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Investigating How Water Type and Temperature Affect Fitness of Freshwater Pulmonate, Physella hendersoni

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INVESTIGATING HOW WATER TYPE AND TEMPERATURE AFFECT FITNESS OF FRESHWATER PULMONATE, *Physella hendersoni*

BY

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ABSTRACT

Precursors to natural selection and evolution start with small species differences at the population level. Observing how species fitness is affected by environmental changes is key to understanding these population differences. This is because individual populations have often been found to be more suited to their native environment than environments occupied by other conspecific populations. Freshwater mollusks, *P. hendersoni* in particular, were used to observe how fitness of an individual population varies among a variety of conditions experienced by the species as a whole. This species of freshwater pulmonate thrives in spring environments with hard and cold water, but has also been found in soft water environments with varying temperature. *P. hendersoni* from a soft water lake environment (Lake Munson in Tallahassee, FL) were placed in varying temperatures and calcium concentrations and their eggs masses and number of eggs per mass were counted, as well as weight gain over 4 weeks. It was found that the snails in soft water had increased fitness in terms of egg production, and were less sensitive to the effects of temperature than snails in hard water. These results suggest that the Lake Munson population is becoming locally adapted to that area, which has implications for early signs of natural selection and evolution.

INTRODUCTION

Charles Darwin's theory of evolution states that natural selection is the driving force of evolutionary change (Hereford 2009; Linhart and Grant 1996). Natural selection favors adaptations in organisms that lead to higher fitness in their environments. Early precursors to these adaptations usually start with small differences between species at the population level (Hereford 2009; Heard and Hauser 1995). In the right circumstances, these small differences can lead to speciation, selection for different traits, and evolution of a species.

Populations of the same species often differ when their native environments have different biotic and abiotic factors that alter their fitness (Hereford 2009; Heard and Hauser 1995). Populations with traits that maximize their fitness in a given environment are considered to be locally adapted to that environment. Local adaptation has been observed in many different instances, and it has been shown that in many cases members of the same species are more suited for and adapted to their own native environments as opposed to other ones where the species also exists (Hereford 2009; Van Zandt and Mopper 1998; Linhart and Grant 1996). Diversification of many plant species comes from natural selection due to environmental constraints (e.g. soil toxicity, light, pollination vectors, etc.), and can change plant characteristics from leaf character, phenology, competitive ability, to heavy metal tolerance (Linhart and Grant 1996). Other environmental constraints that affect diversification of aquatic species in particular are traits such as temperature and calcium concentration. This makes mollusks appropriate species for measuring the effects of temperature and calcium concentration on fitness.

Mollusks make up the second most diverse animal phylum, but freshwater mollusks are considered to be the most imperiled group of invertebrate taxa (Lydeard *et al.* 2004). Nonmarine mollusks have experienced the highest number of documented extinctions of any major taxonomic group, but they are critically important in their environments because they regulate primary production, decomposition, nutrient cycling, and water clarity (Lydeard *et al.* 2004). This makes them interesting to test the effects of water type and temperature on, as nonmarine mollusks are heavily influenced by these qualities. Specifically, their distribution is widely influenced by factors such as calcium concentration, temperature, oxygen, food, and habitat availability (Lodge *et al.* 1987). Calcium concentrations for most freshwater mollusks need to be above 5 mg/L because of calcium's importance in shell growth and thickness, and temperature is important because of its effects on developmental rates, fecundity, and start and stop of reproduction (Lodge *et al.* 1987).

The effects of water type and temperature on fitness were investigated by raising one particular freshwater pulmonate, *Physella hendersoni* under different water conditions. *P. hendersoni* naturally occur in both Newport Sulfur Spring and Lake Munson in North Florida. *P. hendersoni* is a small, freshwater mollusk that occurs naturally in the Southeastern United States, and feeds on periphyton and detritus (Burch, 1988). *Physella hendersoni* naturally flourish in Newport Sulfur Spring, due to the spring's constant temperature and calcium rich water, which is important for shell growth. These snails have been found in Lake Munson as well, where the water isn't nearly as hard (calcium concentrations are around 1-1.5 mg/L). The shallow lake water also experiences seasonal temperature changes, which has been found to be detrimental to freshwater snail populations since temperature fluctuations lead to nutrient drawdown, and *P. hendersoni* prefers nutrient rich shallows (Hunt and Jones 1972). One of the physical barriers of *P. hendersoni* includes dissolved calcium amounts in the water, so they require concentrations greater than 3 mg/liter (Brown 1991). Due to the stark environmental differences, the question asked was: how does temperature and calcium concentration affect the fitness of the Lake Munson population of *P. hendersoni*? It was hypothesized that if this population was placed in environmental conditions similar to their native environment, as well as environmental conditions similar to the Newport Sulfur Spring environment, that fitness would be greater in their native environment. This is because of the evidence towards populations of the same species having higher fitness in their current environments (Hereford 2009; Van Zandt and Mopper 1998; Linhart and Grant 1996). Fitness would be greater in their native environments if they consistently laid more eggs and eggs per mass in an environment consistent with conditions in Lake Munson, as well as experiencing greater growth, or the ability to maintain growth rate regardless of temperature and level of calcium concentration.

METHODS

In order to test the effects of temperature and water type on fitness of the Lake Munson population of *Physella hendersoni*, approximately 60 snails were collected from the shallow area around a recreational boat ramp on Lake Munson. They were then kept in the lab in a 20 gallon tank for approximately four weeks to allow them to reproduce while minimizing maternal effects. This parental generation was fed *ad libitum* and kept under constant temperature and light conditions, with routine water changes several times a week. The offspring were routinely removed as they hatched from their eggs and placed in smaller 1 gallon tanks for approximately 21 days to allow them to reach sexual maturity. Sexual maturity was determined by size, which was previously determined to be 4.57+/- 1.28 mm for the Lake Munson population (Gray 1987).

Once growing to reproductive size, the *P. hendersoni* were separated into treatments. Overall, four pulmonates were designated to a replicate and there were four replicates per treatment condition. The four treatments were: cold temperature and hard water, cold temperature and soft water, warm temperature and hard water, and warm temperature and soft water. Warm temperature treatments were kept in a lab maintained at 81 degrees Fahrenheit, and cold temperature treatments were kept in a lab maintained at 73 +/- 1 degrees Fahrenheit. Hard water came from well water filled from tap in the lab, with calcium concentrations around 120 mg/L and soft water was collected from Trout Pond and stored in carboys in the lab (calcium concentration about 1-1.5 mg/L) in both the warm and cold rooms. Aside from water type and temperature, all other factors were kept constant. *P. hendersoni* were kept in 1 gallon tanks with removable lids, and fed whole fish flakes a*d libitum.* Tanks were aerated with one small air stone per tank, allowing minimal bubbles, and water level was constantly kept at about one-third full. A consistent light/dark schedule was maintained by automatic timers in each lab room.

P. hendersoni were given a one week acclimation period, and then studied for four whole weeks using several different metrics to determine fitness. Firstly, they were individually weighed for an initial weight using a digital scale and were measured in grams. Each pulmonate was then individually weighed two more times; once after two weeks of data collection and again after four weeks of data collection. Weights were averaged for each tank due to the impossibility of identifying each individual pulmonate in a treatment.

Each replicate was also checked for egg masses twice a week for four weeks. Data was recorded on the number of masses per replicate as well as an average amount of eggs laid per mass. Egg masses were counted first, and then at least three masses were randomly chosen to be counted for individual number of eggs (unless the amount of egg masses was less than three, then all masses were counted for number of eggs). Afterwards, all eggs were removed from the treatment tanks and placed in separate tanks so they would not be recounted or accidentally hatch inside of the treatment tanks. In cases where an egg mass would hatch before it was counted, the tank was replaced.

Growth data was analyzed using a general linear model, using the log of growth as a response variable in order to compare total growth across treatments. Total egg numbers were also analyzed using a general linear model, with and without interaction of temperature and water type. The number of masses and average number of eggs per mass was analyzed using a repeated measures design. Residuals of log total growth and total egg numbers were also examined in order to determine if there was a trade-off between growth and reproduction, once treatment effects were taken into account.

RESULTS

Growth

There was no significant difference in *P. hendersoni* growth over four weeks when comparing both temperatures using ANOVA ($F(1, 12) = 1.73$, NS). There was also no significant difference in *P. hendersoni* growth over four weeks when comparing water types using ANOVA ($F(1.12) = 0.75$, NS). However, there was a strong interaction between temperature and water type on total *P. hendersoni* growth ($F(1,12) = 5.22$, $P < 0.04$) (*Fig. 1*). Greatest *P. hendersoni* growth was observed at lower temperature and hard water, which was 2.5 times the *P. hendersoni* growth at higher temperature and hard water (*Fig. 1*). Soft water *P. hendersoni* growth in both temperatures was intermediate between the extremes of hard water (*Fig. 1*).

Eggs per Mass

There was no significant difference in *P. hendersoni* egg production over four weeks when comparing temperature using ANOVA ($F(1, 12) = 0.36$, NS). However there was a significant difference in *P. hendersoni* egg production over four weeks when comparing water type ($F(1.12) = 8.17$, $P < 0.015$). Soft water treatments of *P. hendersoni* consistently produced more eggs at both temperatures (*Fig. 2, 4*). In the lower temperature, there was about a two-fold difference in *P. hendersoni* egg production in soft water than hard water, and a 35% increase in *P. hendersoni* egg production in soft water than hard water at the higher temperature (*Fig. 2, 4)*. There was no significant interaction between water type and temperature on *P. hendersoni* egg number ($F(1,12) = 1.02$, NS). The same results were obtained using a repeated measures ANOVA when comparing water type, temperature, and water type and temperature by half-week instead of all four weeks (Temperature: $F(1,12) = 0.36$, NS; Water Type: $F(1,12) = 8.17$, $P < 0.015$; Water type x Temperature $F(1,12) = 1.02$, NS).

Egg Masses

Similar results were seen when analyzing number of *P. hendersoni* egg masses over four weeks. There was no significant difference in number of *P. hendersoni* egg masses over four weeks when comparing temperature using ANOVA ($F(1,12) = 0.43$, NS). Like egg production, there was a significant difference in number of egg masses produced by *P. hendersoni* over four weeks when comparing water type (*Fig 3)*. Specifically, soft water *P. henersoni* produced consistently more egg masses than hard water snails ($F(1,12) = 7.01$, $P < 0.03$) (*Fig 3)*. There was no significant interaction between water type and temperature on number of egg masses produced by *P. hendersoni* ($F(1,12) = 1.68$, NS). Similarly, when using a repeated measures ANOVA to analyze number of egg masses per week instead of over all four weeks, the same conclusions could be drawn (Temperature: $F(1,12) = 2.27$, NS; Water Type: $F(1,12) = 9.98$, $P < 0.01$; Water x Temperature $F(1,12) = 0.95$, NS).

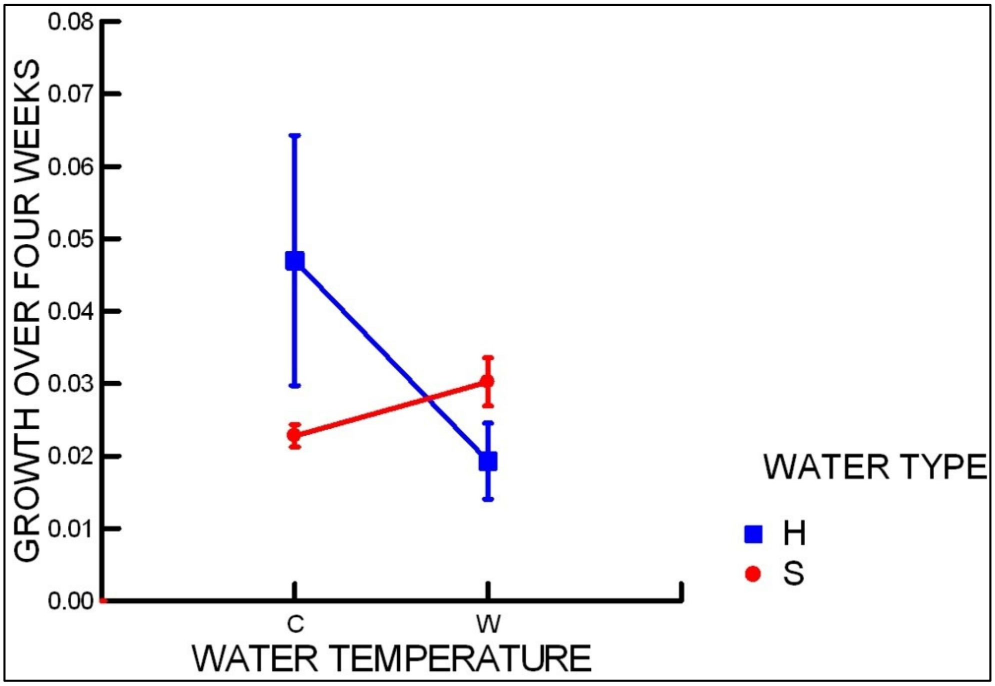

Figure 1: The comparison of P. hendersoni growth over 4 weeks versus water temperature. Water temperature is defined as either cool (C) or warm (W). Water type was either soft (S, red) or hard (H, blue). P. hendersoni in hard water grew much more slowly in warm than cool temperature, and conversely in soft water grew more rapidly in warm than cool.

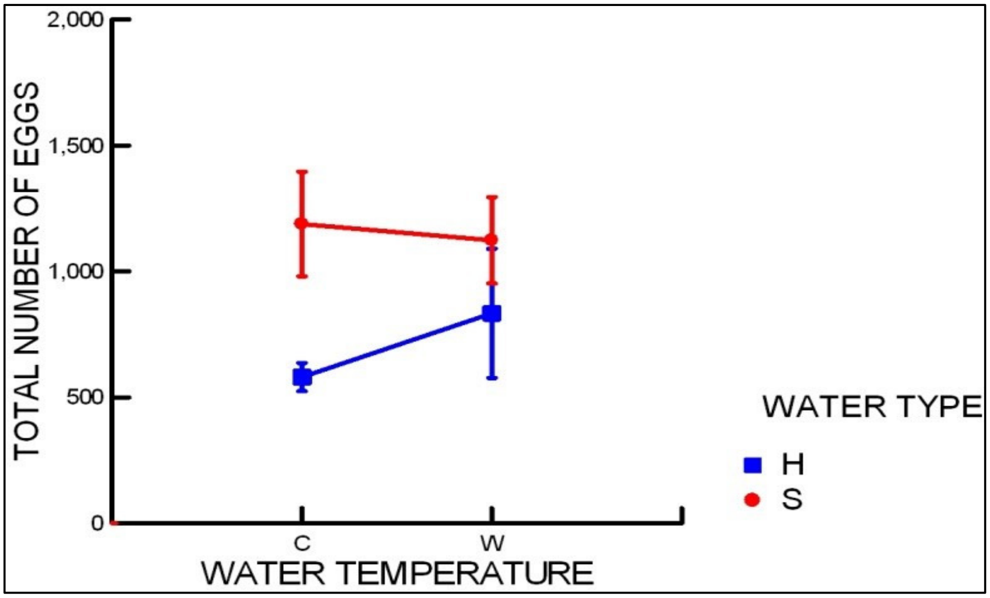

Figure 2: Total number of eggs laid per water type versus water temperature. P. hendersoni in soft water consistently laid more eggs than hard water overall, regardless of temperature.

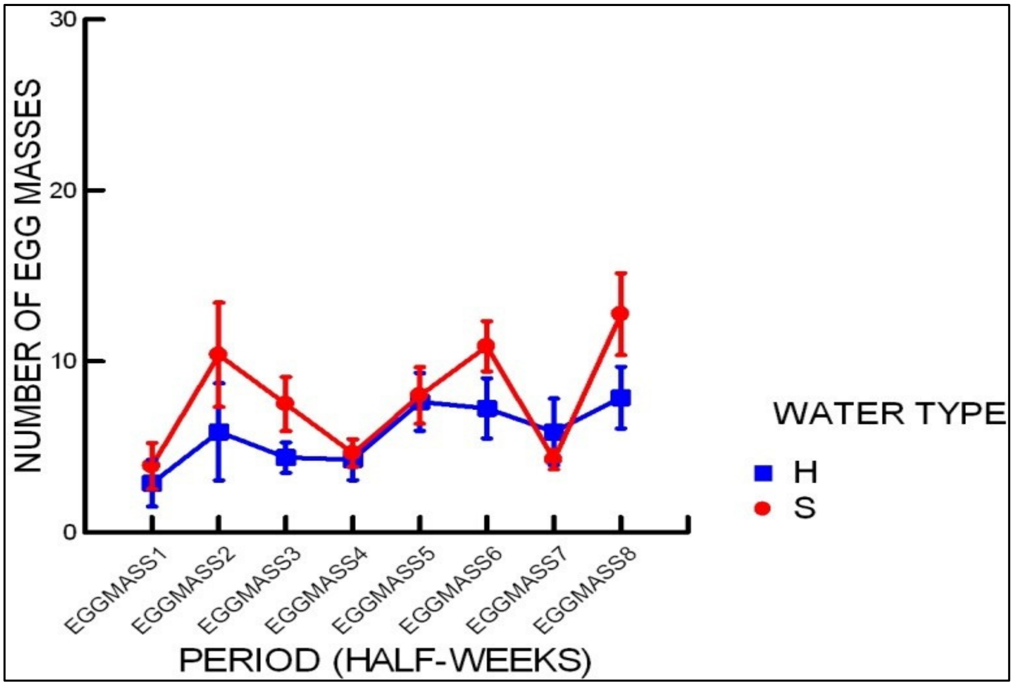

Figure 3: Number of egg masses per water type versus each half-week period. Soft water P. hendersoni consistently laid more egg masses over the 8 periods.

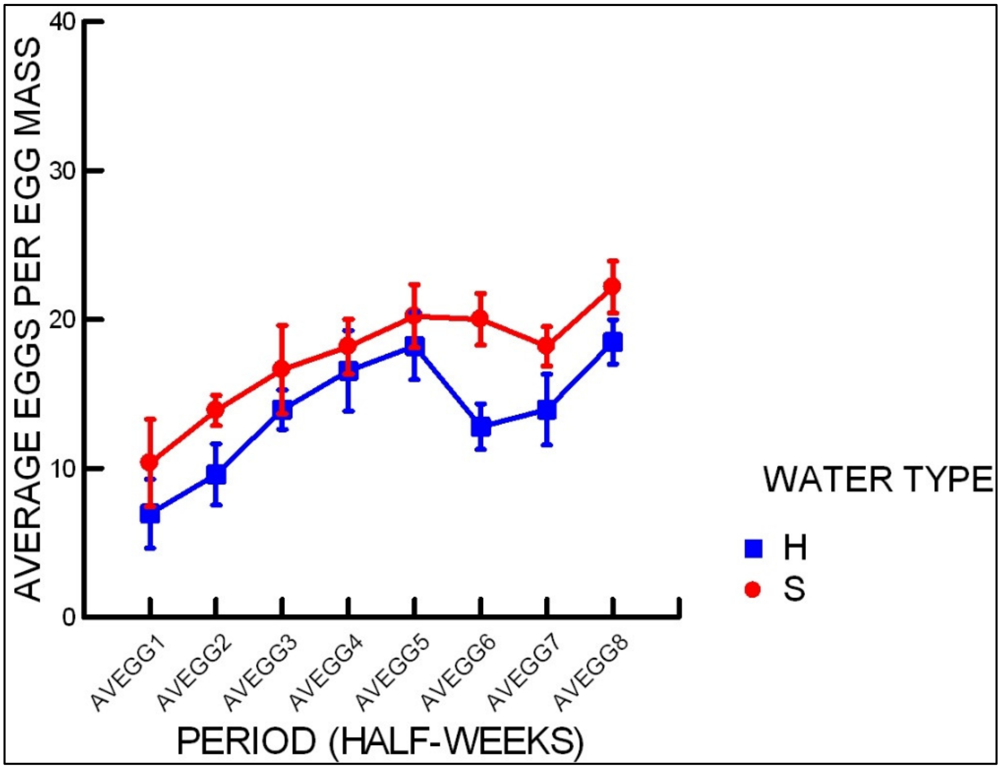

Figure 4: Average eggs per mass per water type versus each half week period. Soft water P. hendersoni consistently laid more eggs per mass over the 8 periods.

DISCUSSION

The snails in soft water treatments of *P. hendersoni* laid significantly more egg masses and eggs per mass than the snails hard water treatments. It was also shown that *P. hendersoni* in hard water were much more affected by temperature in terms of growth, while the soft water *P. hendersoni* treatments were not. Laying more eggs provides the evidence that the *P. hendersoni* in soft water treatments had a higher fitness than the *P. hendersoni* in hard water treatments. Fitness is an organism's ability to survive and reproduce in a population, so it can be concluded that overall laying more eggs provides these pulmonates with the advantage to having more offspring to survive and carry on future generations. It can also be argued that the Lake Munson population is adapted to living in an area with frequent temperature fluctuations, as it was shown that they grew more rapidly in warm temperatures and soft water as opposed to warm temperatures and hard water. Overall the combination of soft water and warm temperature seemed to be beneficial (or at least not detrimental) to the fitness of this population.

A further argument for local adaptation of this population is the level of calcium in Lake Munson. These levels are around 1-1.5 mg/L (Gray 1987), which is true of most soft water lakes around the Tallahassee area. It was previously thought that freshwater mollusks require at least 5 mg/L of calcium in order to thrive, so this provides evidence for shifting baselines towards lower calcium level extremes (Lodge *et al.* 1987).

An organism preferring the conditions of their native environment as opposed to an environment where they are also found has been observed in many other instances (Hereford 2009; Van Zandt and Mopper 1998; Linhart and Grant 1996). For example, many species of snakes and snapping turtles are found to be solitarily freshwater species, some populations are showing changes in salinity tolerance and moving into more saline environments, eventually changing the salt tolerance of their offspring (Dunson 1986; Dunson and Mazzotti 1989). These adaptations at the population level are key to understanding early evolutionary change.

These conclusions are limited, however, by the fact that only one population of *P. hendersoni* was studied and there is no comparison population. Traditionally, conclusions claiming local adaptation are made based on how both native and novel populations fair in reciprocal environments. Although there is certainly evidence pointing in the direction of the possibility of local adaptation, there needs to be the comparison between native hard water and cold temperature populations.

There were also the limitations of only measuring certain aspects of fitness. First, age at first reproduction of this population of *P. hendersoni* was not measured in this study. In a growing population, small decreases in age at first reproduction could generate large increases in fitness. Second, longevity was not measured in this study. Differences in adult survival rates could play major roles in affecting fitness. In particular, if the increased mass in cooler hard water were to increase survival rates, it may be that the direction of fitness differences between hard and soft water would change.

Adaptations can then eventually lead to speciation and further diversification. Further studies should be geared towards understanding other environmental factors that affect this population of *P. hendersoni*, and then also investigating if the effects are the same on other populations of the same species. Reciprocal transplant studies should be performed on populations in the Tallahassee area of both native and novel environments, testing for effects of temperature, calcium concentration, but also levels of oxygen, phosphates, nitrates and nitrites, as these all have been shown to affect freshwater mollusk diversity and abundance (Lodge *et al.* 1987).

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