Optimizing Laboratory Culture Conditions for *Physa acuta* Snails

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Abstract: Heavy metals have been a part of our aquatic environment for many decades. To test toxic effects on the snail *Physa acuta*, we had to refine methods for culturing them in the lab. Thus, we conducted a short feeding study and crowding study to explore the effects of varying amounts of food and different tank populations on snail growth rate. We determined the optimal amount of food per snail to be 30 mg. We were unable to detect a significant difference between tanks with 3 and 5 snails and both groups continue growing over the 18-day trial.

Keywords: Snails, Culture, Metals, Diet, Crowding

Introduction

Occurring as natural trace components in the aquatic environment heavy metal levels have increased due to several reasons such as industrial, agricultural and mining activities (Awheda et al. 2015). Copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb) are among the most commons heavy metals that pollute the environment, especially in areas with high anthropogenic pressure (Nica et al. 2012). Factors that contribute to potential health effects need to consider a greater range of combined exposures which makes the risk assessment process more complex compared to the assessment of single chemicals (Sillins and Hogberg, 2011).

Oklahoma's most northeastern incorporated city, Picher is located eight miles north of Miami on U.S. Highway 69 in Ottawa County. The Picher area was the most productive mining field in the Tri-State Lead and Zinc District (Oklahoma, Kansas, and Missouri) and produced more than $20 billion in ore from 1917 to 1947. Over 50 percent of the lead and zinc metal consumed in World War I came from the Picher Field. The city's first deep water well was provided by O.S.Picher, this purchase provided the beginning of a municipal water system. The leasing system employed for mining dictated that an ore reduction mill be built on each forty-acre tract. In 1927, there were 248 mills operating in the Picher Field, and this continued until the late 1930s when centralized milling resulted in mill consolidation. In 1967, when lead and zinc mining finally ceased, pumping water from the mines ceased and they began to fill with water, accumulating 76,800 acre-feet of mine water underground. In 1973, this contaminated water began to seep from the mines. In 1983, the Picher area became part of the U.S. Environmental Protection Agency's Superfund Site program and remains the number one Superfund Site in America. Due to the possibility of collapsing mine shafts, the Environmental Protection Agency (EPA) determined that the town was dangerous to inhabit. Today, Picher, Oklahoma no longer exists as a community (Matthews and Wood, 2007).

Lead and other metals from the environment accumulates in freshwater invertebrates. (Balogh et al,1988). Those accumulated metals may be measured and the information used as sensitive indicators

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of environmental contamination (Pyatt et al, 1997) The molluscan shell also accumulates heavy metals, although its capacity to retain them over long term has not been demonstrated (Beeby and Richmond, 1989). Biological monitoring uses selected biological responses, like behavior changes, that derive from being exposed to various chemicals in the environment. This monitoring is used for evaluating exposures and health risk compared to an appropriate reference (Sillins and Hogberg, 2011)

The suitability of snails as test organisms depends on proper husbandry methods. These include feeding rate and density. Our objective was to determine optimum per capita food and crowding effects on growth rate of snails.

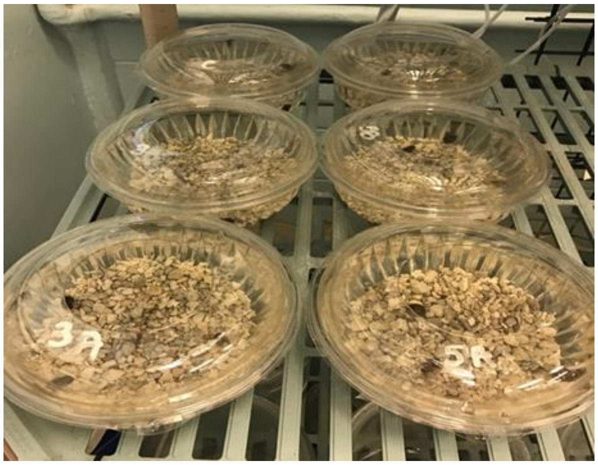

Figure 1 – Experimental setup

Methods

For the food study, we gathered 5 snails per tank, one tank per treatment group. Each tank had 800ml distilled water and 200ml crushed coral (Figure 1). There were 7 different levels of food (2.7, 32, 63, 95, 126, 189, 252mg). The trial was run for 7 days. We changed the water as needed but not on a set schedule. Mass of the snails was measured 4 times, once every two days for each container. In our crowding study, we had 2 different population sizes (3 and 5), 3 snails in 3 tanks, 5 snails in 3 tanks, each tank containing 800ml of distilled water and 200ml of crushed coral. Water was changed every 3 days of this 18-day trail. Artificial light was provided from 6am-9pm every day. We used a Mettler Toledo balance to weigh the food (± 0.1 mg) and snails (± 0.1 mg) for the experiment. JMP Pro 13 was used for statistical analyses of data.

Results

Snails receiving 2.7 mg of food grew minimally within the seven-day study, while the snails that received 32 mg had the highest growth rate (Figure 2). The 32 mg group had an average initial mass per snail of 205 mg, and each day the snails grew an average of 12 mg (Figure 2). A mixed model approach was used to perform a repeated measures analysis of our data. There was no significant difference in the growth rate of snails in tanks with a population of 3 as compared to 5 ($p=0.1299$) (Figure 3). The 3-snail group shows on average an 20% more growth than the 5-snail group.

Discussion

It should be noted that we had a human error during the feeding experiment. In Figure 2, it should have been 15 mg food per capita instead of 2.7 mg. Our results in Figure 2 show that rates above 32 mg tended to have lower growth rates when it came to daily food per capita. We noticed that treatment groups getting more than 95 mg of food tended to have an increase of water fouling which then could have an effect on the growth rate. Another item that could have caused an effect on the growth rate in this study is the weight of the snails at the

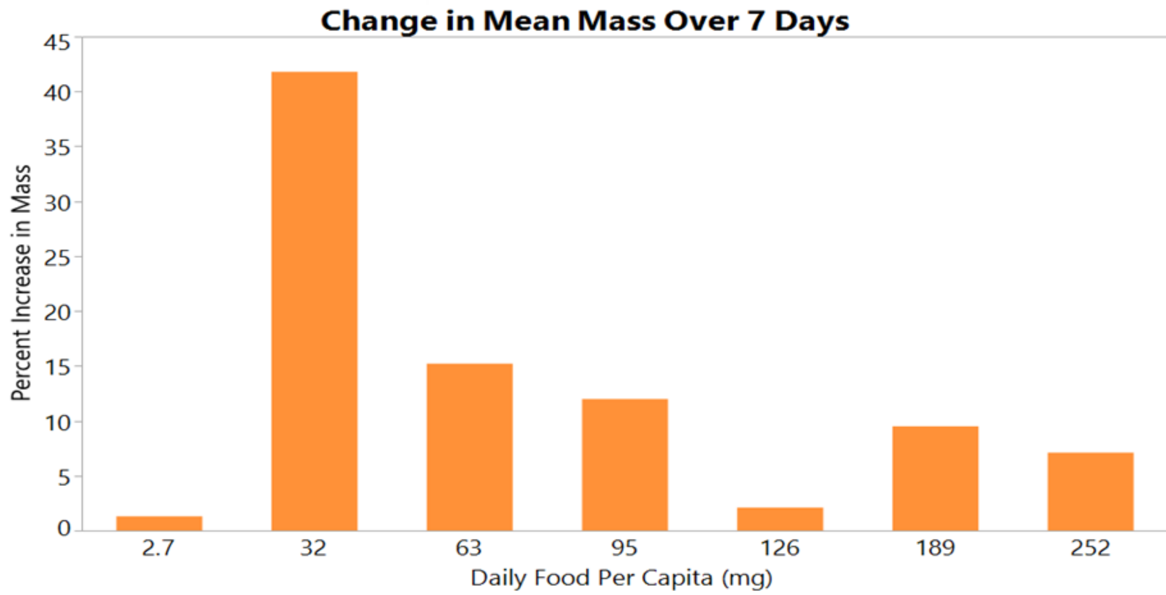

Figure 2 - Change in mass of our treatment groups over 7 days. Water in all of the treatment groups that received 95mg of food or more became fouled within 1-2 days.

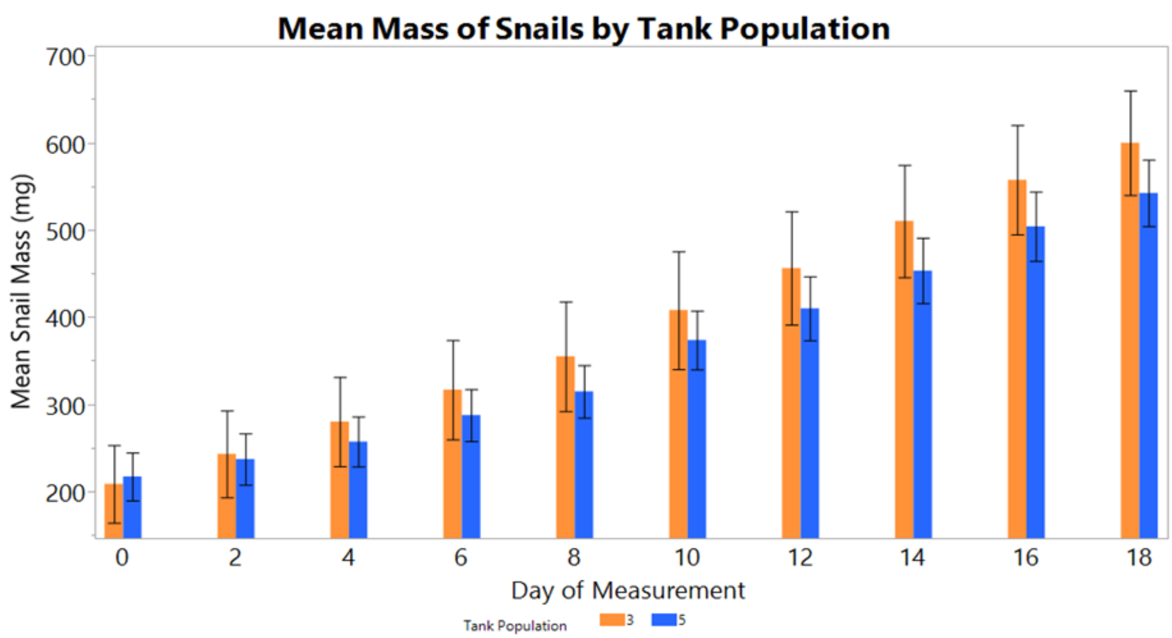

Figure 3 - Change in mean (SE) mass of snails in tanks at either 3 or 5 snails per tank.

beginning of the experiment. The 32mg treatment group had the lowest average initial mass compared to the other treatment groups. Which could have caused them to have the most efficient growth rate.

In Figure 3, the difference of growth rate between 3 & 5 overall is not significant, and both groups continue to exhibit good growth rates after 18 days. There may be a pattern that shows the 3-snail group grows more than the 5-snail group. Without having a longer trial, we were unable to determine whether the crowding effect would be significant. Lacking evidence of a significant crowding effect, we intend to proceed with a 5-snail-per-tank protocol in a future test.

For a future test, you should measure each snail to have an average mass to start the experiment with such masses is not a variable when measuring growth rate. The data you gain from measuring each snail, you will be able to run more in-depth statistical analyses. Our results are important because they help us determine the appropriate amount of food and snail per container for the most efficient growth for our future toxicity tests.

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