Evaluating interspecific competition effects on morphological variation of

Orconectes rusticus

by

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Abstract

Morphological variation in response to environmental conditions is widespread among macroinvertebrates and can affect ecology and evolution in organisms. Interspecific competition may affect morphological variation of plastic traits that can confer a competitive advantage. Invasion of the rusty crayfish, *Orconectes rusticus*, in the Monocacy River provides the opportunity to test the hypothesis that interspecific competition affects morphological variation. Two populations of *O. rusticus* were examined at two separate sites, one site dominated by *O. rusticus*, and the other site where *O. rusticus* and *O. virilis* were present. Crayfish were photographed, and the body and chelae were analyzed with geometric morphometrics. Male *O. rusticus* had significantly larger carapace length, abdominal and chelae width, and different body shape when in competition with *O. virilis*. Shape in female crayfish was significantly different between sites, but metrics of size (carapace length, abdomen width, and chelae width) were not significantly different. These results suggest that interspecific competition in crayfish species plays a role in influencing morphological variation within river ecosystems.

Acknowledgement

I would like to thank my project advisor, Dr. Eric R. Annis, as well as Dr. Sue Carney, without their guidance and assistance this research project would not have been possible. I would also like to thank the members of Dr. Annis' lab who collected the specimens used and made this project possible.

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INTRODUCTION

Morphological Variation

Phenotypic plasticity arises from a change in an organisms' phenotype in response to fluctuating environmental conditions. It may present as morphological, behavioral, or physiological attributes, and can enhance fitness over generations (Bradshaw 1965; Hoverman et al. 2005; Relyea 2002). Environment-specific gene expression has been shown to underlie plasticity across a range of environments (Snell-Rood et al. 2010). Organisms exhibit plasticity in response to environmental conditions and interspecific interactions such as predation, temperature, and food availability (DeWitt 1998; Kishida et al. 2010; Robinson & Parsons 2002; Snell-Rood et al. 2010). Though not mutually exclusive, plasticity can be both adaptive and non-adaptive, with adaptive plasticity producing a phenotype that is favored by natural selection, and non-adaptive plasticity producing a phenotype due to environmental heterogeneity (Ghalambor et al. 2007; Murren et al. 2015).

The predator-prey relationship, combined with environmental conditions, has been shown to induce plastic responses of multiple phyla in aquatic environments. Crayfish predators further alter interspecific competition among crayfish species. Selective fish predation of *Orconectes virilis* as a result of aggressive defensive behavior by *Orconectes rusticus*, may enhance *O. rusticus* fitness, leading to larger sized morphs that reduce predation risk (Garvey et al. 1994; Stein & Magnuson 1976). Crayfish predators can alter crayfish behavior based on size, with larger crayfish inhabiting deep river sections, and smaller crayfish inhabiting shallow river sections (Davis & Huber 2007). Further examples of morphological plasticity include increases in amphibian larvae tail depth and apertural tooth induction in marine gastropods in response to

chemical cues released from predators (Appleton & Palmer 1988; Kishida et al. 2010; Reylea 2002). Predatory cues can produce alternative phenotypes in organisms, with water bugs inducing wider shells and crayfish inducing narrow and high shells in *Helisoma trivolvis* snail populations and fish inducing rotund shell growth and crayfish inducing elongate shell growth in *Physa heterostropha* snail populations (DeWitt 1998; Hoverman et al. 2005).

Factors such as natural selection, environmental stability, and trophic complexity also contribute to phenotypic plasticity in a variety of organisms (Kerschbaumer et al. 2014; Kishida et al. 2010; Pfennig 1992). Natural selection acts on plastic traits of an organism, with cichlid fish exhibiting mean shape differences between sympatric and non-sympatric populations (Kerschbaumer et al. 2014). Environmental stability also affects plasticity, with marine phytoplankton raised in varying levels of CO_2 environments showing an increased frequency of phenotypes able to tolerate high levels of CO_2 (Pfennig 1992; Schaum et al. 2015). Trophic complexity can influence morphological plasticity; for example, morphology of the freshwater drum *Aplodinotus grunniens* is dependent on a combination of body size and habitat differences (Jacquemin & Pryon 2013; Kishida et al. 2010). Multiple predators may also alter plasticity, as evidenced in frog tadpoles responding by increasing defensive morph phenotypes when faced with a combination of predators, salamander and dragonfly (Kishida et al. 2009).

Morphological plasticity in crayfish species has been demonstrated in multiple ecological contexts including growth, behavior, life-history, and physiological trait changes. *Orconectes rusticus* specimens from an invasive population have a higher growth rate than specimens from the native range, indicating invasion events can be enhanced through plasticity dynamics (Sargent & Lodge 2014). Behavioral plasticity has been documented in crayfish, with higher feeding activity and reduced antipredator behaviors used by invasive crayfish during an invasion

event (Reisinger et al. 2017). Fighting dominance hierarchy can alter plasticity of behavior responses to mechanical stimuli, with winning crayfish switching stimuli response from "dart" to "turn" post-fight, regardless of size, and losing crayfish exhibiting only a "dart" response to stimuli post-fight (Fujimoto et al. 2011). Water velocity has induced plastic responses in crayfish, with *O. rusticus* developing smaller chelae in response to increasing water velocity (Perry et al. 2013). Relocation can also induce plastic responses in crayfish. The British white-clawed crayfish *Austropotamobius pallipes* exhibited increases in both carapace and areola width post relocation, due to environmental conditions of low oxygen availability and plasticity constraints of total width resulting in increased areola width (Haddaway et al. 2012). Crayfish have been shown to exhibit plastic responses to a variety of conditions and are ideal for researching the effect of competition on morphological variation.

Crayfish in Maryland

Maryland has nine native crayfish species: *Cambarus acuminatus, C. bartonii, C. carinirostris, C. diogenes, C. dubius, Fallicambarus fodiens, Orconectes limosus, O. obscurus,* and *Procambarus acutus.* There has been a shift in species distribution as invasive species of crayfish have been introduced, with the aquarium, live-bait, aquaculture trade, and anglers being identified for the inadvertent introduction of the non-native crayfish species *O. virilis* and *O. rusticus* into Maryland ecosystems (Kilian et al. 2010). Introduction of non-native species has led to the continuing decline of native crayfish species, including *O. limosus* and *C. bartonii*, and invasions are a leading threat to native ecosystems (Kilian et al. 2010; Wilson et al. 2004). The Monocacy River system is a tributary of the Potomac River, and it has come to be dominated first by *O. virilis* and more recently by *O. rusticus* in the northern part of river since this species was first documented by the Maryland Department of Natural Resources in 2007 (Kilian et al.

2010). *Orconectes rusticus* is an efficient benthic egg predator that feeds at a higher rate, for longer time periods, and exhibits more aggressive behavior than *O. virilis* (Morse et al. 2013; Capelli & Munjal 1982). Differences in habitat type can influence crayfish competition in systems. *Orconectes rusticus* is the dominant crayfish species in cobble habitats, and competition between it and native species increases predation rates of native species, which are forced out of refugia (Taylor & Redmer 1996). The rusty crayfish is abundant in the Monocacy River and is advancing further downstream while simultaneously displacing *O. virilis* (Selckmann 2016). *Orconectes rusticus* displaces native crayfish through interspecific competition advantages, reduced predator vulnerability, and aggressive behavior (Wilson et al. 2004). According to Crandall & De Grave (2017), *Orconectes rusticus* has been reclassified as *Faxionus rusticus*, however for consistency in this study *O. rusticus* is used throughout.

Morphological Plasticity and Competition

Competition between species may drive phenotypic plasticity in aquatic organisms. Size differences between crayfish are an important element in interspecific competition, with more robust crayfish being an important factor in determining territorial dominance, obtaining resources, and reproductive success. *Orconectes rusticus* has larger chelae than competitors, which may confer selective predation protection (Roth & Kitchell 2005; Wilson et al. 2007). While crayfish chelae size is important, it is not a determination of chelae strength. This can alter confrontation outcomes between crayfish; as chelae size is inversely correlated with chelae strength, both size and strength of chelae play a role during territorial disputes (Wilson et al. 2007). However, the effect of interspecific competition on morphological plasticity in *O. rusticus* chelae has not yet been studied.

Orconectes rusticus exhibits morphological plasticity of chelae and carapace size and shape due to varying water velocity (Perry et al. 2013). To our knowledge, no research has examined the effect of competition on morphologic plasticity in this species. We tested the hypothesis that wild populations of *Orconectes rusticus*, in the presence of interspecific competition, exhibit morphological variation developing larger chelae and carapace size relative to conspecifics that are not in competition with another species of crayfish.

MATERIALS AND METHODS

Organisms

Specimens were collected in June 2009 by Dr. Annis and members of his lab at two sites in the Monocacy River system: MD Rt. 140 crossing coordinates: 39°40'34.78"N, 77°14'7.47"W and Mummaford Road crossing, 39°37'22.15"N, 77°17'59.54"W. The control group (MD Rt. 140 crossing) consisted of crayfish collected from a location where *O. rusticus* were dominant and comprised approximately 97% of the population where competition between the species was negligible. The experimental group (Mummaford Road crossing) consisted of crayfish collected from a location where distributions overlapped, with *O. rusticus* at 72% and *O. virilis* at 27% (E.R. Annis personal communication). The specimens stored in ethanol post collection and were approximately ten years old at the time of analysis. Not all specimens collected were usable due to degradation and ethanol evaporation. A total of 189 specimens were analyzed. Site 1 (M1- Rt. 140 crossing) contained 65 males and 61 females, and site 2 (M2-Mummaford Road crossing) contained 40 males and 23 females. For site 2, all collected specimens were examined and all intact specimens were included in the analysis.

Photographs and Digitization

Graph paper was placed underneath a 203 mm x 254 mm Plexiglass sheet. A 50 mm x 50 mm section of modelling clay was used to position crayfish specimens directly under the camera (Samsung NX3000), and a level was used create a level surface. A ruler and each crayfish specimen were placed on sections of modeling clay to reduce measurement variation by allowing for appropriate scaling during digitization. The camera was placed on a tripod at a 90° angle, and a level was used prior to each photograph being taken to reduce potential distortion.

Multiple photographs were taken of each specimen, and the clearest was selected and used for analysis. The photographs were edited using GIMP 2.8.22 software to adjust contrast and brightness. After editing, the photographs were placed into tpsDig2 software and landmarks were added to each specimen. Forty-one landmarks in total were digitized onto each specimen in the same order (Figure 1). Specimens were organized by site and sex for analysis, and specimens were sampled across five habitat types defined predominately by flow velocity from slowest to fastest: pool, vegetation, glide, run, and riffle (adapted from DiStefano et al. 2003).

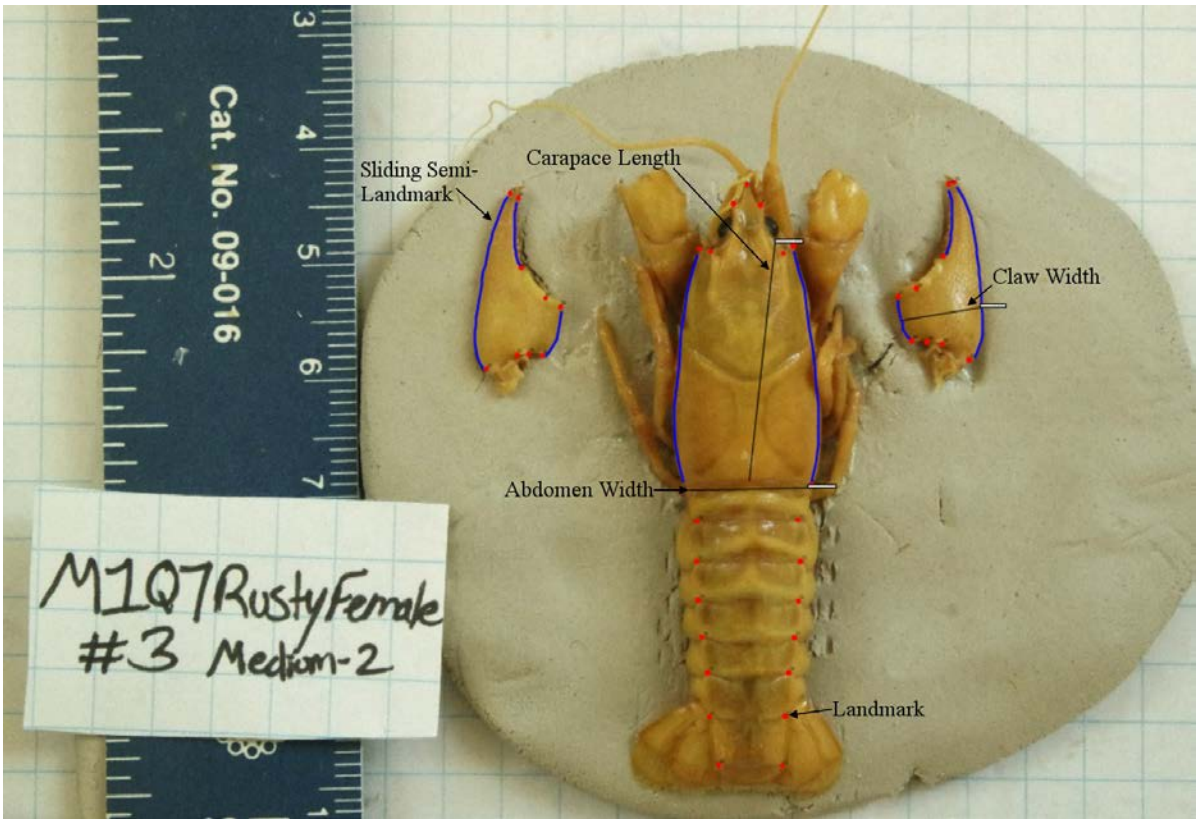

Figure 2: *Orconectes rusticus* specimen showing landmark and measurement data. Red points = landmarks. Blue lines = sliding semi-landmarks. Measurement lines of carapace length, abdomen width, and claw width noted with arrows.

Morphometric Analysis

The data were imported into MorphoJ and analyzed for size and shape variation. Specimen carapace and chelae morphology were kept together to look at a high-level analysis of the individuals as a whole organism, rather than separating each into its own analysis. A Procrustes fit takes the landmarks of the data and minimizes the shape difference between the specimens by placing the data in the tangent space via orthogonal projection. Procrustes superimposition was performed; this removes the effect of size on the variation in shape. This allows the shape variation to be observed without the confounding effect of size. The data were analyzed for outliers; none were found by the software and all specimens were included in the analysis. Covariance matrices were then created and analyzed to determine if a linear relationship existed. A covariance matrix is a table of unstandardized measures which uses deviations between the observed data and the mean value to find the average cross-product deviation, known as the covariance. This descriptive measure is useful for determining if there is a linear relationship between two variables. Covariance matrices use unstandardized measures in the analysis and creation of the matrix; these can be used to understand direction, but not the strength, of the relationship.

Principal component analysis (PCA) was performed on the covariance matrices to further analyze the amount of variance explained by the principal components for each site independently. PCA is a multivariate technique that identifies linear components of a data set that explain the maximum total variance, until 100% of the variance is accounted for. Principal Components are combinations of original variables by their ability to explain the variance. The Eigenvalue is a measure of the amount of variance explained by each principal component. Covariance matrices were contrasted to determine if any relationship existed. Procrustes

ANOVA was used to test for any shape differences among the specimens. A Procrustes ANOVA attempts to assess the amount of variation among individuals, asymmetry, and measurement error.

Specimens' carapace length (CL), abdomen (AW), and chelae width (CW) were measured in tpsDig2. The measurements were compiled into a Microsoft Excel file. Female measurements were log transformed to normalize the data; male measurements were normally distributed and were not log transformed. All data were imported into SPSS v24 for analysis. In SPSS, a one-way ANOVA was performed comparing carapace length, abdomen width, and chelae width of M1 versus M2 Males and M1 versus M2 Females. The one-way ANOVA determines whether there are statistically significant differences between the means of the independent variables.

RESULTS

The covariance matrices between M1 and M2 males and M1 and M2 females showed a strong positive linear relationship between the groups. The covariance matrix correlation for M1 and M2 Males was 0.84, with a P-value < 0.0001 (Figure 2). The covariance matrix correlation M1 and M2 Females was 0.89 with a P-value < 0.0001 (Figure 3). The Procrustes coordinates in Figures 1 and 2 are the covariance of each data point for M1 and M2 divided by the standard deviation of both variables for all points examined.

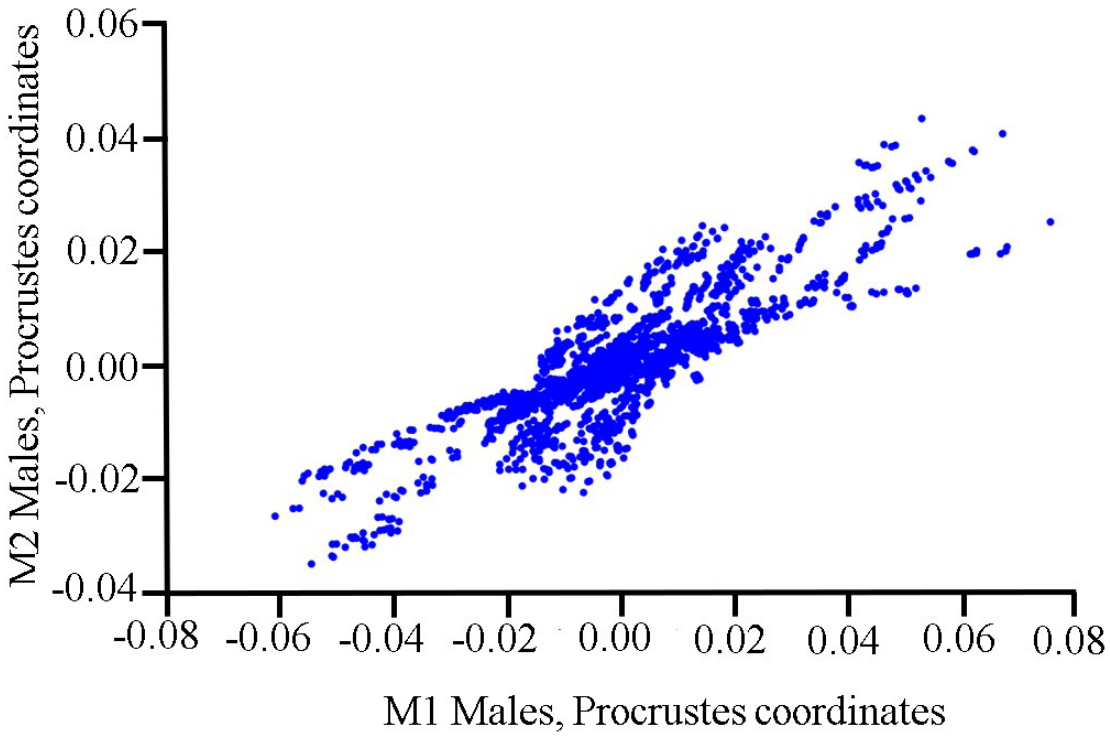

Figure 2: Covariance matrix analysis showing the strength of the linear relationship between M1 and M2 Males.

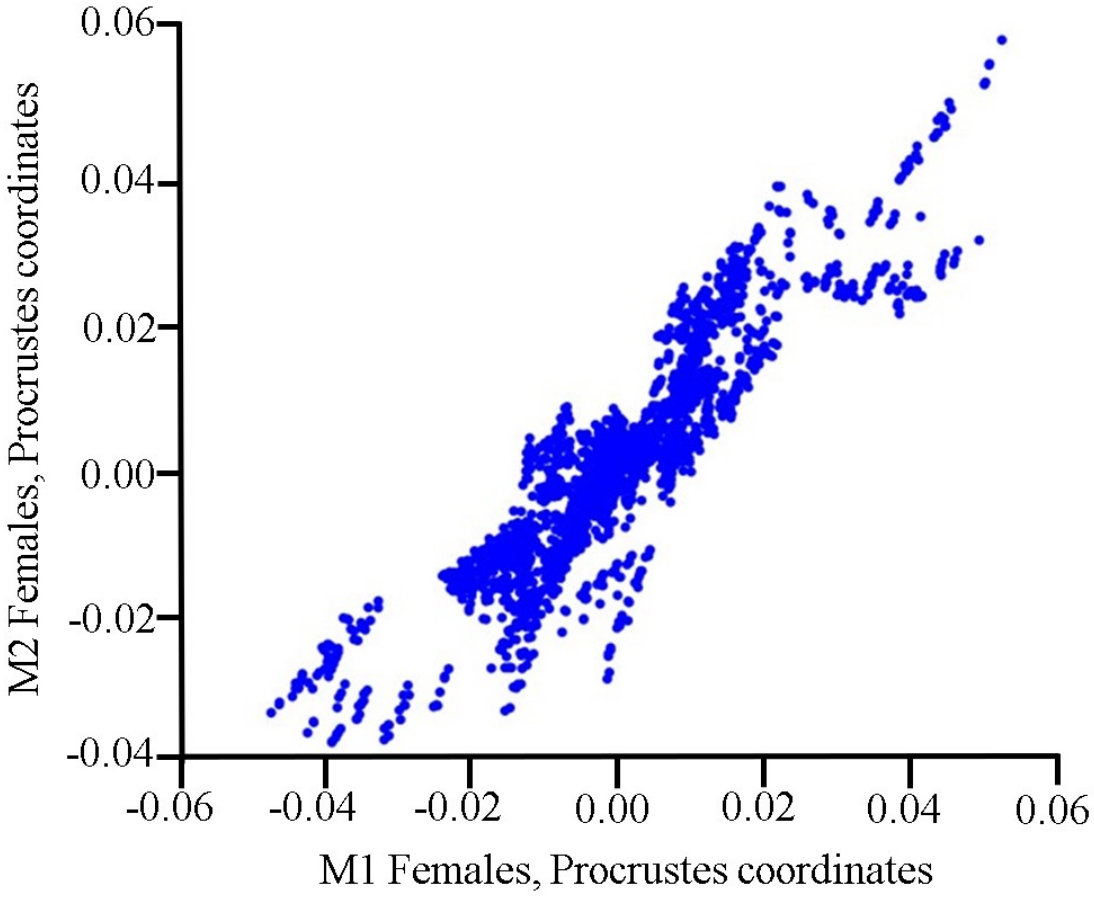

Figure 3: Covariance Matrix analysis showing the strength of the linear relationship between M1 and M2 Females.

In the Principal component analysis of males, PC1 accounted for 61.40% for M1, and 58.91% for M2, of the variance for the populations. PC1 accounted for 62.07% for M1 Females and 50.75% for M2 Females of the variance for the populations (Table 1). In the combined dataset, PC1 accounted for 64.92% for males, and 61.71% for females of the observed variance (Table 1). The Principal components PC1 and PC2 were graphed and separated by site, looking at the most variance explained between the two principal components. The dominant habitat type for males was run in M1 and run/riffle in M2 (Table 2). The dominant habitat type for females was vegetation in M1 and even distribution between vegetation, run, and pool for M2 (Table 2).

Both males and females showed a range of PC1 versus PC2 scores variance for both sites that did not overlap, with the difference being most apparent with PC1 (Figures 4 and 5).

Table 1: Principal Component Analysis of M1 and M2 Males and Females.

Site	PC1 Variance	PC2 Variance
M1 Males	61.406	24.51
M2 Males	58.912	22.226
M1 Females	62.075	24.907
M2 Females	50.751	38.996
M1 & M2 Males	64.922	22.732
M1 & M2 Females	61.711	27.247

Table 2: Habitat Type Count of specimens in the analysis and average flow data for each habitat type with standard deviation. M1 = "Rt.140 crossing". M2 = "Mummaford Road crossing".

Category	Total Counts	Average Flow Rate M1 (m/s)	Flow Rate SD	Category	Total Counts	Average Flow Rate M2 (m/s)	Flow Rate SD
M1 Males				**M2 Males**			
Glide	11	0.18	0.06	Glide	3	0.39	0.13
Vegetation	17	0.14	0.09	Vegetation	2	0.20	0.09
Run	20	0.58	0.13	Run	13	0.76	0.25
Pool	9	0.01	0.02	Pool	9	0.09	0.03
Riffle	8	1.04	0.32	Riffle	13	0.92	0.22
M1 Females				**M2 Females**			
Glide	9	0.18	0.06	Glide	0	0.39	0.13
Vegetation	25	0.14	0.09	Vegetation	6	0.20	0.09
Run	15	0.58	0.13	Run	6	0.76	0.25
Pool	8	0.01	0.02	Pool	6	0.09	0.03
Riffle	4	1.04	0.32	Riffle	5	0.92	0.22

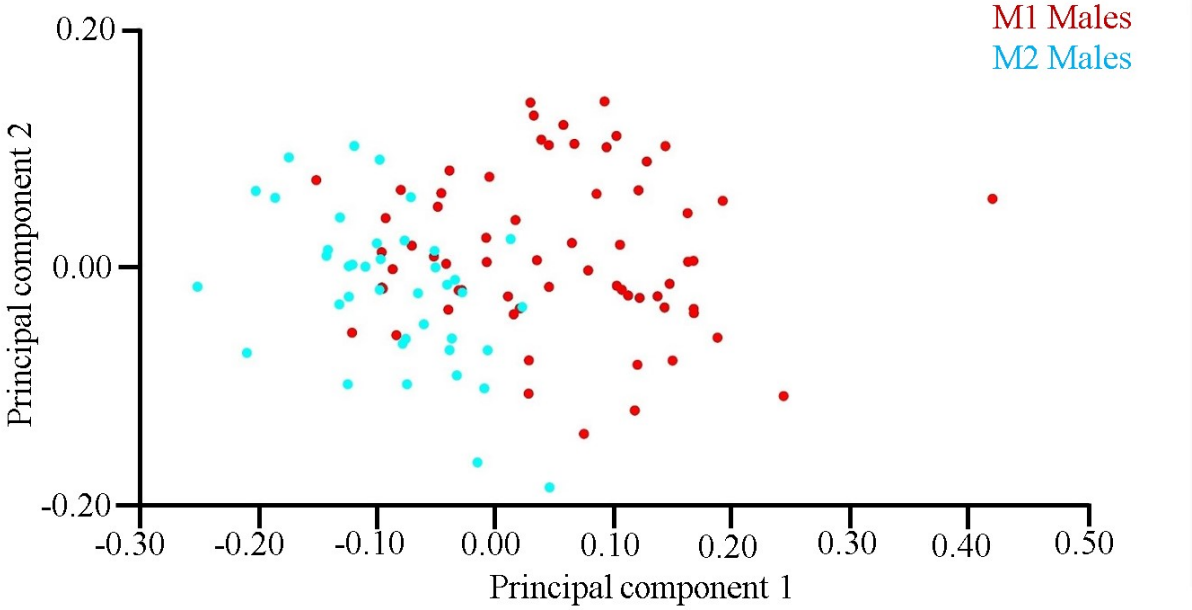

Figure 4: PC1 versus PC2 by site for Males. Red = M1 Males. Blue = M2 Males.

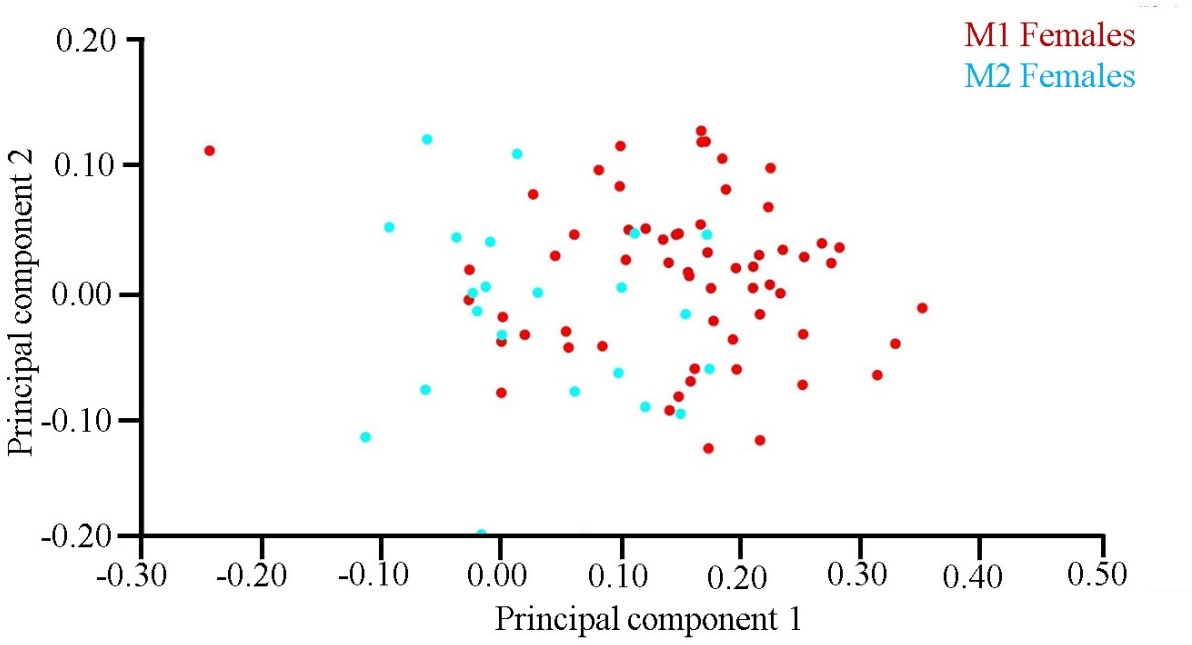

Figure 5: PC1 versus PC2 by site for Females. Red = M1 Females. Blue = M2 Females.

Procrustes ANOVA was performed on the M1 and M2 male, and M1 and M2 female, datasets. Centroid size and shape differed between sites M1 and M2 males (Centroid: $F = 16.87$, $df = 1$, $P < 0.0001$; Shape: $F = 33.48$, $df = 78$, Pillai's trace = 0.93, $P < 0.0001$). Centroid size and shape differed between sites M1 and M2 for centroid size and shape for females (Centroid: $F = 5.22$, $df = 1$, $P < 0.0249$; Shape: $F = 15.19$, $df = 78$, $P < 0.0001$, Pillai's trace = 0.97, $P = 0.2138$).

Male carapace length, abdomen width, and chelae width were significantly larger in M2 versus M1 males (CL: $df = 1$, $F = 12.04$, $P = 0.001$; AW: $df = 1$, $F = 15.32$, $P < 0.001$ CW: $df = 1$, $F = 9.99$, $P = 0.002$; Figure 6). No difference was detected in these metrics between females from M1 versus M2 (CL: $df = 1$, $F = 2.391$, $P = 0.126$; AW: $df = 1$, $F = 2.238$, $P = 0.138$; CW: $df = 1$, $F = 1.674$, $P = 0.199$; Figure 6).

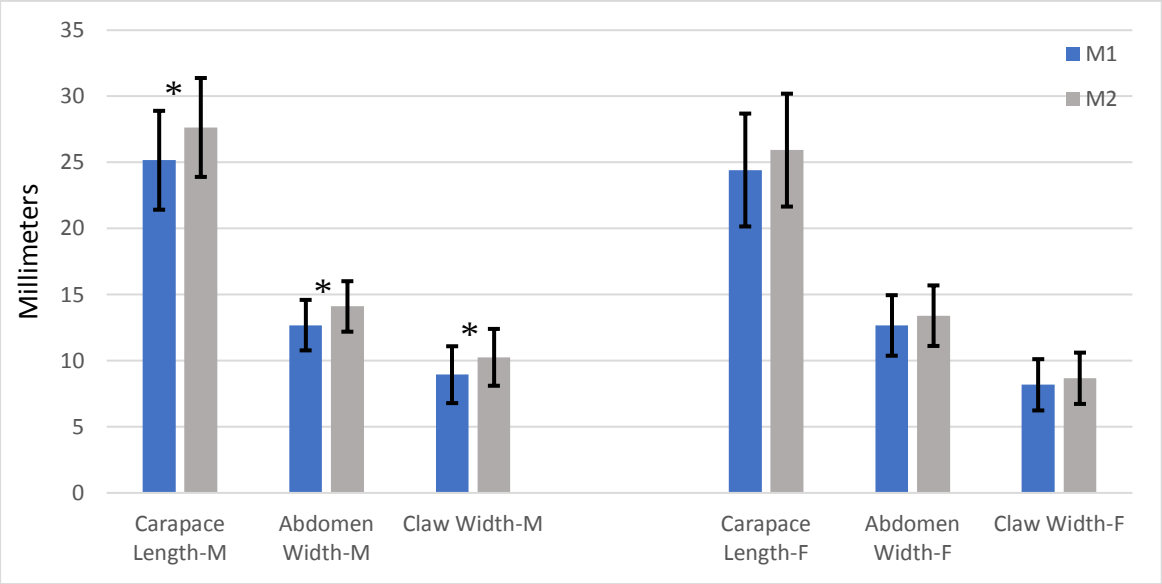

Figure 6: Average carapace length, abdomen width, claw width measurements of M1 and M2 specimens. Left: Male data. Right: Female data. Error bars = Standard deviation. Star = Significant results ($P < 0.05$).

DISCUSSION

Through morphometric analysis, I identified significant differences in shape and size of males between sites that could be consistent with the hypothesis that competition is a factor in the morphological variation observed between sites for *O. rusticus*. A significant difference was not observed in the individual metrics of size for females, but there was a significant difference observed from the Procrustes ANOVA. Reduced sample size in the female group likely played a role in differences observed between the measurement metrics and the Procrustes ANOVA metric used to analyze the specimens. Males are typically more aggressive than females and more likely to engage in agonistic interactions, which may help explain the pronounced difference found between sites for male specimens (Bruski & Dunham 1987). The observed size differences in *O. rusticus* males between sites are potentially due to morphological variation driven by competition with *O. virilis*, as the abundance of this competitor was one variable between sites. The significant differences in shape identified are likely due to differences in the measurement metrics, through allometry, where the variation in shape is associated with variation in size (Claude et al. 2003; Klingenberg & Lobon 2013).

This morphological variation found between sites may provide a competitive advantage for *O. rusticus*. Size differences of crayfish carapace, abdomen, and chelae can confer meaningful selective advantages for species engaged in interspecific competition. The shape and measurement differences observed between sites may signal selection or population processes such as phenotypic variation (Bookstein & Mitteroecker 2013; Roff & Mousseau 2005). Competition between *O. rusticus* and *O. virilis* may lead to genetic and morphological variation, through allele concentration at the invasion front and increased robustness overall (Bourne et al. 2018). Large body size, chelae width, and abdomen width are important for food utilization,

establishing dominance, shelter acquisition, copulation, and escape response in *O. rusticus* (Davis & Huber 2007). Detrital food patches are limited resources, and larger more robust *O. rusticus* can better defend these patches through more intensive fighting (Bergman & Moore 2003). Predator selection of crayfish prey can be influenced by chelae size, with larger chelae size and aggressive chelae display potentially reducing fish predation on crayfish (Roth & Kitchell 2005; Vlach & Valdmanova 2015; Garvey et al. 1994). Larger carapace length of *O. rusticus* can yield a competitive advantage in shelter acquisition, with larger crayfish establishing dominance by evicting smaller crayfish, which exposes them to fish predators (Davis & Huber 2007; Garvey & Stein 1993; Martin & Moore 2008; Roth & Kitchell 2005). More robust body size of *O. rusticus* enable it to reproductively interfere with *O. virilis,* increasing reproductive success of larger *O. rusticus* (Garvey & Stein 1993). Larger abdomen width can improve acceleration during caridoid (tail-flip) responses, which may be advantageous in predator escape response (Krasne et al. 2014).

Although competition is likely driving the observed differences found, there are alternative hypotheses that could account for the findings of this study. One alternative hypothesis is flow velocity, which is a potential confounding variable that was not accounted for during the study. The M2 site was recently invaded at the time of collection. *O. rusticus* invades higher flow rate habitats first (riffle and run), and then expands to the lower flow rate habitats in a river ecosystem (E.R. Annis personal communication). Research into flow velocity on morphologic plasticity has found that higher flow velocities lead to short and broad shaped chelae and shorter more fusiform body shape, where low flow velocities lead to long and narrow shaped chelae, and wider and more robust body shape (Perry et al. 2013). Prior research on flow velocity used an average control of 0 meters per second (“m/s”) velocity, average low velocity of

0.25 m/s and an average high velocity flow of 0.48 m/s (Perry et al. 2013). In this analysis, M1 males were from run habitat with a flow velocity of 0.58 m/s, and M2 males were collected from a run habitat with a flow velocity of 0.76 m/s. M1 males in riffle habitat experienced a flow velocity of 1.04 m/s, and M2 males experienced 0.92 m/s flow velocity. Our flow velocities were higher than what Perry and colleagues (2013) documented as influential to morphological plasticity. Based on the higher flow velocities, I would expect to M2 males to exhibit more fusiform body shape as well as shorter and broader chelae. Individuals at the M2 site showed the opposite, they were found to have larger, more robust, carapace, abdomen, and chelae measurements, which lends support to the hypothesis that competition, not flow velocity could be affecting *O. rusticus* in this analysis.

Other potential explanations include rapid evolution, adult dispersal downriver, founder effect, and habitat type. Rapid evolution can occur when a mutation occurs quickly enough to exhibit a measurable effect in a few generations, whereas phenotypic plasticity is a change to a single genotype which produces different phenotypes in response to environmental cues within a single generation (Yamamichi et al. 2011; Ghalambor et al. 2015). The differences observed here point to phenotypic plasticity, rather than rapid evolution, as phenotypic plasticity would occur within a generation in response to competition cues, and rapid evolution would occur over a few generations in response to environmental stress (Ghalambor et al. 2015). It is unlikely that more than 1-2 generations of *O. rusticus* had passed at the M2 site given the rate of downstream expansion (3 km yr^{-1}) and the rate of transition (1-2 yr) from *O. virilis* dominated to *O. rusticus* dominated at individual sites (E.R. Annis, personal communication). The founder effect could also be an explanation for the observed differences between sites, with the morphology of

specimens collected at site M2 on the leading edge of the invasion front. This invasion front could reflect the morphology of those individuals that elected to migrate downstream.

There is a sampling bias in my study toward lower flow regimes in M1 and M2 males, which could also account for the differences seen, as habitat type was not accounted for during the analysis. All specimens were obtained from five different habitat types: glide, vegetation, run, pool, and riffle. I would expect crayfish occupying run and riffle habitats to exhibit more fusiform body shape and chelae than those crayfish occupying a pool or vegetation habitat, as higher flow velocity can alter morphological variation in crayfish (Perry et al. 2013). Crayfish can move distances of tens of meters per day, with recorded movements of up to 30 meters per day (Byron & Wilson 2001). The two sites selected for analysis were 13 kilometers apart from one another, with each site being 100 meters long (Selckmann 2016). Though crayfish move during the day, this should reduce habitat type bias, as each site was within the range expected for a single *O. rusticus* specimen to move during a single day. This should not have significantly affected the sampling effort. The size of the river at the time of collection was 25 meters across, so I would not expect a single crayfish to move more than the width of the river in a single day.

While the results of the experiment were significant and potentially align with the hypothesis, there are potential sources of error that warrant consideration. Differences in the female groups during the Procrustes ANOVA, but not the size metrics analysis, could be due to sampling error of including measurements from sexually immature Form I versus mature Form II individuals in the population, which is a subsampling bias error that was not accounted for in the study. This may have increased the chance of detecting erroneous size differences among the groups where none exist. The underlying mechanism of the variation observed in my study is unknown and speculative in nature, and though competition is potentially driving the plasticity

observed, flow velocity cannot be ruled out as a potential plasticity mechanism, and further research will need to be performed to elucidate the different effects of water velocity and interspecific competition on morphologic plasticity.

Further investigation into this phenomenon should use size matched individuals, rather than different sized specimens, to determine if the effect observed here is reproducible. This study looked at the whole crayfish. Future studies should look at chelae and carapace separately to elucidate the morphological differences observed in this study. Studying the habitat specific differences between the sites would also be interesting to examine, as this could shed light onto the effects of habitats on morphological plasticity in *O. rusticus*. Ontogenetic studies would also determine if age is a factor in the morphologic variation observed, with lab analysis to look at trait inducibility. Genetic analysis of competing populations of species will yield new insight into the underlying mechanisms of interspecific morphologic variation, as well as the type of plasticity observed.

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