Fluctuating Chelae Asymmetry and Territorial Agonistic Behavior in Female *Orconectes virilis*

Natasha Thapa, Soverno Chen, and Elizabeth Woelmer

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Jordan Price

Abstract

Fluctuating asymmetry (FA) refers to small deviations from perfect symmetry in bilateral traits and is believed to be a product of genetic and environmental stresses. Additionally, FA is believed to be negatively correlated to higher fitness. Hence, FA suggests that symmetrical individuals should outcompete asymmetrical individuals. However, this concept is highly controversial. We investigated the relationship between multiple variables (chelae and antennae asymmetry, average chelae length and width, and carapace length) and outcomes of agonistic encounters among weight-matched female North American freshwater crayfish (*Orconectes virilis*). Crayfish were collected from Burt Lake near Pellston, Michigan. Our results indicate asymmetric chelae and antennae traits, as well as chelae length and width and carapace length, are not reliable predictors of winners and losers in agonistic interactions between female *O. virilis*. This suggests that FA is not negatively correlated to a higher fitness, but rather, another morphological feature, chemical signal, or other factor is influential in fight outcomes.

Introduction

Fluctuating asymmetry (FA) refers to small, random deviations in the biological symmetry of living things (Møller & Pomiankowski, 1993). Used in fighting, mate choice, and competition for resources (Møller & Pomiankowski, 1993), FA is thought to reflect higher levels of environmental stress experienced by an individual during development. Moreover, FA was positively correlated to lower fitness, reducing the ability of an organism to survive and reproduce (Söderman 2007) and to the likelihood of losing in agonistic interactions between organisms such as shore crabs (Sneddon & Swaddle 1999). In that case, FA may hint at fitness through fight outcomes of winner and loser in agonistic encounters.

Agonistic fighting behavior is a mechanism for many organisms, including crayfish, to fight for resources within a territory, leading to territorial disputes (Martin and Moore, 2007). Resource availability (Chibucos et al., 2015) and dominance in crayfish social hierarchy (Herberholz, 2003) dictate fight outcomes in agonistic bouts for access to shelter, territory and protection from predators. Since winning fights would allow crayfish to acquire larger territory with more resources (Emlen & Oring, 1977), those with more resources would have a greater chance of surviving long enough to reproduce successfully.

In these agonistic encounters, crayfish use their chelae as the primary tool to fight for motives such as territory (Stein, 1976). In that case, FA in chelae may indicate fitness through fight outcomes, winners or losers. According to Rutherford (1995), chelae asymmetry in male *Orconectes rusticus* was not a reliable predictor of winners and losers in agonistic interactions though chelae length was correlated with winning fights. However, chelae asymmetry and its effect on fight outcome in female crayfish, to our knowledge, has not been studied elsewhere.

Because FA is positively correlated with lower fitness (Møller & Pomiankowski, 1993), the fight outcome between female North American freshwater crayfish (*Orconectes virilis*) may be affected by the degree of asymmetry expressed in their chelae. In this study, we mainly tested the relationship of overall chelae asymmetry and fighting ability in agonistic interactions between female crayfish. We hypothesized that individuals with more asymmetry in overall chelae would lose more fights than less asymmetrical individuals, so chelae asymmetry would be a reliable predictor of crayfish fight outcomes. Alternatively, our null was that overall chelae asymmetry is not a reliable predictor of fight outcomes, so there would be no statistical significance between overall chelae asymmetry and fight outcome.

Methods

Animal Collection

Crayfish species *Orconectes virilis* were collected on July 22, 2017 between 10:00 PM and midnight at Maple Bay in Burt Lake of Pellston, Michigan. We used flashlights to illuminate the bottom of the lake at knee-deep height for any crayfish. Once crayfish were spotted, they were scooped up with a hand net and deposited into a bucket filled with water. 125 crayfish were collected, transported into a cooler aeration system, and remained in the cooler until the following morning.

Social Status Removal and Isolation

On the morning of the 23rd of July at the UMBS Stream Lab in Pellston, Michigan, we took the crayfish out of the cooler aeration system and divided them into males and females based on their anatomical reproductive parts. A total of 76 female and 49 male *Orconectes virilis* were obtained. In order to have a higher sample size, we used only females and gave the males to another research group. We placed each female crayfish into its own container labeled with a specific number (ranging from 1-76) and isolated them from the rest of the crayfish for one week. The purpose of isolation was to ensure that prior social history was erased. Crayfish have the ability to recognize previous fight opponents by chemical signaling (Schneider et al,. 2001) and remember if they won or lost (Velden et al., 2008), so they will not fight past opponents with much duration or intensity if there is already a clear dominance hierarchy established from a past fight. Therefore, we isolated the crayfish for one week to eliminate the probability that the crayfish came into contact before the study was conducted. All crayfish (in their respective containers) were placed into a 2 x 1 x 1 meter trough. The trough was filled with water pumped from the East Branch of the Maple River, distributed through PVC pipes and valves into the trough, and then discharged back into the river again.

Measurements

Morphological measurements were taken for each crayfish during the time of isolation. On the 26th and 28th of July at the Stream Lab, we took crayfish out of their containers to measure carapace length, left/right antenna length, left/right claw length, and left/right claw width using digital calipers. Figure 1 below demonstrates chela length and width measurements. All measurements were taken to the nearest 0.01 mm.

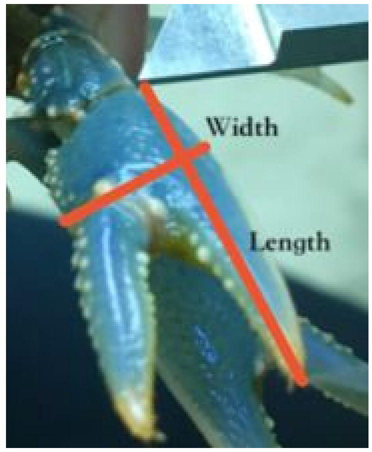

Figure 1. This image shows how chelae length and width were measured (mm) for the right and left chela of each crayfish. Length was measured from the tip of the chela to the beginning of the carpopodite (segment right before the chela). Width was measured at the widest part of the chela.

After the measurements concluded, the crayfish were returned into their containers and placed back into the trough. After the one-week isolation ended, we weighed each crayfish using a Sartorius Basic scale balance and recorded the weights on August 2nd, 2017. Crayfish were paired based on similarity of less than or equal to 10% weight difference, since size is positively correlated with winners in crayfish fights (Pavey & Fielder, 1996).

Experimental Arena and Protocol

Shortly after crayfish were paired by weight, fight trials began on August 2nd, 2017 at the Stream Lab. A 10-gallon Plexiglas tank was filled with 5 gallons of water to be used as the fighting arena. Next, we secured an open-celled egg crate at the center of the tank to act as a divider between the two opposing crayfish. Each crayfish of the fighting pair was dropped on either side of the divider for five minutes to allow them to acclimate to the new environment and establish their territory. After five minutes, we removed the divider from the tank so the two crayfish could fight until we saw signs of a winner and loser or, if a fight outcome was not determined within the allotted 15 minutes, the fight was recorded as unresolved. With a stopwatch, we recorded the initial 5-minute isolation time on either sides of the tank and the 15-minute fighting time to the nearest hundredth of a second. The clearest sign of winner and loser that occur in any crayfish fight is when the winner chases away the loser, which backs away or retreats in a submissive posture with its tail tucked underneath itself (Bergman and Moore, 2003; Moore, personal communication). Winners and losers were recorded based on the identification number on the container in which the crayfish lived. After the fight, each crayfish was put into isolation again. Because crayfish release chemical signals into the water during agonistic encounters (Schneider et al., 2001), we replaced the water in the Plexiglas tank after each trial with new water to prevent confounding variables in upcoming fight trials. Crayfish fight trials and procedures were conducted on August 4th, 5th, and 6th, 2017.

Calculating Asymmetry

Table 1. This table shows the formulas used to calculate chelae length and width asymmetry, chelae asymmetry overall, antenna length asymmetry, chelae length, and chelae width.

Function	Formula
Chelae Length Asymmetry	$\lvert \textit{Right Length} - \textit{Left Length} \rvert$
Chelae Width Asymmetry	$\lvert \textit{Right Width} - \textit{Left Width} \rvert$
Chelae Asymmetry Overall	$\frac{\textit{Length Asymmetry} + \textit{Width Asymmetry}}{2}$
Antenna Length Asymmetry	$\lvert \textit{Right Length} - \textit{Left Length} \rvert$
Chelae Length	$\frac{\textit{Right length} + \textit{Left length}}{2}$
Chelae Width	$\frac{\textit{Right width} + \textit{Left width}}{2}$

Asymmetry values were calculated for chelae and antennae in each crayfish. We calculated chelae length asymmetry by taking the absolute value of the right claw length minus the left claw length (Table 1). Chelae width asymmetry was calculated by taking the absolute value of the right claw width minus the left claw width (Table 1). We calculated antennae length asymmetry by taking the absolute value of the right antenna length minus the left antenna length (Table 1). We averaged the two asymmetry values (length and width) to get an overall or general chelae score of asymmetry for each crayfish (Table 1). Asymmetry values were then categorized into winners or losers. The closer the mean value is to zero, the more symmetrical the trait is (Møller & Pomiankowski, 1993).

Data Analysis

In Excel, standard error bar graphs were constructed to show the mean values of asymmetry in chelae length, chelae width, and antenna length for winners and losers with standard error bars representing the variability in these means. We created a standard error bar graph to show the overall chelae asymmetry for each fight outcome category to see if there was statistical significance in chelae asymmetry between winners and losers. Additional standard error bar graphs were constructed to show chelae length, chelae width, and carapace length for winners and losers to see if any of these variables showed statistical significance between winners and losers. Chelae length for each crayfish was found by averaging the length of the right and left claw. Chelae width was found in the same manner.

In SPSS, we conducted several paired t-tests to determine if the mean difference in winners and losers was different from 0, indicating statistical significance. Paired t-tests were conducted for the seven pairs (Table 2).

Table 2. This table shows the pairs that were run through a paired t-test in SPSS.

Paired t-test Groups
Winner (overall chelae asymmetry) - Loser (overall chelae asymmetry)
Winner (chelae length asymmetry) - Loser (chelae length asymmetry)
Winner (chelae width asymmetry) - Loser (chelae width asymmetry)
Winner (antennae length asymmetry) - Loser (antennae length asymmetry)
Winner (carapace length) - Loser (carapace length)
Winner (chelae length) - Loser (chelae length)
Winner (chelae width) - Loser (chelae width)

Results

Paired t-tests revealed that overall symmetry had no significant impact on fighting ability (Figure 2; p=0.298) and the same applied to all other variables measured: chelae length asymmetry (Figure 3A; p=0.428), chelae width asymmetry (Figure 3B; p=0.168), antennae length asymmetry (Figure 6; p=0.669), chelae length (Figure 4A; p=0.582), chelae width (Figure 4B; p=0.603), and carapace length (Figure 5; p=0.120). However, FA tended to be biased for losers (Figures 2, 3, and 6). Carapace length, and chelae length and width showed the opposite pattern, with winners displaying a higher average mean. For all seven pairs, the standard error bars greatly overlapped, indicating no significant difference between winners and losers, with the greatest overlap occurring with antennae length asymmetry (Figure 6). One fight was not resolved.

Discussion

Overall, we failed to reject our null hypothesis since the FA of winners and losers did not differ significantly. Additionally, length asymmetry, width asymmetry, antennae asymmetry, chelae length, chelae width, and carapace length also did not differ significantly between winners and losers. Thus, our study supported the conclusions from Rutherford (1995) in which chelae asymmetry was not an accurate indicator of fight outcomes, though Rutherford's study utilized male *Orconectes rusticus* crayfish. Rutherford (1995) found statistically significant results in chelae length as a reliable predictor of fight outcomes, but our study did not support chelae length as a predictor of fight outcomes in agonistic interactions (Figure 4A). Our findings, which concluded no statistical significance in all aspects of symmetry measured, were not uncommon compared to the results of studies such as Swaddle & Witter (1994) and Dufour & Weatherhead (1998) in which symmetrical individuals did not always outcompete asymmetrical ones. Moreover, our results did not support the findings in the Møller and Pomiankowski (1993) study which linked FA to higher overall fitness during sexual selection and better genetic quality. FA itself is highly controversial and some studies have pointed out confirmation bias within studies regarding this concept such as the Kozlov & Zvereva (2015) study which found that participants who believed certain downy birch leaves originated from a polluted site were more likely to report higher FA values than those who believed that the leaves originated from an unpolluted site. Thus, this confirmation bias could have leaked into other studies centering around FA.

Our study eliminated several morphological variables that could have been reliable predictors of winners and losers in agonistic relationships; however, it is possible that female *O. virilis* crayfish may exhibit cryptic asymmetry, in which asymmetries of strength are very weakly correlated with asymmetry in size (Angilletta & Wilson, 2012). Due to their exoskeletons, crayfish would be able to 'mask' internal structures such as soft tissue or muscle. Thus, female crayfish may express unreliable signs in which an asymmetrical female could possess higher muscle power than a more symmetrical female crayfish.

A factor that may have influenced our study is that we operated under the assumption that a dominant crayfish has increased survival rates due to greater access to resources and territory. In a previous study by Fero et al. (2007), feeding was unaffected by social status in the observed crayfish populations. This led Fero and his colleagues to the conclusion that dominance does not indicate increased access to resources in male and female *Orconectes rusticus*. Additionally, they found that dominant crayfish occupied shelter less than more subordinate crayfish when the subordinate crayfish were present. This would imply that dominant crayfish are not necessarily dependent on winning fights for increased resources and territory. Rather, maintaining their position in the social hierarchy is more important for other reasons, such as mating, which would allow them to pass on their genes to more offspring.

It must be taken into account, however, some potential problems which may have arisen during our study. For one, we only observed females instead of observing both sexes. Males and females express size dimorphism in their chelae, with males having typically larger chelae than females, as both sexes use their chelae for different purposes (Stein, 1976). Future studies should look into male-male interactions in order to test if FA has any significance in fight outcomes.

Additionally, the fighting trials in our study were conducted in daylight despite the fact that crayfish are nocturnal. Thus, our results may have been different had we fought the crayfish in a more nocturnal setting, as they typically fight more intensely in a natural setting (i.e. in a nocturnal environment with a burrow nearby) (Bergman & Moore, 2003). Our sample size also may not have been large enough. Our study initially contained seventy-six crayfish, but this size was halved due to pairing for fighting trials and some were lost due to unexpected events such as death, molting (too vulnerable to fight), and escape from the container during the isolation period. Although our data was not statistically significant for chelae length and width asymmetry, overall asymmetry, or antenna asymmetry, our data does show a bias for asymmetrical losers, suggesting that a larger sample size could have revealed a statistically significant trend.

In conclusion, chelae and antennae asymmetry, chelae length and width, and carapace length are not reliable predictors of winners and losers in agonistic interactions between female *O. virilis* crayfish. This suggests that another trait may be responsible for influencing fight outcomes. FA, however, may play an influential role in determining fight outcomes in female-male and male-male interactions due to the difference in chelae size between the sexes which are also used for different purposes (Stein, 1976). Therefore, future studies should test male *O. virilis* crayfish and ensure that the fighting trials are conducted in a more nocturnal environment. A large sample size would also need to be used in order to detect any significant patterns. Additionally, it would be interesting to take the study on territorial aggression even further by isolating the crayfish in their own tanks along with burrows in order to reinforce this strong territorial behavior and from thereon determine which plays a stronger role in fight outcomes: FA or simply social hierarchy.

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Figures

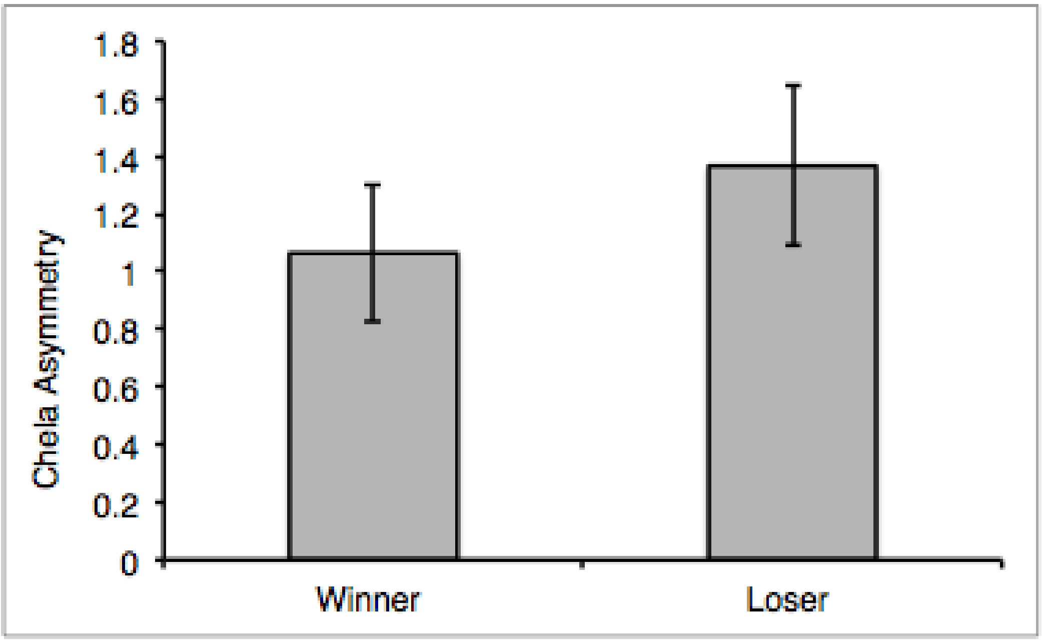

Figure 2. Comparison between overall chelae asymmetry of winners or losers of fights. There was no significant difference between fight outcome and chelae asymmetry (paired t-test; $p=0.298$). Please note axis scale. Larger values along the y-axis indicate higher asymmetry.

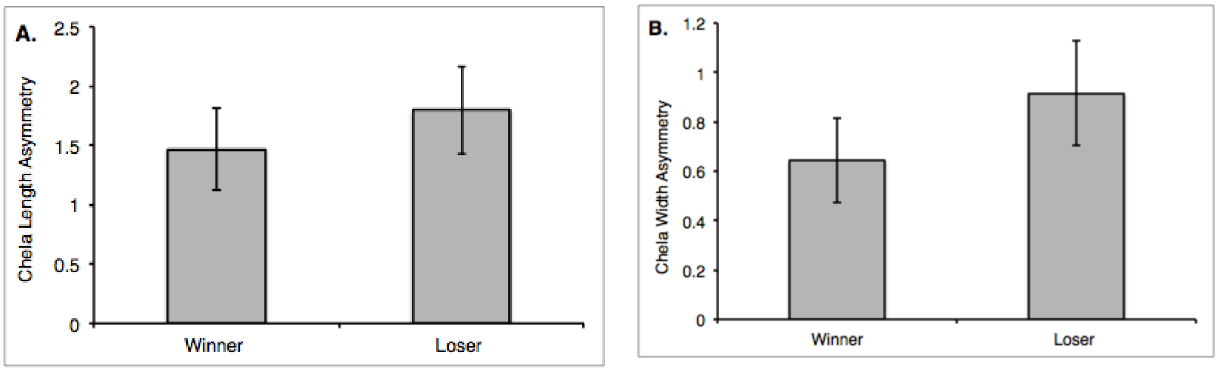

Figure 3. Comparison between chelae length asymmetry (A), chelae width asymmetry (B), of winners and losers. There was no significant difference between fight outcomes and chelae length and width asymmetry (paired t-test; (A) $p=0.428$, (B) $p=0.168$). Please note axis scale. Larger values along the y-axis indicate higher asymmetry.

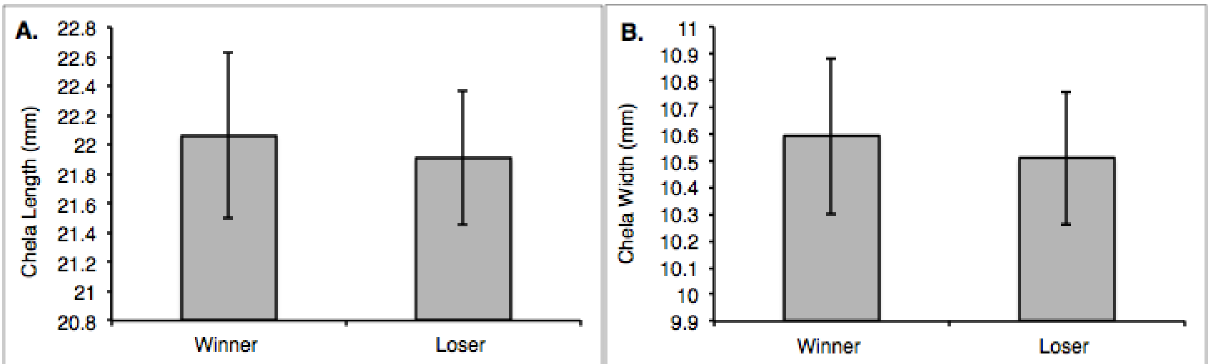

Figure 4. Comparison between chelae length (A), chelae width (B), of winners and losers of fights. There was no significant difference between fight outcomes and chelae length nor width (paired t-test; (A) p=0.582, (B) p=0.603). Please note axis scale.

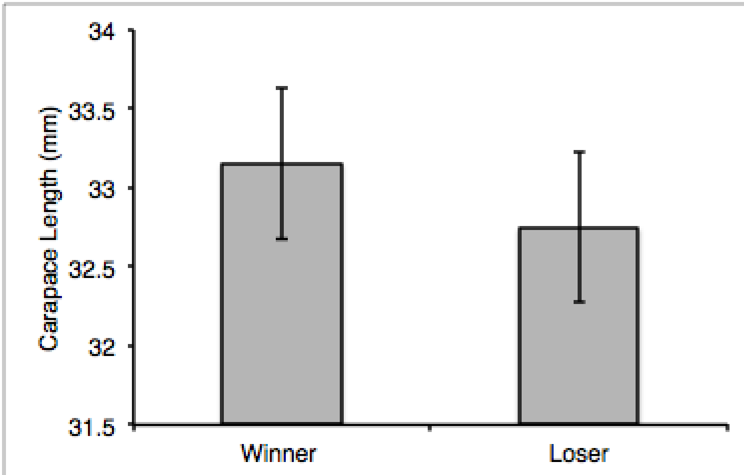

Figure 5. Comparison between carapace length (mm) of winners and losers of fights. There was no significant difference between fight outcome and carapace length (paired t-test; p=0.120). Please note axis scale.

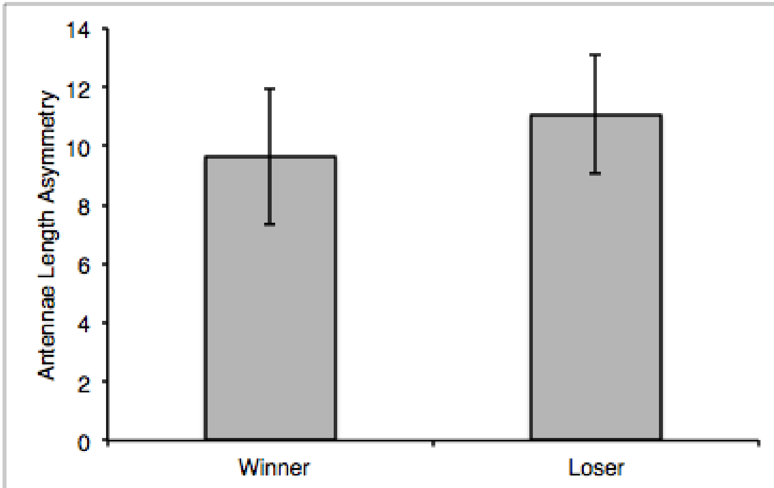

Figure 6. Comparison between antennae length asymmetry and fight outcome. There was no significant difference between fight outcome and antennae length asymmetry (paired t-test; p=0.603). Please note axis scale. Larger values along the y-axis indicate higher asymmetry.