EVALUATING THE EFFECTS OF CHELIPED MORPHOLOGY ON THE MATING BEHAVIOR OF TWO INVASIVE CRAYFISH, THE LOUISIANA CRAYFISH (PROCAMBARUS CLARKII) (GIRARD, 1852) AND THE VIRILE CRAYFISH (FAXONIUS VIRILIS) (HAGEN, 1870)

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by

Hayden C. Hays

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EVALUATING THE EFFECTS OF CHELIPED MORPHOLOGY ON THE MATING BEHAVIOR OF TWO INVASIVE CRAYFISH, THE LOUISIANA CRAYFISH (PROCAMBARUS CLARKII) (GIRARD, 1852) AND THE VIRILE CRAYFISH (FAXONIUS VIRILIS) (HAGEN, 1870)

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DEDICATION

I would like to dedicate this thesis to a few special individuals who have helped me and guided me through the last 3 years. My roommate, Greg Davila, who has helped me through this crazy process and has always been there when I was having a bad day. Also, for helping me find my way in the church through introducing me to my Chi Alpha and First Assemblies of God community. One huge thing I have learned thanks to Greg: "I can do all things through Christ, who gives me strength" (Phil. 4:13).

A couple of teachers who have shown exceptional care in helping me get to where I am today. Dr. Diane Neudorf has been the best advisor I could ever ask for and has always been interested in learning more about the wonderful world of crawfish sex. Mrs. Deirdre Dykes, my high school biology and environmental science teacher, is the original inspiration for my interest in biology. The interesting experiments and her, often, wacky teaching style were so captivating that I just knew the field of biology was right for me.

Finally, to my loving family. My mom and dad (and I can't forget my dog Ryder!) have been so incredibly supportive, both financially and mentally, throughout this whole process. I couldn't have made it through grad school without the support of my entire family. I also want to extend a special dedication to my deceased grandma (Nana). She has been one of my biggest supporters my entire life, no matter the situation. Her passing at the beginning of my graduate career was a hard obstacle to overcome, and was a large focus of my life for the first year of graduate school. As I write this, I can't help but recall all of the fond memories of my time with her, and all the love and encouragement she gave me. This one is for you Nana!

ABSTRACT

Hays, Hayden C., *Evaluating the effects of cheliped morphology on the mating behavior of two invasive crayfish, the Louisiana crayfish (Procambarus clarkii) (Girard, 1852) and the Virile crayfish (Faxonius virilis) (Hagen, 1870)*. Master of Science (Biological Sciences), August, 2019, Sam Houston State University, Huntsville, Texas.

The Louisiana crayfish (*Procambarus clarkii*) is a large-bodied crayfish that is native to northeastern Mexico and the south-central United States, with a breeding season ranging from June to October in colder climates or June through November in warmer climates, with the inclusion of a second mating in the spring. This system combined with the r-selected characteristics of this species allows it to produce a massive amount of offspring, increasing its potential to become invasive. The Virile crayfish (*Faxonius virilis*) is native to the Great Lakes region of the United States, and shares similar life history traits with *P. clarkii*. Despite the extensive research on the invasive potential of these organisms, the visual behavioral aspects of the mating system have been mostly neglected. This study has investigated how cheliped autotomy influences female choice and copulation behavior of these two species of crayfish. Previous studies showed that *P. clarkii* females preferred males of larger body size or larger chelae size, but not which played a larger role. In other crayfish species, chelae asymmetry has shown no impact on mate choice, but no studies have looked at the impact of chelae loss in mate choice. Chelae loss or injury is fairly common in crayfish due to predatory and intraspecific interactions. I investigated 3 physical traits: chelae presence, function, and chelae-body size ratio in female mate choice. Females were given a choice between two different males to determine the importance of these traits in male mating success in two different experiments. Females of both species showed no preference for males based on any of the visual traits examined. Copulations were infrequent and not indicative of female preference for a particular male phenotype. Chelae function and chelae-body size ratio impacted male copulation attempts for *P. clarkii*. This suggests that further research must be done to include other visual, and chemical, stimuli to truly understand the crayfish mating system and the role of female choice.

KEY WORDS: Crayfish, *Procambarus clarkii*, *Faxonius virilis*, Invasive species, Mating behavior, Female choice

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I would also like to thank several other faculty and staff members, primarily my committee (Drs. Sibyl Bucheli and Jerry Cook). They have been very helpful in growing my academic interests, and leading through my graduate experience. Others include, Dr. Jeff Wozniak for his initial acceptance and guidance through part of my time at Sam. I am very thankful for the work he did to bring me in to the program, as I wasn't sure I would be qualified to start a Master's degree elsewhere. Dr. Chad Hargrave for his assistance with equipment for the initial attempts to catch crayfish (which turned out largely unsuccessful). Dr. Amber Ulseth, who I view as an honorary committee member, has been the best stats/R-guru I could ever hope for. Steven Koether has been the most amazing boss ever! He has gone out of his way to help me, and to be someone I can always go to just to chat or discuss any issues I might have. Steven is one of the most supportive and genuine people at Sam. To Tara Martin, Ericka Dupclay, and Lauren Schrodt for their support on the backside of project design, purchasing, data collection, and all other administrative tasks involved. Also, to Alan Byboth for all is help designing/constructing equipment, project design ideas, and use of facilities and other equipment. Alan has always been helpful to me, and has rarely asked for help in return; he has been an incredible resource for me.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

CHAPTER I

Introduction

The Louisiana Crayfish

The Louisiana crayfish, *Procambarus clarkii* (Girard, 1852), 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 the overconsumption of these organisms (Scalici & Gherardi, 2007; Cruz *et al.*, 2008). It has also been known to carry the oomycete, *Aphanomyces astaci*, a common freshwater disease-causing microbe that is also an invasive species (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; Aquiloni & Gherardi, 2008b).

The species is highly promiscuous, choosing multiple mates each breeding season (Aquiloni & Gherardi, 2008a; Aquiloni & Gherardi, 2008b). It is an r-selected species in that it produces many small offspring, with the exception of including minor maternal care (Pianka, 1970; Aquiloni & Gherardi, 2008d). The breeding season typically ranges from June to October in colder climates, but contains an additional breeding season from August through November in warmer climates (Sommer, 1984; Gutiérrez-Yurrita & Montes, 1999; Chucholl, 2011). Furthermore, *P. clarkii* has the ability to disperse overland as well as through aquatic settings, allowing for a rapid, and far spread, range expansion (Herrmann *et al.*, 2018). The combination of these factors allows *P. clarkii* the ability to produce a massive amount of offspring, increasing its invasive potential.

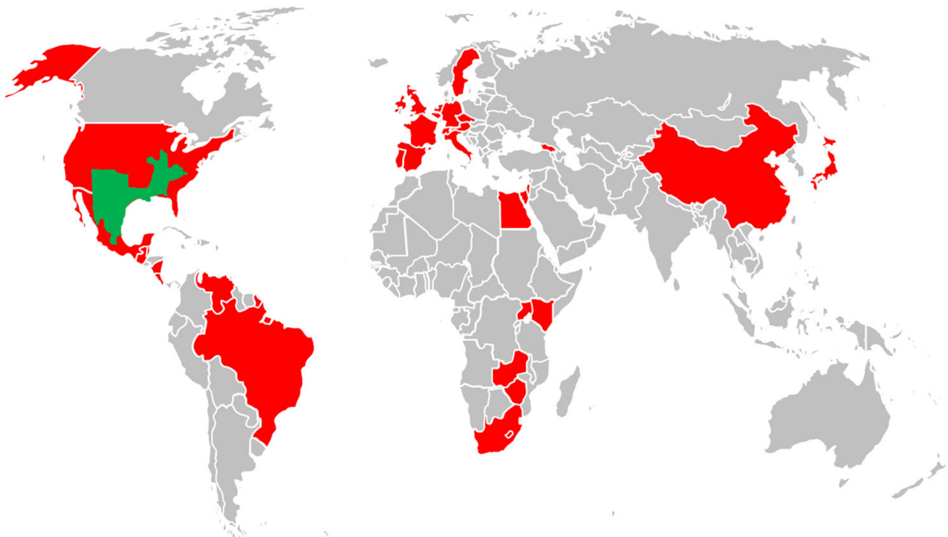

Figure 1. Distribution map for the Louisiana crayfish, *Procambarus clarkii*. This map displays the distribution of the Louisiana crayfish. The green region depicts the native range of the species, while the red regions display areas this crayfish has spread and become established (Loureiro *et al.*, 2015).

The Virile Crayfish

Concurrently, the Virile crayfish, *Faxonius virilis* (Hagen, 1870), is also widely distributed in the United States, ranging from Montana east to the Great Lakes and south along the Midwest into Oklahoma and Arkansas, with the highest densities centered on the Great Lakes region (Larson *et al.,* 2018). However, disjunct populations have spread to other parts of the country due to their use as bait for recreational fishing (Killian *et al.,* 2012). The species has successfully established in a few locations outside of the United States, all in Europe (Ahern *et al.*, 2008).

The spread of this organism has led scientists to question whether to categorize *F. virilis* as an invasive species, or as an introduced species (Phillips *et al.*, 2009a). *F. virilis* often outcompetes congeneric crayfishes in introduced environments, as is the case with the Spinycheek crayfish, *Faxonius limosus* (Rafinesque, 1817), in which *F. virilis* is the better competitor for shelter and resources, as a result of range expansion (Glon *et al.,* 2018). Previous research has also shown that a reintroduction of *F. virilis* after it has been completely removed from a community closely resembles an invasion action (Phillips *et al.*, 2009b). As the crayfish returns, it disrupts the newly established community through outcompeting current conspecifics and overconsumption of prey organisms. Despite the present data, current research points to the need for more information to determine if this species must be controlled, and what management strategies would be most effective.

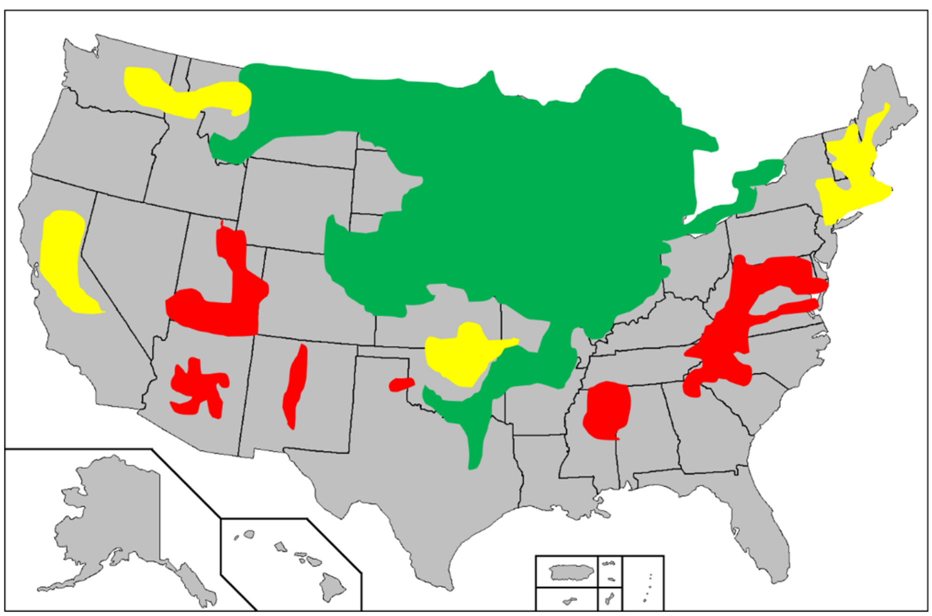

Figure 2. Distribution map for the Virile crayfish, *Faxonius virilis*. This map displays the distribution of the Virile crayfish. The green region depicts the native range of the species, while the yellow and red regions display areas this crayfish has spread, with the latter being more established (Simon & Burskey, 2014).

Female Mate Choice

According to parental investment theory, parents expend time, energy, and other important resources to benefit their offspring at a cost to their future fitness (Trivers, 1972). Previous research shows that female crayfish can modulate their egg production as a function of mating with different sized males. Mating with larger males typically results in fewer, larger eggs (Aquiloni & Gherardi*,* 2008b).

Female choice likely plays a more important role in the mating system of crayfish than male choice (Aquiloni & Gherardi*,* 2008b). This discovery 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 requires an immense amount of information that must be quickly assimilated, or the organism may risk a loss to its fitness. Therefore, it follows that information must also come from multiple sources, and interpreted using different systems of sensation, simultaneously (Partan & Marler, 2005). Crayfish rely most heavily on both visual and chemical cues in deciding between 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, as well as certain environmental cues (Aquiloni & Gherardi, 2008c). However, it is difficult to determine which sense is more important when assessing mating behavior due to the multimodal fashion of crayfish cognition and sensation.

The majority of research has 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.*, 2002; Aquiloni & Gherardi, 2008c). Most species of crayfish, including both *P. clarkii* and *F. virilis*, display a hierarchical dominance system, which is broken into alpha and beta individuals of both sexes. The dominance status of crayfish is determined exclusively through chemical cues, and is so advanced that individuals can determine the outcome of past conflicts with the same individual (Schneider *et al.*, 1999). Crayfish also rely on chemoreception, for sex determination, through the use of their antennules. When crayfish antennules are ablated, the expected result would be a decrease in the number and duration of mating attempts as seen in previous crustacean studies (Corotto *et al.*, 1999). According to Corotto *et al.* (1999) and Acquistapace *et al.* (2002), 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. These papers provide the crucial link to the importance of crayfish vision. Crayfish vision is highly complex among the invertebrates, and has the added component of color vision (Wald, 1964; Tuthill & Johnsen, 2006).

Despite more recent interest in these organisms, little is known about the visual traits females prefer when choosing their mates (Aquiloni & Gherardi*,* 2008a; Aquiloni & Gherardi*,* 2008b). Aquiloni & Gherardi (2008a) found a preference of females for large males, while males preferred virgin females in *P. clarkii*. 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. This suggests that females will mate with beta males hesitantly, and in the process will adjust the amount of reproductive resources expended accordingly

Few studies have explored the effects of chelae loss, function, and chelae-body size ratio as visual cues on mating behavior in the crayfish, *P. clarkii* and *F. virilis*, among other species. Each of these traits can be used as a metric to measure the mating behavior as a function of some natural interaction. For example, both chelae ablation and function are substitutes 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). Overall, my study is concerned with visual stimuli in crayfish mating response exclusively. The main objective of my research was to determine the role of various visual stimuli on female mate choice in two crayfish species, and to better understand aspects of their mating system, which could be useful in the management of these highly influential species.

Questions and Hypotheses

Overarching Research Questions. How do visual stimuli, in the context of chelae morphologies, affect mating behavior in the Louisiana crayfish (*Procambarus clarkii*) and the Virile crayfish (*Faxonius virilis*)? More specifically, what is the result of cheliped ablation, chelae functionality, and chelae-body size ratio in male crayfish on mate choice by female crayfish?

Hypothesis 1. I hypothesize that female crayfish will show a preference for chelae presence, when given the choice between no chelae, 1 chelae, and 2 chelae, in choosing a mate.

Prediction. Female crayfish will choose males with fewer chelae (0 or 1) less frequently than "intact" males.

Hypothesis 2. I hypothesize that female crayfish will show a preference for chelae functionality, when choosing between functional and non-functional chelae, in choosing a mate.

Prediction. Female crayfish will choose males with dysfunctional claws less frequently than functional males.

Hypothesis 3. I hypothesize that female crayfish will show a preference for a certain chelae-body size ratio, when choosing between a small and large chelae-body size ratio, in mate choice.

Prediction. Females will choose males with large chelae-body size ratios more frequently than smaller chelae-body size ratio males.

CHAPTER II

Methodology

The Study Species

Procambarus clarkii (Girard, 1852) is a common crayfish found, originally, throughout the Southeastern United States with the highest densities in Louisiana and East/Southeast Texas (Taylor *et al.,* 2007). Due to the high density of *P. clarkii* in Texas, mates are easily found, allowing for female individuals to be selective of their mates. As an r-selected species, it would be expected that these crayfish would produce a large number of offspring with low survival rates and low maternal care. However, this species displays the exception of extended maternal care (Aquiloni & Gherardi*,* 2008b). The combination of the r-selected nature of this species with their long mating season (June through November) can enhance the invasive potential of *P. clarkii* (Scalici & Gherardi*,* 2007). Concurrently, *Faxonius virilis* (Hagen, 1870) is also widely distributed in the United States, ranging from Montana East to the Great Lakes/New York and South along the Midwest into North Texas (Larson *et al.,* 2018). However, disjunct populations have spread to other parts of the country due to their use as bait for recreational fishing (Killian *et al.,* 2012). This has led scientists to question whether or not to categorize *F. virilis* as an invasive species, as well.

Crayfish were obtained from Carolina Biological Supply Company (Carolina Biological) and Louisiana Crawfish Company (Louisiana Crawfish). The crayfish from both Carolina Biological and Louisiana Crawfish are cultivated and caught wild. All crayfish came from Louisiana, USA. Of the *P. clarkii* specimens, 60 came from Carolina

Biological and 30 came from Louisiana Crawfish, whereas all 90 *F. virilis* specimens came exclusively from Carolina Biological.

Crayfish were kept in a LivingStream (LS-120; Frigid Units Inc.) aquarium system with 3 divided sections. Each section of the system contained a gravel substrate and multiple PVC pipe segments (3” bore; 4” long). The pipe was placed in the tank in order to provide shelter for the crayfish specimens. The air conditioning unit for the system was set at 25ºC for the entirety of the project. Once received, individuals were processed (depending on the visual trait in question) and sorted into 3 groups of 30 crayfish (per species): females, treatment males, and control males. The 3 groups of crayfish were placed into one of the 3 divided sections of the system for a one week period. This initial period was necessary to reset their previous social experiences, to provide enough time for any eggs to form in previously mated females, and to separate the two sexes entirely.

Male Treatment

The male crayfish for the cheliped ablation and chelae function experiments underwent further processing before they were utilized (Table 1). The chelae were removed for the treatment males in the cheliped ablation experiment, 2 weeks prior to the experiment. Crayfish were randomly sorted into 3 groups, 2 chelae, 1 chelae, or 0 chelae. Once sorted, chelae were removed using wire cutters for the treatment crayfish, with the ablation occurring as close to the body as possible. The chelae 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 and dysfunctional/treatment). Once males were determined, clear plastic hair ties were wrapped around the chelae of treatment males to incapacitate them. Males in the chelae-body size ratio were differentiated as either small (chelae shorter than 6 cm. and bodies larger than 8 cm.) or large chelae-body size (chelae longer than 6 cm. and bodies shorter than 8 cm.) ratio categories.

Female Preference

The first portion of the project solely measured female preference. Observations took place between the months of August 2018 through January 2019. This was accomplished by placing one female in the center of an aquarium (10-gallon) with two males, one on each side (one treatment and one control). The males were separated into 2 clear plastic terrariums (16" x 13"), whereas the female was able to move freely around the center of the tank. These terrariums allowed the female to visually perceive both of the 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 test aquarium for 5 minutes to acclimate to the water temperature (25ºC – 30ºC). After this acclimation period, the female was placed on the start point to move freely around the aquarium for 20 minutes. Markings were placed on the sides of the tank to delineate which male was "chosen" (Figure 3). During the 20 minute time period, the female was able to wander around the tank to examine the males. 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. 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

The second experiment occurred immediately after the Female Preference experiment and allowed for 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 (Figure 4). The water temperature was kept within the same range as the previous experiment, 25ºC – 30ºC. Observations lasted a maximum of 30 minutes, unless there was an active copulation event. If copulation was ongoing, the crayfish were allowed to finish in order to optimize their chances of successful reproduction and offspring production. For each trial, 3 behaviors were recorded for analysis, number of mating attempts by each male, time until copulation, and duration of copulation. Mating attempts were categorized as an approach of the male towards the female from behind, with the initiation of a copulation attempt. 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 mating attempts were compared.

Statistical Analysis

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 distributed normally, as per the results of the Shapiro-Wilk test (Table 2). Treatments that received a p-value of less than 0.05 were determined to be normally distributed. Following summary statistics, it was determined that 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%). Therefore, the data was analyzed by comparing the number of successes for the hypothesized traits to the total number of events, against a 50% probability. It also allows for the analysis of non-parametric data in the form of either counts or proportional data. The test 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-squared tests for the 30 data points of the Chelae 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 $\alpha = 0.05$.

Table 1

Experimental breakdown for male treatments

	Cheliped Ablation	Chelae Function	Chelae-Body Size Ratio
Number of Males	**45 Total:** 15 with 0 chelae, 15 with 1 chelae, and 15 with 2 chelae	**30 Total:** 15 functional and 15 dysfunctional	**30 Total:** 15 small ratio and 15 large ratio
Number of Females	**30**	**30**	**30**

Note. This table displays the number of males and females used. Males are further separated into the different experimental treatments of concern.

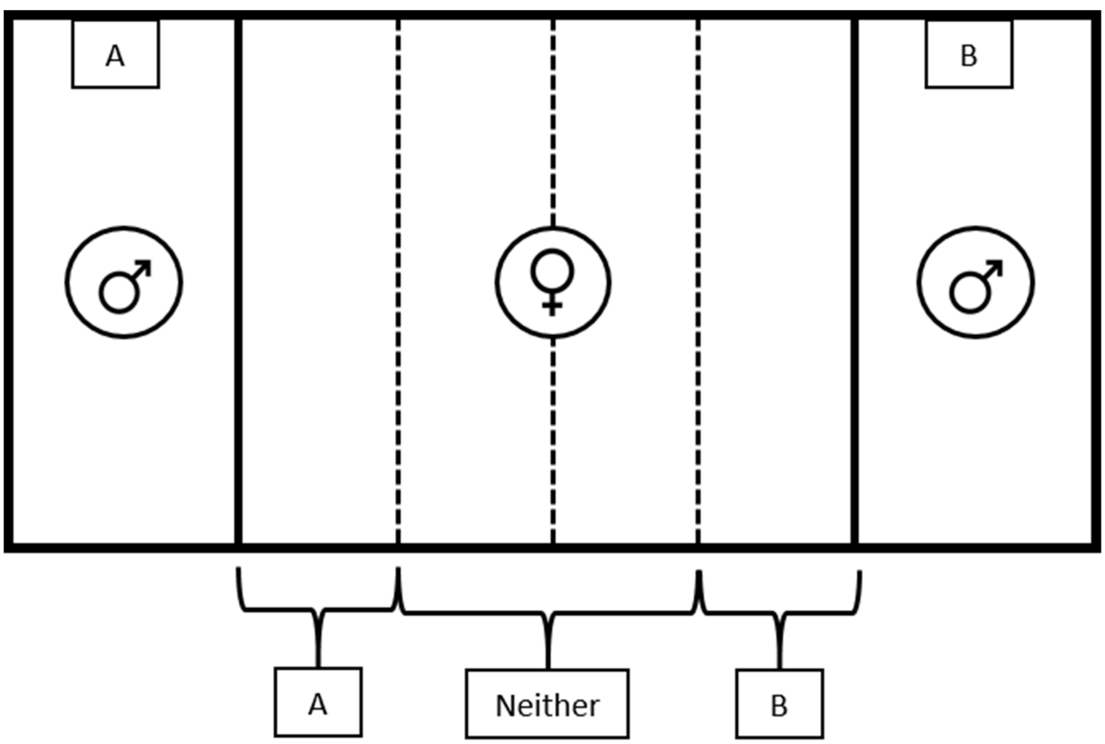

Figure 3. Experimental aquarium for testing Female Preference. Bold lines indicate the barriers separating the males from the female. The dashed lines indicate the boundaries for female preference for male crayfish. For example, if the female crossed the dashed line separating "Neither" and "B" while moving towards male B, this would count as a visit to male B.

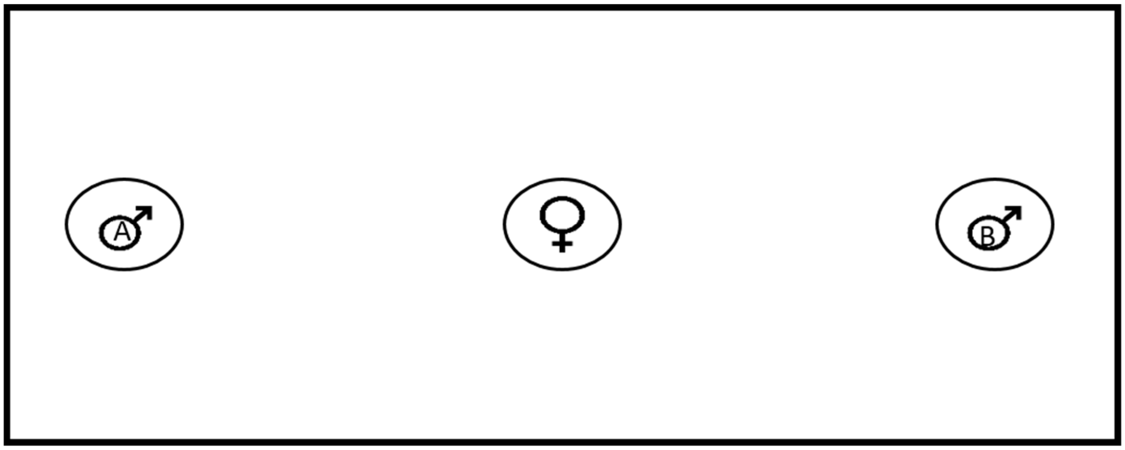

Figure 4. Experimental aquarium for testing Copulation Behavior. Both males and the single female were able to move freely around the tank.

Table 2

Shapiro-Wilk test for Normality results for both species of crayfish

			p-values	
			P. clarkii	*F. virilis*
Female Preference	Cheliped Ablation	0 Chelae	0.767	0.494
		1 Chelae	0.008**	0.158
		2 Chelae	0.141	0.267
	Chelae Function	Functional	0.182	0.078
		Dysfunctional	0.486	0.305
	Chelae-Body Size Ratio	Small Ratio	0.368	0.006**
		Large Ratio	0.051	0.185
Copulation Behavior	Cheliped Ablation	0 Chelae	<.001**	0.043
		1 Chelae	<.001**	0.033**
		2 Chelae	<.001**	0.003**
	Chelae Function	Functional	0.010**	0.170
		Dysfunctional	<.001**	0.168
	Chelae-Body Size Ratio	Small Ratio	0.009	0.001**
		Large Ratio	0.103	0.001**

*Note. All of the significance (p) values for each male treatment tested for the Female Preference and Copulation Behavior data (** denotes statistical significance)*

CHAPTER III

Results

Female Preference

There was no difference in the number of visits from female crayfish according to male treatment for both crayfish species (Table 3). The only case in which the p-value was close to significance was for chelae function in *F. virilis* ($X^2 = 43.179$; $p = 0.057$). Females did not appear to settle on either male during the experiment, seemingly wandering between the two sides of the tank the entirety of the 20 minute test. This is supported by the similar mean values for the Female Preference data. This is representative of a lack of female preference for any of the male treatments.

Copulation Behavior

I 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 I analyzed the number of copulation attempts to determine if particular males were more likely to initiate copulation with females. On average, female *P. clarkii* received more mating attempts from 2 chelae, functional, and large chelae-body size ratio treatment males (Table 4). Female *F. virilis* received the opposite, more mating attempts from 0 and 1 chelae, dysfunctional, and small chelae-body size ratio treatment males. However, none of the treatments for *F.virilis* were significantly different, whereas both chelae function and chelae-body size ratio were significantly different for *P. clarkii* (Table 4; Figure 5). 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 greatest physical condition guarding the female from the impaired male. However, 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 29 successful copulations, 10 from *P. clarkii* and 19 from *F. virilis* (Figure 6). Despite the number of mating attempts for each trial, a maximum of 1 copulation would occur in a single trial. Previous research suggests extra pair mating is common in *P. clarkii* (unknown for *F. virilis*), which was not observed during this experiment (Aquiloni & Gherardi, 2008b). The mean time for copulation of the different treatments saw a similar trend as the number of copulations (Figure 7). *F. virilis* males typically had longer copulation events than *P. clarkii*, with males of the more successful treatments having longer copulations than the impaired males.

Table 3

Female Preference data (means ± SE) for the binary choice test between males of two different male treatments

		Mean Visits	
	Treatments	*P. clarkii*	*F. virilis*
Cheliped Ablation	0 Chelae (n = 15)	7.400 ± 0.861	4.467 ± 0.639
	2 Chelae (n = 15)	7.133 ± 0.761	4.733 ± 0.539
	X^2 (df = 15)	1.898	5.002
	p-value	1.000	0.992
	1 Chelae (n = 15)	6.333 ± 1.245	4.333 ± 0.494
	2 Chelae (n = 15)	6.200 ± 0.932	4.200 ± 0.571
	X^2 (df = 15)	3.451	3.846
	p-value	0.999	0.998
Chelae Function	Functional (n = 30)	5.633 ± 0.635	4.133 ± 0.414
	Dysfunctional (n = 30)	6.100 ± 0.600	4.667 ± 0.413
	X^2 (df = 30)	9.074	43.179
	p-value	0.999	0.057
Chelae-Body Size Ratio	Small ratio (n = 30)	5.300 ± 0.541	4.300 ± 0.521
	Large ratio (n = 30)	4.767 ± 0.486	4.100 ± 0.478
	X^2 (df = 30)	18.172	17.065
	p-value	0.956	0.972

*Note. Female visit data (means ± SE) of male treatments in the Female Preference experiment (** denotes statistical significance)*

Table 4

Copulation attempts (means ± SE) for the interaction between males of two treatments and the female.

		Mean Mating Attempts	
	Treatments	*P. clarkii*	*F. virilis*
Cheliped Ablation	0 Chelae (n = 15)	1.867 ± 0.350	5.800 ± 1.147
	2 Chelae (n = 15)	3.800 ± 0.641	3.600 ± 0.505
	X^2 (df = 15)	16.964	19.824
	p-value	0.321	0.179
	1 Chelae (n = 15)	1.800 ± 0.355	5.400 ± 1.159
	2 Chelae (n = 15)	1.933 ± 0.182	4.600 ± 0.950
	X^2 (df = 15)	8.819	12.551
	p-value	0.887	0.637
Chelae Function	Functional (n = 30)	5.833 ± 0.751	5.133 ± 0.486
	Dysfunctional (n = 30)	3.500 ± 0.555	5.300 ± 0.528
	X^2 (df = 30)	49.824	33.433
	p-value	0.013**	0.304
Chelae-Body Size Ratio	Small ratio (n = 30)	5.900 ± 0.818	4.100 ± 0.578
	Large ratio (n = 30)	6.600 ± 0.826	3.700 ± 0.512
	X^2 (df = 30)	67.462	27.44
	p-value	<0.001**	0.600

*Note. Male mating attempts (means ± SE) of male treatments in the Copulation Behavior experiment (** denotes statistical significance)*

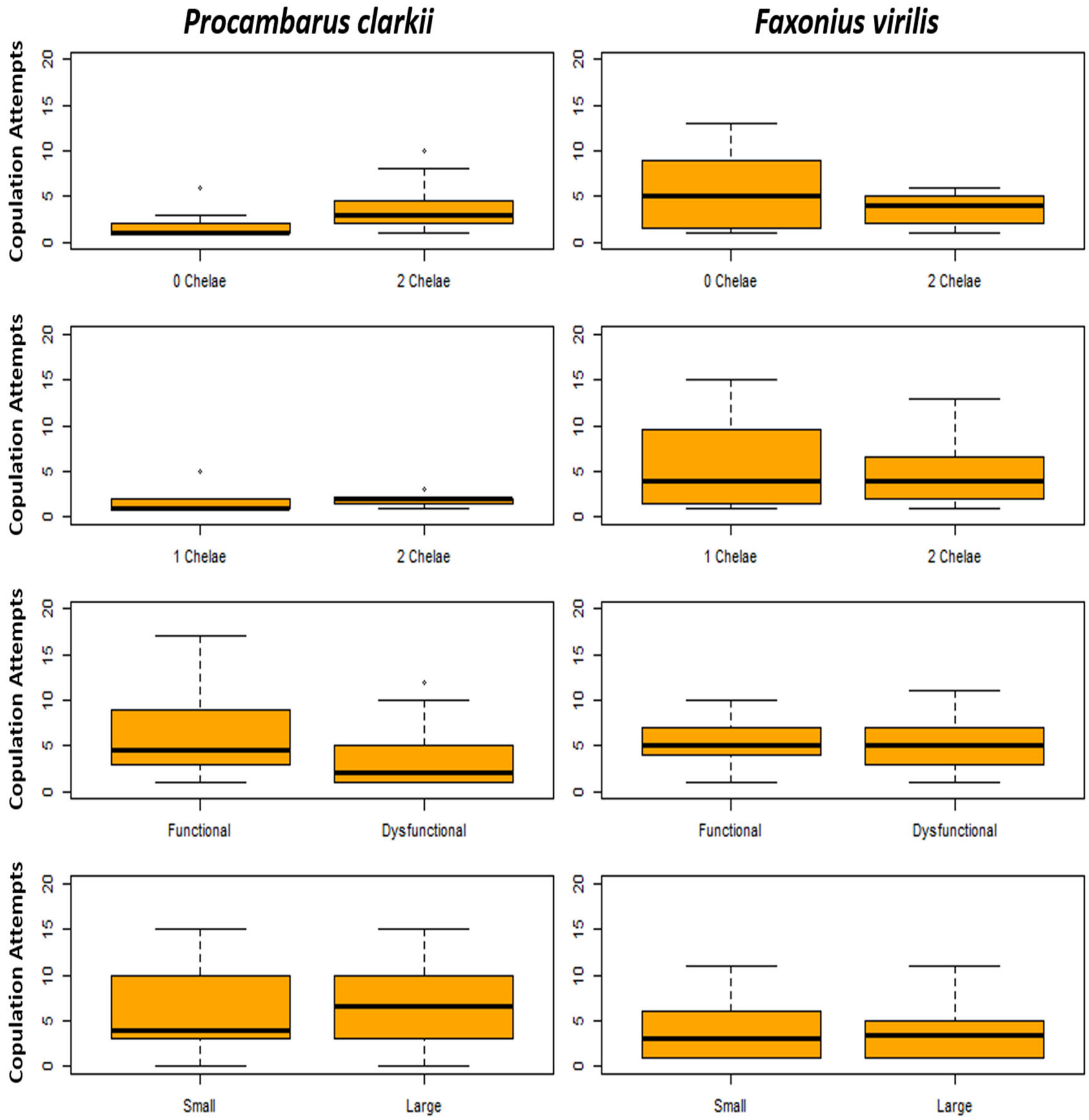

Figure 5. Male mating attempts during the Copulation Behavior experiment for both species of crayfish, *Procambarus clarkii* and *Faxonius virilis*. The box plots show the difference between the medians for each treatment

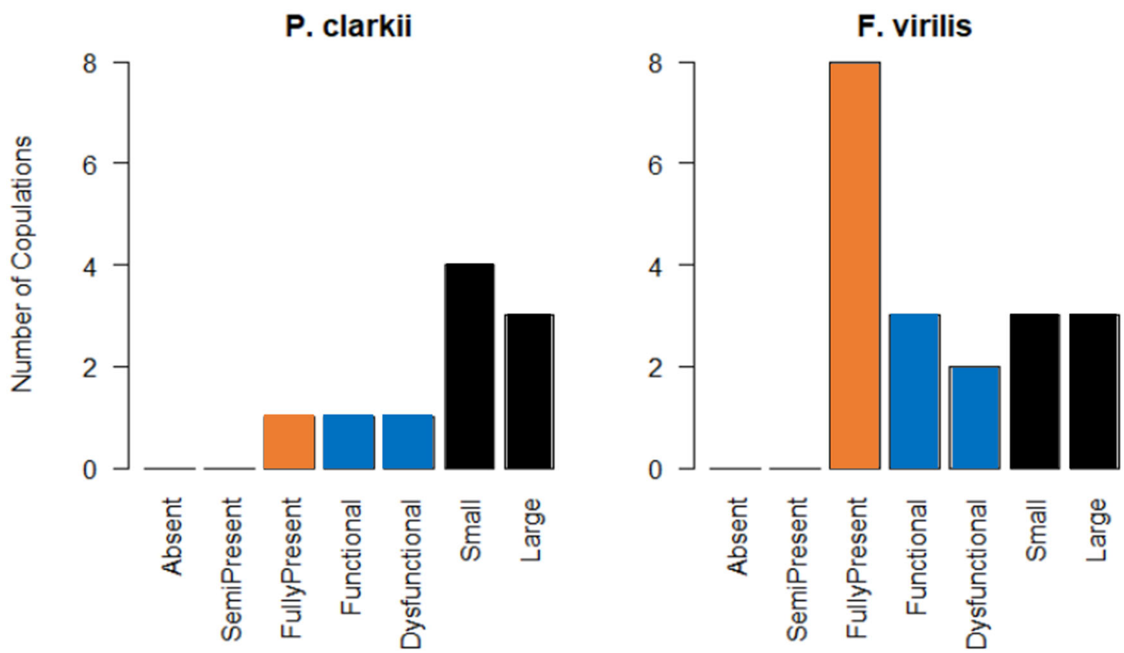

Figure 6. Number of successful copulations achieved by males of each treatment for both species of crayfish. The graph on the left depicts the number of copulations for *P. clarkii*, while the graph on the right is for *F. virilis*. The x-axis codes can be interpreted as: Absent – 0 Chelae, SemiPresent – 1 Chelae, FullyPresent – 2 Chelae, Functional – Functional chelae, Dysfunctional – Dysfunctional chelae, Small – Small chelae-body size ratio, and Large – Large chelae-body size ratio

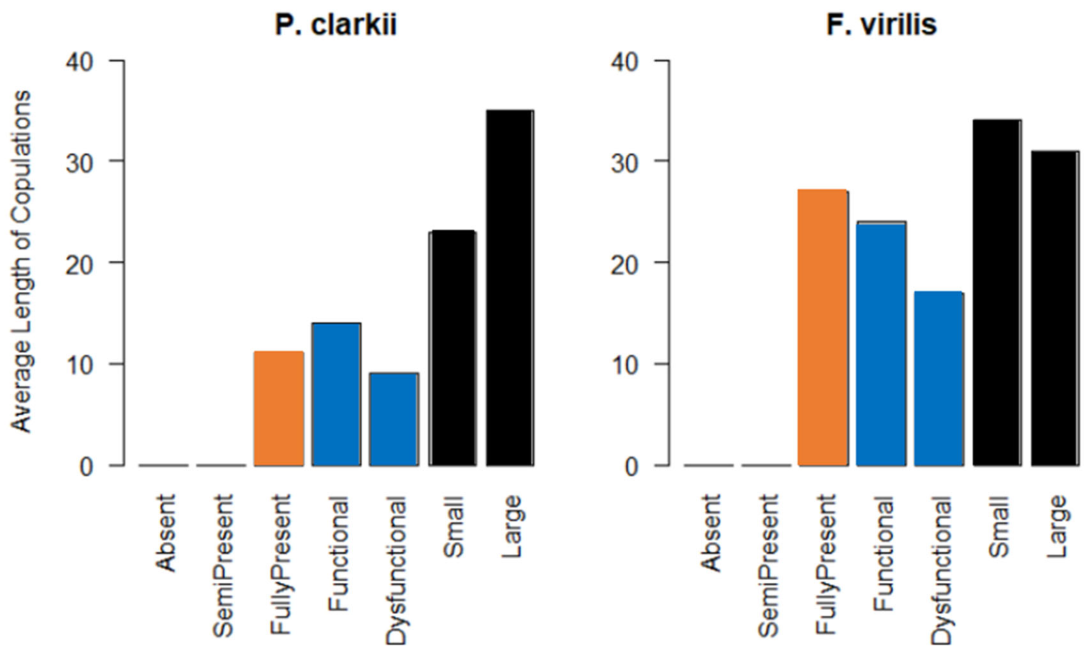

Figure 7. Average length (in minutes) of copulations achieved by males of each treatment for both species of crayfish. The graph on the left depicts the length of copulations for *P. clarkii*, while the graph on the right is for *F. virilis*. The x-axis codes can be interpreted as: Absent – 0 Chelae, SemiPresent – 1 Chelae, FullyPresent – 2 Chelae, Functional – Functional chelae, Dysfunctional – Dysfunctional chelae, Small – Small chelae-body size ratio, and Large – Large chelae-body size ratio

CHAPTER IV

Discussion

Female Preference

I tested the effects of male physical traits in female mate choice in two species of invasive crayfish to determine which traits could play a role in the management and aquaculture of these organisms. I hypothesized that the female crayfish would display a clear preference for male phenotypes corresponding to peak physical condition (2 chelae present, functional chelae, and a large chelae-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 wander around the tank, never seeming to choose a male. It was for this reason I chose to count the number of visits as opposed to the amount of time the female spent with each male. This design also follows Aquiloni & Gherardi (2008a). Theoretically, the number of visits to a male crayfish should show the same trend as the amount of time spent with each male, as has been demonstrated in many animal models (Pogany *et al.*, 2018; Witte, 2006; Zinck & Lima, 2013).

Another factor to consider would be the chemical cues that influence the mating behavior of these crayfish species. As mentioned previously, there are many chemical cues that crayfish use to communicate amongst one another (Schneider *et al.*, 1999). These pheromones are responsible for communicating the locations of food sources, predators, or potential shelters, as well as determining the presence of a potential mate. The Female Preference experiment was largely unsuccessful in determining if there was a female preference for certain male phenotypes based on visual stimuli alone. The wandering behavior exhibited by females could be a result of the physical barriers keeping the crayfish from communicating chemically. My findings suggest chemical cues may be an essential component in mate choice by female *P. clarkii* and *F. virilis*.

Male Copulations and Copulation Attempts

Copulations were not frequent (Figure 6) 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 2 chelae were able to successfully copulate with females when compared to males with 0 and 1 chelae, respectively. A similar trend can be seen with the functionality treatments, functional males were more successful for *F. virilis*, and had the same amount of success as dysfunctional males for *P. clarkii*. This seems to indicate that there is a physical barrier, as opposed to a behavioral cue for crayfish copulation. This follows, in that male crayfish use their chelae to seize, turn, and hold females when attempting to achieve copulation (Stein, 1976). However, dysfunctional males were able to achieve copulations, indicating some level of female cooperation or choice.

Generally, males in peak physical condition (2 chelae present, functional chelae, and a large chelae-body size ratio) attempted copulation with female crayfish more frequently than males with the impaired traits. Male *P. clarkii* with functional chelae (Table 4; Figure 5), as well as those with the large chelae-body size ratio treatment, attempted copulation more frequently with female crayfish.

Males with chelae ablation were not able to achieve copulations although they attempted to do so (Figure 5). 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 and 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 (Figiel & Miller, 1995). 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, chelae loss, as I have demonstrated, likely impacts male mating success.

Chelae self-autotomy must have a high evolutionary significance in the Decapoda, as many individuals display this trait and can utilize the ability readily. Cleaver (1949) and Davis *et al.* (1978) were concerned with chelae autotomy in crabs, and determined that chelae removal impacted mortality rates, but did not greatly reduce life expectancies unless both chelae were autotomized. In other clades of the Kingdom Animalia, many organisms, ranging from other arthropods to lizards to mammals, display the same trait, often as an escape mechanism when dealing with potential predators (Bateman & Fleming, 2003; Bingham *et al.*, 2000; Juskaitis, 2005; Vervust *et al.*, 2011).

Previous research on the visual aspects of crayfish mating behavior have looked at chelae and body size almost exclusively (Aquiloni & Gherardi, 2008a), 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). Chelae-body size ratio was included as a male treatment to try and tease apart this theory, and to determine if body size matters as opposed to chelae size. According to my results, even though female *P. clarkii* did not visit males more often when they were of the large ratio treatment, statistically speaking, these males attempted copulation more often than the small ratio males. Therefore, males with larger chelae and smaller bodies attempted mating more than the opposite, which could point to more of a preference for larger chelae than larger bodies.

Finally, this study attempted to breakdown the overall crayfish mating system for these two species. Previous literature emphasizes the importance of female choice as the major driver of their mating system (Aquiloni & Gherardi*,* 2008b). However, this study concludes that male choice plays a significant role in the crayfish mating system. 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). If a female does not prefer the male, she has the option of escaping the male's hold and fleeing the immediate area. However, if the male is dominant and a larger individual, he will hold the female firmly enough to prevent her escape. Therefore, male choice also plays a governing role in the crayfish mating system.

Implications and Future Directions

Global Change Biology has become highly important to the scientific community over the last many decades, with much research focused on the drivers of change, and their impacts on native species and global biodiversity (Tylianakis *et al.*, 2008). Of these drivers, invasive species have been identified as one of the key causes of global change, resulting in the extirpation of native species from their native regions (Traill *et al.*, 2010).

In addition to the driving nature of invasive species, they are also a result of global change. As the planet undergoes a period of increased warming, non-native organisms are able to spread further into new ecosystems, filling any open niche, or outcompeting native species (Traill *et al.*, 2010). Therefore, invasive species can be thought of as a never-ending global feedback loop.

However, the largest contributor to this feedback loop would be human-introduced invasive species through a number of vectors including, bait trade, ornamental plants, pet trade, or as biological control agents (Foxcroft *et al.*, 2008; Nathan *et al.*, 2014; Nel *et al.*, 2004; Smith *et al.*, 2009). These vectors have devastating effects on native species and have rapidly led to the growing list of organisms of concern worldwide. For example, when introduced into new environments, the Louisiana crayfish has brought with it the crayfish plague, which has decimated native crayfish (Aquiloni *et al.*, 2011). The introduction of this species has also led to a crash in the rice market for multiple nations worldwide (Barbaresi & Gherardi, 2000; Souty-Grosset *et al.,* 2016). Therefore, research on the human-drivers and methods to control these drivers has become increasingly imperative in recent years.

To conclude, the majority of the data gathered in this study does not point to a preference in the female 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. It is also clear that there are some behavioral differences in the two crayfish species. However, more data on the mating system of these invasive crayfish, including chemical cues, long-term interactions, and any other extraneous factors, needed for the management and improved aquaculture of these organisms.

Finally, this research has created many new questions to focus on in the future. More research on the visual system of crayfish is imperative to fully understand these organisms, and to learn and create novel strategies for interacting with them in the future. Some areas of concern should include male preferences, different colorations, ectosymbiont effects, dominance and competition effects, extra pair mating, and the allocation of reproductive resources. Each of these ideas have become clear through the process of this study and could potentially have an influence on the crayfish mating system.

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VITA

Hayden Hays

Education

Master of Science – Biological Sciences **August 2019**
Sam Houston State University, Huntsville, TX
Thesis Title "Evaluating the effects of cheliped morphology on the mating behavior of two invasive crayfish, the Louisiana crayfish (*Procambarus clarkii*) (Girard, 1852) and the Virile crayfish (*Faxonius virilis*) (Hagen, 1870)"
Committee Sibyl Bucheli, Jerry L. Cook, Diane L. Neudorf (Chair and Advisor)

Bachelor of Science – Biological Sciences **December 2015**
Colorado State University, Fort Collins, CO

Research Interests

- Behavioral ecology, particularly mating systems and sexual selection
- Study and management of invasive species and their effects on the environment, and vice versa
- Use of new technologies in biological research, i.e. eDNA, distribution modelling, GIS, etc.
- Invertebrate zoology, mostly crustaceans, mollusks, and echinoderms
- Aquatic systems, including marine, wetlands, rivers, lakes, and streams
- Herpetology, primarily members of the Order Testudines

Research Experience

Graduate Research Assistant **2017 – 2018**
Animal Behavior Lab, Department of Biological Sciences, Sam Houston State University, Huntsville, TX

- Assistant to Dr. Diane L. Neudorf with ongoing research (8+ years) to determine nesting behavior/activity, reproductive success, and fledgling success across an urban-rural gradient in the Carolina wren (*Thryothorus ludovicianus*).
- Attended regular lab meetings and assisted with regular lab duties, gear maintenance, packing and moving of equipment and facilities, and data collection.

Graduate Research Assistant **2016 – 2017**
Aquatic Ecosystems Lab, Department of Biological Sciences, Sam Houston State University, Huntsville, TX

- Assistant to Dr. Jeffery Wozniak with ongoing research (4 years) to determine the habitat quality, Blue crab (*Callinectes sapidus*) distribution and abundance, and feeding behavior of the endangered Whooping crane (*Grus americana*) at the Aransas National Wildlife Refuge.
- Assisted with 2 Earthwatch trips, 1 adult session and 1 youth session (IGNITE). Trip duties included packing, loading, and unloading gear, operating university vehicles, vegetation surveys, water quality measurements, capture and cultivation of macroinvertebrates, wildlife identification, food preparation, and wilderness first aid.
- Assisted with an ECO-IMPACS (Enhancing Career Opportunities – Integrative Mathematical Program for Analyzing Coastal Systems) field trip that provided undergraduate students with an opportunity to collect water, vegetation, and soil samples.
- Organization, cleaning, and maintenance of lab space and equipment, including the research boat

Teaching Experience

Head Graduate Teaching Assistant **2018 – 2019**
Foundations of Science Lab, Department of Biological Sciences, Sam Houston State University, Huntsville, TX

- Taught 4 lab sections each semester, ranging from 2 in person labs and 2 online labs, to 3 in person labs and 1 online lab
- Mentored new lab instructors, both graduate and undergraduate
- Assisted in lab preparation meetings

Graduate Teaching Assistant **2016 – 2019**
Foundations of Science Lab, Department of Biological Sciences, Sam Houston State University, Huntsville, TX

- The course was designed to guide a non-science audience through the exploration of scientific principles and methods, distinguish the differentiation between science vs pseudoscience, and impart fundamental scientific knowledge
- Taught 3 or 4 lab sections each semester
- Attended regular lab preparation meetings

Assistant Editor and Subject Matter Consultant **2018**
Science for Citizens, National Blended Course Consortium, American Association of State Colleges and Universities, Washington, D.C.

- This project was a Teagle Foundation grant-funded initiative to create a blended course for a first-year undergraduate, non-science audience
- This course uses case studies and critical thinking-building skills to evaluate claims tied to medicine, environmental policy, UFOs, extra-sensory perception (ESP), and mysterious creatures like the Loch Ness Monster. Throughout the course, students are gradually introduced to scientific

concepts and processes, as well as the differentiation of science from pseudoscience

- Worked for the faculty fellow responsible for the program to design and implement 2 units: *Physics and the Paranormal* and *Evolution*

Camp Instructor **2008 – 2011**

Worth Ranch Boy Scout Camp, Boy Scouts of America, Palo Pinto, TX

- Responsible for educating boys between the ages of 11 and 18
- Topics of concern included bird study, environmental science, fish and wildlife management, fishing, forestry, geology, entomology, mammalogy, oceanography, botany, herpetology, soil and water conservation, and meteorology
- Assisted with daily operations of the camp

Other Relevant Experience

Field Station Technician **2018**

Center for Biological Field Studies, Department of Biological Sciences, Sam Houston State University, Huntsville, TX

- Assisted with the maintenance and care of field station equipment, facilities, and wildlife
- Coordinated and assisted in the completion of the 1st and 2nd annual departmental BioBlitz events. Worked at the ornithology, herpetology, and fish/aquatic invertebrate tables
- Aided with any ongoing research projects held at the Center for Biological Field Studies, some of which include, the capture and maintenance of Ground skinks (*Scincella lateralis*), construction and placement of bird feeders and bird boxes, setting mist nets and periodically checking them for various bird species, and the introduction and management of Louisiana crayfish (*Procambarus clarkii*).
- Assisted with facility security

Interpretive Specialist Intern **2015**

Region 2, Texas Parks and Wildlife Department – State Parks Division, Rockport, TX

- Presented interpretive programming to park visitors
- Produced interpretive programs based on the natural and cultural resources in the region
- Assisted with daily park maintenance
- Worked in the office answering questions, phone calls, and handling check-in procedures
- Assisted the Regional Natural Resources Specialist with natural resource management of various invasive species at Goose Island State Park
- Conducted and assisted with day camps at various parks, some of which include Goliad State Park, Goose Island State Park, Mustang Island State Park, and Lake Corpus Christi State Park

Research Awards & Grants

Student Research Grant, $1,000 **April 2018**
Society of Wetland Scientists

Student Research Grant Chapter Award, $750 **April 2018**
Society of Wetland Scientists, South Atlantic Chapter

3 Minute Thesis Research Award **March 2018**
The Graduate School, Sam Houston State University

Joey Harrison Student Research Award **December 2017**
Department of Biological Sciences, Sam Houston State University

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Publications

Couch, S., Gillespie, M., **Hays, H.**, Hill, J., Koether, S., Ludwig, M., Realzola, E., Rose, L., Rowe, M., & Shaffer, D. (In review). Star Trek and the Laws of Nature. In *Foundations of Science Laboratory Manual*. Huntsville, TX: Sam Houston State University.

Couch, S., Gillespie, M., **Hays, H.**, Hill, J., Koether, S., Ludwig, M., Realzola, E., Rose, L., Rowe, M., & Shaffer, D. (In review). The Haunting: Do the Laws of Nature Apply to Paranormal Phenomena? In *Foundations of Science Laboratory Manual*. Huntsville, TX: Sam Houston State University.

Koether, S., Jenkins, K., & **Hays, H.** (2018). Evolution. In *Science for Citizens*. San Francisco, CA: Smart Sparrow. Retrieved from https://www.blendedcourses.org/courses/science-for-citizens/

Koether, S., & **Hays, H.** (2018). Physics and the Paranormal. In *Science for Citizens*. San Francisco, CA: Smart Sparrow. Retrieved from https://www.blendedcourses.org/courses/science-for-citizens/

Research Presentations

Invited Presentations

2019, Cnidarians: An inventory of creatures of the Deep. **Hays, H.** Sam Houston State University, Huntsville, TX.

2018, Sexual selection, mating systems, and social behavior. **Hays, H.** Sam Houston State University, Huntsville, TX.

2017, Mating behavior and reproductive success as a function of cheliped autotomy in the Red swamp crayfish, *Procambarus clarkii*. **Hays, H.** Huntsville High School, Huntsville, TX.

Oral Presentations

2019, The effect of cheliped function on male mating success in the Louisiana crayfish, *Procambarus clarkii*. **Hays, H.** & Neudorf, D.L. Texas A&M University – Corpus Christi Student Research Forum.

2019, The effect of cheliped function and color on male mating success in the red swamp crayfish, *Procambarus clarkii*. **Hays, H.** & Neudorf, D.L. Texas Academy of Science.

2018, Mating behavior as a function of cheliped and color morphologies in the Red swamp crayfish, *Procambarus clarkii*. **Hays, H.** & Neudorf, D.L. Society of Wetland Scientists – South Central Chapter Fall Meeting.

2018, Mating behavior as a function of cheliped and color morphologies in the Red swamp crayfish, *Procambarus clarkii*. **Hays, H.** & Neudorf, D.L. Texas A&M University Ecological Integration Symposium.

2016, Determining density and distribution of Mudflat fiddler crabs (*Uca rapax*) in coastal salt marshes. **Hays, H.** & Wozniak, J.R. Texas A&M University – Corpus Christi Student Research Forum.

Poster Presentations

2017, Mating behavior as a function of cheliped autotomy in the Red swamp crayfish, *Procambarus clarkii*. **Hays, H.** & Neudorf, D.L. Texas A&M University – Corpus Christi Student Research Forum.

2017, Assessing periphyton dynamics Determining whole ecosystem metabolism in coastal saltwater ponds. **Hays, H.** & Wozniak, J.R. Texas Bays and Estuaries Meeting.

2016, Invasion of the Red king crab (*Paralithodes camtschaticus*). **Hays, H.** Colorado State University Marine Biology Student Poster Session.

Honors and Awards

Presidential Graduate Fellowship, nominated **2019**
Department of Natural Resources Management, Texas Tech University, Lubbock, TX

Outstanding Graduate Student Award **2019**
The Graduate School, Sam Houston State University, Huntsville, TX

Outstanding Graduate/Doctoral Student Sammy Award **2019**
Sam Houston State University, Huntsville, TX

Outstanding Graduate Student Award, nominated **2019**
Department of Biological Sciences, Sam Houston State University, Huntsville, TX

Outstanding Assistantship Award, finalist **2019**
The Graduate School, Sam Houston State University, Huntsville, TX

Outstanding Student Sammy Award, nominated **2019**
Sam Houston State University, Huntsville, TX

Student Presentation Award, $600 **2018**
Society of Wetland Scientists – South Central Chapter, Madison, WI

The Graduate School General Scholarship, applied **2018**
The Graduate School, Sam Houston State University, Huntsville, TX

Graduate Achievement Scholarship, applied **2018**
College of Science and Engineering Technology, Sam Houston State University, Huntsville, TX

Eagle Scout Rank Award **2008**
Troop 214, Boy Scouts of America, Haslet, TX

Professional Affiliations

Animal Behavior Society **2018 – present**

International Association of Astacology **2018 – present**

The Crustacean Society **2018 – present**

Society of Wetland Scientists – South Central Chapter **2017 – present**

Ecological Society of America **2017 – present**

Texas Academy of Science **2016 – present**

International Phycological Society **2017**

Extracurricular Activities

Honorary Executive Board Member, Food Pantry **2018 – 2019**
College of Health Sciences, Sam Houston State University, Huntsville, TX

Member, Chi Alpha Campus Ministry **2018 – 2019**
Sam Houston State University, Huntsville, TX

Webmaster, BSGSO **2018 – 2019**
Department of Biological Sciences, Sam Houston State University, Huntsville, TX

Member, BSGSO **2016 – 2019**
Department of Biological Sciences, Sam Houston State University, Huntsville, TX

Community Engagement and Outreach

Food Distributions, Food Pantry **2018 – 2019**
College of Health Sciences, Sam Houston State University, Huntsville, TX

Feed the Growl Volunteer, Food Pantry **2018**
College of Health Sciences, Sam Houston State University, Huntsville, TX

Hunger and Homeless Awareness Week Volunteer, Food Pantry **2018**
College of Health Sciences, Sam Houston State University, Huntsville, TX

Big Thicket Trash Clearing, BSGSO **2018**
Big Thicket National Preserve, Kountze, TX

ForestFest Outreach, Huntsville State Park **2018**
Texas Parks and Wildlife Department, Huntsville, TX

Outreach Presenter, BSGSO **2017**
Huntsville High School, Huntsville, TX