Summary of Findings from New Zealand Mud Snail Research at FES

As you already know, New Zealand mud snails (NZMS) were discovered at the Utah Division of Wildlife Resources Loa State Fish Hatchery (Wayne County, UT) in November 2007. Beginning in January 2008, researchers at the Fisheries Experiment Station began to research techniques that can be used to eradicate NZMS from Loa and other methods that can be used to prevent NZMS infestation at other hatcheries in the state.

The goal of this document is to summarize the results of the NZMS research that has been conducted by FES. We are deliberately keeping the findings presented here concise, however, feel free to contact Randy Oplinger (randyoplinger@utah.gov or 435-752-1066 x 206) if you have any questions or would like more details.

1) **What is the risk of stocking fish from the Loa State Fish Hatchery?**
   - We used a syringe to insert 243 NZMS into the stomachs of 8-10 inch rainbow trout at Loa. Only 4.5% of these snails survived digestion.
   - We know that roughly 1 in 500 fish at Loa have a NZMS in their stomachs. Given this information, we estimate that 1 live snail could be excreted into the wild for every 11,000 fish stocked out of Loa.
   - Our results indicate that fish need to be held in a NZMS-free raceway for at least 96 h to ensure passage of NZMS through the digestive tract.
   - Other studies have shown that closer to 50% of ingested NZMS survive digestion. Our fish were housed in fed in a manner that representative of typical hatchery operation and this may explain some of the discrepancies between our results and those of other studies.
   - Previous studies have noted excretion of high numbers of neonates after ingestion. This was not assessed in our study.

2) **Can Epsom Salt be used to expedite the excretion of ingested NZMS?**
   - We tested whether a 20,000 mg/L Epsom Salt + 7,000 mg/L NaCl solution could be used to expedite the excretion of ingested NZMS. To apply this treatment, the solution was created in a tub and fish were immersed in the solution for 15 minutes.
   - We found that the excretion rate and survival of the snails did not differ among the fish treated with the Epsom salt immersion when compared to the control (fish immersed in hatchery water). Therefore, Epsom salt cannot be used to expedite the excretion of ingested NZMS.

3) **What is the light preference of NZMS?**
   - This experiment was conducted to determine whether NZMS will willingly occupy darkened areas such as the inside of pipes or waste removal systems.
   - We found that it was equally likely that snails will occupy both dark and light regions. Regardless, snails have a strong preference to occupy areas with available forage. Thus, when eradicating snails from Loa, we need to

consider both light places and darkened places. Particular emphasis should be placed on areas where forage might be present.

4) **What effect does salt have on NZMS?**
   - For this experiment, snails were placed on a glass plate that sat in a tub of water at a 45 degree angle. Salt was then added and the number of snails that released from the glass plate was recorded.
   - 59% of the snails subjected to an 11 g/L salt solution released whereas 33% exposed to a 5.5 g/L solution released.
   - The snails quickly respond to salt and it is possible that it can be used to "flush" snails off of surfaces such as pipes or raceway walls.
   - Unfortunately, the presence of salt had no effect on the survival of NZMS.

5) **What is the toxicity of Iodine, Formalin, Potassium Permanganate, and Hydrogen Peroxide to NZMS?**
   - For iodine, our estimated LD_{50} for a 15 min treatment is 187 ppm. Our estimated LD_{90} is 3,400 ppm. The concentration required during a 15 minute exposure to kill 100% of NZMS is > 10,000 ppm.
   - For formalin, our estimated LD_{50} for a 15 min treatment is 12,200 ppm. Our estimated LD_{90} is 30,700 ppm. The concentration required during a 15 minute exposure to kill 100% of NZMS is 60,000 ppm.
   - For potassium permanganate, our estimated LD_{50} for a 15 min treatment is 35 ppm. Our estimated LD_{90} is 5,700 ppm. The concentration required during a 15 minute exposure to kill 100% of NZMS is 25,000 ppm.
   - For hydrogen peroxide, our estimated LD_{50} for a 15 min treatment is 2,200 ppm. Our estimated LD_{90} is 3,400 ppm. The concentration required during a 15 minute exposure to kill 100% of NZMS is 7,500 ppm.
   - Alternatively, 1 min in a 3% hydrogen peroxide solution will kill 97% of NZMS, but, 15 min is the shortest duration in a 3% solution that will kill 100% of NZMS.
   - In general, the concentrations of these chemicals required to kill 100% of NZMS are greater than what can be safely applied to our fish or eggs. Therefore, we cannot use any of these chemicals to disinfect our wild eggs.
   - Also, these chemicals would likely have an effect on non-target organisms if released into our effluent.
   - Regardless, if fish or eggs aren't present and steps are taken to de-toxify the effluent stream, it is possible that these chemicals can be used to disinfect Loa. These chemicals can also be used to disinfect gear.

6) **To disinfect eggs from wild egg takes, can we use Hyamine, household ammonia, copper sulfate, Pinesol or 409?**
   - We tested these chemicals on some eggs to determine their toxicity. All of these chemicals have been previously shown to be lethal to NZMS.
   - 100% of the eggs treated with Hyamine, ammonia, and 409 died within 24 h.
   - The eye-up, cripple, and hatch rates of eggs treated with a 504 ppm copper solution (as copper sulfate) and Pinesol (50% dilution) did not differ from the

control (hatchery water). But, some of the data indicates that these chemicals could be potentially dangerous to eggs. As a result, we do not recommend the use of these chemicals until further testing is conducted.

7) **Can a long-term, low dose drip of either potassium permanganate or hydrogen peroxide be used to kill NZMS?**
   - We established 12 groups of 100 NZMS. Four of these groups received a 2 ppm dose of potassium permanganate, another 4 received a 150 ppm dose of hydrogen peroxide, and the remaining groups did not receive any chemical and thus served as a control. Snails received the chemical treatment daily for 14 days. They were only exposed to the chemical for 2 hours each day. Thus, this treatment simulates a chemical drip that might be established at a hatchery.
   - At the end of the 14 day trial, on average, 94 of the 100 snails in each of the potassium permanganate groups were dead, all 100 in each of the peroxide groups were dead, and on average, 24 of the 100 in the control groups were dead.
   - Therefore, the chemicals appeared to elevate snail mortality. I see a couple of problems with the use of these chemicals, though. First, I think that dripping them for 2 weeks might be pretty expensive. Also, while the concentrations that I tested should be fish safe, I think that they are at the upper limit that fish can safely handle. Therefore, these chemicals might have an adverse effect on the fish at Loa. Finally, the effects of these chemicals when released into the effluent are un-known.

8) **Can we anesthetize snails and would this decrease chemical concentrations required for 100% NZMS mortality?**
   - We assessed the anesthetic properties of MS-222, Epsom salt, ethanol, and menthol on NZMS.
   - None of these chemicals successfully induced anesthesia.

9) **Previous studies have immersed snails into chemical solutions. What chemicals are effective at killing NZMS if they are applied to the snails as a fine spray?**
   - We determined whether household ammonia, copper sulfate, Commerical 409 Cleaner, De-greaser, and Disinfectant, Hyamine, hydrogen peroxide or Pinesol are effective at killing NZMS when these chemicals are sprayed onto snails using a squirt bottle (about 1 mL sprayed onto air dried snails). 15 min after exposure, the chemical was rinsed off and the NZMS were placed back in water.
   - Copper sulfate (504 ppm as copper), Hyamine (3,880 ppm), and hydrogen peroxide (30,000 ppm) all killed 100% of NZMS.

10) **Can copper strips be used to prevent the upstream movement of NZMS?**
   - We determined whether NZMS can detect the presence of copper in water and if they avoid crossing copper strips. We tested 1, 2, 3, and 4 inch wide strips.

- NZMS readily crossed all four copper strip widths tested.
- We tested brand new copper strips in this trial. It is possible that older strips that show signs of corrosion are more effective at inhibiting NZMS movement. We will test this in the future.

11) What specifications would you recommend when designing an electric barrier to prevent the upstream movement of NZMS?

- If DC current is used, we would recommend designing a barrier that produces > 0.80 mA/cm^2. If AC current is used, we would recommend designing a barrier that produces > 0.25 mA/cm^2.
- Corrosion is a major issue that affects the performance and durability of an electric barrier. We recommend constructing electrodes using either stainless steel alloy 303 or alloy 304. Also, barriers electrified with AC current are more durable than those electrified with DC current.
- We highly recommend the use of current density (mA/cm^2) as a benchmark of barrier performance. The use of current (mA) is misleading because if current is held constant but a barrier increases in size, the current density and hence the strength of the "charge" that NZMS experience decreases. Therefore, larger barriers need to emit more current. The use of current density terminology provides a more universal mean of discussing barrier performance.

12) Have you designed an effective electric barrier?

- We have constructed and tested two raceway sized barriers. One was constructed using copper pipe and was powered by a 12 volt deep cycle battery. This barrier corroded and fell apart within 4 weeks of installation and was not effective at preventing snail movement.
- The second barrier was powered by an AC power supply (27 volts, 310 mA, 0.40 mA/cm^2). The electrodes were constructed using stainless steel banding that was mounted to a 2 x 4. Snails were marked with the fluorochrome calcein and stocked downstream of the barrier. After 4 weeks, snails were collected upstream of the barrier and assessed for the presence of the fluorochrome mark. Any marked snails collected would have crossed the barrier. Unfortunately, this barrier was not 100% effective at preventing the upstream movement of NZMS. Marked snails were collected upstream of the barrier. The barrier did, however, slow the rate of passage (0.9% of collected snails were marked versus 9.7% of snails marked in a non-electrified control raceway).
- One unfortunate problem with electric barriers is that there are numerous dead zones that may allow NZMS to cross these barriers. So, even a well designed barrier might not stop the upstream movement of 100% of snails.

13) Have you tested other barriers intended to prevent the upstream movement of NZMS?

- We constructed a flume out of plywood that was intended to accelerate the velocity of water to a point greater than NZMS can tolerate (flume velocity =

30 cm/s, maximum velocity that NZMS can withstand = 20 cm/s). This flume looked like a Parshall flume and was installed in a raceway at Loa. Fluorochrome marked snails were placed downstream of the flume and after 4 weeks, snails were collected upstream of the flume and the presence of the mark was assessed.

- Unfortunately, 22% of the snails upstream of the flume possessed the mark and thus the barrier failed. The percentage of marked snails in this raceway was much greater than the percentage marked in an adjacent control raceway (3.5% possessed mark). We still have not isolated the reason why the percentage is so much greater in the treatment (flume) raceway.

14) Can NZMS leave the water to crawl around any in-stream barriers?

- We have conducted several tests assessing the out of water movement abilities of NZMS. The end result of all of these experiments is that NZMS move little when they are out of the water.
- We have observed that when out of the water, NZMS tend to move slowly and in a random pattern. We think that it is un-likely that NZMS can set their sights on the other side of a barrier and travel in a straight line to their destination. Instead, they tend to zig-zag and frequently back-track on their previous path.

15) Can we use bait to attract and trap snails?

- We have found that NZMS can be successfully trapped. Our traps are simply a funnel attached to a plastic beaker. The best bait that we have discovered is bread dough.
- To make the bread dough bait, first dump 1 packet of yeast into 0.5 cups of hot water, mix, and let sit for 5 min. Then, add enough flour (around 0.75-1.0 cups) to the yeast/water mixture to make a dough that is thick enough to handle. Then, dump this onto a floured surface and knead for 5 min. Then, take the kneaded dough and place it into a greased bowl. Cover the bowl with a moistened paper towel. Let sit for 2 hours. Then knead for about 30 sec to remove any air bubbles. Break the dough into desired size pieces and bake at 400ºF for about 5 min. The goal here is to harden the surface but leave the middle gooey (it holds up better underwater if you do this). Regular bread rapidly disintegrates underwater.
- A funnel trap baited with 25 g of bread dough should catch 200-500 snails in a 24 h period. Unfortunately, we think that the dough only draws in snails that are within a couple of feet of the trap. So, future research will attempt do optimize the trap design so we can draw snails in from a further distance.

16) How many snails do you thing are in the raceways at Loa?

- Based on mark-recapture estimates using the fluorochrome marked snails from the AC electric barrier study and some extrapolation, we estimate that there are between 150,000 and 29,181,000 NZMS in the raceways at Loa. This estimate is very crude and violates a lot of mark-recapture assumptions. I think that there are more than 150,000 NZMS in the raceways but also think

that there are a lot less than 29,181,000 NZMS. I'd guess there are 1,000,000-5,000,000 snails. Regardless, these estimates show that there are a lot of snails at Loa that need to be removed. These estimates do not include snails in the main springs.

17) What do we think can be done to remove NZMS from Loa?

- For the short term, we think that the following steps can be taken:
  - All aquatic vegetation can be removed from the main springs and un-used raceways.
  - Progressively use the de-watering/disinfection protocol to remove NZMS from all of the raceways. Install basket filters on all of the raceways after disinfection to prevent NZMS re-entry.
  - Continue searching for NZMS in springs that are not currently infested.
  - Ensure that the new snail species is not found in the main springs. Also, search out new habitats for this species. Perhaps there are other springs on the Loa property or on nearby properties that harbor this snail?
- In the future, we recommend:
  - Trying to "vacuum filter" NZMS out of the spring sediment
  - Re-construct the main springs, placing them underground

18) What are your future research plans?

- Our two main priorities are to look at methods of eradicating NZMS from the Loa head springs and the development of barriers that can be installed at our other hatcheries to prevent NZMS infestation.
- We would like to continue testing various traps with the hopes of devising something simple that can attract large numbers of snails (> 2,000).
- We plan on continuing our work on the electric barrier and plan on testing new, novel barriers that could also be used to prevent the upstream movement of NZMS.