

IMPACTS OF CHELIPED MORPHOLOGY ON MATE SELECTION OF  
AN INVASIVE CRAYFISH, THE RED SWAMP CRAYFISH  
(*PROCAMBARUS CLARKII*)

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**Abstract.**—The red swamp crayfish, *Procambarus clarkii*, is a large-bodied crayfish that is native to northeastern Mexico and the south-central United States. The extensive breeding season and the r-selected characteristics of this species allow it to produce a large number of offspring. Despite the extensive research on the invasive potential of this organism, some behavioral aspects of the mating system have not been well-studied. We investigated how cheliped morphology impacts female choice and copulation behavior by manipulating three male traits: chela presence, function, and chela-body size ratio. Females showed no preference for males based on any of the visual traits examined, and copulations were infrequent and not indicative of female preference for a male phenotype. Chela function and chela-body size ratio impacted male copulation attempts, suggesting male dominance and aggression as a potentially significant component in the mating system of *P. clarkii*. Finally, our we encourage further research to include other visual and chemical stimuli to truly understand the crayfish mating system and the role of female choice.

Keywords: cheliped ablation, female choice, male choice, mating systems

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The red swamp crayfish, *Procambarus clarkii* (Girard, 1952), is a large crayfish that is native to northeastern Mexico and the south-central United States, with the highest densities recorded in the wetlands and swamps of Louisiana and east Texas (Taylor et al. 2007). Since the mid-twentieth century, *P. clarkii* has found its way onto every continent except Antarctica and Australia (Aquiloni & Gherardi 2008a). Throughout Europe, this crayfish has decimated mollusk, fish, and amphibian communities through their overconsumption and has also been known to carry the oomycete, *Aphanomyces astaci* (crayfish plague), a common freshwater disease-causing microbe that is also an

invasive species (Scalici & Gherardi 2007; Cruz et al. 2008; Aquiloni et al. 2011). Despite the extensive research on the invasive potential of this organism, the breeding system has been partially neglected (Gherardi et al. 2006; Aquiloni & Gherardi 2008a, 2008b).

*Procambarus clarkii* is a highly promiscuous organism, choosing multiple mates each breeding season, and is also an r-selected species producing many small offspring; however, unlike most r-selected species, *P. clarkii* provides considerable maternal care for offspring during the first few weeks (Pianka 1970; Aquiloni & Gherardi 2008a, 2008b, 2008d). The mating season typically ranges from June to October in colder climates and from August to November in warmer climates (Sommer 1984; Gutiérrez-Yurrita & Montes 1999; Chucholl 2011; Loureiro et al. 2015). Furthermore, *P. clarkii* has the ability to disperse overland as well as through aquatic settings, allowing for a rapid, widespread, range expansion (Herrmann et al. 2018). As global temperature increases, and aquatic habitats become scarcer, these factors increase the invasive potential of *P. clarkii*.

It is widely postulated that female choice likely plays a more important role in the mating system of crayfish than male choice (Aquiloni & Gherardi 2008b). This idea meshes well with traditional sexual selection theory, in that the female is more limited reproductively (Emlen & Oring 1977). Females are limited in gamete production, while males are limited by their access to females for mating. This system predicts that females will be more particular in choosing better quality mates.

Decision-making processes require an immense amount of information that must be quickly assimilated, or the organism may risk a loss to its fitness. It follows that information must also come from multiple sources, interpreted using different systems of sensation simultaneously (Partan & Marler 2005). Crayfish rely most heavily on visual and chemical cues in deciding among multiple potential mates (Aquiloni & Gherardi 2008c; Aquiloni et al. 2011; Dunham & Oh 1996; Schneider et al. 1999). These senses are used to interpret information on an individual's dominance status, relative health, parasite load,

strength, and environmental cues (Aquiloni & Gherardi 2008c). It is difficult to determine, however, which sense is more important when assessing mating behavior due to the multimodal fashion of crayfish cognition and sensation.

Most research efforts have focused exclusively on the chemical cues that affect crayfish mating systems, as opposed to visual stimuli (Corotto et al. 1999; Schneider et al. 1999; Acquistapace et al. 2011; Aquiloni & Gherardi 2008c). Most species of crayfish, including *P. clarkii*, display a hierarchical dominance system, determined exclusively through chemical cues (Schneider et al. 1999). Crayfish also rely on chemoreception for sex determination through the use of their antennules. However, according to Corotto et al. (1999) and Acquistapace et al. (2011), the lack of antennules in either sex did not significantly impact either of these characteristics, suggesting that crayfish have the ability to rely on visual cues in the absence of chemoreception when determining sex of a conspecific. This interaction provides a crucial link to the importance of the highly complex visual system in crayfish.

Despite more recent interest in these organisms, little is known about the visual traits females prefer when choosing their mates (Aquiloni & Gherardi 2008a, 2008b). Previously, Aquiloni & Gherardi (2008a) found a preference of females for large males, while males preferred virgin females in *P. clarkii*. Furthermore, a later study by the same authors confirmed that females preferred larger males and concluded that females actively allocated their reproductive resources (number and size of offspring) as a result of their choice (Aquiloni & Gherardi 2008b). This suggests that females will mate with beta males hesitantly, and in the process will adjust the amount of reproductive resources expended accordingly.

To the best of our knowledge, no studies have explored the effects of chela loss, function, and chela-body size ratio as visual cues on mating behavior in the crayfish, *P. clarkii*, among other species. These traits can be used as a metric to measure the mating behavior as a function of some natural interaction. For example, both chela ablation

and function are proxies for body damage that could result from intraspecific, agonistic interactions, as well as predation by another organism. This damage could be detrimental to the survival and fitness of the crayfish, as chelae are used by the male to hold the female in place during reproduction (Aquiloni & Gherardi 2008a).

The main objective of our study was to determine the role of various visual stimuli on female mate choice and to better understand aspects of the mating system of *P. clarkii*, which could be useful in the management of this highly invasive species. We hypothesized that female crayfish will show a preference for: 1) chela presence, when given the choice between no chelae, 1 chela, and 2 chelae, 2) chela functionality, when choosing between functional and non-functional chelae, and 3) chela-body size ratio, when choosing between a crayfish with small chelae and a large body versus large chelae and a small body in mate choice.

## MATERIALS & METHODS

Crayfish were obtained from Carolina Biological Supply Company and Louisiana Crawfish Company, all cultivated or caught wild from Louisiana, USA; crayfish were received in multiple batches from August–November 2018 to replace individuals lost from natural mortality events. Mean carapace length (CL) of female crayfish was 3.6 cm, whereas male crayfish were about 3.8 cm. Observations took place between the months of August 2018 and January 2019 (August–November 2018, n=60; December 2018–January 2019, n=30). Crayfish were kept in a LivingStream aquarium system (LS–120; Frigid Units Inc.), within a greenhouse facility with natural light conditions, containing three divided sections. Each section of the system contained a gravel substrate and multiple polyvinyl chloride (PVC) habitats (8 cm by 11 cm). Crayfish were fed once per week with algae wafers (Kyorin Food Industries, Ltd.) *ad libitum*. The chiller unit for the system was set at 25°C for the entirety of the project; this temperature was selected to prolong the mating season for *P. clarkii* and corresponds to ambient

water temperature during warmer months in their natural range (Daniels et al. 1994). Increased photoperiod (i.e., extended periods of sunlight) enhances the effects of warm water temperatures in prolonging the mating season for *P. clarkii*; we did not alter photoperiod in our trials, as water temperature is more significant in female development (Daniels et al. 1994). Well-water was the source of all water utilized for the project. Once received, individuals were processed (depending on the visual trait in question) and sorted into three groups of 30 crayfish for each trial (new crayfish were ordered for each visual trait in question): females, treatment males, and control males (all males utilized were Form I, as Form II males are not reproductively active). Each group of crayfish was placed into one of the three divided sections of the system for a one-week acclimation period. This initial period was necessary to reset any previous social experiences, to provide enough time for any mature eggs to be noticed in previously mated females, and to separate the two sexes entirely; any females displaying mature eggs were removed from the experiment.

The male crayfish for the cheliped ablation and chela function experiments underwent further processing before they were utilized. For the chela ablation experiment, males were randomly sorted into three groups: 2 chelae (n=15), 1 chela (n=15), or 0 chelae (n=15). Once sorted, chelae were removed using wire cutters for the treatment crayfish, with the ablation occurring as close to the body cavity as possible. Chelae were removed two weeks prior to the experiment. The chela function experiment underwent similar processing. First, the treatment selection occurred just as it did in the cheliped ablation experiment, with the exception of only two groups, functional/control (n=15) and dysfunctional/treatment (n=15). Once males were determined, clear plastic hair ties were wrapped around the chelae of treatment males to incapacitate them. With respect to chela-body size ratio, males were differentiated as either small, with chelae shorter than 6 cm and bodies larger than 8 cm (total length; n=15), or large, with chelae longer than 6 cm and bodies shorter than 8 cm (total length; n=15). For the cheliped ablation, chela function, and chela-body size

ratio experiments, different females and treatment males (n=30 each) were tested per experimental treatment. Control males were used multiple times within each experiment, but were not used in consecutive trials (control males were used once per day at most); it is unlikely this would affect the results of the study as control males were novel to each female.

*Female preference experiment.*—The first portion of the project solely measured female preference. This was accomplished by placing one female in the center of an aquarium (40-liter) with two males, one on each side (one treatment and one control). The males were separated into two clear plastic terrariums (40 cm by 33 cm), whereas the female was able to move freely around the center of the tank. These terrariums allowed the female to visually perceive both males, while blocking all chemical sensation. To begin, the female was allowed to sit in the test aquarium filled with water at the same temperature as the holding aquarium for 5 min to acclimate to the water temperature (25–30°C). After this acclimation period, the female was placed on the start point to move freely around the aquarium for 20 min. Markings were placed on the sides of the tank to delineate which male was “chosen” (Fig. 1). During the 20-min time period, the female was able to explore the tank to examine the male crayfish. Whenever a female crossed one of the dashed lines, the observer recorded this as a visit for the corresponding male. Whenever the female was stationed in the neutral area, neither male received a visit score. This was implemented to truly distinguish between the female choosing a male and simply exploring the aquarium. The number of visits for each male received was calculated to determine the “chosen” male, or the individual that received the most visits. This method is modified from Aquiloni & Gherardi (2008b).

*Copulation behavior experiment.*—The second experiment occurred immediately after the female preference experiment and allowed females to interact and potentially copulate with preferred males. This was achieved by removing the plastic terrariums and placing both of the males in the original aquarium with the female, allowing for complete interaction. The water temperature was kept

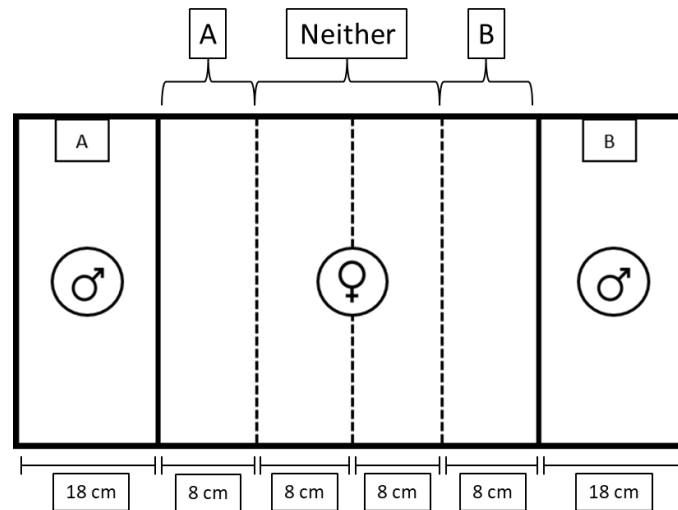


Figure 1. Experimental aquarium for testing female preference. Bold lines indicate the barriers separating the males from the female. The dashed lines indicate the boundaries for female preference for male crayfish. For example, if the female crossed the dashed line separating “Neither” and “B” while moving towards male B, this would count as a visit to male B.

within the same range as the previous experiment (25–30°C). Observations lasted a maximum of 30 min. For each trial, three behaviors were recorded for analysis: number of copulation attempts by each male, time until copulation, and duration of copulation. Copulation attempts were categorized as an approach of the male towards the female from behind, followed by an attempt by the male mounting the female and attempting to flip the female onto her back. Attempts were recorded to determine if males of particular treatments were more likely to initiate copulation. If the female fled from the male or did not remain still, the female was deemed non-receptive. Finally, duration of copulation was the amount of time the male and female remained embraced, with the male on top of the female, and ended when both crayfish separated. Copulation behavior was evaluated as the male that successfully achieved copulation. In the absence of copulation, male copulation attempts were compared.

*Statistical analyses.*—All statistical analyses were completed using the program R. Data were first checked for normality and homogeneity of variance visually using histograms, density-distribution plots, and

Shapiro-Wilk tests for each of the experimental treatments. Some of the treatments for both female preference and copulation behavior data were not distributed normally, as per the results of the Shapiro-Wilk test ( $P > 0.05$ ). Chi-square goodness-of-fit tests were the most appropriate to analyze both the female preference and copulation behavior data. This test compares the observed frequencies to an expected distribution, usually random, which in this case was 0.5 (or 50%). The data was analyzed by comparing the number of successes for the hypothesized traits to the total number of events, against a 50% probability. The test also allows for the analysis of non-parametric data in the form of either counts or proportional data. and assumes that experimental individuals are random subsets of a population, which was followed with the randomization of test subjects (Whitlock & Schluter 2015). The “prop.test” function in R was used to complete this analysis for each of the male treatment components simultaneously. For example, the function would run 30 chi-square tests for the 30 data points of the chela function data. Overall, this tested the hypothesis that there is a preference for a treatment, as opposed to a completely random situation. The level of significance at which the null hypothesis was rejected is  $\alpha = 0.05$ .

## RESULTS

*Female preference experiment.*— There was no significant difference in the number of visits from female crayfish to males that differed in cheliped ablation, chela function, or chela-body size ratio (Table 1). Females did not appear to settle on either male during the experiment, randomly wandering between the two sides of the tank the entirety of the 20-minute test.

*Copulation behavior experiment.*—We predicted that females would copulate with preferred males, but females did not appear to choose a particular male during the female preference experiment. Furthermore, copulations were not plentiful enough to analyze statistically, so we analyzed the number of male copulation attempts to



Table 1. Female preference data (means  $\pm$  SE) for the binary choice test between males of two different treatments.

	Treatments	Mean Visits	$\chi^2$	<i>P</i> -value
Cheliped Ablation	0 Chela (N=15)	7.400 $\pm$ 0.861	1.898	1.000
	2 Chelae (N=15)	7.133 $\pm$ 0.761		
	1 Chela (N=15)	6.333 $\pm$ 1.245	3.451	0.999
	2 Chelae (N=15)	6.200 $\pm$ 0.932		
Chela Function	Functional (N=30)	5.633 $\pm$ 0.635	9.074	0.999
	Dysfunctional (N=30)	6.100 $\pm$ 0.600		
Chela–Body Size Ratio	Small ratio (N=30)	5.300 $\pm$ 0.541	18.172	0.956
	Large ratio (N=30)	4.767 $\pm$ 0.486		

determine if particular males were more likely to initiate copulation with females. Female *P. clarkii* received more copulation attempts from males with functional chela and from large chela-body size ratio treatment males (Table 2). Throughout the experiment, males did not immediately attempt copulation with the female. In most cases the males and the female would wander around the aquarium for a few minutes before interacting with each other. These interactions occasionally included fighting between the males, with the male in the better physical condition (control) guarding the female from the impaired male (treatment). Nevertheless, it was common for impaired males to still attempt copulation despite an unsuccessful outcome. In a few of the trials, the female did not appear readily open to copulation with one of the males (often the largest individuals), however the male was persistent enough to force copulation.

Throughout the experiment, there were ten successful copulations (Table 3). Two copulations were from males that were fully intact (e.g., still had both chelae intact). Males with chela ablation did not achieve copulations, however, one dysfunctional male was able to copulate. Males with a smaller chela-body size ratio had four successful copulations compared to three copulations in males with a larger chela-

Table 2. Copulation attempts (means  $\pm$  SE) for the interaction between males of two treatments and the female in the copulation behavior experiment (\* denotes statistical significance).

	Treatments	Mating Attempts	$\chi^2$	P-value
Cheliped Ablation	0 Chela (N = 15)	1.867 $\pm$ 0.350	16.964	0.321
	2 Chelae (N = 15)	3.800 $\pm$ 0.641		
	1 Chela (N = 15)	1.800 $\pm$ 0.355	8.819	0.887
	2 Chelae (N = 15)	1.933 $\pm$ 0.182		
Chela Function	Functional (N = 30)	5.833 $\pm$ 0.751	49.824	0.013*
	Dysfunctional (N = 30)	3.500 $\pm$ 0.555		
Chela-Body Size Ratio	Small ratio (N = 30)	5.900 $\pm$ 0.818	67.462	<0.001*
	Large ratio (N = 30)	6.600 $\pm$ 0.826		

body size ratio. Functional chelae males and dysfunctional chelae males were able to copulate equally (one copulation each). Despite the number of copulation attempts for each trial, a maximum of one copulation would occur in a single trial.

## DISCUSSION

*Female preference.*—We tested the effects of male physical traits in female mate choice in a species of invasive crayfish. We hypothesized that female crayfish would display a clear preference for male phenotypes corresponding to peak physical condition (two chelae present, functional chelae, and high chela-body size ratio) over the traits that depict a physical impairment. However, this prediction was not supported by the data. During the experiment, it was common for females to move around the tank, never seeming to favor a particular male. The wandering behavior exhibited by females could be a result of the physical barriers keeping the crayfish from communicating chemically or possibly the artificial testing environment did not allow females to properly assess males. However, other crayfish studies have used similar testing apparatus in mate choice experiments (Aquiloni & Gherardi 2008a, 2008b, 2008c). It is possible that altering water temperature but not photoperiod, or completing trials outside of the

Table 3. Successful copulations for each treatment in the Copulation Behavior experiment.

	Treatments	Successful Copulations
Cheliped Ablation	0 Chela (N = 15)	0
	1 Chela (N = 15)	0
	2 Chelae (N = 15)	1
Chela Function	Functional (N = 30)	1
	Dysfunctional (N = 30)	1
Chela–Body Size Ratio	Small ratio (N = 30)	4
	Large ratio (N = 30)	3

mating season, could have impacted female behavior; however, we think this is unlikely, as female crayfish displayed the same wandering behavior throughout the entirety of our study. Therefore, our findings suggest chemical cues may be an important component in mate choice by female *P. clarkii*, but more studies are needed.

*Male copulations and copulation attempts.*—Copulations were not frequent, and it was difficult to discern whether females were choosing particular males with which to copulate or males were simply forcing copulation. Only males with two chelae were able to successfully copulate with females when compared to males with no chelae or with one chela, respectively (Table 3). This seems to indicate that there is a physical barrier, as opposed to a behavioral cue for crayfish copulation, regarding the number of chelae present. This follows, in that male crayfish use their chelae to seize, turn, and hold females when attempting to achieve copulation (Stein 1976). However, one dysfunctional male was able to achieve a copulation, indicating some level of female cooperation or choice.

Generally, males in peak physical condition (two chelae present, functional chelae, and high chela-body size ratio) attempted copulation with female crayfish more frequently than males with the impaired traits. Male *P. clarkii* with functional chelae, as well as those with a high chela-body size ratio, attempted copulation more frequently with female crayfish.

Males with removed chelae were not able to achieve copulation, although they attempted to do so. In nature, predation can result in the loss of chelae and, on occasion, the loss of function in chelae (Rutherford et al. 1995). This is understandable as crayfish utilize their chelae primarily for food and mate acquisition, competition with congeneric species, and protection from predation. Figiel & Miller (1995) determined that limb damage was dependent on the density of conspecifics for many crayfish species, most notably *P. clarkii*, and that survival rates also correlated positively with the amount of limb damage. When compared to other members of the order Decapoda, it is common for these organisms to lose their chelae with relatively low mortality levels (Figiel & Miller 1995; Juanes & Smith 1995; Smith 1992). However, chela loss, as we have demonstrated, likely impacts male mating success.

Previous research on the visual aspects of crayfish mating behavior have looked at chelae and body size independently (Aquiloni & Gherardi 2008a, 2008d), suggesting that size does matter (bigger is better). These findings follow evolutionary theory in the form of parental investment theory, in that females will choose mates that will maximize their reproductive output, which maximizes their fitness (Trivers 1972). Typically, males that are larger in size are chosen more often, as larger organisms should be able to defend territories from predators/competitors and provide food resources better than smaller individuals (Bovberg 1953; Stein 1976). Chela-body size ratio was included as a male treatment to tease apart this theory and to determine if body size matters as opposed to chelae size. According to our results, even though female *P. clarkii* did not visit males more often when they were of the high ratio treatment, these males attempted copulation more often than the low ratio males, suggesting male aggression might play a larger role than female preference.

Aquiloni & Gherardi (2008b) emphasized the importance of female choice in the mating system of *P. clarkii* whereas our study points to the significance of male dominance. Theoretically, females have the ability to “choose” a mate by deciding whether or not to allow males to approach, seize, turn, and hold them (Stein 1976). However, large–

bodied males, as well as those with a higher chela–body size ratio, could overpower the female and prevent escape.

Male aggression further compounds the issue of potential female preference throughout our study; the majority of our findings do not point to a female preference for certain male physical traits. Male *P. clarkii* are more likely to approach females and attempt copulation when they have functional chelae, and have larger chelae compared to their body size, indicating the ability of males to dominate females. Nevertheless, more data on the mating system of these invasive crayfish, including chemical cues, long-term interactions, and any other extraneous factors, are needed for the management and improved aquaculture of these organisms. Aquaculture systems that rely on crayfish as a food source for other organisms, or human consumption, can focus on optimizing production of *P. clarkii* by stocking individuals with large bodies or large claws.

Furthermore, research on life history traits (e.g., reproductive phenology) of invasive species can lead to better management practices. For example, *P. clarkii* introductions to new locations have resulted in the spread of the crayfish plague (*Aphanomyces astaci*) and damage to local crops (e.g., rice) worldwide (Barbaresi & Gherardi 2000; Aquiloni et al. 2011; Souty-Grosset et al. 2016). A better understanding of the mating system in *P. clarkii* could provide clues towards containment measures to limit further spread and damage. Additionally, research on human causes of invasion and methods to limit the spread of invasive species has become increasingly necessary in recent years. Research efforts surrounding invasion ecology must take species behavior into account, as behavioral research can be beneficial in the management of invasive organisms, which often display different behaviors in invaded compared with native ranges (Carere & Gherardi 2013; Chapple et al. 2012).

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