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# Can phenotypic plasticity buffer organisms from environmental change?

**Arielle Wimmer[1*] and Amy Krist[2]**

1 **M.S. Student, Department of Zoology and Physiology, University of Wyoming, Laramie, WY**
2 **Associate Professor, Department of Zoology and Physiology, University of Wyoming, Laramie, WY**
* **Author for correspondence: ellewimmer@yahoo.com**

**Abstract** Anthropogenic activities are greatly altering the natural environment. Environmental changes can occur rapidly, such as when non-native species are introduced to new locations. Faced with these rapid changes, organisms may not be able to evolve quickly enough to persist. Phenotypic plasticity, the ability of individuals to alter their traits within their lifetimes in response to variable environments, is a possible mechanism to buffer organisms against large and rapid environmental change. Evolutionary theory predicts that extreme, novel conditions induce more variable traits (greater phenotypic plasticity) than natal habitats. This mechanism may increase the chances that populations will survive rapid environmental change because some individuals will produce traits that are appropriate to the new environment. These theoretical predictions have rarely been tested in nature, yet if true, this resiliency in the face of environmental change could mean the difference between population persistence and local extirpation. To test these predictions in nature, I contrasted the amount of among-individual variation of six populations of freshwater snails between their home (natal) environment and five novel, non-natal environments. I reared snails from all populations in both natal and non-natal environments containing novel crayfish predators and novel environmental conditions (e.g. water chemistry, temperature, flow rate, etc.). Crayfish induce phenotypic plasticity in the morphology, behavior, and life histories (e.g. rates of growth and reproduction) of multiple types of freshwater snails. Non-natal conditions also differ physiochemically from the natal environment and thus are also novel conditions that may induce changes in trait variability. Currently, I am completing the final stage of data collection: assessing variation in shell shape, shell structure, and growth rates in response to these novel environments. Ultimately, my research will enhance our understanding of how organisms respond to environmental changes and elucidate a potential mechanism of population resilience to anthropogenic alterations.

## Introduction

Environments are changing rapidly with anthropogenic activity and climate change, and organisms must be able to cope with these changing conditions in order to survive and for populations to persist. It is important to understand how these species respond to changing environments because of the increasing frequency at which species shifts are occurring in natural systems. Species may respond to environmental changes via shifts in phenology, abundance, and distributions (Parmesan and Yohe, 2003). Distribution shifts can be catalyzed by invasive species (predators, competitors, parasites, or macrophytes) driving native species out of their native environments. Unfavorable conditions such as limited resources or increased temperature can also make home ranges undesirable and cause distribution shifts (Wingfield et al., 2015; Kilvitis et al., 2017). Distribution changes demand that organisms cope with novel conditions

and often extremely novel conditions in their new habitats. Phenotypic plasticity can facilitate organism survival in changing and novel environments (Crispo, 2008).

Organisms may take advantage of phenotypic plasticity to survive range shifts. Phenotypic plasticity can increase the range of environmental conditions under which an organism can survive and grow (Richards et al., 2006). Plasticity can promote establishment and persistence in a new environment before directional selection has the opportunity to operate (Ghalambor et al., 2007; Crispo, 2008; Lande, 2009). Phenotypic plasticity can be a temporary and quick way to adjust to the environment without requiring genetic alteration. However, genetic alteration may come later if directional selection is acting on the trait in question (Ghalambor et al., 2007). Genetic changes relating to plasticity can occur because phenotypic plasticity is subject to evolution by natural selection (Richards et al., 2006; Lande, 2009; Davidson et al., 2011). Local adaptation on some traits related to fitness in natal habitats can occur, provided that natal habitat conditions are consistent and there is little gene flow (Crispo, 2008). The result of selection on relevant traits is often a subsequent reduction in plasticity for those traits through a process called genetic assimilation (Crispo, 2008). In contrast, traits unrelated to fitness in natal habitat conditions are neither selected for nor against and may not be expressed under natal conditions (Waddington, 1942; Buckley et al., 2010). Therefore, these traits can undergo any number of genetic mutations and collect hidden variation in trait value without being restricted by selection pressure. The subsequent hidden variation is often referred to as 'cryptic genetic variation' (Ghalambor et al., 2007). In theory, when organisms are placed in a novel environment with a new environmental stimulus or cue, previously hidden or unexpressed traits will become expressed (Ghalambor et al., 2007). Therefore, theory predicts that organisms should display higher variation in certain traits in novel habitats than in their natal habitats (Waddington, 1942; Ghalambor et al., 2007; Crispo, 2008; Buckley et al., 2010). However, this prediction has rarely been tested in natural systems.

For this study, we analyzed phenotypic variation of snail populations in the pulmonate family Physidae when exposed to novel habitat conditions. To ensure that non-natal habitats were novel, we chose habitats with and without crayfish predators. We conducted surveys during the summer of 2020 to locate 6 sites with Physid snails, 3 of which contained crayfish that lived in close proximity to the snails. The other 3 sites lacked crayfish. Crayfish and snail interactions have been extensively studied and crayfish chemical cues have been shown to induce phenotypic plasticity in many snail species, including those from the family Physidae and other pulmonate families. Crayfish can induce phenotypic plasticity in the morphology (Dewitt et al., 1999; Krist, 2002; Stevison et al., 2016), behavior (Alexander Jr and Covich, 1991; Alexander and Covich, 1991; Dewitt et al., 1999; Dickey and McCarthy, 2007), and life history traits (Crowl and Covich, 1990) of snails when they are exposed to a crayfish chemical cue from the water where the crayfish fed on a snail of the same family. Crayfish induce morphological changes due to their methods of feeding on snails. Crayfish use two different methods to consume snails: they either reach inside the opening of the shell (called the aperture) to grab the soft tissue out, or they crush the shell to retrieve the soft tissue (Dewitt et al., 1999). When exposed to the effluent from crayfish, a freshwater snail *Elimia* (family Pleuroceridae) grew smaller aperture openings than conspecifics raised without crayfish effluent (Krist, 2002). Snails in the family Physidae grew thicker shells in the presence of crayfish effluent (Stevison et al., 2016). Additionally, a pulmonate snail in the family Physella exhibited later sexual maturity in populations exposed to crayfish chemical cues (Crowl and Covich, 1990). Because crayfish can induce phenotypic plasticity changes in snails, we predicted that snails from natal habitats lacking crayfish would exhibit greater phenotypic plasticity in habitats where crayfish predators occur. Also, we recognize that non-natal habitats represent novel abiotic conditions as well. Thus, we also predict that non-natal habitats, especially those that differ the most physiochemically from natal conditions, will induce increased variation in phenotypic plasticity.

## Methods

### Snail and crayfish surveys

During the summer of 2020, we conducted surveys at sites within 60 miles of Laramie, WY (University of Wyoming imposed travel restrictions because of the COVID-19 pandemic) to locate sites with snails and to detect crayfish living in the same habitat. We discovered *Physa* snails (family Physidae) at six sites: Gelatt Lake (*P. acuta*), Crow Creek at Hereford Ranch (*P. acuta*), the Laramie River at the Laramie Greenbelt (*P. acuta*), Leazenby Lake (*P. acuta*), ponds at Happy Jack Recreation Area (*P. gyrina*), and Alsop Lake (*P. acuta*; identifications made by Dr. Robert Dillon of the Freshwater Gastropods of North America). Using minnow traps and dog food as bait, we assessed whether or not crayfish were present at each of the six sites. We captured crayfish at two of the sites: Crow Creek and the Laramie River. While we did not capture crayfish at Gelatt Lake, we coordinated with the Wyoming Cooperative Fish and Wildlife Research Unit which discovered crayfish at this location within 200 meters of the habitat where we found snails. Previous studies have shown that crayfish can travel up to 221 meters in 2 days (Byron and Wilson, 2001), so crayfish may encounter and interact with snails in their habitat at Gelatt Lake. The three other field sites (Leazenby Lake, ponds at Happy Jack Recreation Area, and Alsop Lake) have no history of crayfish, and we, in coordination with the Wyoming Cooperative Fish and Wildlife Research Unit, did not capture crayfish at these locations.

### Snail housing and rearing

In the summer of 2020, we collected approximately 20 snails from each of the six sites in and around Laramie, Wyoming. We brought the snails to the Krist lab at the University of Wyoming where they were housed in individual cups with full water changes biweekly. In the spring of 2021, we bred these snails to obtain half-siblings that were later used for our reciprocal transplants in each of the six sites. We successfully bred the following number of families (offspring of a single snail) per population: 2, Alsop Lake; 3, Laramie River; 4, Gelatt Lake and Happy Jack ponds; 7, Crow Creek; and 10, Leazenby Lake.

### Field experiment: reciprocal transplants

In the summer of 2021, we set up reciprocal transplant experiments in each of the six sites. For the reciprocal transplants, we reared juvenile populations from each of the six sites at each location for two weeks. We identified each snail by painting them with various colors of nail polish. At each site, these snails were then placed in modified square plastic sandwich containers (289 $cm^2$) with mesh windows on the tops and sides that allowed clean, oxygenated water to flow through. Each of the cages held six individuals from different families. We housed three snails per family in separate cages at each of the sites. Some families that we raised in lab did not produce enough half siblings to have full replication at each of the sites; for these families, we placed as many individuals in the field as possible and used extra lab-reared snails as replacements for these missing individuals to maintain a constant density of six snails per container. We also attempted to rear individuals from different populations in each cage; however, some populations had much higher reproductive success than others and as a result had a larger number of families. Subsequently, nine out of the fifteen cages had multiple individuals from the same population.

Twice a week, we cleaned the mesh windows to remove algae, silt, and debris, and we removed dead snails as we encountered them. We added consistent amounts of food in each cage at every site in the form of submerged vegetation or algae, depending on the food naturally available at each site. To quantify the physiochemical environment at each site, we measured pH, temperature, conductivity, flow rate, and dissolved oxygen three times over the course of each experimental block. We also scrubbed epiphyton from submerged vegetation and algae to quantify chlorophyll a and to determine nutrient ratios (C:N, N:P, and C:P) to assess food quantity and quality among sites.

Due to time constraints, we staggered our experiments into several temporal blocks over the course of the summer of 2021. For of the temporal blocks, we ran experiments at two separate sites over the

same two-week increment where we alternated our visits of the sites so that maintenance and research at each site occurred every other day. Block 1, Gelatt Lake and Alsop Lake, took place from July 28th to July 13th, block 2, Crow Creek and Leazenby Lake, ran from July 15th to July 30th, and block 3, Happy Jack ponds and Laramie River, occurred from August 2nd to August 17th.

### Data collection

At the onset and end of each experiment in each temporal block, we measured shell length and aperture opening width for each of the snails in the block. We measured both of these traits with a Leica microscope with an ocular micrometer. Our measurements for shell length were taken from a consistent angle for each shell where the shell was placed with the aperture facing upwards and the shell slightly rotated to maximize the length. Our aperture width is a measurement of the width of the aperture opening as viewed from a direct angle above with the aperture facing upwards. We are currently developing mass-length regressions for each population to assess the relationship between shell length and biomass (following Benke et al., 1999). We will use the biomass of each individual to calculate specific growth rates of each juvenile in each lineage and population during their duration of the experiment at each field site. We also used an image-processing microscope after the conclusion of the field trials to analyze shell morphology for each of the snails in the experiment. We will analyze photographs using ImageJ (Schneider et al., 2012) to determine measurements for the following morphological traits: shell length, shell width, aperture length, aperture width, and aperture area. We used the same shell angles for image processing as described above for the previous measurements taken after the field experiment. We will create ratios of lengths to widths to determine the shapes of each of the shells in our experiment; these shapes can range from rotund to elongate following DeWitt et al. (2000). We will also use a minimum compressive force experiment as outlined in Stevison et al. (2016) in order to determine shell thickness for each individual in the experiment.

### Statistical analyses

To assess variation among sites in physiochemical properties, we will use Principal Component Analysis (PCA) to separate sites along a few principal components. We will contrast variation among lineages and populations in growth rates, shell morphology, and shell thickness between natal environments and non-natal environments using analysis of variance and coefficients of variation.

## Preliminary results

We do not have any results yet, since we are still processing data for analysis.

## Conclusions

We have no results to elaborate on yet. However, I can reveal my predictions.

Based on theory, we expect to see lower variation in phenotypic plasticity among populations exposed to their natal environment than in novel environments. We expect to see the highest level of trait variation in environments that are more ‘extreme’ (possibly environments with novel crayfish predators).

Failure to document the predicted patterns of phenotypic plasticity could occur for several reasons. First, the sites in this study may not represent extreme enough conditions to induce the expected patterns in variation. Secondly, we may not be able to detect increased variation in some traits due to physiological limitations on those traits (Chevin et al., 2010) or lack of underlying genetic variation due to selection pressure in the natal habitat. Some traits are strongly selected for or against in natal habitats (canalized), in turn reducing underlying genetic variation and the potential for phenotypic variation, even in novel habitats (Ghalambor et al., 2007; Lande, 2009). It is possible that some of the measured traits in this study are canalized in certain populations, and there is an additional possibility that many of these traits are under physiological limitations that can reduce the range of possible phenotypes.

## Future work

Depending on our results, it would be interesting to rear the snails in the lab under extreme conditions to measure the extent of phenotypic plasticity. In lab conditions, we can also expose the snails for a longer duration and measure traits that require more time to develop such as age and size at first reproduction and fecundity.

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