fish or transport water from infested hatcheries could spread NZMS to uninfested water bodies. Steelhead and rainbow trout (*Oncorhyncus mykiss*) will volitionally feed on NZMS inhabiting raceways or holding tanks (Bruce 2006). Snails consumed by fish may pass through the intestinal tract of a fish unharmed (Bondesen and Kaiser 1949; Haynes et al. 1985; Vinson 2004; Bruce 2006). In addition, the use of biocides to remove pests and clean springs is often not feasible due to regulatory requirements, and risks to other biota of special concern such as threatened or endangered species. Without a way to remove snails from the hatchery supply water the potential risk of introducing an invasive species has caused hatcheries to discontinue shipments of fish to uninfested water bodies (Mark Olsen, Willow Beach NFH Project Leader, personal communication).

Research to control or remove snails from infested hatcheries has been limited. Bruce (2006) suggested depuration strategies for fish from infested hatcheries and found that snails can be retained in the gut of starved fish for as long as 48 hours and remain alive. However to ensure that NZMS are not transported out of an infested fish hatchery fish need to be raised or depurated and transported in NZMS free water (Bruce 2006). NZMS could be filtered from hatchery inflow to provide a NZMS free water source.

Cyclonic separation is used in many industries to separate particles from waste or inflow systems. Hydrocyclone filtration uses centrifugal force to remove particles of a higher specific gravity than water from a pressurized water source (Kraipech et al. 2006), and filtration can be relatively low cost when compared to other treatment methods especially those with moving parts (Ortega-Rivas 2004; Williams and Evans 2007). Hydrocyclones

have been widely used in mining (Trawinski 1976) and food industry (Ortega-Rivas 2004) but have never been considered for separation of biological material in fish hatcheries.

The objectives of this study were to test the efficacy and filtration efficiency of a hydrocyclone to remove all life stages of New Zealand mudsnails; and to model and compare the passage of water and snails through the filtration system.

Methods

Study Location, Operational Design, and Instrumentation

The hydrocyclone used in this study was a 15.25 cm diameter standard hydrocyclone Krebs model U6-gMAX (FLSmidth Krebs, Tucson, Arizona). We used two different vortex finders to allow for a total flow of 367 L/min (5.7 cm aperture) or 257 L/min (3.8 cm aperture). The apex opening was 1.27 cm and allowed approximately 13.44 L/min to the underflow (Figure 1.1). Design pressure for hydrocyclone operation was 69 kPa for both flow rates.

The hydrocyclone intake was placed in Len Lewis spring on the northeast side of Hagerman National Fish Hatchery, Hagerman, Idaho (Figure 1.2). The location was chosen to obtain a 9.1 m drop from the water intake to the hydrocyclone and achieve the required head pressure (69 kPa) for efficient operation (Figure 1.2).

The hydrocyclone was supplied with water traveling through 72 m of 7.6 cm diameter flexible fire hose and 12 m of 7.6 cm PVC placed from the spring source to the filtration site (Figure 1.3). A Signet 8150 Flow Totalizer (George Fisher Signet, Inc., El Monte, California) was mounted in the PVC pipe to monitor instantaneous flow rate (L/min) and totalize flow over operations. The water that entered the hydrocyclone was separated into two streams, underflow and overflow. Water at the underflow was constricted by the

manufacturer design for a flow of approximately 13.44 L/min and was where high-density particles were removed. Water directed to the overflow (approximately 353 or 243 L/min) was piped through a 7.6 cm PVC pipe to deliver filtered water through a 5 cm reduction manifold controlled with gate valves to provide ports for testing filtration efficiency. The overflow was directed using drops to spill into one of two tanks and then flow back to the spring water moving to the hatchery. Flow through the hydrocyclone was adjusted with a gate valve at the hydrocyclone overflow and pressure with a similar valve at the inflow of the hydrocyclone.

Water Transit

We modeled the transit rate of water through the hydrocyclone by both step down and pulse tracer concentration methods at 367 L/min and 257 L/min. Step-down tests were conducted to establish the flow pattern and the characteristics associated with possible mixing in the hydrocyclone using residence time distribution analysis. Residence time analysis models flow in a non-ideal reactor and requires either a pulse or step change in tracer concentration introduced into the reactor (Danckwerts 1953). The reactor in this study was the hydrocyclone and water intake. Pulse tests were conducted to verify the time necessary for collecting snails for establishing the snail transit pattern through the hydrocyclone.

Step-down trials – Step-down trials were conducted in October 2007. A concentrated solution of NaCl was introduced through the hopper at approximately 20 L/min to raise NaCl concentration of the water exiting the hydrocyclone to a stable conductivity reading for one minute. To achieve a step down in tracer concentration, the flow of tracer solution was terminated and concentration decay was monitored via conductivity. Conductivity was

measured and recorded at one-second intervals with a YSI 556 MPS multi-probe meter (YSI, Inc., Yellow Springs, Ohio) in a 4L container designed to capture the water exiting the underflow and keep the YSI conductivity probe covered.

Pulse trials – Pulse trials were conducted on May 14, 2008. A concentrated NaCl solution was introduced as a tracer through the hopper bucket. 73.4 g of NaCl were dissolved in 20 liters of spring water in the hopper and released into the intake hose over a one-minute to raise the inflow water approximately 200 ppm. The tracer was introduced through the hopper at approximately 0.33 L/sec for one minute to dilute into 367 or 257 liters according to the flow rate.

To monitor the movement of the tracer through the intake and the hydrocyclone, grab samples were taken every ten seconds from the overflow and underflow starting at 60 seconds for 367 L/min flow rate and at 80 seconds for 257L/min. Conductivity of the samples was measured after the test with a YSI 556 MPS multi-probe meter. The conductivity of water exiting the hydrocyclone at the underflow was recorded before each trial and used as baseline conductivity.

For both methods, water with tracer from the overflow was piped into the Main Spring head box where it was diverted into pipes feeding one of the hatchery buildings. Water from the underflow was piped away from the spring and applied to the ground to avoid any potential disturbance of the threatened Bliss Rapids snails (*Taylorconcha serpenticola*) in the spring below the hydrocyclone test site.

Snail Transit

Snails used in studies were collected from Len Lewis spring adjacent to the north boundary of HNFH. Snails were collected once a week and kept in a 20-L bucket with native

vegetation and spring water in a walk in refrigerator at the HNFH laboratory. Snails were separated by size: (adults (>2 mm); juveniles (1mm to 2 mm); neonates (<1mm). Samples of 100 adult, 100 juvenile, or 50 neonate snails were separated into 120 cc plastic cups and kept in a walk-in refrigerator at 5°C for 1-2 days prior to using for hydrocyclone analysis. Lights were on inside the refrigerator for approximately 9 hours per day.

To collect neonates, 5-10 adult snails were placed in 120 mL cups with well water for 2-3 days and held at room temperature (~21°C). After 2-3 days the contents of the cups were passed through two stainless steel sieves (850 µm, 90 µm) to separate and collect adults and neonates. Neonate NZMS collected on the 90-µm sieve were rinsed into water with 5% Rose Bengal to improve identification of them in collections (Sigma-Aldrich Co., Dallas, Texas) and held at 5°C for 24-hours. After 24 h, dyed neonate snails were separated with a sieve, rinsed, and placed into fresh well water to be counted and used for trials.

The transit of three sizes of snails through the hydrocyclone was tested and modeled at both 367 L/min and 257 L/min. Trials began with introduction of 100 adult or 50 juvenile or neonate snails. Ring nets (80 µm mesh; 30.5 cm diameter, with cod ends fitted with 80µm mesh) were placed at the overflow and underflow of the hydrocyclone to collect snails (Figure 1.3). Time intervals of collection were determined by snail size and hydrocyclone flow rates (Table 1.1). The overflow ring net remained in place for the duration of the test. Nets were removed from the underflow and clean nets replaced to filter contents at selected time intervals. When removed the nets were placed in clean plastic bags until sample removal. Samples were collected from ring nets by rinsing contents carefully with clean water down to cod end of the net. Contents of the cod end were placed into 250 mL plastic jars and fixed in 10% buffered formalin for at least 24 hours and then transferred to 40% 2-

propanol for storage and quality control at the University of Idaho. Using a dissecting microscope the number and size of snails collected at each time interval were counted and a subset of 10% of samples was recounted for quality control.

Snail transit tests were conducted in July and October 2007. After analysis of data from July the collection intervals were decreased in October samples to provide more accurate models (Table 1.1). Cumulative percent recovery from similar time intervals for trials from both months was comparable. Since intervals in October trials resulted in better models these values were used for final analysis.

Statistical Analysis

Water transit – For tracer step-down trials, conductivity measurements were normalized to a percent of the maximum conductivity value ($\Delta C/\Delta C_{max}$)(Watten et al. 2000). Mean residence time ($\bar{t}$) and variance (σ^2) for each step-down test were calculated using the equations:

$$\bar{t} = \frac{\Sigma\, t_i C_i}{\Sigma\, C_i}$$

$$\sigma^2 = \frac{\Sigma\, t_i^2 C_i}{\Sigma\, C_i} - \bar{t}^2$$

where t_i represents the one second time intervals and C_i represents the normalized conductivity measurements (Levenspiel 1984). Using the mean residence time and variance for each test the vessel dispersion number ($D/\mu L$) was calculated to characterize the amount of mixing leading to deviation from a plug flow pattern. The vessel dispersion number ranges in value from zero to infinity with numbers greater than zero characterizing a greater degree of mixing within the reactor (Levenspiel 1984). The equation for the vessel dispersion number was,

$$2(D/\mu L) = \frac{\sigma^2_t}{\bar{t}^2}$$ (Levenspiel 1984).

For pulse trials conductivity measurements were normalized to a percent of the maximum conductivity value ($\Delta C/\Delta C_{max}$)(Watten et al. 2000) and plotted as normalized conductivity and as cumulative percent conductivity across time. The normalized conductivity plot was used to visually compare overflow and underflow tracer passage. A four-parameter logistic curve was fit to the cumulative conductivity points (SigmaPlot 9.0, Systat Software, Inc. 2004) for comparison to the depletion pattern of snails.

Snail transit - Filtration efficiency was determined by the calculating the percent of NZMS collected at the underflow vs. the overflow. To compare the transit of the three snail sizes at both flows, the percent of snails recovered at each time interval was normalized by calculating the percentage of snails recovered at the time interval (ΔR) by the total number of snails recovered during that test ($\Delta R/\Delta R_{max}$), similar to the process for normalizing tracer concentrations. The cumulative normalized percentages were graphed and a four parameter logistic curve was fit to each data set. The model for the curves was,

$$y = min + ((max - min)/ (1 + (x/EC50)^{Hillslope})) ,$$

where min is the lowest observed value, max is the highest observed value , EC50 represents the time to 50% of the NZMS dose recovery, Hillslope is the greatest absolute value of the slope of the curve, x is the elapsed time of the test, and y is the percent recovery. Logistic curves for each of the snail sizes was visually compared to logistic curves for pulse tracer tests to verify that collection intervals were appropriate for snail tests.

Parameter estimates from logistic curves were compared using MANOVA with the model,

$$X_{lkr} = \mu + \tau_l + \beta_k + \gamma_{lk} + \varepsilon_{lkr},$$

where μ is an overall mean, τ_l was the flow effect, β_k was the size effect, γ_{lk} was the flow/size interaction, and ε_{lkr} is the random error. Analysis was conducted using proc glm in SAS 9.1 (SAS Institute 2002-2003). If a significant effect was detected ($P<0.05$) then individual parameter estimates were compared using ANOVA on individual parameter estimates. The linear model for this test was,

$$y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk},$$

where i was flow, j was snail size, k was replicate, and τ_i, β_j, and $(\tau\beta)_{ij}$ represent the effects of flow, size, and the flow-size interaction respectively. When parameter estimates were significantly different, a Tukey test was used to separate the combined flow*size groups. For flow*size groups that were not different a median value was used for a combined logistic model defining transit. The combined logistic model was tested against all of the logistic parameters from all individual snail transit tests to assure that the model differed significantly from zero with proc nlin in SAS release 9.1.

Results

Water Transit

Step-down trials – Four step-down trials were conducted at each flow. Mean residence time ± SD for 367 L/min was 105.31 ± 5.38 seconds and the mean dispersion number ($D/\mu L$) was 0.055 ± 0.008 (Table 1.2, Figure 1.4). Mean residence time for 257 L/min was 120.18 ± 2.42 seconds with a $D/\mu L$ of 0.021 ± 0.007 (Table 1.2, Figure 1.4). The vessel dispersion numbers are >0.01 indicating a large deviation from plug flow, in effect making the hydrocyclone an imperfect plug flow reactor (Levenspiel 1984).

Pulse trials – Two pulse trials were conducted at each flow. Conductivity measurements from grab samples from the overflow and underflow across the duration of the tests were overlaid and indicate that the movement of tracer was the same to both the overflow and underflow (Figure 1.5). Tracer arrived at both the underflow and overflow between 60 and 70 s for 367 L/min flow and left the system during the 130-140 s interval for a total detection time of approximately 70 s (Table 1.3). Tracer was detected in samples in the 90 to 100 s interval and left the system in the 160 to 170 s time interval again for a total detection time of approximately 70 s (Table 1.3). Theoretical time to arrival based on the volume of the intake hoses and the hydrocyclone (394.12 L) and flow rates was 64.4 s and 91.9 s for 367 L/min and 257 L/min respectively, indicating that flow was unimpeded to the hydrocyclone. The 70 s duration of tracer detection compared to the 60 s introduction time indicates that some mixing may have occurred in the hydrocyclone and intake lines.

Snail Transit

The hydrocyclone was effective in removing all three sizes of snails to the underflow. The recovery into the underflow represented 99.3% of adults, 95% of juveniles, and 69% of neonates. No snails were recovered in the nets filtering the overflow water. Portions of the samples introduced into the hydrocyclone were destroyed as noted by the presence of shell fragments in the nets when incomplete sample recovery occurred.

Transit rates of all sizes of NZMS were faster at 367 L/min than at 257 L/min (Figure 1.6). At both flows neonate NZMS were recovered faster than the adult or juvenile NZMS (Figure 1.6). Adult and juvenile NZMS transit rates do not differ at either flow.

Logistic curves were fit to the cumulative percent recovery for all tests with high R^2 (Table 1.4). Comparison of logistic models of water tracer to snails indicated that sampling

intervals were started and ended at appropriate time intervals (Figure 1.5). We found an effect attributed to flow rate ($P<0.001$) and NZMS size ($P<0.001$) on parameter estimates from the logistic curves with minimal interaction between flow and snail size (Table 1.5). Analysis of variance of individual parameter estimates indicated no significant differences were observed for the min or max parameter estimates for either size or flow or for the interaction between size and flow (Table 1.6). The EC50 and hillslope parameters were significantly different between tests of flow ($P< 0.001$) and size of snails ($P = 0.0025$, Table 1.6). A Tukey tests grouped adults and juveniles by similar EC50 value within flows (Figure 1.14, Table 1.7). Adults and juveniles were grouped within and between flows with a similar Hillslope value (Figure 1.15, Table 1.7). Neonate groups from both flows were grouped with a similar Hillslope value (Figure 1.15, Table 1.7). A single combined four-parameter logistic model using the median group values was tested against the parameter values from individual snail transit tests (Table 1.4) and differed significantly from zero (Table 1.8, $P<0.001$). The combined logistic model accurately represented the data we collected and gives a good approximation of percent of snail recovery dependent on flow by time from introduction into the test hydrocyclone system.

Discussion

This test was the first ever use of a hydrocyclone to remove invasive species in hatchery applications. We found the hydrocyclone effective in removing the NZMS from infested inflow waters and can be used effectively to provide a water source free of invasive species. By giving hatcheries the option to produce snail free fish and transportation water they can possibly reduce the risk of spreading harmful non-native species and reduce limitations placed on them by regulating agencies.

All tests of filtration with introduced snails provided snail free overflow water. Other studies have reported success at filtering biological material such as yeast, blood cell separations, or parasite cultures from solution using a hydrocyclone (Ortega Rivas 2004; Williams and Evans 2007). Based on the average specific gravity of 1.33 for live neonate NZMS and information given by the manufacturer of the hydrocyclone used for this study the D50, or the size that 50% of the particles are removed through the underflow (Frachon and Cilliers 1999; Arterburn 2006), would be approximately 36 microns and the D98, 98% particle removal to the underflow, would be 80 µm (Michael Trew, Krebs Engineers, personal communication). Dried neonate NZMS are greater than or equal to 150 µm. Given these calculations our test hydrocyclone should remove all of the sizes of NZMS.

Despite the high level of filtration efficiency we did not recover all of the snails introduced into the hydrocyclone. The loss of adult NZMS in the system may be attributed to sampling and/or counting error, but juvenile and neonate snails were likely crushed moving through the filtration system. A principle concern with using hydrocyclones to remove biological material from suspension is the destruction due to the shear force from the tangential feed into the vortex chamber of the hydrocyclone (Svarovsky 1984, Williams and Evans 2007). Williams and Evans (2007) experimented with a hydrocyclone for removing entomopathogenic nematodes from rearing solution and found that with a small hydrocyclone and short vortex cone damage to the nematodes was minimal. Their claim was that a short residence time in the hydrocyclone led to a reduced exposure to shear forces and a low level of damage to nematodes (Williams and Evans 2007). With a large hydrocyclone and high flows such as in this study there may be a high enough shear force and residence time in the hydrocyclone to destroy some biological material. In both overflow and

underflow samples we observed pink fragments after tests using neonates. Neonates were dyed pink with Rose Bengal dye and pink fragments may have been parts of neonate NZMS destroyed in the filtration process. According to our observations, smaller sizes of NZMS, especially neonates, are delicate and easily crushed. There were 72 m of intake hoses and pipes that NZMS traveled through before reaching the hydrocyclone increasing their residence time and exposure to turbulence and/or friction throughout the water line leading from the water intake to the hydrocyclone. It is probable that smaller juvenile and neonate NZMS were fragmented and/or abraded by a long residence time in hoses and high shear forces in the hydrocyclone. The result was a lower percent recovery at the underflow and zero whole NZMS in the overflow.

At both flows tested in this study we found that adult and juvenile NZMS had a similar transit pattern and their data were combined for the final logistic transit model. Neonates however showed a faster transit pattern than the larger two classes of NZMS (Figure 1.7). The size for 98% particle removal (D98) to the underflow for the 15.25 cm hydrocyclone we tested is approximately 80 µm (Arterburn 2006). A D98 of 80µm indicates that the hydrocyclone should remove the representative particle sizes of NZMS at a high efficiency. Within a hydrocyclone there are two vortices, the primary vortex that removes particles as they move into the hydrocyclone and a secondary vortex that consist of water and less dense particles pulled up through the vortex finder to the overflow (Trawinski 1976; Matveinko and Dueck 2006). Laverack (1980) and Matvienko and Dueck (2006) reported that large particles removed by the primary vortex move slowly down the side of the vortex chamber and then the vortex cone as they form a more viscous layer than the surrounding slurry. Smaller, less dense particles may not be filtered out initially by the primary vortex but

experience a higher level of centrifugal force in the smaller and more tightly spinning secondary vortex (Trawinski 1976). Particles removed by the secondary vortex are removed to the outer part of the primary vortex and removed quickly through the underflow experiencing less friction on the side of the vortex cone (Trawinski 1976). Neonate NZMS may have a low enough specific gravity to be filtered out in the secondary vortex rather than the primary vortex. This may lead to a decreased residence time for the smaller particle sizes that the neonate NZMS represent.

The shape of the particle is also important to the filtration rate and efficiency of hydrocyclone. Particles that are flattened may tend to concentrate more towards the overflow (Trawinski 1976). Trawinski (1976) discussed how flat particles would be removed through the overflow regardless of how coarse they may be. As NZMS grow they become more elongated and conical. While their specific gravity dictates that they should be filtered out very efficiently, the deviance with age from a spherical shape may cause an increase in the residence time of the adult particles.

Residence time of snails in the hydrocyclone was shorter for of the 367 L/min flow than for the NZMS than for the 257 L/min flow. The obvious explanation for the difference in residence times between water and snails at the two flows is that the higher flow is more effective at flushing the particles out of the hydrocyclone. Despite the turbulence and mixing in the hoses and hydrocyclone at the higher flows, the force of the water at the higher flow pushes the NZMS of all sizes through the hydrocyclone more effectively. At the lower flow rate, the added friction from the hoses acts on the larger snail particles. The conclusions from all trials of snail transit and filtration were that the hydrocyclone remained effective at

both flows, and removed all sized particles including the smallest and least dense neonate NZMS.

Other research on hydrocyclone filtration provides data on recovery efficiencies of a range of particle sizes with a range of hydrocyclone sizes. Models built using recovery efficiencies aid in determining the effective filtration size of a hydrocyclone. However, few studies report a transit model for materials. The test system used in our study was uniquely constructed for the conditions at Hagerman National Fish Hatchery. Installations at other facilities would need to consider water temperature, gravity drop, line pressure, as well as the hydrocyclone unit. If the needed gravity drop or line pressure is not readily available by the location of the water source in relation to the hydrocyclone, the required pressure can be generated using a water pump. The logistic model constructed in this study to describe snail transit is specific to this site, but provides a useful tool for discussion and future construction and implementation of this technology as a solution to invasive species infestations in general.

The calculated vessel dispersion numbers were >0.01 and indicate that there was a significant amount of mixing that occurred in our hydrocyclone system. However the duration of tracer detection in pulse trials indicates that minimal mixing occurred. In a perfect reactor (or hydrocyclone in this case), tracer passing through in a plug flow pattern would all arrive and leave the reactor at the same time. Longitudinal mixing would be marked by a quick rise in tracer concentration at arrival and a more gradual depletion as the tracer leaves. Longitudinal mixing is characteristic of fluid moving through a pipe as viscosity of the fluid creates laminar flow (Danckwerts 1953). Due to the low viscosity of water (1.3 cP at 10°C) there would be some mixing simply due to laminar flow in the hoses

even if they were completely straight (Dankwerts 1953). The hose from the water intake to the hydrocyclone had several bends and different gradients in its course that could contribute to mixing especially at the higher flow tested. Adding salt to the water as a tracer presumably increases the density and thus the viscosity of the water, however, once diluted the salt mixture would not contribute significantly to a viscosity change and mixing rate. The two types of water tests had measurements taken in different ways. The step-down tests conductivity measurements were taken instantaneously with a YSI probe from a 4L bucket at the underflow mixed flow reactor while the pulse test measurements were taken from grab samples. The 4L bucket had its own residence time distribution and its characteristics are more like those of a mixed flow reactor. This caused a rise in the vessel distribution number and made our tests appear to have more mixing than the pulse trials. Our hydrocyclone system most likely causes minimal mixing and more closely mimics a plug flow system with some laminar flow in the intake line.

Recommendations

This study was conducted with relatively high snail concentrations compared to what would naturally drift into the hydrocyclone. Tests also only lasted for short periods of time (maximum of four minutes per test). To further evaluate the feasibility of hydrocyclone filtration to remove invasive NZMS longer-term evaluations of efficiency on snail removal should be conducted with naturally occurring drift rates.

To achieve the amount of flow for long-term evaluation on a raceway at Hagerman National Fish Hatchery, several hydrocyclones would be coupled together with a manifold. Hydrocyclone models with 25.4 cm diameter can filter up to 1136 L/min and remove particle sizes represented by the smallest neonates (Michael Trew, Krebs Engineers, personal

communication). Flow per raceway at peak production at Hagerman National Fish Hatchery approaches 4542 L/min at peak production (Mark Olsen, Willow Beach NFH Project Leader, personal communication). Four hydrocyclone units could be coupled together to provide enough water to a raceway. Drift nets would be placed in the raceway at various distances from the inflow and at various depths to evaluate if any NZMS, particularly neonates, are found drifting into the raceway with the filtered water.

Water is a limited resource at HNFH and spring flows have been decreasing by 0.03 m^3s^{-1} since 1999 (HNFH 2006). Technology that provides clean water and enables HNFH to maintain production levels on less water or increase production on the water available is desirable. Modifying existing raceways to create mixed cell rearing units may provide a solution to rearing more fish on the same volume of water while quickly removing fecal solids from rearing space (Watten et al. 2000, Bruce 2006). This technology modifies rectangular raceways to giver them hydraulic characteristics of circular tanks. Mixed cell raceways have self-cleaning properties and can cause a reduction in oxygen consumption by conditioning fish through forced swimming (Watten et al. 2000). Mixed cell raceways in combination with hydrocyclone filtration may be a feasible option for raising a greater number of fish on a limited clean water source and an evaluation of the efficiency of removing NZMS is needed.

The underflow rate of the hydrocyclone is approximately 13.44 L/min. As this contains NZMS it is contaminated and not useful for rearing fish to be stocked in uninfested locations. One method to accomplish reuse or reduction in water wasted at the underflow is by fitting the underflow with a grit pot designed to catch the filtered particles from the hydrocyclone (Puprasert 2004). Puprasert (2004) reported that this design reduced filtration

efficiency of their hydrocyclone by only 5% while returning most of the filtered water to the overflow. Further evaluation of large-scale filtration should involve tests using a grit pot fitted to the underflow to evaluate performance in removing and collecting NZMS from the inflow.

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Table 1.1. Intervals used for collecting nets from the hydrocyclone underflow to determine passage of NZMS by size and test flows.

Time interval (seconds)					
367 L/min			257 L/min		
Adult	Juvenile	Neonate	Adult	Juvenile	Neonate
60	60	60	120	120	90
75	75	75	135	135	105
90	90	90	150	150	120
105	105	105	165	165	135
120	120	120	180	180	150
150	150	150	210	210	165
180	180	180	240	240	180

Table 1.2. Residence time distribution characteristics ± SD of the hydrocyclone at 367 L/min and 257 L/min flows. All calculations are based on four observations from tests conducted in October 2007.

Flow	Mean residence time ($\bar{t}$, sec)	Mean variance (σ^2, sec^2)	Mean dispersion number ($D/\mu L$, dimensionless)
367 L/min	105.31 ± 5.38	1215.54 ± 127.63	0.055 ± 0.008
257 L/min	120.19 ± 2.42	610.43 ± 186.70	0.021 ± 0.007

Table 1.3. Summary of time of first detection and total times (from marker release to return to baseline conductivity) in seconds of hydrocyclone pulse type water particle transit tests from May 2008.

Test	N	Time Interval (sec)
First detection		
367 L/min	2	60-70
257 L/min	2	90-100
Total time		
367 L/min	2	130-140
257 L/min	2	160-170

Table 1.4. Parameter estimates for a four parameter logistic curve for each test completed on the hydrocyclone including the squared residual value and standard error of the estimate.

Flow	Size	Range (Min – Max)		EC50	Hillslope	R^2	SE
367 L/min	Adult	<0.001	1.00	111.404	-21.850	1.00	0.006
		<0.001	1.01	110.812	-10.519	0.990	0.092
		<0.001	0.98	105.431	-14.512	0.993	0.081
	Juvenile	<0.001	0.99	100.378	-23.787	0.999	0.013
		<0.001	0.99	108.211	-13.498	0.996	0.059
		<0.001	1.03	114.665	-9.395	0.989	0.097
		<0.001	1.00	109.609	-11.048	0.991	0.091
	Neonate	0.0175	1.00	93.827	-17.754	0.999	0.017
		0.0483	1.00	96.708	-40.677	0.999	0.039
		0.0044	1.00	86.832	-20.296	0.999	0.011
		0.0009	0.95	85.525	-21.049	0.992	0.105
257 L/min	Adult	<0.001	0.98	141.798	-10.878	0.988	0.129
		<0.001	1.01	153.544	-15.483	0.998	0.048
		<0.001	0.99	150.317	-22.915	0.999	0.035
		0.0094	1.02	163.310	-22.713	0.997	0.060
	Juvenile	0.0035	0.98	161.596	-31.091	0.999	0.035
		<0.001	0.99	150.126	-20.509	0.995	0.081
		<0.001	0.94	149.759	-15.747	0.985	0.131
		<0.001	0.99	150.126	-20.509	0.995	0.081
	Neonate	<0.001	1.00	126.296	-36.325	1.00	0.0011
		0.0439	1.00	126.934	-44.101	0.999	0.0359
		0.0171	1.00	121.496	-55.966	0.999	0.014
		<0.001	0.99	132.078	-29.881	1.00	0.0027

Table 1.5. Summary statistics from MANOVA on the four parameter estimates from the logistic curves fit to the cumulative percent recovery of three sizes of NZMS at two flow rates.

Variable	DF	Wilks' Lambda	F	P
Flow	4	0.035	95.24	<0.0001
Size	8	0.1002	7.55	<0.0001
Flow*Size	8	0.382	2.16	0.0627

Table 1.6. Summary statistics from individual ANOVA for all four parameter estimates from logistic curves fit to the cumulative percent recovery of three sizes of NZMS at two different flows from a hydrocyclone.

Parameter	Variable	Mean square	F	*P*
Minimum				
	Model	0.0003	1.61	0.2115
	Flow	0	0	0.9657
	Size	0.0006	3.97	0.385
	Flow*Size	0	0.07	0.93
Maximum				
	Model	0.0005	1.45	0.2578
	Flow	0	0.3	0.5922
	Size	0.0002	0.49	0.6204
	Flow*Size	0.001	2.87	0.0846
EC50				
	Model	2528.51	70.66	<0.001
	Flow	9662.71	270.03	<0.001
	Size	1249.91	34.63	<0.001
	Flow*Size	42.27	1.18	0.3308
Hillslope				
	Model	391.05	5.86	0.0025
	Flow	444.41	6.66	0.0195
	Size	644.23	9.65	0.0016
	Flow*Size	97.88	1.47	0.2585

Table 1.7. Model parameters for the four parameter logistic curve defining NZMS transit through the hydrocyclone filtration system at HNFH.

Parameter	Model Parameters Group Size*Flow (L/min)	Value
Min	All	0
Max	All	1
EC50	Neonate*367	92.5404
	Adult/Juvenile*367	107.6668
	Neonate*257	126.7769
	Adult/Juvenile*257	153.51
Hillslope	Neonate*367/257	-33.1026
	Adult/Juvenile*367/257	-15.7471

Table 1.8. Summary of model test of non-linear model fit for the four-parameter logistic curve calculated for NZMS hydrocyclone transit. Low p-value indicates that the model differs significantly from zero.

Source	DF	Mean square	F	*P*
Model	1	4.1361	435.86	<0.001

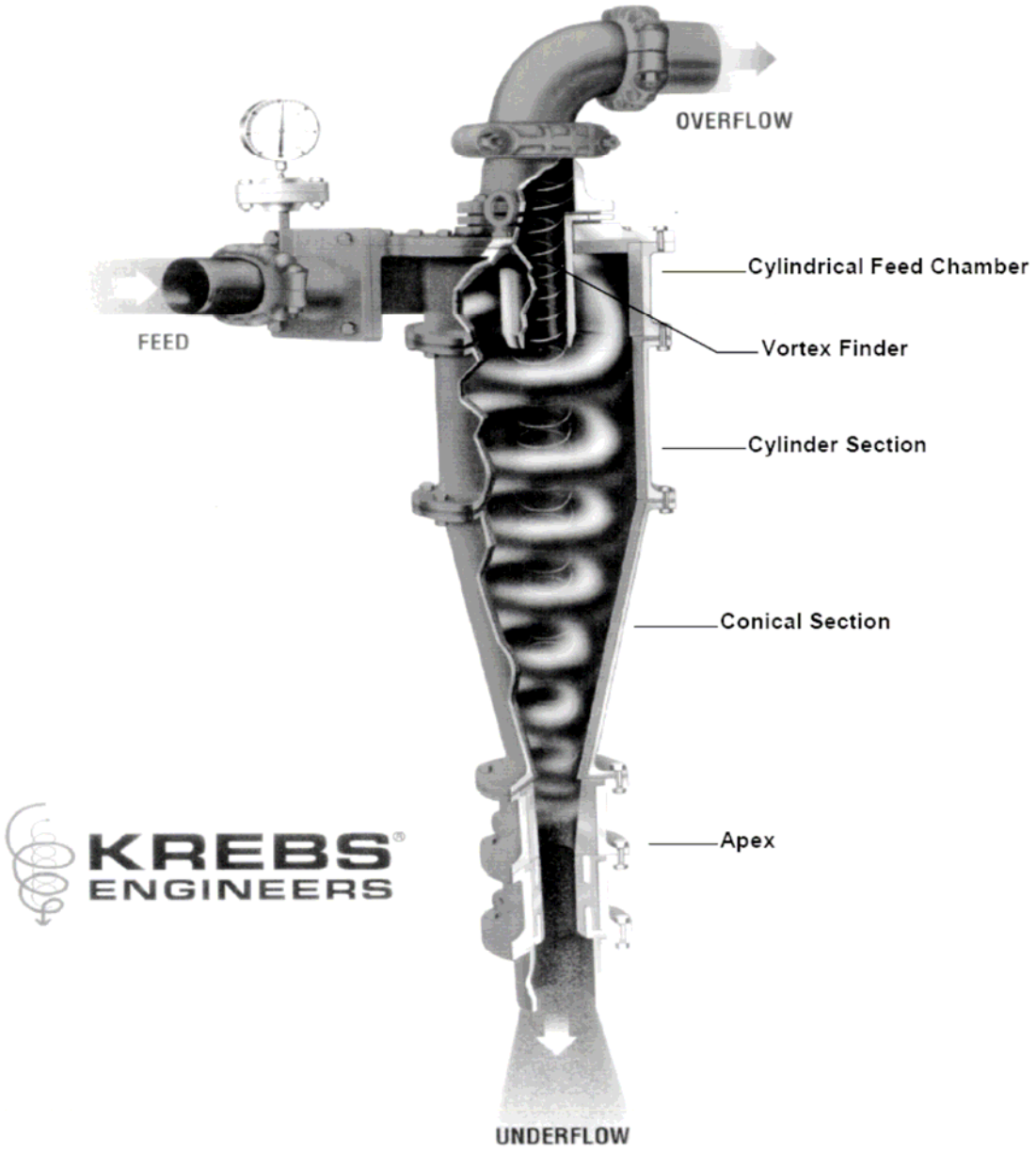

Figure 1.1. Cut-away schematic of a hydrocyclone (Courtesy of FLSmidth Krebs).

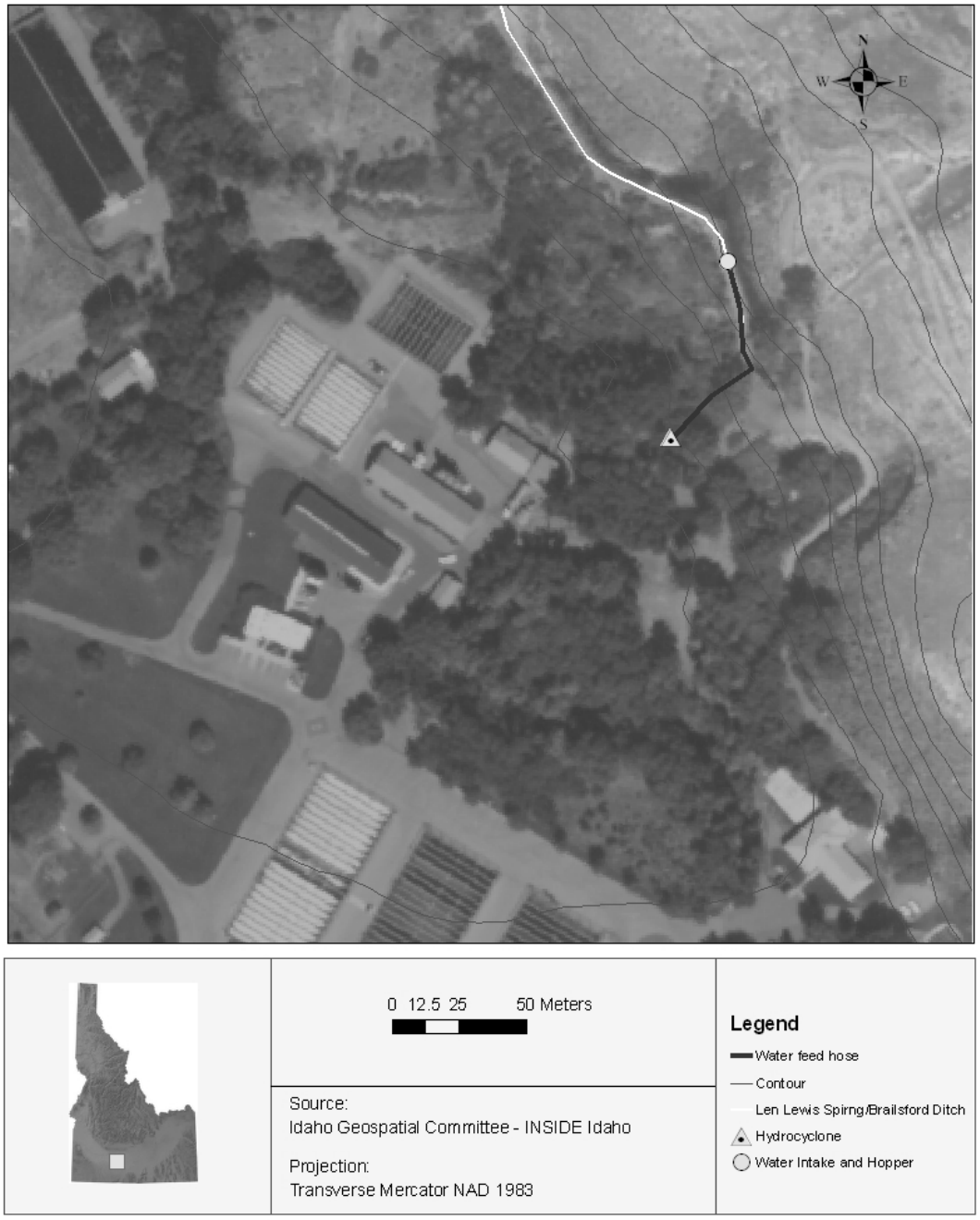

Figure 1.2. Relief map of hydrocyclone location, spring water intake, and feed line layout.

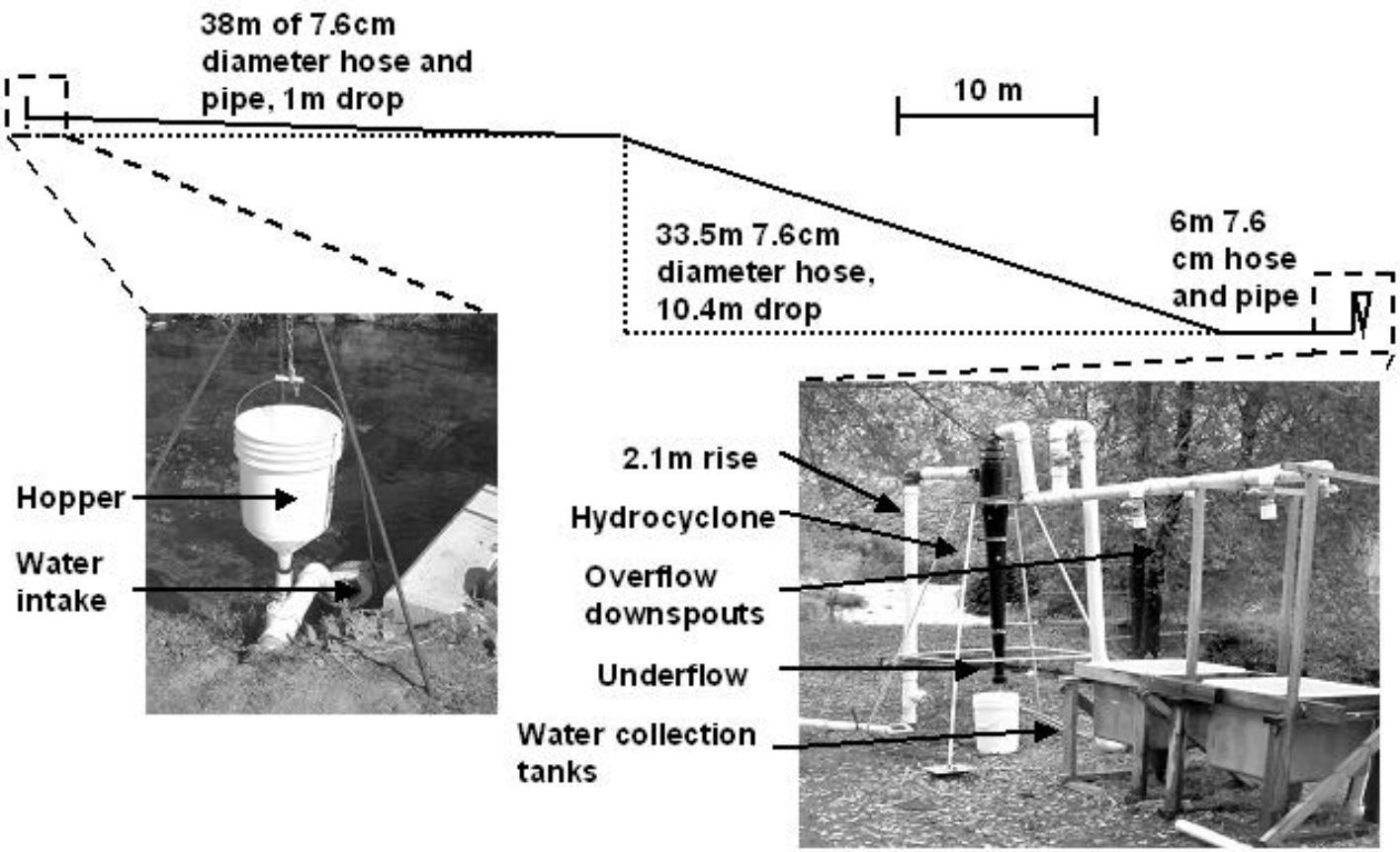

Figure 1.3. Scaled schematic diagram of the test hydrocyclone and from water intake to the hydrocyclone indicating drops and distances.

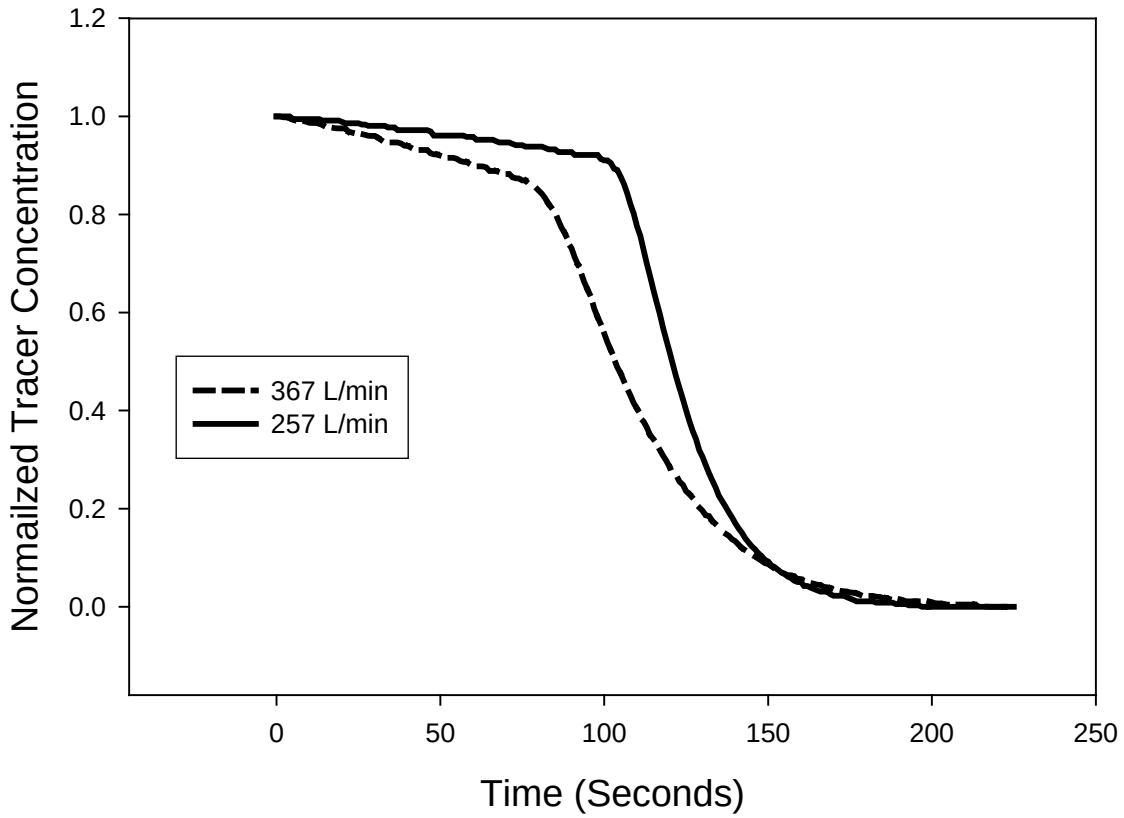

Figure 1.4. Average normalized tracer concentration for step-down trials during October 2007.

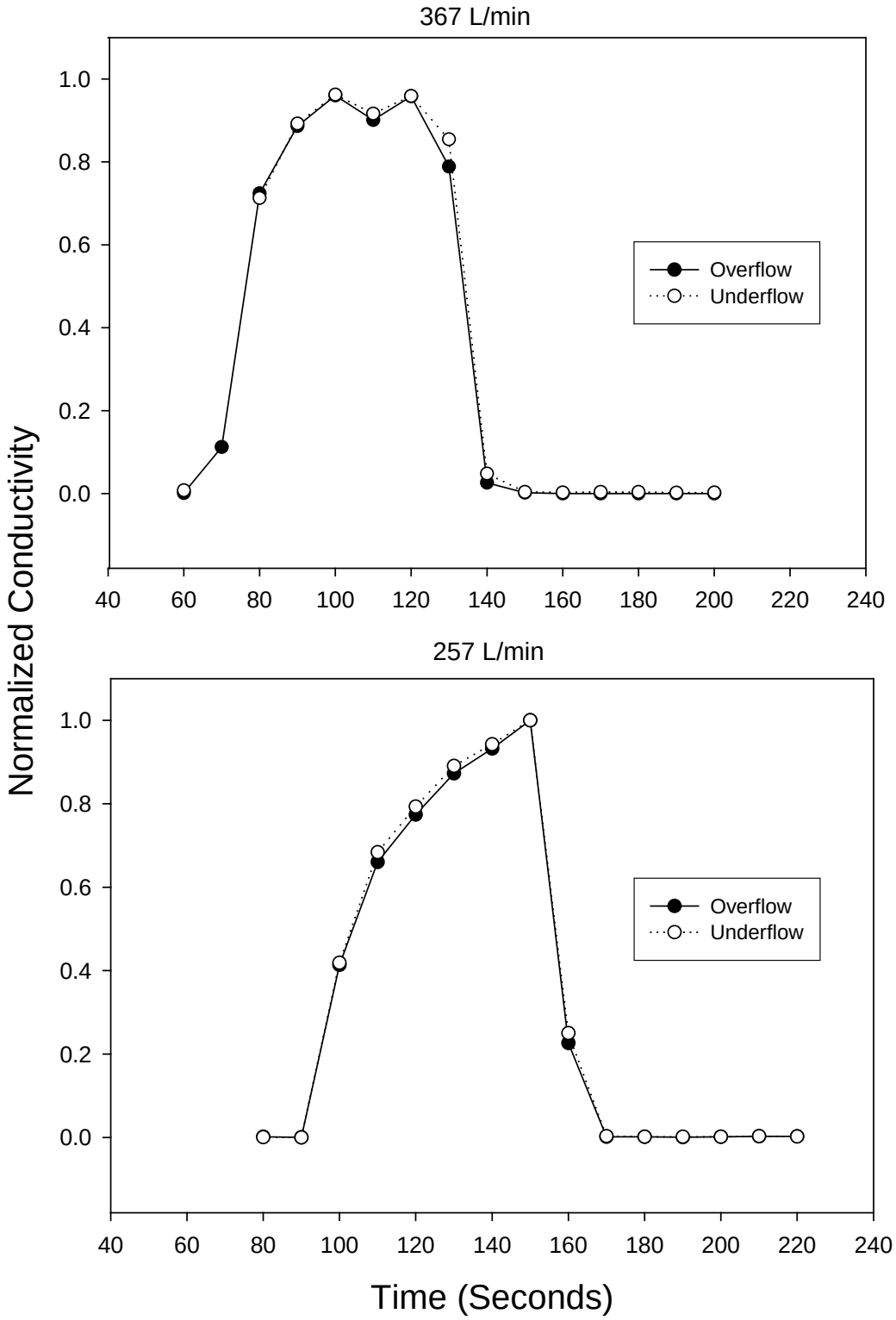

Figure 1.5. Average normalized tracer concentration across time for underflow and overflow samples at both flows. Visual analysis indicates that tracer movement to the underflow and overflow is the same. Tests were conducted in May 2008.

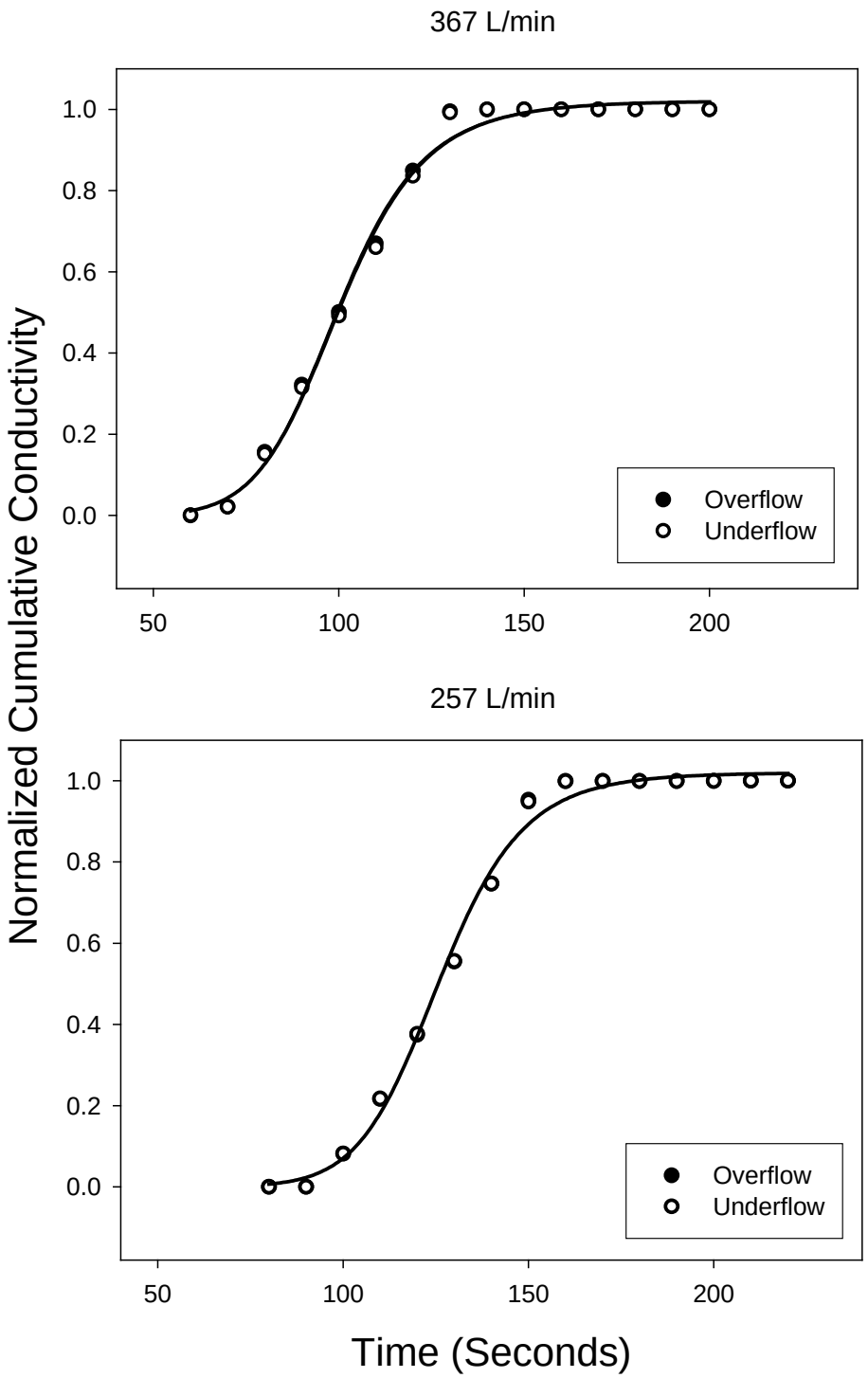

Figure 1.6. Average normalized cumulative conductivity units for water with tracer at 367 L/min and 257 L/min with a four parameter logistic curve fit by SigmaPlot 9.0 for comparison against snail hydrocyclone transit.

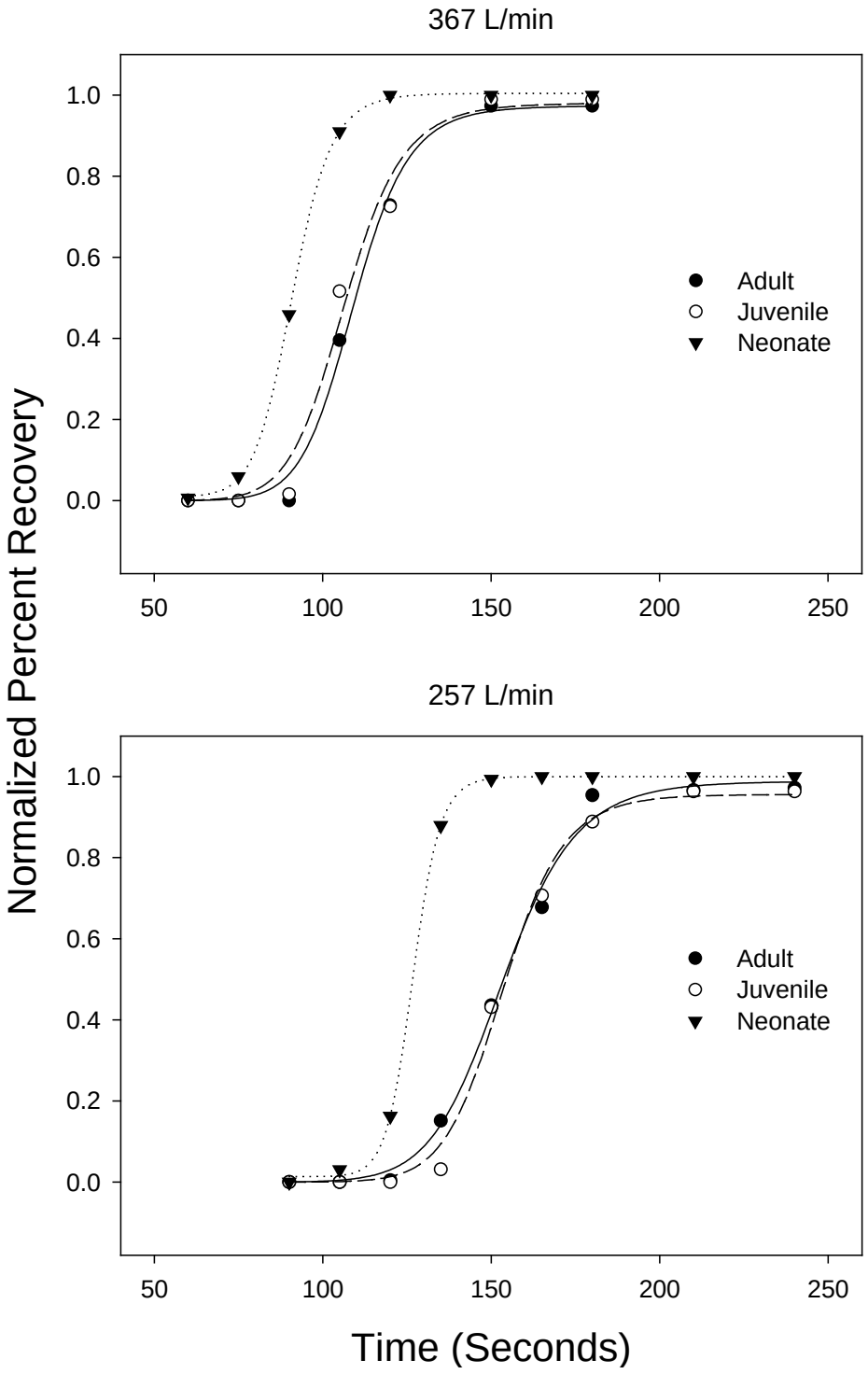

Figure 1.7. Average cumulative normalized percent recovery of NZMS at 367 and 257 L/min with a four parameter logistic curve fit by SigmaPlot 9.0.

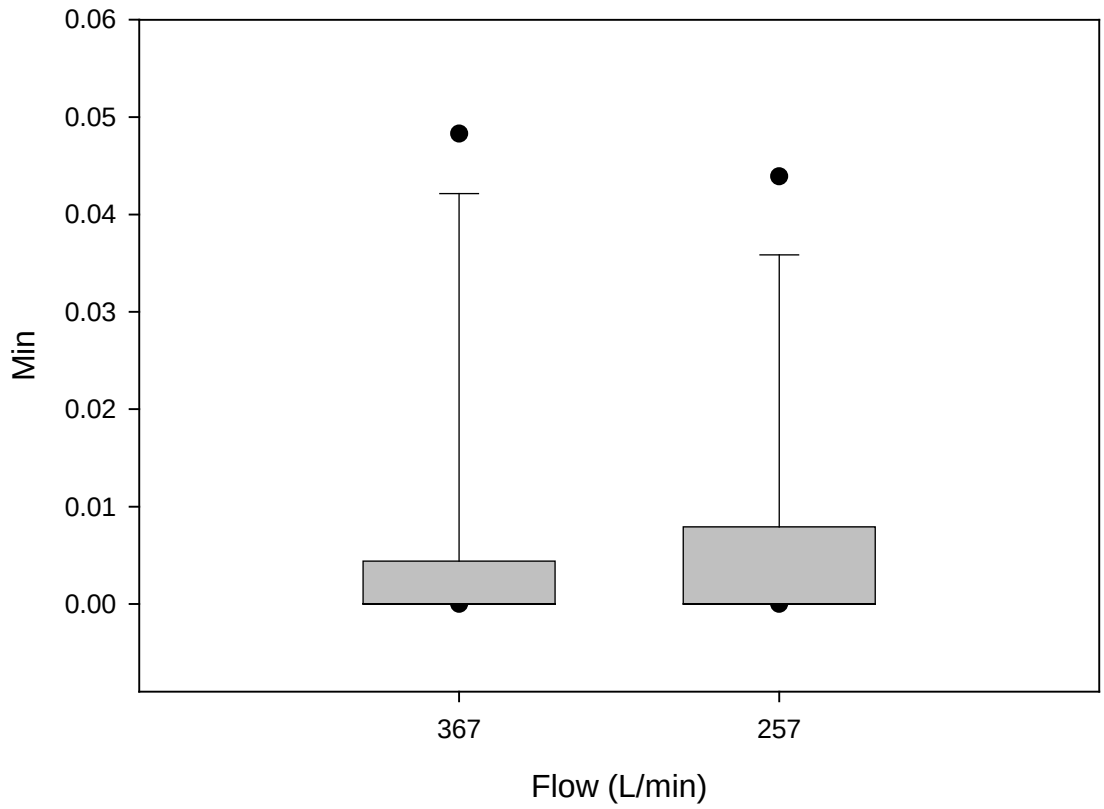

Figure 1.8. Comparison of the min parameter estimate between flows.

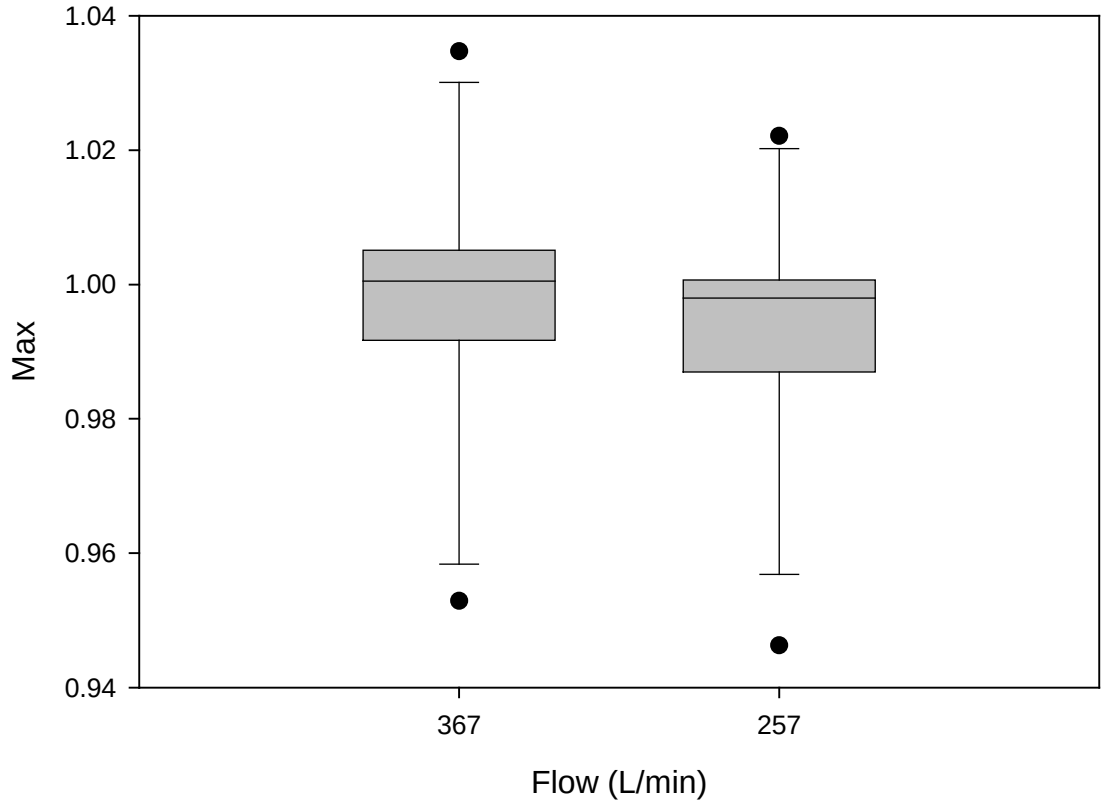

Figure 1.9. Comparison of the max parameter estimate between the two flows.

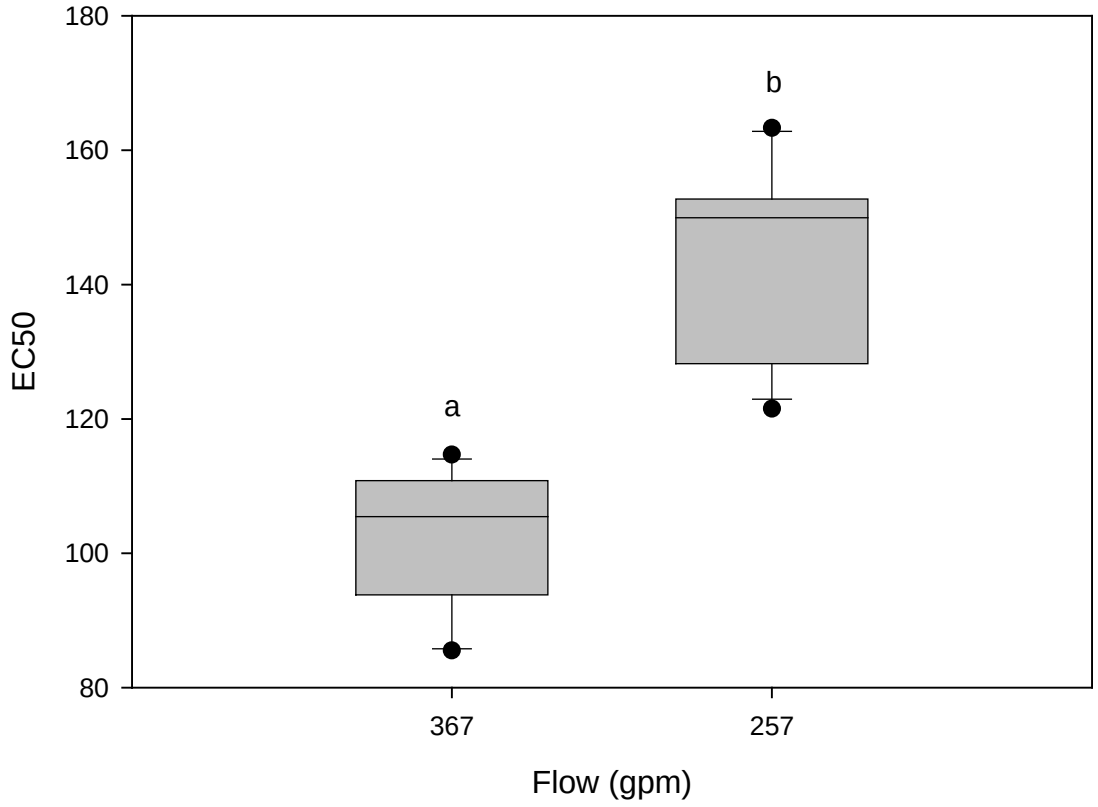

Figure1.10. Comparison of the EC50 parameter estimate between the two flows. Different letters indicate significant differences.

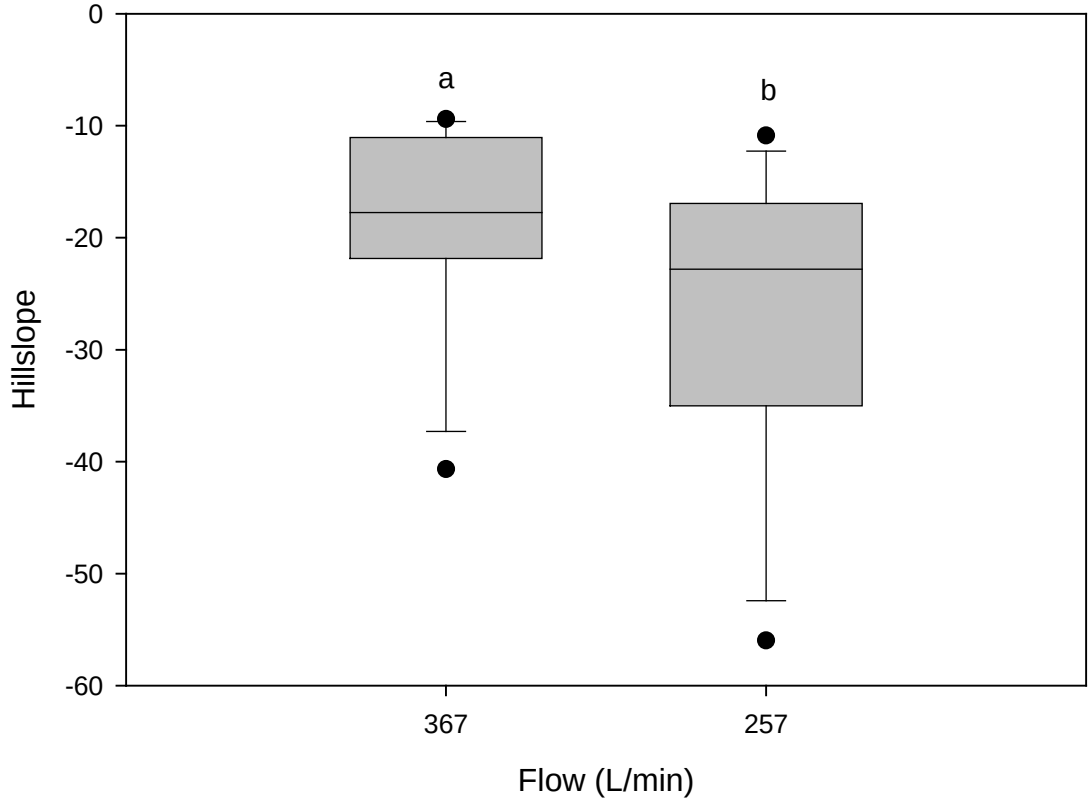

Figure 1.11. Comparison of the hillslope parameter estimate between flows. Different letters indicate significant difference.

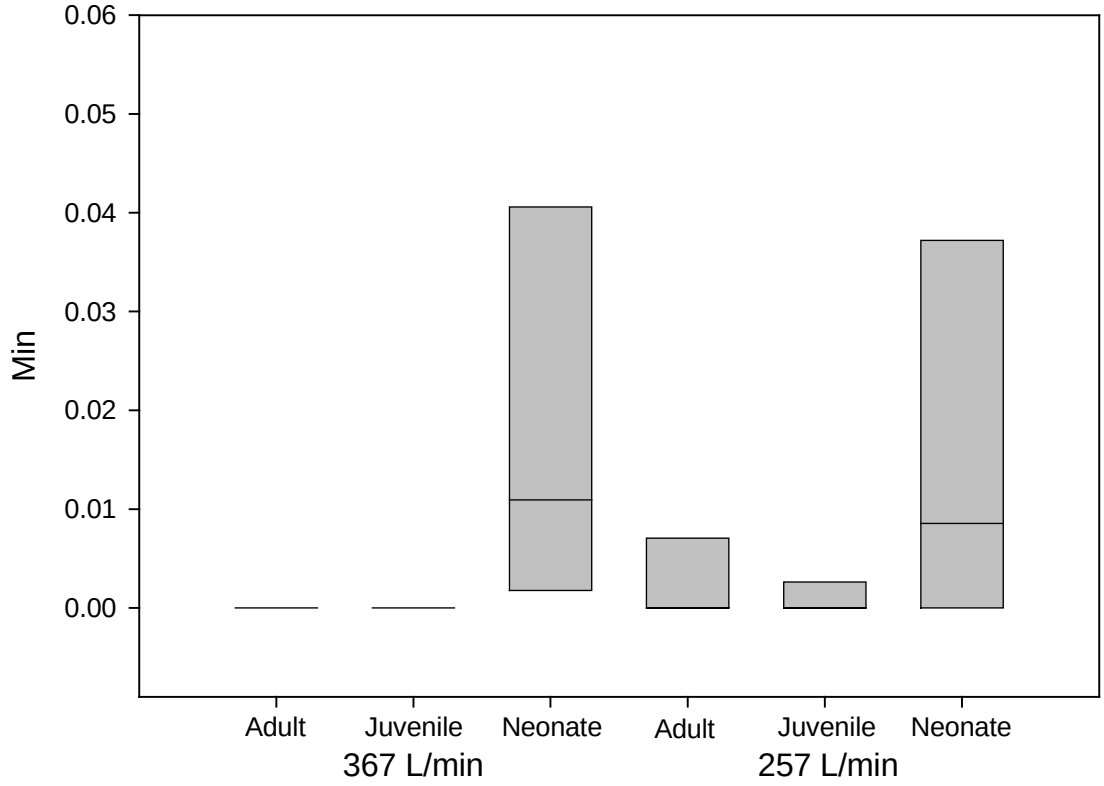

Figure 1.12. Comparison of the min parameter estimate between flows and sizes. There was no significant difference in this comparison.

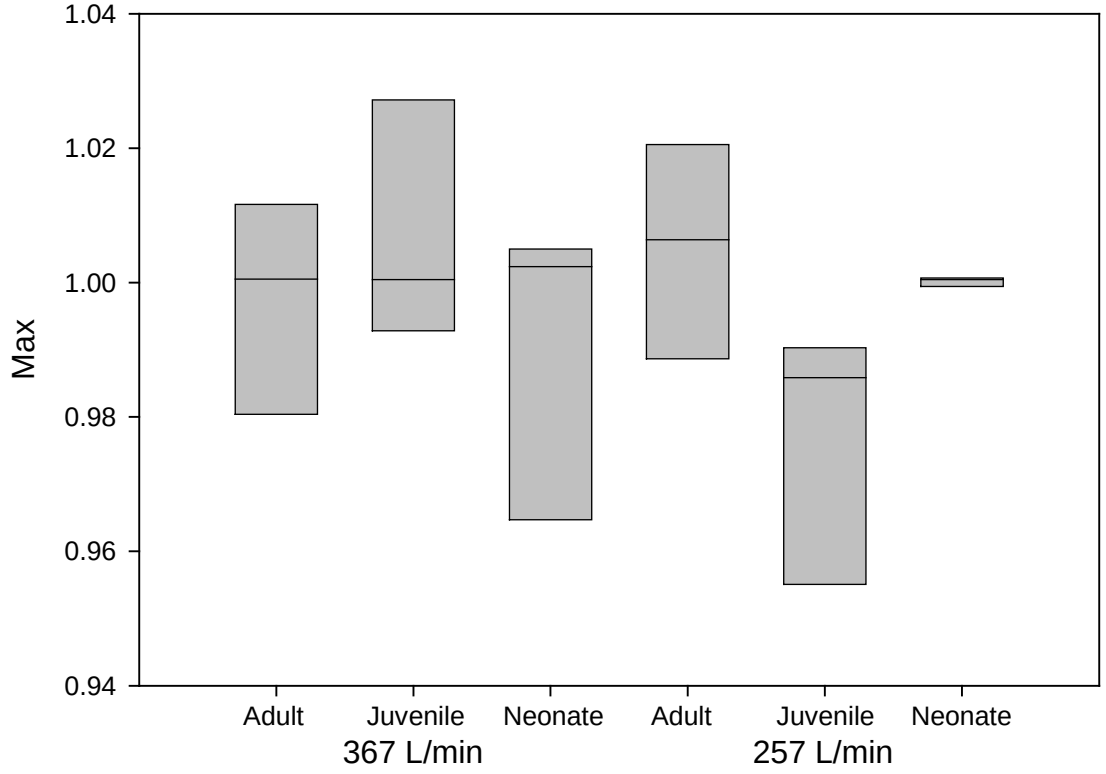

Figure 1.13. Comparison of the max parameter estimate between flows and sizes. There was no significant difference in this comparison.

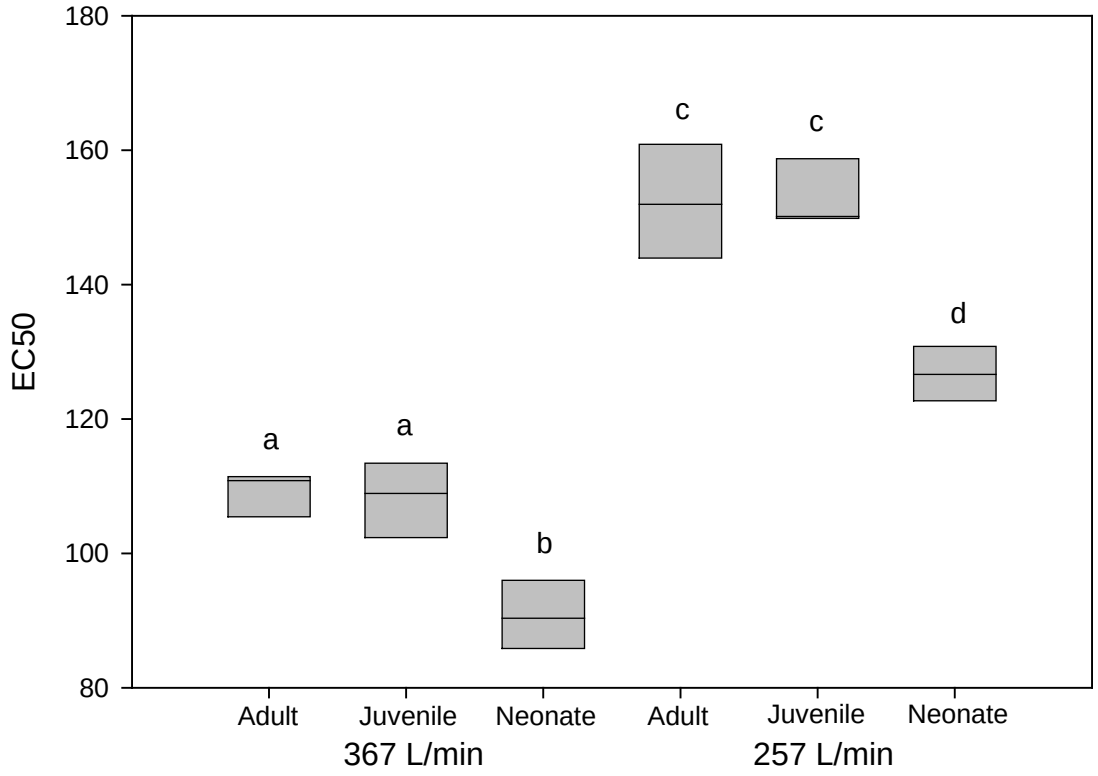

Figure 1.14. Comparison of the EC50 parameter estimate by flows and size. Different letters note significant differences.

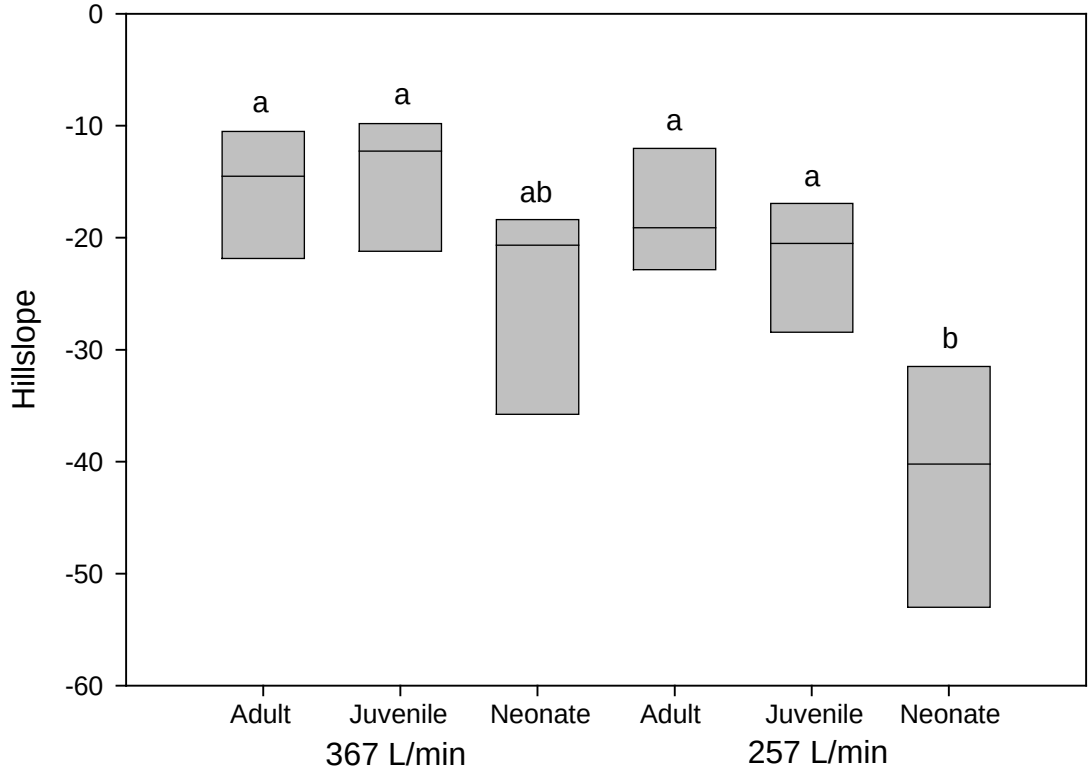

Figure 1.15. Comparison of the hillslope parameter estimate by flows and sizes. Different letters note significant differences.

CHAPTER II - TOXICITY OF CARBON DIOXIDE TO NEW ZEALAND MUDSNAILS: IMPLICATIONS FOR CONTROL IN FISH HATCHERIES

Abstract

We conducted laboratory studies of the toxicity of carbon dioxide to NZMS as a method for killing NZMS. Three different sizes of NZMS based on life history characteristics were exposed to CO_2 under 100 kPa pressure at several time intervals and three different temperatures to determine survival based on temperature units. Probit models were used to compare the different sizes of NZMS ability to survive pressurized CO_2 treatment. Adult and juvenile NZMS did not differ in their response to CO_2 treatment. Both of those sizes differed from the smallest size neonate. Probit models predicted an estimated 50% lethal time of exposure (LT_{50}) at 59.4 °C-hours for the adult and juvenile combined model and 5.4 °C-hours for the neonate size. Additional CO_2 exposure tests were conducted on adult NZMS in a non-pressurized environment at 15°C. A probit model was constructed using survival rate across temperature units and produced an LT_{50} of 100.9 °C-hours. When compared to the adult and juvenile combined probit model for pressurized CO_2 treatment, the probit model for adult NZMS exposed to non-pressurized CO_2 treatment did not differ. These results suggest that pressurized CO_2 treatment may be effective at treating and killing NZMS collected in a hatchery and aid in eliminating the potential for further spread.

Introduction

Invasive species cost the US federal government over $137 billion per year in management and remediation (Pimental 2000). With climate change and globalization of commerce and human travel, the risks of introducing species that cause ecological and monetary damage will continue to increase (Dukes and Mooney 1999).

The New Zealand mudsnail (*Potamopyrgus antipodarum,* NZMS) has been introduced from its native range in New Zealand to three new continents in the last 100 years; Australia, Europe, and most recently in North America (Zaranko et al. 1997). In North America, the NZMS was first discovered by D.W. Taylor in routine benthological surveys in the Nature Conservancy's Thousand Spring Preserve near Hagerman, Idaho in 1987 (Bowler 1991). Since its introduction in Idaho, the NZMS has spread to all of the states in the western U.S. with the exception of New Mexico (Gustafson et al. 2002). A separate invasion has occurred since in the Great Lakes area (Grigorovitch et al. 2003) and moved into Wisconsin and Minnesota (Minnesota Department of Natural Resources 2008).

The spread of NZMS has been facilitated its life history characteristics. The snails reach a maximum length of 5-6 mm and are prosobranch snails (with opercular plate can seal the opening of the shell). This characteristic provides protection for snails during unfavorable conditions such as lack of water for periods of 48 h (Richards et al. 2004) to 50 days (Winterbourn 1970b). The small size and the prosobranch characteristics enhance the likelihood that NZMS can remain on wading gear, animal hair, or feathers for periods of time and be transferred between watersheds to new locations. In addition, most of the populations of the NZMS in North American are reported to be asexually reproducing females (Wallace 1992) that can brood up to 120 embryos at a time(Winterbourn 1970b, Schreiber et al. 1998,

Richards and Lester 2000, Richards 2004). In addition, the snails are ovoviviparous, and release live young (Winterbourn 1970a; Bowler 1991; Richards et al. 2004). Therefore only one transplanted snail can found a new population of snails.

Several fish hatcheries have become infested with NZMS. The snails thrive in constant temperature springs, or in effluent areas or downstream areas that are enhanced by constant temperature well waters released from the hatcheries (James 2006). Infested hatcheries pose a possible vector for the spread of NZMS to uninfested water bodies via fish or fish transportation water. Rainbow trout and steelhead (*Oncorhynchus mykiss*) are found to volitionally feed on NZMS in raceways or holding tanks (Bruce 2006). NZMS consumed by fish may pass through the intestinal tract unharmed (Bondesen and Kaiser 1949; Haynes et al. 1985; Vinson 2004; Bruce 2006). The risk of introduction of snails from fish or water sources is sufficient that hatchery facilities have discontinued transporting fish to uninfested water bodies (Mark Olson, Willow Beach NFH Project Leader, personal communication).

Control of the spread of NZMS has been studied using extreme temperatures with some shown success (Richards et al. 2004). Richards et al. (2004) found that snails died on a dry substrate after 1 h of exposure to 40°C with low humidity and after 2 h at –3°C. James (2006) also found that snails could not survive in sub zero temperatures in water. Dwyer et al. (2003) explored the toxicity of chlorine (500 to 3000 mg/L) and copper sulfate (100 and 1000 mg/L) to NZMS at short durations and found 30% to 60% mortality. Bruce (2006) explored fish depuration strategies as a method for controlling snail movement from the hatchery and found that rainbow trout and steelhead can retain volitionally consumed snails in the gut and potentially transfer them to waters where they are stocked. For depuration to work hatcheries need an NZMS free water source for raising and/or depurating fish as well as

for transportation (Bruce 2006). There is a need for a well-documented treatment method that is easy and safe to use at hatchery.

Toxicity of CO_2 has been explored on other invasive bivalve mollusks Zebra mussel (*Dreissena polymorpha)* and Asian clam (*Corbicula fluminea*) as a method to prevent water intake pipe fouling in coal fired power plants (Elzinga and Butzklaff 1994, Rau 2003, Watten et al. 2005). Watten et al. (2005) and Rau (2003) found that exposure to high dissolved gas pressures of CO_2 lead to byssal thread detachment and death in Zebra mussels and Asian clams. Elzinga and Butzklaff (1994) demonstrated narcotizing effects of CO_2 on Zebra mussels at doses of 100 mg/L and lethal effects at 500 mg/L. Carbon dioxide treatments pose a feasible use as a control substance for NZMS based on its success with other mollusks, its low cost, and safe and easy use (Watten et al. 2005).

The objective of this study was to explore the toxicity of carbon dioxide to three sizes of NZMS at three water temperatures, and to provide suggestions for using CO_2 as a lethal treatment for snails collected from infested hatchery water supplies.

Methods

Snail Collection and Care

Snails used in the study were collected from Len Lewis spring at the Hagerman National Fish Hatchery (HNFH) by hatchery staff and shipped via overnight courier in a cooler with native vegetation and wrapped in moist paper towels to the UI Wetlab. In the laboratory, the snails were maintained in two-liter plastic containers at a density of approximately 500-1000 snails per container, and maintained at 15°C with artificial daylight following the natural daylight cycle for the springs where they were collected. A small amount of native vegetation shipped with the snails was placed in each container. Every two

days at least half of the water was drained from the containers and replaced with fresh dechlorinated water at 15°C.

Snails used in experiments were classified into three life stages for testing by total shell length by separating a series of stainless steel sieves. Adult (>2mm), juvenile (1mm to 2mm), and neonate (<1mm) NZMS were collected on 1.18mm, 850µm, and 90µm sieves respectively.

Neonates were collected from adult NZMS placed in 120 mL plastic cups (5 snails per cup) with approximately 50 mL fresh dechlorinated water for 48 hours.

To ensure that residence in the lab did not negatively affect the physiology of the snails and their robustness to the testing procedures, they were kept in the lab for a maximum of four weeks (Table 2.1). Unused NZMS were disposed of by placing them in 10% bleach solution for 48 hours at the end of each three to four week period.

Pressurized CO_2

CO_2 was applied to NZMS in a test system of 5 identical 6 L wide mouth pressure tanks (type 304; McMaster-Carr, Aurora, Ohio), each attached to a pressure pump (Gast Manufacturing, Inc., Benton Harbor, MI). Each tank had four ports utilized for (1) inlet and (2) outlet for the pressure pump, (3) inlet for CO_2 gas, and for a (4) pressure gauge (Figure 2.1). The pump removed headspace gas through the outlet port and sparged it through water in the tank through plastic air diffusers attached to the inlet port. CO_2 gas was introduced into the headspace of four of the five tanks via a common manifold to equalize pressure among the tanks. The fifth tank was connected to compressed air valve to serve as a control. The pumps were mounted to a shelf on the laboratory wall directly above a flow through

trough where the tanks were placed. The flow through trough served as a water bath to control the temperature of the tanks during tests.

Pressure tanks were filled with 3 L of dechlorinated, aerated well water. Tanks were then closed, and pressurized to 100 kPa with CO_2 for treatment tanks and 100 kPa compressed air for control tanks. The tanks were precharged for 15 minutes with the pumps circulating headspace gas under pressure. After the precharge, water quality measurements were taken in the water of each tank. Two to four groups of 10 NZMS in 41.5 mm stainless steel mesh balls (500µm mesh) were placed into each of the tanks for adult and juvenile tests. For neonate tests, all available neonates were placed directly into the test tanks. Each tank was closed and re-pressurized with the pumps running for ten minutes. Pumps were then turned off and the tanks were allowed to sit for the desired test duration at 100 kPa pressure.

Tests were conducted at target temperatures of 8°, 15°, and 20°C. Test temperatures were controlled using a flow through water bath at the target temperature. Water bath temperature was recorded at 10-minute intervals during tests with Hobo H8 temperature loggers (Onset Computer Corp., Bourne, MA). The data from temperature monitoring in the water bath were then used to calculate an hourly temperature unit for each group of snails tested by multiplying the average temperature over the duration of the test interval by the duration of exposure in hours.

Duration of exposure to pressurized CO_2 varied by size of snail and test temperature due to the varied response from the snails. Test intervals for adult snails were 1, 3, 6, and 8 h for all temperatures and tests at each interval were repeated three times. Test intervals used to model juvenile snails were hourly for 10 h for tests at 8°C, hourly for 8 h for tests at 15°C. For tests at 20°C, intervals of 1, 3, 6,and 8 h were selected. The tests were repeated from 1 to

3 times at each interval of duration. For tests with neonates intervals varied for each temperature tested. For tests at 8°C, snails were evaluated at 0.5, 1, 2, 3, and 4 h. Test intervals used to evaluate toxicity at 15°C were 0.5, 1, 1.5, and 2 h. For tests at 20°C, intervals were 0.5, 1, 2, and 3 h. Test with neonates were also repeated three times for each duration of exposure with the exception of 0.5 and 4 h tests at 20°C that were only repeated twice. Four time intervals were tested at any one time, and predetermined time intervals were assigned at random to tanks on a test-by-test basis.

At the end of each duration of exposure to CO_2, the selected tank was isolated from the CO_2 manifold via a needle valve in the feed line. Using another valve on the tank, the gas pressure was slowly released over approximately one-minute. At this point CO_2 content in the headspace gas was measured and mesh balls were then removed from the tank. The snails from each mesh ball were placed in cups with fresh dechlorinated water and held at 15°C for recovery. Tank water was disposed of after autoclave sterilization or 48 hours in a -18° freezer.

Survival of snails was assessed by observation under a dissecting microscope at 24 and 48 h post exposure. Snails were observed for movement and/or physical behavior. If there was no movement after 48 h or snails were gaping from shells, they were scored dead. For tests with neonates snails that were not found after the tests were scored dead.

Non-pressurized CO_2

To test the toxicity of CO_2 saturation at atmospheric pressure to adult size NZMS the same pressure tank apparatus was used but the sparged CO_2 was vented from the tanks. The tanks were modified for non- pressurized CO_2 tests by removing the inlet hose to the circulating pumps and attaching the CO_2 manifold to the pump inlet. Five groups of ten adult

NZMS in mesh balls were placed in each CO_2 test tank and in the control tank. Carbon dioxide or mixed atmospheric air (for control) was sparged through the airstones at ½ L/min for the duration of the tests. At each time interval one mesh ball was removed from each tank. Snails were removed at intervals of 1, 3, 6, 8, 10,and 12 h of exposure. Tests at durations of 1, 3, 6, and 8 h were repeated four times, and tests at 10 and 12 h durations were repeated three times. After each test interval, snails were removed from the mesh balls and placed into clean dechlorinated water to recover and survival was assessed as in trials with pressurized CO_2. All tests were conducted at 15°C

Water Quality Measurements

Water quality characteristics were measured at three points during testing; 1) before the start of the test, 2) when snails were introduced into the tanks (pressurized tests only), and 3) when snails were removed from the tanks. The first water quality was measured as a single common measurement from one tank; and measurements taken at subsequent points were from individual test tanks. Dissolved oxygen and pH were monitored with Hach HQ10 and SensION meters (Hach Co., Loveland, CO). Water temperature inside the tanks was recorded using the HQ10 meter. Alkalinity was determined using standard methods (2320, American Public Heath Association 2005) on a 100 mL sample taken from tanks at the three sampling points described above. Headspace CO_2 composition was measured at the end of each test with a Portable Carbon Dioxide Plus Toxic Gas/Oxygen Analyzer (CEA Instruments, Inc., Emerson, NJ). Percent of CO_2 from headspace gas was used to calculate dissolved gas pressure (kPa and mmHg) and concentration (mg/L) of CO_2 using the equation:

$$C_i \text{ (mg/L)} = (1000K_i * B_i * X_i * (BP\text{-}PH_2O))/760,$$

where C_i is the concentration of CO_2 in mg/L, K_i is the ratio of the molecular weight to molecular volume for CO_2, Bi is the Bunsen coefficient, X_i is the mole fraction of CO_2, BP is the local barometric pressure during the test plus the additional pressure added for our testing procedure, and PH_2O is the vapor pressure in the test tank (Colt 1984).

Data Analysis

The proportion of snails surviving trials was analyzed with probit models plotting survival against the Celsius degree hours of exposure at a given test temperature. The number of hours of exposure was multiplied by the average temperature for each test group to create a degree hour unit (°C-hours) and eliminate inconsistencies caused by variability in test temperature. Median lethal °C-hours of exposure to pressurized CO_2 for each snail size was determined and maximum likelihood estimates of a median lethal °C-hours (LT_{50}) were generated with corresponding 95% CI using the equation:

$$\Phi^{-1}(\chi) = \alpha + \beta\chi,$$

where $\Phi^{-1}(\chi)$ is the cumulative distribution of mortality for the standard normal distribution, α is the estimated parameter for the intercept, β is the estimated parameter for the slope, and χ is the $\log_{10}$ (°C-hours). Slopes and intercepts of the probit models were compared using Walds' test to determine if the probit models were different among sizes and if sizes could be combined into a larger group. All analyses were conducted with SAS release 9.1 (SAS Institute 2002-2003).

Results

Pressurized CO_2

The survival of NZMS exposed to pressurized CO_2 exhibited a dose response. Survival of adult and juvenile snails was similar with survival decreasing from 100% to 0%

over a range of exposures from 6.86 to 159.00 °C-hours (Table 2.2, Figure 2.2). Mortality in neonates was more rapid (Table 2.2, Figure 2.2).

Probit models predicted the median lethal exposure time (LT_{50}) for adults as 58.1 °C-hours (43.5-78.6). The LT_{50} and CI for juveniles and neonates were 46.2 °C-hours (35.7-58.4) and 5.4 °C-hours (2.9-7.6) respectively (Figure 2.3). Since our goal was complete mortality we report the LT_{99} (95% C.I.) predicted by the probit for adults, juveniles, and neonates respectively: 257.3 (154.5-857.7), 322.0 (195.1-841.5), 120.2 (69.0-358.5) (Figure 2.3).

The slope and intercept of the adult and juvenile models did not differ significantly (Table 2.4, Figure 2.3). The slope of the neonate model differed from both the adult and juvenile models (Table 2.4 Figure 2.3).

Adult and juvenile survival data were combined into one probit model (Figure 2.4) for a predicted LT_{50} of 59.5 °C-hours (42.4-87.7). The predicted combined LT_{99} was 249.9 °C-hours (141.8-1225.3). Confidence intervals for the combined adult and juvenile model were wider than the individual models indicating that there may be some effect of the snail size and individual models serve better for predicting lethal effects. The adult/juvenile combined model did not differ from the neonate model in slope but did differ in intercept (Table 2.5).

The observed temperatures in all tests conducted with pressurized CO_2 were lower than our target temperatures. Average temperatures ± SD for tests at 8°C, 15°C and 20°C were 7.30 ± 0.51°C, 14.23 ± 1.0 °C, and 19.38 ± 0.66 °C, respectively. The water quality measured across all trials was consistent before charging with CO_2. Alkalinity and pH averaged ± SD 137.8 ± 9.0 and 7.32 ± 0.11 respectively. Charging water with CO_2 dropped

the pH to approximately 5.15 ± 0.22. Alkalinity remained relatively unchanged after charging. The DO measurements varied with temperature, and colder temperatures had higher dissolved oxygen (Table 2.6). Water quality was measured when snails were removed from tanks (Tables 2.7 and 2.8). Juvenile tests closely resembled adult tests and are not reported.

Non-Pressurized CO_2

Adult NZMS exposed to non-pressurized CO_2 also showed a dose response for survival. Survival decreased from 100% to 0% with exposures from 14.1 to 182.64 °C-hours. The probit model predicted LT_{50} at 100.9 (83.5-122.9) and LT_{99} at 458.8 (295.5-1141.9)(Figure 2.5). The slope and intercept of models for pressurized and non-pressurized treatments in adults did not differ due to overlapping confidence intervals (Table 2.10). However, the LT_{99} level was higher for non-pressurized CO_2 exposure.

In these non-pressurized tests the observed temperatures were 14.74 ± 0.71 °C. The pH and dissolved oxygen declined in a similar manner as in the pressurized treatments with final measurements averaged over each interval of 4.63 ± 0.31 and 0.36 ± 0.30 respectively. Average dissolved gas pressure was 583.80 ± 149.90 mmHg or 77.83 ± 19.99 kPa. Average dissolved concentration of CO_2 was 1,523.23 ± 392.15 mg/L.

Discussion

Both pressurized and non-pressurized CO_2 treatments were effective at killing all life stages of NZMS. Neonate snails were more susceptible to CO_2 treatment than the larger sizes of snails, and thus, treatments targeting 0% survival can be based on the lethal effects of CO_2 to the largest size NZMS. Non-pressurized CO_2 treatments were effective for killing NZMS, but the treatment required a longer exposure and substantially more CO_2. Non-

pressurized treatments used over 2,500 L CO_2 and pressurized treatments used less than 250 L. If a constant source of CO_2 emissions were available the non-pressurized treatment could be feasible.

When CO_2 is introduced into treatment water as in these experiments it reacts to form carbonic acid (H_2CO_3); a weak acid. CO_2 saturation reduces the pH of the treatment water considerably depending on the alkalinity of the water. The average pH of CO_2 saturated water during our experiments was 5.00 ± 0.12. The effect would be more pronounced in soft water because of the reduced carbonate buffering system (Watten et al. 2005). The average alkalinity of the water used for testing was 135.8 ± 15.2. Reduced pH may have deleterious effects on the NZMS survival primarily associated with respiratory dysfunction. High partial pressure of CO_2 can cause the inability of aquatic mollusks to regulate blood and hemolymph pH across gill surfaces (Byrne and Dietz 1997). With high CO_2 concentrations in the treatment water NZMS would experience respiratory acidosis leading to hypercapnia in the blood (Cameron 1989). Aquatic mollusks may use shell carbonates to osmoregulate blood pH (Byrne and Dietz 1997). With high levels of environmental and metabolic CO_2 building up in the NZMS blood, an osmoregulatory system based on shell carbonates may break down quickly especially if readily available shell material is etched by acidic water to buffer the acidic environment.

The range of temperature tolerance of NZMS is relatively wide for an aquatic species. Upper lethal tolerances have been demonstrated up to 32.4°C (Quinn et al. 1994) and 33.6°C (Cox and Rutherford 2000). Richards et al. (2004) found subzero temperatures were lethal to NZMS after 1 h and James (2006) found 100% mortality after 96 h of exposure to 0°C. The temperature range in our experiment was between 8° and 20°C, well within the range of

tolerance for NZMS. However, as a poikilotherm, the surrounding temperature and individual body size dictates metabolism of NZMS (Precht 1973). We demonstrated that as temperature units accumulate, survival in saturated CO_2 treatment decreases. This is presumably an effect of respiratory metabolism as a result of size and temperature.

McMahon (1995) found that aquatic mollusks had lower rate of survival when exposed to anoxic conditions with 100% CO_2 versus anoxic conditions with 100% N_2. The difference in survival between CO_2 and N_2 demonstrates some toxic effect of the CO_2 on the aquatic organisms versus other gasses. Application of 100% CO_2 under pressure in our study resulted in DO concentrations being reduced to below 2 mg/L and in many cases below 1 mg/L (Table 2.8 and 2.10). The exact mechanism of toxicity in our studies remains unclear, as to whether mortality was due to high CO_2 concentrations, low pH, or low DO concentrations. Further explorations into the effect of anoxic conditions with other gasses would clarify this relationship.

In addition, exposure to high gas partial pressures can cause gas bubble disease. Gas bubble disease is non-infectious physical trauma resulting from elevated dissolved gas pressures (Bouck 1980). The main effect of the hyperbaric gas exposure is the formation of gas bubbles in the blood and tissues causing emboli and emphysema resulting in physiological dysfunction (Bouck 1980). Carbon dioxide gas measurements in the headspace of the pressure tanks were not taken in a reproducible manner and calculations of dissolved gas pressures are most likely lower then the actual dissolved gas pressures. However the calculated dissolved gas pressures ranged from a minimum of 809 mmHg (108 kPa) to a maximum of 1440 mmHg (191 kPa). Gas bubbles can form only if the sum of the dissolved gas pressures is higher than the hydrostatic pressure (Buock 1980). Nebeker (1976)

demonstrated lethal effects gas bubble disease on *Daphnia magna*, the crayfish *Pacifastacus leniusculus,* and stoneflies *Pteronarcys californica* in air super saturated water (122.5% to 145%) after 96 hours. The atmospheric pressure in the lab during the times of testing, which represent the hydrostatic pressure of the snails, rarely exceeded 700 mmHg (93 kPa). Subtracting the hydrostatic pressure from the dissolved gas pressure experienced by the snails leaves a pressure deficit. The pressure deficit gives rise to the possibility of gas bubble disease even at the low end of the dissolved gas pressure calculations. The likely contribution to toxicity of CO_2 was the rapid depressurization of the pressure chambers. We conducted no studies to evaluate the difference in a slow acclimation procedure.

Elzinga and Butzklaff (1994) reported lethal effects of CO_2 on Zebra mussels at 196 and 290 mg CO_2/L after 48 hours of exposure at 18°C and 22°C respectively. CO_2 exposure concentrations in this study had a wide range because of the inaccuracy of the headspace gas sampling. In our experiments, CO_2 concentrations ranged between 2000 and 4500 mg CO_2/L. Despite the possibility for error in CO_2 concentrations, elevated concentrations explain a more rapid time to lethality than other bivalves and indicate a dose response to CO_2 exposure. LT_{50} for adult NZMS was 58.1 °C-hours and LT_{50} for neonate NZMS was 5.4 °C-hours.

The confidence intervals for our probit models were wide, especially for the 99% lethality. Sources of variation in our tests included the time of sampling and testing. Collections of test snails for the study were made from February 2007 to January 2008. Although the springs at Hagerman NFH are constant water temperature, the day length or season could have affected the viability of these snails. The difference in snail response to treatment according to time of year was not evaluated as shipments were used to target

specific life stages of snails and life stages were not tested consistently throughout the entire year. Snails held in the lab may have a reduced ability to resist any type of toxic treatment as they have been removed and kept out of their habitat possibly affecting physiological robustness. Snails may experience physiological differences at different times of the year (Winterbourn 1970b).

Pressure tanks were designed to be uniform but some variability in water quality measurements were observed likely due to small defects or leaks in the tanks. Inconsistencies in replicate tanks could create variation in survival. The amount of CO_2 dissolved in the test water was dependent on the pressure of the tanks. While we were able to keep the pressure in the tanks at 100 kPa throughout the duration of the tests, the barometric pressure also plays a role in the amount of pressure on the tanks. Changes in the barometric pressure could possibly play a role in introducing variability in the water quality measurements and affect survival of the NZMS.

Elzinga and Butzklaff (1994) used CO_2 as a narcotic to relax Zebra mussels and Asian clams out of their shells making them more susceptible to other forms of chemical treatment. Investigations of the narcotizing effect of CO_2 followed by another toxic treatment may prove to be a quicker and easier way to treat the snails collected from an infested hatchery.

Copper has a negative effect on snail survival (Dwyer 2003). Preliminary studies in our lab indicate that a 1% copper sulfate dip at high concentrations may kill snails in less than an hour. Copper strips at the effluent of raceways are being experimented with at Hagerman State Fish Hatchery as a barrier (Joe Chapman, personal communication) and at Colorado State University (Chris Myrick, personal communication) and may prove to be effective to keeping snails from entering into a hatchery by volitional upstream movement.

Potassium containing chemicals are acutely toxic to Zebra mussels (Wildridge et al. 1998). Potassium treatments for fish disease outbreaks at Nampa State Fish Hatchery have resulted in a decreased amount of NZMS in raceways (Rick Alsager, personal communication). Potassium compounds may also provide an effective post CO_2 treatment. Further investigation of toxic substances that are safe, quick, inexpensive, and easy to use would be warranted as a part of a treatment method or a barrier.

NZMS are not tolerant of heat above 35°C (Quinn et al. 1994; Cox and Rutherford 2000). As temperature units accumulated in the study reported here, the mortality was increased for a given duration of exposure. The highest temperature tested in trials in our laboratory was 20°C. Elevating the temperature of treatment tanks would likely result in a more rapid time to 100% mortality.

Places such as Hagerman National Fish Hatchery enjoy a very warm climate. As NZMS are not tolerant to the temperatures common to these areas especially during the summer a method that takes advantage of intense solar energy such as a solar oven may produce an effective and quick treatment method for eradicating NZMS collected by filtration from the hatchery.

This study was conducted in small tanks in a laboratory on a small number of snails at a time. The snails that were used were in clean water and free of other debris that may accompany them in the stream feeding the hatchery. Requirements for CO_2 exposure may change with the addition of other biological material and the possibility of increased loading from springs in the hatchery setting. The tanks used in this study held 3 liters of water with 3 liters of headspace. This may not be adequate for the load of snails drifting into and being collected from a hatchery. A hatchery implementing this type of treatment method may need

to use larger tanks to treat the number of snails and debris collected on a daily or weekly basis. The water in our laboratory was hard by most standards and may be somewhat resistant to acidification by CO_2 introduction. The water source of implementing hatcheries would need to evaluate the alkalinity of their source water and adjustments to the treatment length may need to be made.

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Table 2.1. Dates and temperature of pressurized CO_2 tests and corresponding shipment receipt from Hagerman National Fish Hatchery shipments were targeted for adult, juvenile and neonate. The shipment from 17 January 2008 was used for one juvenile test and targeted for neonate snails. All other shipments were used to test one specific life stage.

Shipment Date	Test date	Temperature of test
Adult Snails		
28-Feb-07	6-Mar-07	15
	7-Mar-07	8
	8-Mar-07	20
	19-Mar-07	15
	21-Mar-07	8
	22-Mar-07	20
	26-Mar-07	15
	27-Mar-07	8
	28-Mar-07	20
Juvenile Snails		
5-Apr-07	19-Apr-07	15
	24-Apr-07	15
15-May-07	17-May-07	15
	18-May-07	15
	30-May-07	8
	31-May-07	8
1-Jun-07	5-Jun-07	8
	6-Jun-07	8
	13-Jun-07	8
	16-Jan-08	20
	17-Jan-08	20
17-Jan-08	29-Jan-08	20
Neonate Snails		
12-Dec-07	26-Dec-07	15
	27-Dec-07	15
3-Jan-08	7-Jan-08	15
	8-Jan-08	8
	9-Jan-08	8
	10-Jan-08	8
	15-Jan-08	8
17-Jan-08	23-Jan-08	20
	24-Jan-08	20
	25-Jan-08	20

Table 2.2. Range of °C-hours exposures and the corresponding survival for each size of NZMS for pressurized CO_2 tests. Nine tests were conducted on adult snails, twelve on juveniles, and ten on neonate snails. °C-hours were calculated for tanks by multiplying the test duration by the average water bath temperature. Two to four tanks were used in every pressurized CO_2 test to give up to four degree hour calculations; repeated degree hour exposures and corresponding survival percentages were combined for this table.

Adult		Juvenile		Neonate	
°C hours	%Survival	°C hours	%Survival	°C hours	%Survival
6.68	100.0	7.03	65.0	3.21	52.63
7.14	100.0	14.47	100.0	3.55	34.78
7.43	80.0	18.40	92.5	6.62	50.0
14.09	97.5	19.09	82.5	7.00	60.0
14.14	95.0	19.64	95.0	7.13	16.67
14.58	100.0	19.70	85.0	7.43	24.39
19.09	100.0	25.35	90.0	7.49	28.57
19.23	95.0	28.23	72.5	9.71	46.43
20.19	95.0	33.20	82.5	9.76	41.18
20.70	100.0	40.75	55.0	9.90	33.96
21.47	97.5	42.56	55.28	13.62	57.89
22.29	90.0	49.20	67.22	13.87	44.83
41.32	92.5	50.70	89.44	14.14	48.84
42.27	100.0	57.06	40.0	14.29	21.74
42.33	85.0	57.97	53.61	14.74	27.59
43.53	87.5	58.06	75.0	15.11	18.92
43.54	10.0	58.15	25.0	19.42	23.33
44.52	95.0	59.31	25.0	19.48	15.0
55.56	70.0	76.75	52.5	19.75	35.19
57.66	82.5	83.25	45.0	20.64	19.35
57.80	10.0	85.46	10.0	20.73	29.63
58.61	50.0	88.00	7.5	21.36	40.63
58.98	40.0	98.43	5.0	21.54	14.29
59.84	87.5	113.62	0.0	21.99	12.5
84.41	62.5	115.12	5.0	22.73	2.27
84.86	37.5	117.16	2.5	27.58	5.71
90.90	12.5	124.40	0.0	28.82	2.08
113.09	0.0	151.70	2.5	29.23	0.0
113.54	7.5	153.27	0.0	30.79	5.0
116.41	2.5	155.83	2.5	31.26	15.66
117.16	0.0			38.78	0.0
119.38	5.0			38.78	0.0
119.87	0.0			39.56	1.52
154.70	0.0			58.02	0.0
155.82	2.5			58.20	0.0
159.00	0.0			59.67	0.0

Table 2.3. Probit parameter estimates for survival of NZMS exposed to 100 kPa CO_2 by °C-hours of exposure. Parameter estimates are provided by the three test sizes and with the adult and juvenile sizes combined.

Size	Parameter	N	DF	Estimate	SE	χ^2	*P*
Adult	Intercept	9	1	6.3511	1.3925	20.8	<0.0001
	Log_{10}(°C-hours)		1	-3.6	0.7859	20.98	<0.0001
Juvenile	Intercept	12	1	4.596	0.8063	32.49	<0.0001
	Log_{10}(°Hours)		1	-2.7603	0.4645	35.31	<0.0001
Neonate	Intercept	10	1	1.3113	0.3565	12.73	0.0004
	Log_{10}(°Hours)		1	-1.8689	0.2905	35.45	<0.0001
Adult/	Intercept	21	1	6 .6257	1.6789	15.57	<0.0001
Juvenile	Log_{10}(°Hours)		1	-3.7336	0.9519	15.38	<0.0001

Table 2.4. Chi-square analysis of probit parameter estimates comparing among sizes. The first set of parameter estimates compares adult and juvenile probit models to the neonate model. The second set compares the adult and neonate probit models to the juvenile model. By completing two iterations of this test all pairwise comparisons were made.

Parameter	Estimate	SE	χ^2	*P*
Adult and juvenile versus neonates				
Intercept				
Adult	6 .3511	2.0341	6.14	0.0132
Juvenile	4.596	1.8598	3.12	0.0774
Slope				
Adult	-3.6001	1.3268	1.7	0.192
Juvenile	-2.7603	1.2545	0.5	0.4774
Adult and neonate versus juvenile				
Intercept				
Adult	6.3511	2.2598	0.6	0.4374
Neonate	1.3112	1.8598	3.12	0.0774
Slope				
Adult	-3.6001	1.287	0.43	0.5141
Neonate	-1.8689	1.2545	0.5	0.4774

Table 2.5. Chi-square analysis of probit parameter estimates comparing models for adult/juvenile combined data to neonate data.

Parameter	Estimate	SE	χ^2	*P*
Intercept	5.4181	1.3367	9.03	0.0027
Slope	-3.1480	0.9702	1.74	0.1874

Table 2.6. Average and standard deviation (in parentheses) of water quality measurements at each measuring interval before tests (pre-charge); after the saturation period when snails are placed in tanks (charged) and at the end of trials by temperature. Concentrations of CO_2 were calculated using the percent headspace CO_2 composition.

	Measurement interval	pH	DO (mg/L)	Alkalinity (mg/L)	[CO2] (mg/L)
8°					
	Pre-charge	6.96	5.60	144.00	
		(1.07)	(3.43)	(13.76)	
	Charged	4.82	1.93	131.30	
		(0.63)	(1.82)	(16.52)	
	End	4.75	1.24	128.89	3975.86
		(0.69)	(0.75)	(16.62)	(512.19)
15°					
	Pre-charge	7.46	6.43	126.47	
		(0.31)	(2.81)	(18.52)	
	Charged	5.12	1.17	120.07	
		(0.22)	(1.15)	(26.15)	
	End	5.05	0.67	126.53	3108.38
		(0.15)	(0.42)	(18.98)	(457.66)
20°					
	Pre-charge	7.36	4.77	133.63	
		(0.08)	(2.86)	(7.58)	
	Charged	5.06	1.70	126.89	
		(0.09)	(1.49)	(14.24)	
	End	5.01	0.73	131.55	2756.04
		(0.09)	(0.42)	(15.25)	(380.02)

Table 2.7. Average and range (in parentheses) water quality measurement values taken at the end of each pressurized test for adult snails. Averages are calculated from three tests at each temperatures and duration.

Temperature (°C)	Duration (h)	pH	DO (mg/L)	Alkalinity (mg/L)	CO_2%
8°	1	4.89 (4.83 - 4.92)	1.3 (0.3 - 2.6)	120.3 (103 - 135)	76.9 (65.6 - 93.2)
	3	4.87 (4.84 - 4.91)	0.8 (0.4 - 1.6)	113.3 (103 - 127)	88.9 (84.0 - 93.8)
	6	4.90 (4.88 - 4.91)	1.9 (1.3 - 2.6)	122 (117 - 130)	79.7 (66.6 - 86.8)
	8	4.88 (4.86 - 4.92)	1.4 (0.3 - 2.6)	118.3 (102-133)	95.3 (93.2 - 96.8)
	8 (Control)	7.31 (7.25-7.39)	16.5 (12.9 - 18.5)	126.3 (117-142)	0.1 (0.08 - 0.12)
15°	1	5.06 (4.82 - 5.38)	0.5 (0.4 - 0.6)	110.3 (107 - 116)	77.8 (70 - 91.4)
	3	5.05 (4.85 - 5.39)	0.37 (0.2 - 0.5)	112 (110 - 116)	90 (84.0 - 98.0)
	6	5.10 (4.96 - 5.30)	1.03 (0.7 - 1.2)	112 (108 - 117)	76.5 (63.4 - 91.2)
	8	4.93 (4.87 - 5.05)	0.4 (0.3 - 0.5)	110.3 (109 - 112)	94.5 (92.6 - 97.0)
	8 (Control)	7.24 (6.89 - 7.59)	14.8 (13.3 - 16.8)	132.3 (118 - 144)	0.09 (0.06 - 0.10)
20°	1	4.95 (4.84 - 5.07)	0.77 (0.3 - 1.5)	110.3 (107 - 116)	84.0 (68.0 – 97.8)
	3	5.06 (4.93 - 5.19)	0.7 (0.5 - 1)	112 (110 -116)	78.7 (57 - 93)
	6	4.97 (4.85 - 5.14)	0.4 (0.2 - 0.6)	112 (108 - 117)	87.5 (76.6 – 99.8)
	8	4.98 (4.9 - 5.05)	0.57 (0.4 - 0.9)	132.3 (118 - 144)	80.8 (66.8 – 88.4)
	8 (Control)	7.43 (7.37 - 7.5)	12.5 (11.3 - 13.6)	110.3 (109 - 112)	0.37 (0.06 – 1.0)

Table 2.8. Average and range (in parentheses) water quality measurement values taken at the end of each pressurized test for neonate snails. Averages are calculated from three tests at each temperatures and duration with the exception 0.5 and 4 hours at 8° that only had two tests.

Temperature (°C)	Duration (h)	pH	DO (mg/L)	Alkalinity (mg/L)	CO_2%
8°	0.5	5.00 (4.97 - 50.2)	1.9 (1.1 - 2.6)	158.5 (157 - 160)	68.3 (58.6 - 78.0)
	1	5.01 (4.95 - 5.08)	1.9 (1.4 - 2.5)	159 (152 - 164)	78.2 (75.8 - 80.0)
	2	4.98 (4.88 - 5.06)	1.4 (0.9 - 2.0)	163 (167 - 158)	82.7 (78.0 - 91.8)
	3	4.98 (4.94 - 5.04)	1.5 (1.1 - 1.8)	162 (158 - 165)	92.5 (87.6 - 95.2)
	4	5.00 5.00	0.85 (0.6 - 1.1)	155.5 (155.5 - 160.0)	97.5 (97.4 - 97.6)
	4 (Control)	7.54 (7.41 - 7.66)	18.1 (16.2 - 19.4)	169.7 (155 - 180)	0.13 (0.12 - 0.14)
15°	0.5	5.01 (4.95 - 5.06)	0.83 (0.7 - 1.1)	157.7 (150 - 173)	68.6 (62.6 - 80.2)
	1	5.01 (4.97 - 5.07)	1.1 (0.8 - 1.3)	149.3 (145 - 152)	74.8 (70.2 - 78.8)
	1.5	4.98 (4.87 - 5.05)	0.57 (0.3 - 1.0)	151 (145 - 155)	87.0 (72.4 - 95.8)
	2	5.00 (4.87 - 5.14)	1.2 (0.4 - 1.9)	160 (156 - 164)	78.5 (56.0 - 95.6)
	2 (Control)	7.40 (7.53 - 7.29)	8.0 (5.2 - 12.8)	147.3 (140 - 155)	0.14 (0.1 - 0.2)

Table 2.8 Continued.

Temperature (°C)	Duration (h)	pH	DO (mg/L)	Alkalinity (mg/L)	CO_2%
20°	0.5	5.04 (4.95 - 5.12)	0.90 (0.5 - 1.5)	135.7 (134 - 138)	74.8 (59.8 - 92.6)
	1	5.12 (5.06 - 5.21)	1.2 (1.1 - 1.4)	133 (126 - 140)	71.1 (66.2 - 74.4)
	2	5.07 (4.93 - 5.14)	0.83 (0.3 - 1.2)	139 (137 - 140)	88.9 (83.6 - 96.8)
	3	5.09 (5.05 - 5.14)	1.1 (0.9 - 1.5)	136.7 (131 - 145)	82.4 (78.4 - 84.6)
	3 (Control)	6.97 (5.09 - 8.01)	12.0 (7.9 - 15.7)	131 (127 - 135)	0.12 (0.1 - 0.16)

Table 2.9. Probit parameter estimates for survival of adult NZMS exposed to non-pressurized CO_2 by °C-hours of exposure.

Size	Parameter	DF	Estimate	SE	χ^2	*P*
Adult	Intercept	1	7.0862	1.2968	29.86	<0.0001
	Log_{10}(°Hours)	1	-3.5364	0.6472	29.85	<0.0001

Table 2.10. Chi-square analysis of probit parameter estimates comparing models for pressurized and non-pressurized CO_2 tests on adult snails.

Parameter	Estimate	SE	χ^2	*P*
Intercept	7.0862	3.3570	0.05	0.8267
Slope	-3.5364	1.7296	0.00	0.9707

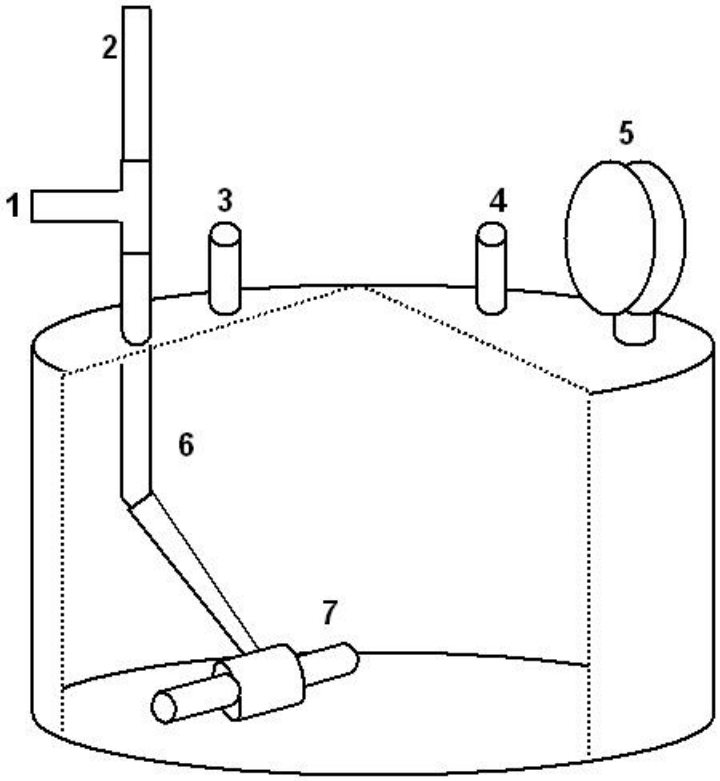

Figure 2.1. Cut-away diagram of tests tank configuration for exposing NZMS to pressurized CO_2. Five identical tanks were used for testing; four were connected through a manifold to equalize CO_2 pressure. All tanks were connected to individual pressure pumps. Numbered parts are: 1) gas inflow from pressure pumps, 2) gas flow rotameter, 3) CO_2 inflow from pressure equalizing manifold, 4) gas outflow to pressure pump, 5) pressure gauge, 6) internal tank stainless steel tubing, 7) 15mm porous plastic gas diffusers.

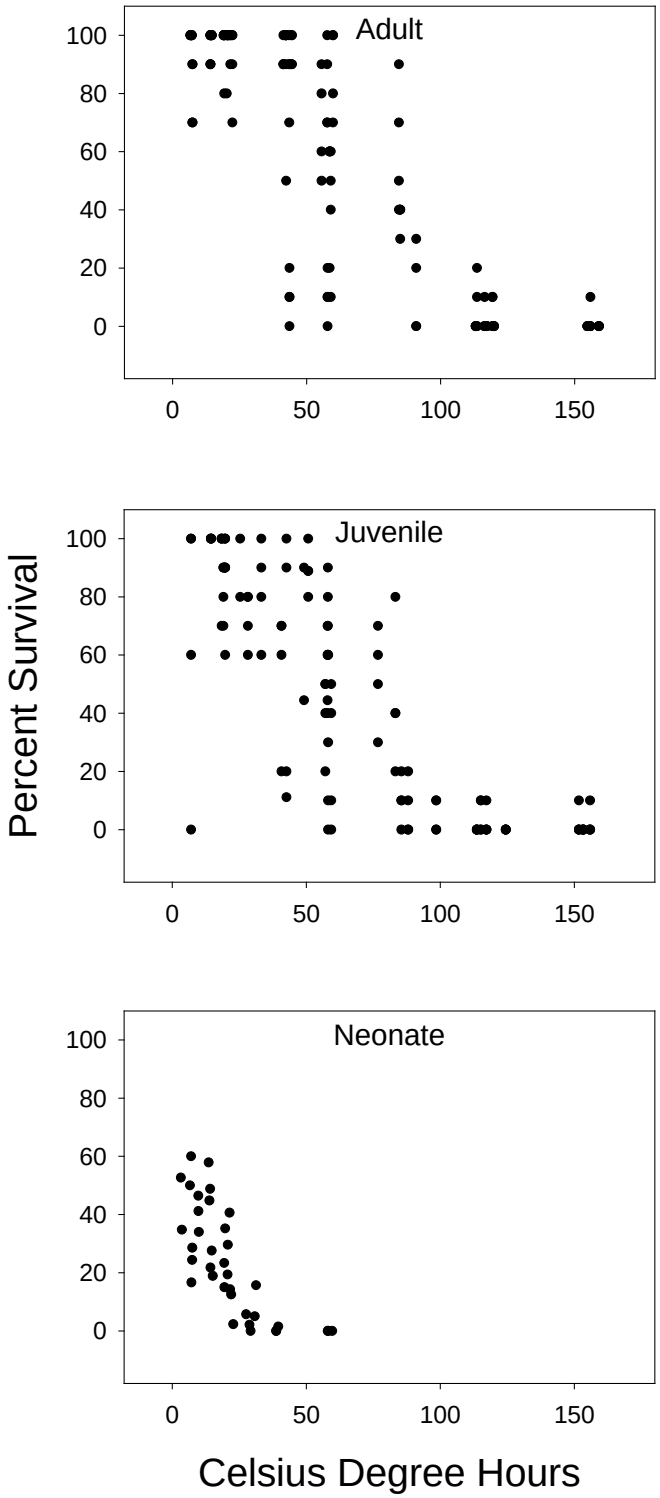

Figure 2.2. Percent survival of all three sizes of NZMS tested by °C-hours of exposure.

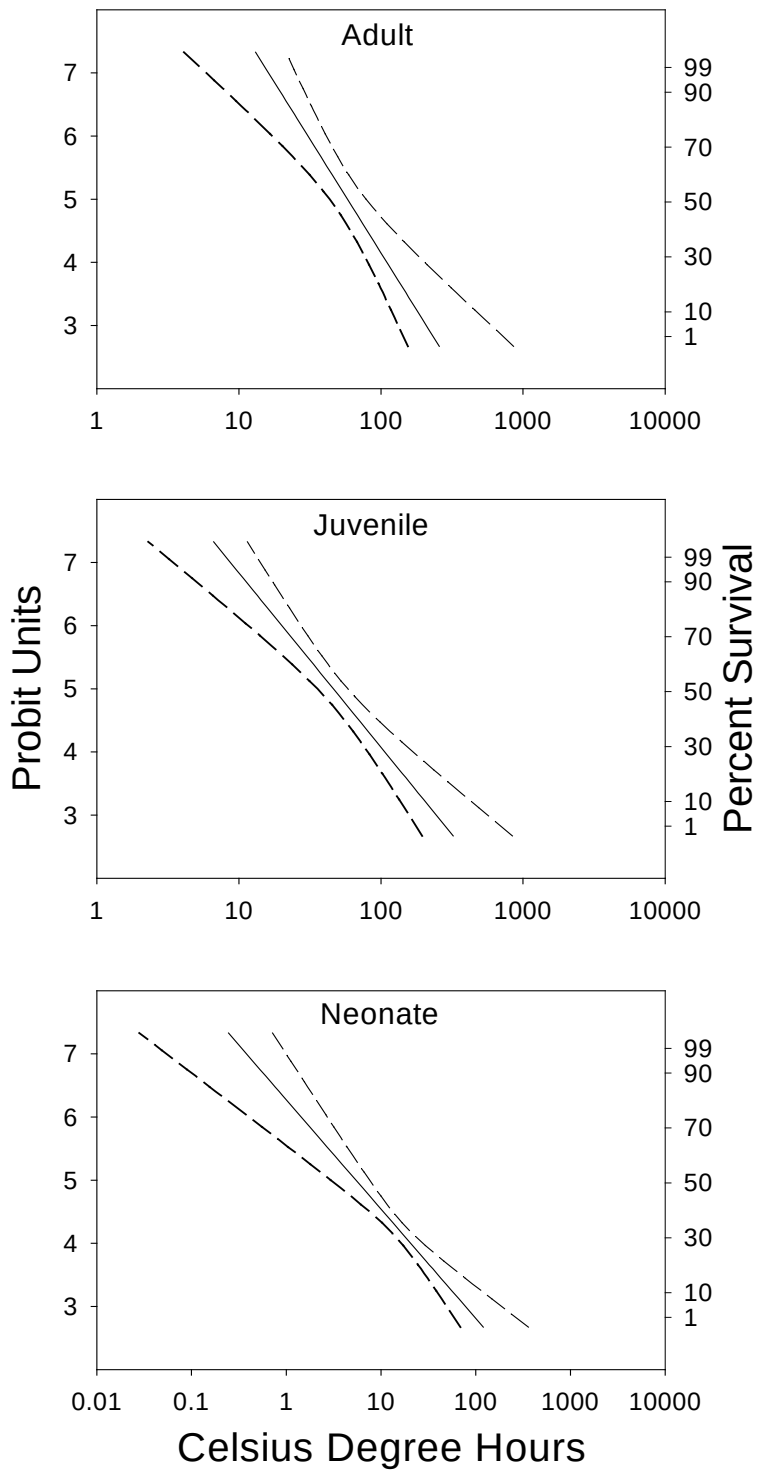

Figure 2.3. Probit estimations of mean and 95% confidence intervals of the survival response of the three sizes of NZMS exposed to 100 kPa CO_2. Parameter estimates of adult and juvenile sizes do not differ significantly. Intercepts of both adult and juvenile sizes differ from the neonate size. Slopes do not differ at among the sizes.

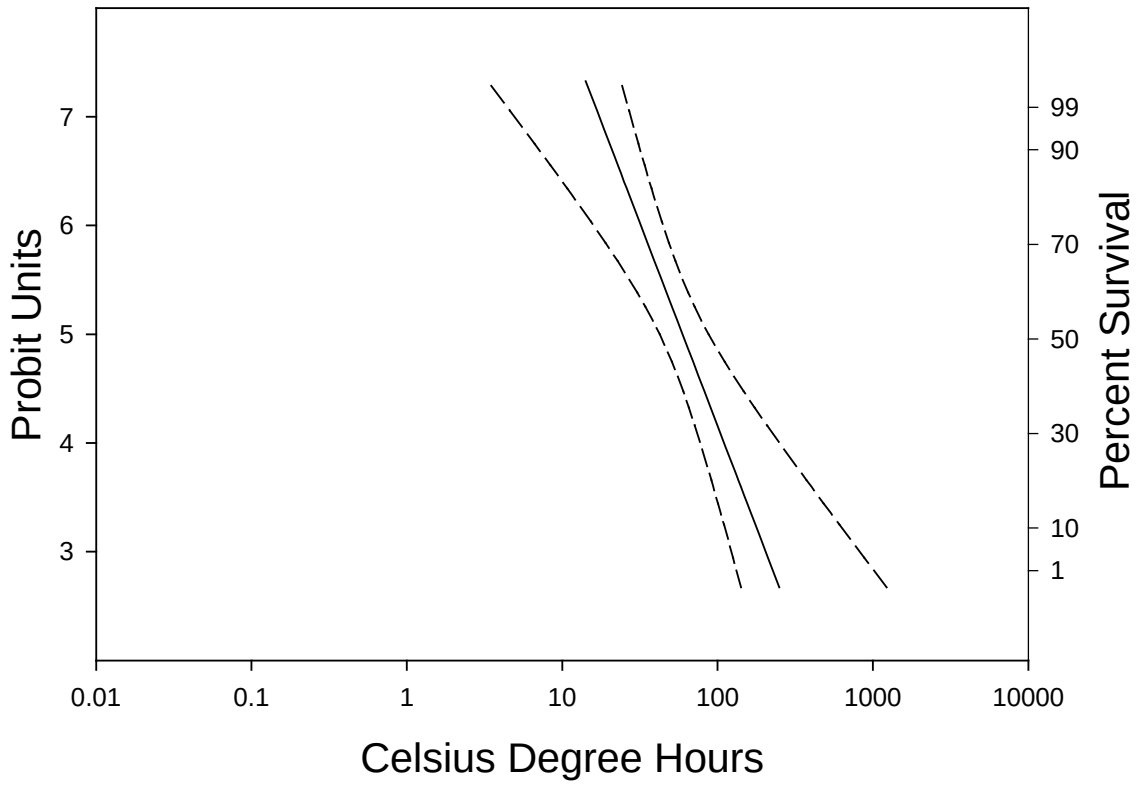

Figure 2.4. Probit estimate of median and 95% confidence intervals of the adult/juvenile combined survival. Parameter estimates of slope differed from the probit model for neonate snails (Figure 2.3).

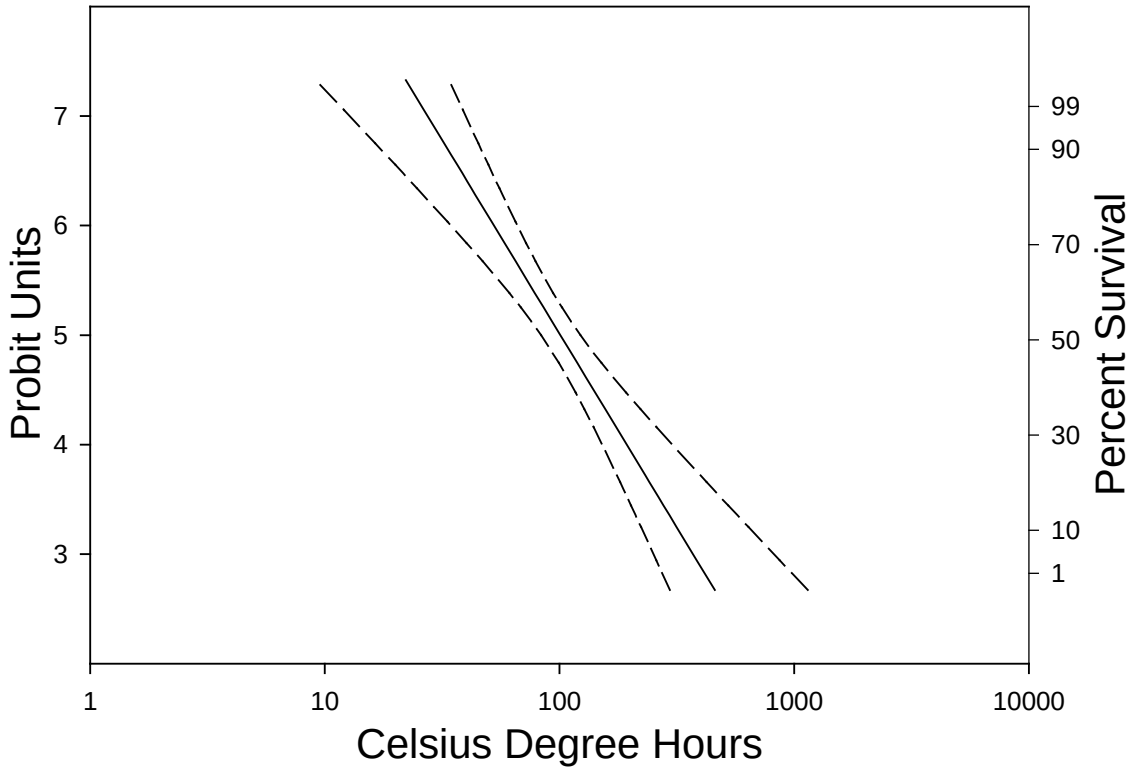

Figure 2.5. Probit estimate with 95% confidence intervals of adult NZMS exposed to non-pressurized CO_2. Parameter estimates did not differ from parameter estimates of adult snails tested with pressurized CO_2.

CHAPTER III – ESTIMATING NEONATE NEW ZEALAND MUDSNAIL DRIFT FROM INFESTED SPRINGS

Abstract

The New Zealand mudsnail (*Potamopyrgus antipodarum*, NZMS) has infested many spring environments in the United States. To explore the timing and rate of downstream drift of NZMS in an infested, constant temperature spring in Hagerman, Idaho, we collected drift samples from a hydrocyclonic filtration apparatus in July and October 2007. We found up to 37 X more neonate snails than juvenile or adults were captured in drift samples. Quantification of the total drift was adjusted for an hourly rate to examine differences between night and day rates and between months. Drift rates were higher in samples collected in October than July. We observed a higher nighttime drift associated with the period immediately following the new moon in July. To further explore if releases of neonates could be related to the diurnal light cycle we conducted a brief laboratory trial with small groups of snails held in static containers at 15°C under continuous light or 20 hours of dark for three consecutive days. We failed to find differences between releases of neonates in these test groups.

Introduction

New Zealand mudsnail (*Potamopyrgus* antipodarum, NZMS) populations in the US are primarily female and reproduce via parthenogenesis (Wallace 1992). One female snail can produce up to 120 embryos in a brood pouch at reproductive maturity (Winterbourn 1970b, Schreiber et al. 1998, Richards and Lester 2000, Richards 2004). This release allows for a potential rapid colonization of favorable habitat to densities reported as high as 500,000 snails m^{-2} (Richards 2002, Hall et al. 2003).

The dispersal of snails between watersheds can be from transportation on fishing gear, boats, or clothing as well as through animal fur, feathers or natural fish movements or fish stocking (Bondesen and Kaiser 1949; Haynes et al. 1985; Vinson 2004; Bruce 2006). The mode of dispersal within streams can be enhanced through fish or animal movements, but the NZMS are capable of moving upstream and downstream (Richards et al. 2001).

Once introduced, the reproductive potential for within stream recruitment to the population from release of embryos is substantial and may be cyclical either with diurnal and/or seasonal releases. Richards and Lester (2000) found that >75% of NZMS over 3 mm shell length in Banbury Springs, Idaho, had neonates in their brood pouch in May and <25% in September, perhaps indicative of a synchronized release of neonates in the summer season (Richards and Lester 2000). Contrary to the results of Richards and Lester, James (2006) found that adult snails in Riley Creek, a spring fed stream in near Banbury springs, had a higher number of neonates in their brood pouches in the winter than in the summer. No studies have been conducted to assess the release of neonate snails in a natural spring.

Understanding reproductive and dispersal patterns of NZMS can help in managing and preventing the spread of NZMS to new locations. This study presents a preliminary

investigation of the dispersal patterns of neonate mudsnails captured in downstream drift filtered by a hydrocyclone in Len Lewis Spring, Hagerman, Idaho.

Methods

Study Location, Design, and Instrumentation

Drift samples were collected from the underflow of a hydrocyclonic filter (15.25 cm diameter hydrocyclone model U6-gMAX; FLSmidth Krebs, Tucson Arizona). We used two different vortex finders to allow for a total flow of 367 L/min (2.25 in aperture) or 257 L/min (1.50 in aperture). The apex opening was 1.27 cm and allowed approximately 13.44 L/min to the underflow (Figure 1.1). Design pressure for hydrocyclone operation was 69 kPa for both flow rates.

The hydrocyclone intake was placed in Len Lewis spring on the northeast side of Hagerman National Fish Hatchery, Hagerman, Idaho. The location was chosen to obtain a 9.1 m drop from the water intake to the hydrocyclone and achieve the required head pressure (69 kPa) for efficient operation.

The hydrocyclone was supplied with water traveling through 72 m of 7.6 cm diameter flexible fire hose and 12 m of 7.6 cm PVC placed from the spring source to the filtration site (Figure 1.3). A Signet 8150 Flow Totalizer (George Fisher Signet, Inc., El Monte, California) was mounted in the PVC pipe to monitor instantaneous flow rate (L/min) and totalize flow over operations. The water that entered the hydrocyclone was separated into two streams: water that was constricted at the underflow by the manufacturer design for flows of approximately 13.44 L/min, and the overflow (approximately 353 or 243 L/min) that was piped through a 7.6 cm PVC pipe to deliver filtered water through a 5 cm reduction manifold controlled with gate valves to provide ports for testing filtration efficiency. The

overflow was directed using drops to spill into one of two tanks and then flow back to the spring water moving to the hatchery. Flow through the hydrocyclone was adjusted with a gate valve at the hydrocyclone overflow and pressure with a similar valve at the inflow of the hydrocyclone.

Snail Drift Collection

Tests were conducted throughout the month of July 2007 and for the week of 21-27 October 2007. Time intervals were defined as day and night. Day test times were conducted between sunrise and sunset. Night tests included the time frame two hours before sunset and two hours after sunrise.

Ring nets (80 µm mesh) were placed at the overflow and underflow of the hydrocyclone to collect particles moving through the systems during each test. Start time and stop time was noted for all tests, and at the end of each, nets were carefully rinsed and the contents collected and preserved. Samples were transferred to University of Idaho where they were examined with aid of a dissecting microscope (10x) to determine the life stages of NZMS collected. Snail life stages were classified by shell length: adult (>2.0mm), juvenile (1mm to 2.0mm), and neonate (<1mm).

Total counts of drifting snails were divided by test duration to calculate an hourly rate of (snails/h). The number of snails captured per hour with the hydrocyclone was then multiplied by the infested hatchery flow of 1,436 L/sec to estimate the potential drift into the hatchery.

Laboratory Tests of Neonate Production

Adult snails were collected on 17 January 2008 from Len Lewis spring at Hagerman National Fish Hatchery, wrapped in wet paper towels, and shipped in a cooler via overnight

courier with a 4L plastic bag of native vegetation to the University of Idaho Wet lab. Snails were acclimated in 2 L containers at a density of approximately 500 to 1,000 snails and held in 1 L of fresh dechlorinated water in an incubator at 15°C under a natural daylight cycle for the latitude of Hagerman, Idaho (42.8175 N). Adult snails were separated with a 1.18 mm mesh stainless steel sieve for testing. Replicate groups of 10 adult NZMS each were placed into 120 plastic cups with approximately 50 mL of fresh dechlorinated water. These cups were separated at random into two groups of 60 cups each. One test group of snails was exposed to continuous light, and the other was exposed to 20 h of dark, 4 h light for 3 days. Neonate snails produced by adults were counted daily by examination with a 10x dissecting microscope for the three days. Snails were maintained at 15°C in an incubator.

Statistical Analyses

Snail drift – Frequency tests were conducted on corrected drift rates to compare: 1) average overall drift rate between the months of October and July, 2) rates between night and day samples within and between the months of July and October. Since sample sizes were unbalanced, we tested differences using several different statistical analyses. We used frequency tests with Pearson's chi-square for equal proportions to examine difference between day and night samples, separated by month and across both months. To adjust for small sample size in October, we reduced the data set from July by randomly removing points from the data set so there was the same number of observations as the October data set. Random data point removal was conducted for 20 iterations for all observations compared between months and for day sample observations between months, and 35 iterations for night sample observations between months. Data were organized into four groups by month and time of sampling and a Kruskal-Wallis test was performed to identify

differences among groups. A visual analysis of neonate drift pattern was conducted by aligning nighttime drift rate with lunar cycle in July and October.

Laboratory tests of neonate production – The number of neonates in each replicate was counted and the daily increase in numbers was used in a repeated measures model to determine if there was a difference in neonate production between light and dark treatments over time. The linear model was:

$$y_{ij} = \mu + \alpha_i + \pi_j + (\alpha\pi)_{ij} + \varepsilon_{ij},$$

where α_i represents the effects of dark or light cycle treatment, π_j represents the effects of day of the treatment, $(\alpha\pi)_{ij}$ represents the day-treatment interaction, and ε_{ij} represents random error. There was little to no mortality during the experiment.

Results

Snail Drift

We captured a similar proportion of drifting neonates in October as found in samples collected in July (Table 3.1). When samples were separated by day or night, we found proportionately more snails drifting in the nighttime samples during July (P=0.001) but not in October (Table 3.1). There was no difference in the rate of snails collected during the day and night between July and October (Table 3.1).

The average drift (± SD) for adult NZMS in July was 0.08 ± 0.14 and 0.05 ± 0.10 snails/h for day and night respectively (Table 3.2). Drift rates for juvenile snails were 0.14 ± 0.27 and 0.10 ± 0.14 per snails/h for day and night, respectively (Table 3.2). When rates were extrapolated to the entire infested hatchery flow and hours of daylight or night, adult drift in July was estimated at 18.43 ± 32.02 and 12.87 ± 24.55-snails/h for day and night

respectively. Juvenile drift rate for total infested hatchery flow was 33.52 ± 62.81 and 23.82 ± 31.93 snails/h for day and night respectively.

In October, we collected no adult or juvenile snails in tests of daytime drift. We estimated a nighttime drift rate of 0.13 ± 0.09 juvenile snails/h, or 34.49 ± 26.25 snails/h when extrapolated to the total hatchery flow (Table 3.2).

A Kruskal-Wallis test was only conducted on neonate drift rates because there were too few adult and juvenile snails collected to analyze. The Kruskal-Wallis test further confirmed a difference between groups according to month and time of day (Table 3.3, P=0.0598). We found that the Fisher's LSD separations showed a higher neonate drift rate for both day and night in October than the day drift rate in July (Figure 3.1).

When neonate night drift rates were examined with the lunar cycle, we observed peak neonate drift five days after the new moon in July (Figure 3.2).

Laboratory Evaluation of Neonate Production

The average number of neonates produced in each cup decreased across the days of observation in both treatments (Table 3.4). There was no difference in overall mean neonate production between the two treatments of continuous light or 20 h of dark in the repeated measures test (Table 3.5). However there was an effect of time within the treatment (Table 3.6, P<0.001) indicating that the decrease in mean daily production throughout the treatment was significant.

Discussion

Natural drift of snails out of Len Lewis Spring was composed mostly of neonates in both months of evaluation. The drift rate was higher in October than July, but the number of samples collected in October was limited. Richards and Lester (2000) reported that >90% of

snails >3.00 mm in Banbury Springs in southern Idaho had neonates in their brood pouches in April and May and fewer than 25% had neonates in their brood pouches in September. Richards and Lester (2000) reported higher frequency of small snails (<1.5 mm) with the post- or non-reproductive adults in September. James (2006) found that in Riley Creek, Hagerman Idaho, adult snails had a higher number of neonates in the brood pouches in the winter than in the summer. In evaluations of NZMS in a higher elevation stream, Loving Creek near Haley, Idaho, fewer numbers of neonates were found in brood pouches of NZMS in the winter samples (James 2006). Neonate drift rates collected from Len Lewis Spring in October support the results of James (2006) and indicate that adults may brood and release neonates at the end of the summer and in to the fall season. Too few adult or juvenile snails were captured to determine a pattern of drift and dispersal among larger snails. Richards and Lester (2000) found that drift of adult snails below vegetated habitats was low probably due to the increased habitat structure available to snails for cover from stream flow. The reach above where hydrocyclone samples were taken was densely vegetated during the summer months and may explain the low numbers of captured adult and juvenile snails.

In the field tests, we observed a higher proportion of neonates drifting during night than in the day. Highest drift rates in July coincided with lunar cycle and peaked 5 d after the new moon (Figure 3.2). Other invertebrates have shown higher nighttime drift rates that are correlated to lunar cycles. Zakai et al. (2006) found that coral planulation was highly correlated with lunar cycle with peak production of dispersing planula 2 to 6 nights after the occurrence of a full moon. The pattern of NZMS drift in July followed a similar but inverse pattern with highest releases following the new moon. While coral use moonlight as a cue for reproduction (Zakai et al. 2006), NZMS may use the lack of moonlight to reproduce or

disperse in low light in order to avoid potential predators. Alldredge and King (1980) showed that some species of lake amphipods and large zooplankton migrated vertically in the water column less during the nights surrounding a full moon presumably as a predator avoidance behavior. Neonates migrating up vegetation into stronger currents would be more susceptible to being swept away and drifting downstream. More data are needed to support the hypothesis that reproduction is influenced by lunar cycle. Moreover, the nighttime collections in our study would have captured drift from dawn and dusk times, and this could have influenced our results.

The laboratory test with two light regimes showed no difference in the overall production of neonates between dark and light. However, snails in the lab were not held in the same conditions as their natural environment and may release neonates as a stress response regardless of day length treatment. Also, the containers that held the test snails were static, snails were handled on a daily basis, and snails were not fed, all factors that contribute to a possible elevated stress and neonate release as a response.

Although the data in this study provides insight to potential reproductive and dispersal patterns of New Zealand mudsnails, larger samples and more controlled studies need to be conducted to better understand neonate release and drift patterns. Previous work indicates that between 28% and 60% of neonates are destroyed in the hydrocyclone during filtration. Thus there would be a need for a correction factor to accurately estimate neonate drift into the hatchery. Laboratory neonate production trials were conducted for only three days on one shipment of snails. It is advisable to improve lab conditions to meet conditions where snails naturally occur to eliminate stress as a cause for releasing neonates. Laboratory

trials should be repeated on different groups of snails across all seasons to further explore the seasonality of NZMS reproduction.

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Table 3.1. Frequency tests with Pearson's chi-square values for single degree of freedom comparisons of drift rates of neonates between months, between day and night within months and between day and night between months. The frequency reported is the total number of neonates per hour captured across all trials of each type.

Comparison	N	Frequency	Percent	χ^2	P
Between Months					
July	13	26.03	47.70	0.1154	0.7341
October	7	28.53	52.30		
Day vs. Night					
July					
Day	6	6.38	24.53	6.754	0.0094
Night	7	19.64	75.47		
October					
Day	3	12.16	42.63	0.6203	0.4309
Night	4	16.37	57.37		
Day by Month					
July	6	6.38	34.42	1.8012	0.1796
October	3	12.16	65.58		
Night by Month					
July	7	19.64	54.54	0.2971	0.5857
October	4	16.37	45.56		

Table 3.2. Average number of NZMS captured in nets estimations for total infested hatchery flow at Hagerman National Fish Hatchery (HNFH). No adult snails were caught or juvenile snails during the day in October so hatchery drift was not estimated. The letter N represents the number of samples taken. Total filtration time across all trials was 257 h.

				Drift	
Month	Time	N	Size	Filtered Mean ± SD	Estimated for HNFH Mean ± SD
July	Day	6	Adult	0.08 ± 0.14	18.43 ± 32.02
			Juvenile	0.14 ± 0.27	33.52 ± 62.81
			Neonate	1.06 ± 1.0	249.68 ± 235.60
July	Night	7	Adult	0.05 ± 0.10	12.87 ± 24.55
			Juvenile	0.10 ± 0.14	23.82 ± 31.93
			Neonate	2.81 ± 2.62	658.45 ± 613.66
October	Day	3	Adult	0	-
			Juvenile	0	-
			Neonate	2.78 ± 1.17	707.38 ± 203.07
October	Night	4	Adult	0	-
			Juvenile	0.13 ± 0.09	34.49 ± 26.25
			Neonate	4.84 ± 0.87	1,223.89 ± 239.30

Table 3.3. Kruskal-Wallis statistics for comparing time of day*month groupings.

Source	N	DF	χ^2	*P*
Time of Day*Month	20	3	7.41	0.0598

Table 3.4. Mean and standard deviation of neonate production for continuous light (light) and 4 h light, 20 h dark (dark) treatments by day of observation in laboratory tests conducted 23 to 25 January 2008. Mean production was calculated for each of the 60 containers with ten adult snails.

	Day 1		Day 2		Day 3	
Trt	Mean	SD	Mean	SD	Mean	SD
Light	1.87	2.25	1.23	1.38	1.133	1.47
Dark	2.12	2.96	1.78	1.91	0.82	1.11

Table 3.5. Summary statistics of within and between subject effects of repeated measures test on neonate production by day of test and treatment. There were 60 containers of ten snails per treatment for an overall N of 120.

Variable	DF	Mean Square	F	*P*
Treatment	1	2.336	0.52	0.4704
Day (time)	2	31.033	8.97	0.0002
Day*Treatment	2	3.459	1.68	0.1886

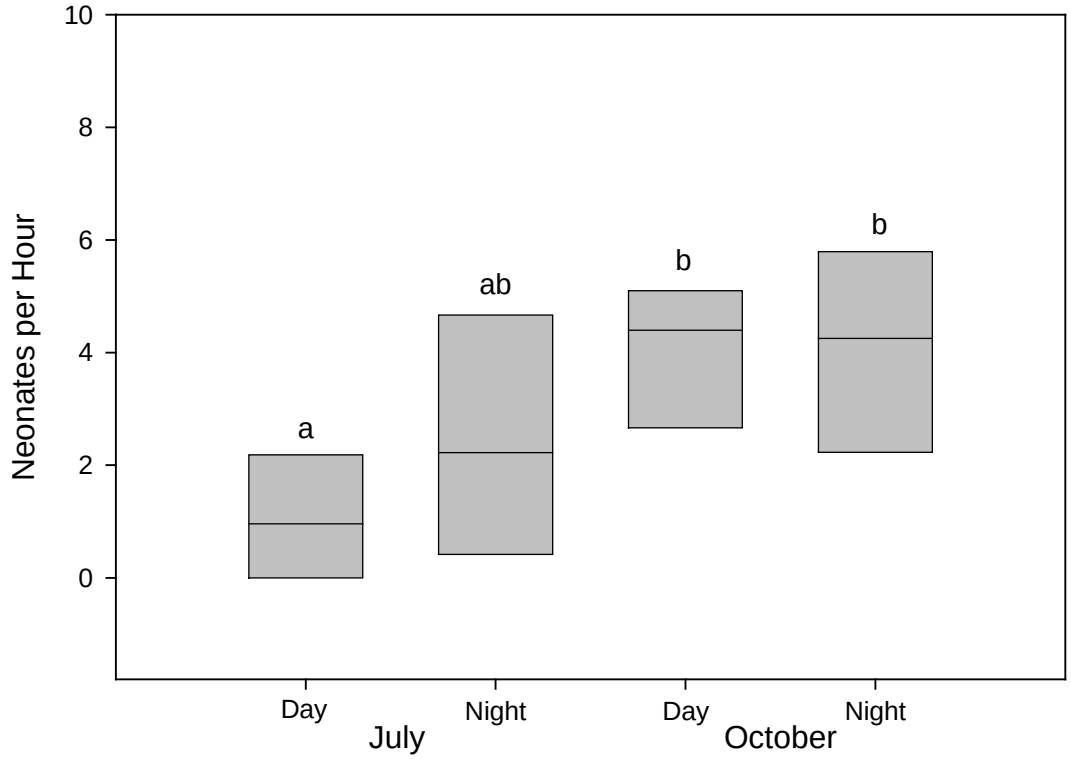

Figure 3.1. Box plot comparison of NZMS drift rate in the months of July and October 2007 by collections from nighttime or daytime hours at Hagerman National Fish Hatchery. Box plots represent the median and range of data from each group. Different letters above box plots indicate significant differences.

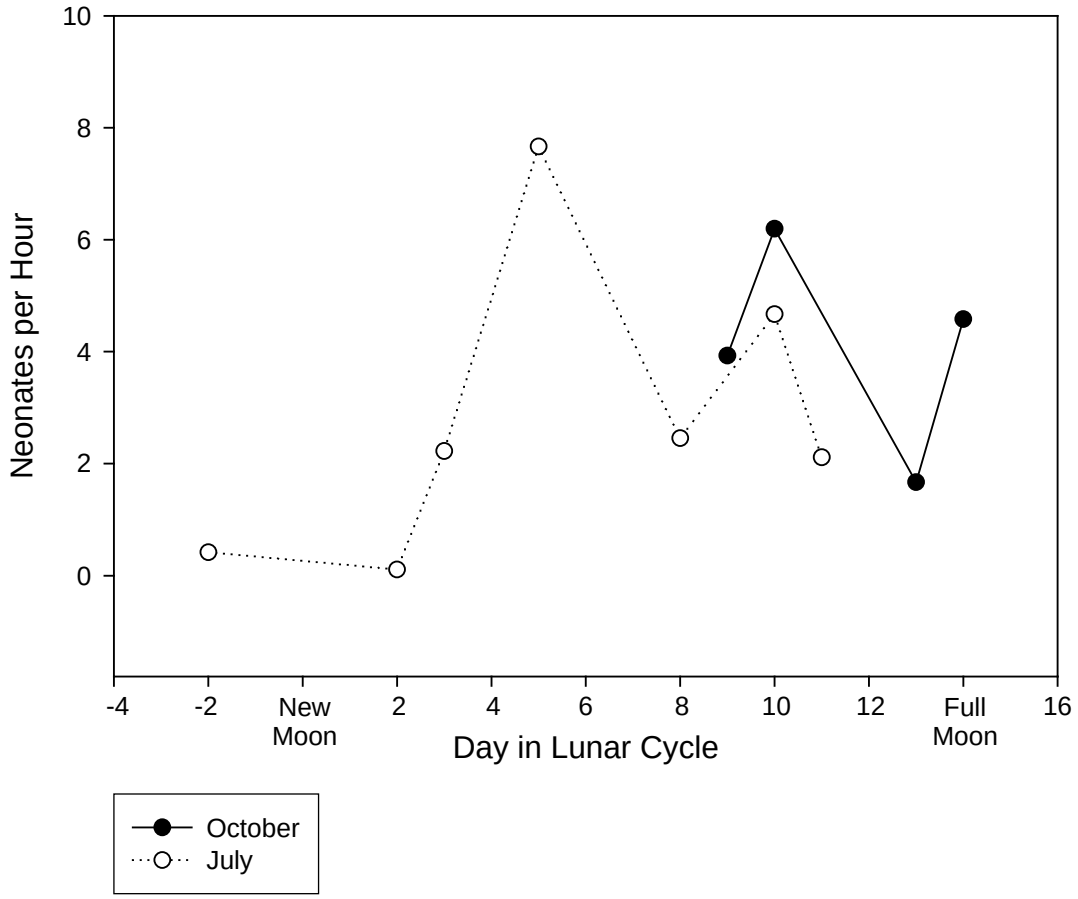

Figure 3.2. Nighttime drift rates of neonate snails according to lunar cycle in the months of July and October 2007.