

Honest and Dishonest Communication in Crayfish

by

Zackary Graham

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved March 2021 by the
Graduate Supervisory Committee:

Michael Angilletta, Chair
Emilia Martins
Kevin McGraw
Stephen Pratt
Robbie Wilson

ARIZONA STATE UNIVERSITY

May 2021

ABSTRACT

Despite theoretical models predicting that signals should only evolve if they convey honest information, dishonest signals may persist. Interestingly, crustaceans have been crucial in furthering biologists understanding of how and why dishonest signals persist; because many crustaceans wield claws that function as dishonest signals. For example, male fiddler crabs have claws that grow to large sizes but are incapable of inflicting severe damage to opponents, thus acting as a dishonest signal of their strength. Although initial work suggested that dishonest signaling was common throughout Crustacea, biologists understanding of the generality of dishonest communication is lacking. To resolve these issues, I combined morphological, behavioral, and comparative studies to investigate whether crayfish engage in dishonest communication. First, I found that regenerated claws in virile crayfish (*Faxonius virilis*) produce 40% weaker pinching forces compared to original claws. These results suggest that claw regeneration in crayfish may be the functional mechanism that produces dishonest signals. Second, I conducted two studies that investigated what traits determine dominance in staged contests; one on intrasexual contests in both male and female *F. virilis*, and a second between intra- and interspecific contests between male *F. virilis* and male red swamp crayfish (*Procambarus clarkii*). In both studies, I did not find support the hypothesis that large but weak claws function as dishonest signals; because claw size did not predict the outcome of signaling interactions and claw strength did not predict the outcome of physical fights. Lastly, I conducted a comparative study between six species of crayfish — three stream-dwelling species that use their claws as weapons and signals, and three burrowing species that use their claws for excavating burrows. Despite all six species

possessing claws that unreliably predicted claw strength, I found no support for the hypothesis that their claws function as dishonest signals in any of these species. Thus, my dissertation results suggest that despite having claws that unreliably predict their strength, such unreliable signals do not equate to dishonest signals. Altogether, my work highlights the importance of collecting behavioral data in studies of dishonest communication and stresses the importance of separating unreliable signals from dishonest signals.

ACKNOWLEDGMENTS

I want to first thank my advisor, Mike, for giving me the freedom to choose the research that most interested me. After pursuing projects across several disciplines, I am grateful for all of your encouragement when I finally found my passion for crayfish. Thank you for always being understanding of our personality differences and making me laugh through my nervousness.

I want to thank my colleagues and committee members for supplying feedback and guidance throughout my dissertation. Emilia, Kevin, and Stephen have all pushed me to think about my research from different angles which has made me a better scientist.

Thank you, Robbie, for all of the encouragement to continue working with crustaceans. Although I was unable to visit Australia during my time at ASU, I hope that I will be able to visit one day. I would also like to thank John VandenBrooks and Jeff Dawson for help getting the force transducer. I especially want to thank Alex Palaoro for being an amazing friend and collaborator over the past few years. Meeting Alex was a turning point in my academic career, and I owe a lot of my accomplishments to his willingness to help me with anything I needed.

I want to thank my parents, Al and Maryann, who have always trusted my decisions and supported everything I wanted in life. My original love for nature (and crayfish) came about during our yearly cabin-camping trips. My parents also supported my research by allowing me to use their cars and garage for two summers of research. I would not have been able to do this work without their support.

I additionally want to thank the infrastructure at ASU that has provided me with emotional and financial support. Thank you RTI and GPSA for funding several of my projects for my dissertation. I also want to thank SOLS for funding two semesters of my completion fellowship which has allowed me to focus on writing in my final year. I would also like to thank all of my undergraduate researchers for helping me throughout the years. Mentoring you all has made this experience so much more rewarding.

Lastly, I want to thank my lab members, Jake, Lauren, and Dylan. Whether we are at happy hour, on a camping trip, or just chit-chatting in the office, I will forever be grateful for our relationships. I want to thank Jake for the memories of our first-year shenanigans and for always letting me talk through my thoughts. I want to thank Lauren for making me laugh and giving me the opportunity to travel and collect lizards. I want to thank Dylan for his friendship and our daily walks around campus. You are all my lifelong friends and the primary reason I was able to maintain my sanity throughout the past 5 years.

TABLE OF CONTENTS

	Page
LIST OF TABLES	vii
LIST OF FIGURES	viii
CHAPTER	
1 INTRODUCTION	1
Study Species	7
Dissertation Overview	8
2 REGENERATED CRAYFISH CLAWS GENERATE WEAKER PINCHING FORCES COMPARED TO ORIGINAL CLAWS	12
Introduction	13
Methods.....	18
Results.....	21
Discussion	22
3 VIRILE CRAYFISH ESCALATE AGGRESSION ACCORDING TO BODY SIZE INSTEAD OF WEAPON SIZE.....	34
Introduction	35
Methods.....	38
Results.....	42
Discussion	43
4 CLAW SIZE PREDICTS DOMINANCE WITHIN AND BETWEEN INVASIVE SPECIES OF CRAYFISH.....	57
Introduction	58

CHAPTER	Page
Methods.....	62
Results.....	68
Discussion.....	69
5 DISTINGUISHING DISHONEST SIGNALS FROM UNRELIABLE SIGNALS: A COMPARATIVE STUDY OF AGGRESSIVE COMMUNICATION IN CRAYFISH.....	80
Introduction.....	81
Methods.....	85
Results.....	92
Discussion.....	93
6 CONCLUSION AND FUTURE DIRECTIONS.....	103
REFERENCES	108
APPENDIX	
A SUPPLEMENTAL MATERIAL	122

LIST OF TABLES

Table	Page
3.1 Principal Components of Claw Size	49
3.2 Probability of Engaging in Aggression.....	50
3.3 Probability of Achieving Dominance	51
5.1 Covariation in Claw Size and Claw Strength	98

LIST OF FIGURES

Figure	Page
1.1 Picture of Crayfish Fighting.....	11
2.1 Picture of Regenerated and Original Crayfish Claws	30
2.2 Original and Regenerated Size Versus Claw Strength.....	31
2.3 Variation in Original and Regenerated Claw Strength.....	32
2.4 Original Claw and Regenerated Claw Muscle Mass.....	33
3.1 Diagram of Linear Claw Measurements.....	53
3.2 Repeatability of Claw Strength.....	54
3.3 Size and Strength in Male and Female Virile Crayfish.....	55
3.4 Probability of Engaging in Aggression.....	56
3.5 Probability of Achieving Dominance	57
4.1 Diagram of Linear Claw Measurements.....	76
4.2 Principal Component Plot	77
4.3 Size and Strength in Male Red Swamp and Virile Crayfish.....	78
4.4 Probability of Engaging in Aggression.....	79
4.5 Probability of Achieving Dominance	80
5.1 Variation in Claw Size and Claw Strength.....	99
5.2 Repeatability of Claw Strength.....	100
5.3 Residual Variation in Max Pinching Strength.....	101
5.4 Escalating Aggression and Achieving Dominance	102
5.5 Theoretical Framework	103

CHAPTER 1

INTRODUCTION

Animals communicate to each other using signals, which are behaviors or structures that have evolved to convey information to receivers (Rendall, Owren, & Ryan, 2009). Communication occurs through various modalities, including visual, acoustic, and chemical (Bradbury & Vehrencamp, 2011). Generally, it is assumed that receivers will attend to signalers because their signals convey reliable or honest information. Thus, substantial research on animal communication revolves around unearthing whether communication between individuals conveys honest or dishonest information (Searcy & Nowicki, 2005). In many cases, signalers honestly communicate information to receivers. For example, male collared lizards (*Crotaphytus sp.*) engage in displays of their conspicuous jaw-adductor muscles. Collared lizards with larger jaw muscles signal their fighting ability to conspecifics because larger jaw muscles correlate to greater bite forces, which predicts dominance in combat (Husak, Lappin, Fox, & Lemos-Espinal, 2006; Lappin, Brandt, Husak, Macedonia, & Kemp, 2006). In this way, the strong correlation between jaw-adductor muscle size and bite force represents an honest system of communication. Despite the ubiquity of honest communication among animals, dishonest signals are present in many systems of communication (Searcy & Nowicki, 2005; Wilson & Angilletta, 2015).

Distinguishing honest signals from dishonest signals is difficult because dishonest signals are designed to go undetected. According to Hughes (2000), researchers should calculate the relative honesty of a signal from the relationship between the magnitude of an

animal's signal and the underlying quality being signaled. The residual variation from this signal-quality relationship indicates the relative honesty (or dishonesty) of a signal in a population. Within this "signal residuals" framework, small signal residuals (and a tight correlation between signal size/magnitude and quality) represent an honest system of communication, whereas large signal residuals (and a poor correlation between signal size/magnitude and quality) represent a potentially dishonest system of communication (Hughes, 2000). For example, in territorial contests, male fig wasps (*Philotrypesis* sp.) communicate fighting ability by displaying their mandibles (Moore, Obbard, Reuter, West, & Cook, 2009). *Philotrypesis* with large mandibles can inflict severe damage onto opponents. Interestingly, there is an atypical male phenotype of *Philotrypesis* that has significantly larger mandibles for a given body size than a typical male. Atypical male *Philotrypesis* jaws are 50% larger than expected for their body size and these males primarily engage in pre-contest signaling, where they can signal their mandible size to smaller opponents. Importantly, when atypical males do engage in combat, they perform poorly and incur more injuries than a typical male. Thus, it has been proposed that atypical males of *Philotrypesis* produce large mandibles as a dishonest signal that dissuades opponents from contesting resources (Moore et al., 2009). The disassociation between the magnitude of the signal (i.e. mandible size) and the underlying ability (or quality) of the signaler (i.e. fighting ability) enables dishonest signaling to occur in this system; in other words, atypical males with negative signal residuals produce a large jaw but perform poorly in fights. Importantly, despite performing poorly when a contest escalates to combat, atypical male *Philotrypesis* can win contests through the dishonest signaling of their mandibles without having to engage in combat (J. Moore et al., 2009).

Interestingly, crustacean systems have been lucrative in furthering our understanding of dishonest communication (Wilson & Angilletta, 2015). The earliest example reported was the bluffing behavior of mantis shrimp (*Gonodactylus bredini*). Like all crustaceans, growth in *G. bredini* is a stepwise process that occurs during a molt. Immediately after a molt, the exoskeleton of a mantis shrimp is soft, and the shrimp faces an elevated predation risk or chance of injury from a fight. Because the exoskeleton has not yet hardened after a molt, mantis shrimp are unable to inflict damage with their hammer-like raptorial appendages. Despite this limitation, these shrimp exhibit their most aggressive behaviors after a molt. For example, when defending a burrow, a recently molted *G. bredini* will feverishly chase competitors and use meral spreads (an aggressive display) as they behave hyper-aggressively despite not being able to inflict damage (Steger & Caldwell, 1983). Deceptive behavior in mantis shrimp is a textbook example of dishonest communication and has even inspired game theorists to model how this behavior remains evolutionary stable (Searcy & Nowicki, 2005). Because the cost of aggression in mantis shrimp is high, the cost of incorrectly calling a signaler's bluff discourages receivers from detecting a dishonest signal. Consequently, the best strategy might be to assume all conspecifics are capable of inflicting damage (Adams & Mesterton-Gibbons, 1995; Számádó, 2000). Furthermore, the dissociation between signaling behavior (i.e. displaying a meral spread) and the quality of the individual (i.e. being able to inflict damage) suggests that dishonest signaling occurs in this species. Moreover, dishonest signaling in mantis shrimp demonstrates a key tenet of signaling theory. That is, if a relatively small portion of individuals within a population signal dishonestly, dishonesty

can remain evolutionarily stable (Searcy & Nowicki, 2005). However, despite the theory predicting that signals should be honest on average, some species exhibit surprisingly high levels of dishonest communication.

Decapod crustaceans such as fiddler crabs use dishonesty when defending territories. Male fiddler crabs (*Uca* sp.) use their enlarged claws as signals during both mate attraction and male-male combat (Callander, Kahn, Maricic, Jennions, & Backwell, 2013; Dennenmoser & Christy, 2013). Male fiddler crabs with larger claws are more likely to win territorial contests and attract mates compared to small-clawed fiddler crabs (Shackleton, Jennions, & Hunt, 2005). Interestingly, the size of a male fiddler crabs' claw is not always an honest indicator of the strength of the claw (Backwell, Christy, Telford, Jennions, & Passmore, 2000). Injuries are common during male-male combat and many fiddler crabs end up autotomizing their enlarged claw. Luckily, like all crustaceans, male fiddler crab claws can regenerate their claws back. However, when they regenerate a claw, they primarily invest energy into the size of the claw, and relatively little energy into claw muscle (Bywater, Seebacher, & Wilson, 2015; Bywater, White, & Wilson, 2014). Although a strong claw gives advantages when fighting occurs, many of the encounters settle through pre-fight communication. Therefore, possessing a large but weak claw enables fiddler crabs to reap the benefits of possessing a large claw, but not invest the metabolic energy that a strong claw requires. Surprisingly, fiddler crab populations can possess up to 40% of males possessing regenerated (large but weak) claws, which demonstrates one of the most common uses of dishonest signaling in a natural population (Backwell et al., 2000; Bywater & Wilson, 2012). Indeed, high rates of

dishonesty observed in natural population has been supported by theoretical models demonstrating that cheaters can be extremely common under specific conditions (Szalai & Számadó, 2009a); such as when the cost of detecting a dishonest signal is high or if the cost of developing a dishonest signal is low.

Additionally, certain crayfish species have been found to deceive opponents during aggressive encounters (Angilletta & Wilson, 2012; Wilson, Angilletta, James, Navas, & Seebacher, 2007). Specifically, although both male and female *Cherax dispar* and *Cherax destructor* have enlarged claws that they use during aggression, only males are known to engage in dishonest communication (Bywater, Angilletta, & Wilson, 2008; Walter, van Uitregt, & Wilson, 2011). In *Cherax*, compared to male claws, female claws are honest signals of their underlying claw strength (Bywater et al., 2008). Interestingly, although all crayfish can regenerate their claws, it is unknown whether or not dishonest communication is linked to claw regeneration like it is in fiddler crabs. During the initiation of a contest, male *C. dispar* confront opponents cautiously and whip each other with their antenna to assess their opponent. If both opponents are of similar size, the next stage of the contest will ensue. This stage involves opponents taking turns tapping their opponents' claws. In only a small portion of encounters when opponents are close in size will direct physical combat ensue (Seebacher & Wilson, 2007). Most aggressive encounters settle without incurring costs through fighting. During the early stages of a fight, relative claw size determines dominance (Wilson et al., 2007). Thus, dishonest slender crayfish with large claws signal their fighting ability to other crayfish without suffering from costs of actual fighting. However, when physical fighting occurs, relative

claw strength is the best predictor of who obtains dominance (Wilson et al., 2007). Thus, some crayfish species effectively build and maintain metabolically cheap claws with relatively little muscle underneath that presumably function as dishonest signals of their strength. Although dishonest signaling has been suggested to occur in every crayfish species which has been investigated thus far (*Cherax dispar*, *Cherax destructor*, *Faxonius palmeri longimanus*, *Procambarus clarkii*), only the *Cherax* species have been confirmed to engage in dishonesty with the support of behavioral trials (Malavé, Styga, & Clotfelter, 2017; Robinson & Gifford, 2019; Walter et al., 2011; Wilson et al., 2007). In *F. palmeri longimanus* and *P. clarkii*, the poor relationship between claw size and claw strength was used as the sole evidence for dishonesty to occur in these species.

To date, much of the research on crustacean dishonesty has focused on the mechanisms and consequences of possessing a large but weak claw (Wilson & Angilletta, 2015). A majority of this work has focused on fiddler crabs because they are a tractable system for field and laboratory-based tests. However, despite crustaceans serving as an important empirical system to study dishonest signaling, our understanding of the taxonomic diversity of dishonest signals throughout crustaceans is lacking; dishonest signals have only been thoroughly investigated in two species of crayfish and half a dozen species of fiddler crabs (Wilson & Angilletta, 2015). Furthermore, researchers have begun to assume that the unreliable correlation between claw size and claw strength (i.e. negative values of signal residuals) can be used as evidence for dishonest signaling, without conducting behavioral observations (Malavé et al., 2017; Robinson & Gifford, 2019). Although some studies present high degrees of variation between a signal (i.e. claw size)

and the underlying quality being signaled (i.e. claw strength), this does not necessarily mean that these signals are used in dishonest communication. Such assumptions that unreliable signaling equates to dishonest signaling are pervasive throughout the field of animal communication – not just within studies of crustaceans (Carazo & Font, 2010, 2013; Caro, West, & Griffin, 2016). Importantly, the behavior of the signal (i.e. claw size) and the underlying quality that is being signaled (i.e. claw strength) must be tested in social contexts to determine whether or not dishonest communication is present. To resolve these issues, my dissertation research focuses on assessing the relative honesty (or dishonesty) of signals of strength throughout several species of crustaceans. Specifically, I first determine whether claw regeneration is the functional mechanism that produces large but weak claws (and potentially dishonest signals) in crayfish, like it does in fiddler crabs. Next, I test whether or not variation in claw size and claw strength in several different species of crayfish are used as dishonest signals by conducting behavioral trials. Thus, my work sheds light on the taxonomic diversity of signal honesty (or dishonesty) in crustaceans through studies of morphology, performance, and behavior in eight crayfish species.

Study Species

Freshwater crayfish are known for their aggressive territorial behaviors (Fig 1.1). They wield enlarged claws that function as signals during competition as they will engage in repeated bouts of claw displays and claw interlocking to resolve a contest (Moore, 2007). In chapter 2, chapter 3, and chapter 4, I studied the virile crayfish (*Faxonius virilis*), which is an invasive species throughout Arizona as it was introduced from the

Northeastern United States in the 1960s. Additionally, in chapter 4, I also studied the red swamp crayfish (*Procambarus clarkii*), which is another Arizona invasive species known for its aggressive behavior. Lastly, in Chapter 5, I studied three species of stream-dwelling crayfish (*Cambarus carinirostris*, *Cambarus robustus*, and *Faxonius obscurus*) and three species of burrowing crayfish (*Cambarus dubius*, *Cambarus monongalensis*, and *Lacunicambarus thomai*) from Pennsylvania and West Virginia. Because crayfish are easy to collect and will readily engage in aggression in a laboratory environment, this makes them an ideal study organism to investigate questions related to relative honesty (or dishonesty) of their claws as signals.

Dissertation Overview

My dissertation aimed to investigate whether a system of dishonest signaling is present throughout several species of North American crayfish species. To do so, I conducted morphological, behavioral, and comparative studies that investigated whether crayfish claws function as dishonest signals of strength in territorial interactions.

In chapter 2, I examined how claw regeneration in male and female *F. virilis* influences crayfish claw strength. In this study, I measured the maximum pinching strength from over 300 crayfish as well as dissected their closer claw muscle tissue. Then, I recorded whether or not each crayfish claw was a regenerated or original claw and analyzed how muscle mass and claw strength was affected by regeneration. This work clarifies whether claw regeneration is the functional mechanism that creates large but weak (and potentially dishonesty) claws in crayfish, as it does in fiddler crabs.

In chapter 3, I examined how claw size and claw strength influenced the outcome of aggression between same-sex territorial encounters in male and female *F. virilis*. To do so, I collected morphology and performance data from 124 *F. virilis* and then conducted 97 staged territorial encounters. Because not all aggressive interactions resulted in direct physical fighting, I was able to determine what traits best predict the outcome of contests that were settled through signaling and which traits best predict the outcome of contests that were settled through direct physical combat. I used the results of this study to interpret whether male and female *F. virilis* use their claws as honest or dishonest signals of strength in intrasexual contests.

In chapter 4, I examined how claw size, claw strength, and body size influenced the outcome of intra- and interspecific male-male contests between two invasive species of crayfish, *F. virilis*, and *P. clarkii*. This chapter served as a follow up to the result in chapter 3, as I wanted to collect more data and conduct fights from another species. Thus, I collected morphology and performance data on 44 male *F. virilis* and 40 male *P. clarkii*. Then, I conducted staged territorial interactions within and between these two species between opponents that spanned a range of sizes to ensure I collected data on encounters that ended in signaling interactions and encounters that ended in physical combat. In total, this resulted in 160 encounters. I used the results of this study to provide evidence for whether male *F. virilis* and *P. clarkii* use their claws as honest or dishonest signals of strength in contests within and between species.

In chapter 5, I examined how divergence in function between burrowing crayfish claws and stream-dwelling crayfish claws has influenced the variation in the size and strength of their claws. To do so, I collected over 300 male and female crayfish spanning 6 species; three of which are burrowing crayfish (*C. dubius*, *C. monongalensis*, and *L. thomai*) and three of which are stream-dwelling species (*C. carinirostris*, *C. robustus*, and *F. obscurus*). Then, I collected data on the size and strength of their claws for all six species to determine whether or not variation was inherent to the relationship of claw size and claw strength, or whether such variation would only be present in stream-dwelling species, thus potentially serving as a dishonest signal. Next, to understand how claw size, claw strength, and body size determine the outcome of signaling interactions and interactions that escalated to combat, I staged 150 encounters: 40 encounters between males of *C. robustus*, 50 encounters between males of *C. carinirostris*, and 60 encounters between males of *F. obscurus*. These methods allowed me to determine whether the claws of these three stream-dwelling species are used as dishonest signals of strength during male-male competition.



Fig 1.1. Image depicting the ritualized contest behavior between two male virile crayfish, *Faxonius virilis*.

CHAPTER 2

REGENERATED CRAYFISH CLAWS GENERATE WEAKER PINCHING FORCES COMPARED TO ORIGINAL CLAWS

Abstract

Many crustaceans possess enlarged and exaggerated claws (i.e. chelae) that are used as both weapons and signals during aggression. Because these claws are used directly in combat, injuries can occur, and the claws can be autotomized and later regenerated. Interestingly, original claws and regenerated claws often differ in both their external morphology and internal musculature. Regenerated claws can reach sizes similar to that of original claws but lack many features that enable them to function as weapons (i.e. muscle mass and tubercles). Furthermore, regenerated (large but weak) claws in crustaceans might serve as dishonest signals, such that individuals with regenerated claws deceptively signal their fighting ability to competitors. Here, I investigate the relationship between claw size and claw strength among virile crayfish, *Faxonius virilis*, to determine how regeneration influences maximal pinching force. Similar to other crustaceans, a regenerated claw of a virile crayfish could regrow to the size of an original claw. However, regenerated claws were weaker than the original claws (36% and 40% weaker in males and females, respectively). Despite the weakness of regenerated claws, I caution not to interpret this pattern as evidence for dishonest signaling without knowing that claw size determines social dominance. Overall, I present the first evidence of how claw regeneration influences pinching performance in crayfish and discuss how these findings relate to the literature on dishonest communication in crustaceans.

Introduction

Sexual selection is credited for the evolution of weaponry that has evolved to increase fighting success and thus gain access to territories, food, and mates (Andersson, 1994; Emlen, 2008). Generally, selection favors weapons that are large and strong compared to weapons that are small and weak; because large and strong weapons enable competitors to manipulate their opponent and monopolize resources. Indeed, weapon size is a strong predictor of the outcome of territorial contests in many taxa (Pinto, Palaoro, & Peixoto, 2019; Vieira & Peixoto, 2013). Further, because contests often involve the potential for weaponry to inflict damage, having a weapon that can produce strong forces capable of damaging opponents can also increase fight success. For example, powerful bite forces produced by lizards and strong claw pinches from crustaceans predict the outcome of contests in these taxa (Hall, McLaren, Brooks, & Lailvaux, 2010; Husak, Lappin, & Bussche, 2009; Sneddon, Huntingford, Taylor, & Orr, 2000a). Thus, wielding a large and powerful weapon confers a clear benefit during competition.

Because weapons have evolved to manipulate opponents and inflict damage during contests, injuries are common in weapon-wielding species (Emlen, 2008; Palaoro & Briffa, 2017). Injury severity occurs across a spectrum; ranging from minor bodily scratches to appendages being partially or completely severed (Candaten, Possenti, Mainardi, Carvalho, & Palaoro, 2020; Emberts, Escalante, & Bateman, 2019; Green & Patek, 2015; Umlers, Tatarnic, Holwell, & Herberstein, 2012). Unsurprisingly, enduring damage can decrease fighting success and mating opportunities. For example, in the hindleg weaponry of male coreid bugs, up to 21.5% of individuals within a population

have missing hindlegs that they have autotomized (Emberts, St Mary, & Miller, 2016). Because male coreid hindlegs are weapons used to exert pinching forces onto opponents during combat, the loss of a hindleg significantly reduces the autotomized individual's fighting ability. In staged laboratory trials, Emberts et al. (2018) found that a male *Narnia femorata* with an autotomized hindleg was 5 times less likely to establish dominance in territorial contests compared to a male with both of its hindlegs intact (Emberts, St. Mary, Herrington, & Miller, 2018). In coreids, because weapon autotomy is permanent, the consequences of losing a weapon can significantly alter an individual's fitness. Similarly, in contests between jumping spiders (*Trite planiceps*), individuals missing forelegs are less likely to win territorial encounters compared to individuals with both forelegs intact (Taylor & Jackson, 2003).

In contrast to the permanent loss of a weapon, other taxa can regenerate and regrow their weapons. Specifically, many crustaceans wield enlarged frontal chelae (hereafter referred to as claws) that are used as weapons and signals during aggression (Mariappan, Balasundaram, & Schmitz, 2000; P. Moore, 2007). Further, the regenerative abilities of crustaceans have fascinated biologists for centuries (Hopkins & Das, 2015). Following injury from aggression, predation events, or a molting error, crustaceans can autotomize and subsequently regenerate their claws throughout subsequent molts (Skinner, 1985; Smith, 1990). Interestingly, regenerated claws often differ in both their external morphology and their internal musculature (Fig 2.1). Regenerated claws will grow back to the expected size of their original claw, although the shape of the regenerated claw is different (Brock & Smith, 1998; Skinner, 1985; Smith, 1990; Yasuda, Matsuo, & Wada,

2014). Original claws are characterized by a large and robust manus, which is the area in which the claw's closer muscle is found. Greater area for muscle enables individuals with original claws to generate strong pinching forces (McLain & Pratt, 2011). Conversely, regenerated claws have a smaller manus and relatively longer fingers (i.e. pollex and dactyl; McLain & Pratt, 2011; Rosenberg, 2002). Further, in original claws, there are often several enlarged calcified bumps (i.e. tubercles) on the inner margins of the pinching fingers. Tubercles increase a claw's biomechanical efficiency (Dennenmoser & Christy, 2013; McLain & Pratt, 2011; Rosenberg, 2002). By contrast, regenerated claws often lack tubercles entirely. Together, a smaller manus (with less space for muscle) and a lack of tubercles in regenerated claws relates to weaker pinching forces being generated in species that have been investigated (Brock & Smith, 1998; Bywater et al., 2015). For example, regenerated fiddler crab claws (*Uca sp.*) will return to the normal size of the autotomized original claws, although regenerated claws are two to three times weaker for a given size compared to an original claw (Bywater et al., 2015; Lailvaux, Herrel, VanHooydonck, Meyers, & Irschick, 2004; Lailvaux, Reaney, & Backwell, 2009; McLain, McBrayer, Pratt, & Moore, 2010). Moreover, because pinching strength influences the outcome of male-male competition in fiddler crabs, individuals with regenerated claws suffer from reduced fighting ability (Backwell et al., 2000; Lailvaux et al., 2009; McLain et al., 2010).

Interestingly, because regenerated claws grow back to the expected size of an original claw, but suffer from reduced pinching performance, some crustacean species with regenerated claws use them as dishonest signals of their fighting ability. For example,

male fiddler crabs use their enlarged claws as signals during both mate attraction and male-male combat (Callander, Kahn, Maricic, Jennions, & Backwell, 2013; Dennenmoser & Christy, 2013). Large-clawed fiddler crabs are more likely to win territorial contests and attract mates compared to small-clawed fiddler crabs (Shackleton et al., 2005). But the size of a male fiddler crabs' claw is not always an honest indicator of the strength of the claw (Backwell et al., 2000); because individuals with regenerated claws primarily invest energy into the size of the claw and relatively little energy into their claw muscle (Bywater, Seebacher, & Wilson, 2015; Bywater, White, & Wilson, 2014). Although a strong claw gives advantages when fighting occurs, many of the encounters settle through pre-fight communication. Therefore, possessing a regenerated (large but weak) claw enables fiddler crabs to reap the benefits of possessing a large claw, but not invest the metabolic energy that a strong claw requires (Bywater et al., 2014). In fiddler crabs, dishonest signaling is clearly linked to claw regeneration, because individuals with regenerated claws can deceive opponents into behaving as if they have a strong claw, but they actually have a weak claw.

Similarly, crayfish deceive opponents during aggressive encounters (Angilletta & Wilson, 2012; Wilson et al., 2007). However, despite several species of crayfish supposedly engaging in a similar system of dishonest communication (Malavé et al., 2017; Robinson & Gifford, 2019; Walter et al., 2011; Wilson et al., 2007), it is unknown whether or not dishonest signaling is linked to claw regeneration in crayfish. Further, in the crayfish species that have been studied, it has been suggested that only males are known to engage in dishonest communication, despite females also having enlarged

claws (Bywater et al., 2008; Malavé et al., 2017; Robinson & Gifford, 2019; Walter et al., 2011). Compared to male claws, female claws are honest signals of the underlying claw strength (Bywater et al., 2008; Robinson & Gifford, 2019; Wilson, James, Bywater, & Seebacher, 2009). In crayfish, it is unknown how claw regeneration influences pinching strength in both sexes and whether claw regeneration is a functional mechanism that produces dishonest signaling.

Here, I investigate how claw regeneration influences the claw strength of male and female virile crayfish, *Faxonius virilis*. Virile crayfish are highly aggressive and both males and females engage in repeated bouts of claw interlocking and claw pinching to determine the outcome of aggression (Graham & Angilletta, 2020; Graham, Padilla-Perez, & Angilletta, 2020). Previous studies have found that there is substantial variation in the size and strength of virile crayfish claws, but the role of claw regeneration was not accounted for (Graham, Padilla-Perez, et al., 2020). Like previously studied crustaceans, I predict that regenerated claws will have relatively weaker pinching strengths compared to an original claw. Moreover, because claw regeneration in crustaceans has been found to play a role in dishonest signaling, my investigation will shed light on whether or not regenerated crayfish claws may be a mechanism that produces dishonest signals of strength. Thus, I calculated size-adjusted strength values for regenerated and original crayfish claws. Strength for a given size (i.e. residual strength) values have been used in crustaceans to identify whether these claws function as dishonest signals (Hughes, 2000; Malavé et al., 2017; Robinson & Gifford, 2019). Residual strength values come from regressing the performance (claw strength) of an individual against the magnitude or size

of their signals (claw size). Thus, if regenerated claws have the potential to function as dishonest signals as they do in other crustacean species, I expect original claws to have greater residuals strength values compared to regenerated claws. That is, I predict that within this population, the expected strength for a given size will be greater in original claws compared to regenerated claws.

Methods

Collection and husbandry of animals

I collected 183 male and 184 female virile crayfish with baited traps from Saguaro Lake, Arizona, U.S.A from March to October 2019. Upon capture, I transported crayfish to a laboratory at Arizona State University. While in the laboratory, I housed each animal in an individual plastic aquarium (20 x 14 x 10 cm) filled with dechlorinated water. Water was dechlorinated using a commercial dechlorinating agent. The A 12:12 h light:dark cycle was in effect throughout the experiment. I acclimated all crayfish to the laboratory conditions for a minimum of 4 days before data collection. All crayfish were intermolt and had fully regrown left and right claws. Individuals with claws that were autotomized or partially regenerated were not included in the study.

Morphology and claw strength measurements

For each crayfish, I quantified the claw length using digital calipers. Claw length was determined from the enlarged tubercle on the heel of the manus to the tip of the pollex. Claw length is used as a proxy of overall claw size throughout this study. Additionally, I identified whether each claw was original or regenerated based on visual observation of

several claw characteristics: including the presence/absence of tubercles, the area of the manus, as well as the ratio of the mesial margin of the palm relative to the dactyl length (Fig 2.1; Brock & Smith, 1998; McLain & Pratt, 2011). Further, to measure claw pinching strength, I used a custom-built force transducer designed to estimate the forces applied to a set of metal plates (see Robinson & Gifford, 2019 for information on the force transducer). I measured the maximal pinching strength of each claw by introducing each crayfish claw to the force plates and allowing each claw to pinch the plates three to five times (Graham & Angilletta, 2020; Wilson et al., 2007). Importantly, studies have shown that claw force measurements from this device are highly repeatable across days (Graham & Angilletta, 2020; Graham, Padilla-Perez, et al., 2020). Following the collection of pinching strength data, I froze each crayfish to later collect data on each claw's closer muscle mass (i.e. the chela closer muscle which is used during pinching; Schenk & Wainwright, 2001). To do so, I thawed individual claws of each crayfish and then the entire closer claw muscle was dissected out of the claw and the muscle's wet weight was recorded using a digital balance.

Statistical analysis

I wanted to test how claw regeneration status and sex influences the maximal pinching strength of male and female *F. virilis* claws. To do so, I fit several general linear mixed models to the data using the lme4 library of the R Statistical Package (Bates, 2010). First, I fit a model with maximum claw strength as the dependent variable and a a three-way interaction interaction between claw length, sex, and regeneration status (original or regenerated) to test how regenerations status and sex influenced the relationship between

claw length and claw strength. Next, I wanted to determine how claw regeneration and sex influence residual claw strength (i.e. strength for a given size). Thus, I used the ‘nmlr’ library in R (Pinheiro et al., 2017) to fit linear models of claw size and claw strength. I used the most likely model based on the corrected Akaike information criterion (AICc) to calculate the residual force of each claw. This residual force serves as an index of dishonest signaling (Hughes, 2000; Wilson et al., 2007). Then, I fit a model with residual claw strength as the dependent variable and an interaction between claw length, sex, and regeneration status (original or regenerated) as the independent variables. Lastly, I wanted to investigate how claws’ closer muscle mass is influenced by sex and regeneration. Thus, I fit a model with maximum claw strength as the dependent variable and an interaction between sex, regeneration status, and the mass of the claw closer muscle as the independent variables. In all models described above, because each individual has two claws, I controlled for individual variation in the dependent variables by including the identity of the crayfish as a random effect.

Then, for each of these analyses, I used the MuMIn library (Bartón, 2018) to perform multimodel averaging. To do so, I used the dredge function which fits a set of models with all possible subsets of fixed factors and their interactions (i.e. the full model) After fitting all possible models, I calculated the Akaike information criterion and Akaike weight of each model, the latter variable being the probability that the model best describes the data. Finally, I calculated the weighted average of each parameter including estimates from all models. I used the resulting values of parameters to calculate the most likely mean for each combination of factors. These means are plotted over the raw data to

interpret effect sizes, model fit, and biological significance. Thus, by using multimodel averaging, I can reduce biases when estimating effect sizes since all likely models are considered instead of one model with less than 100% likelihood (Burnham & Anderson, 2002). Furthermore, this approach eliminates the need to interpret p values because all models (including the null model) contributed to the most likely value of each mean.

Results

In my combined sample of 367 claws, I identified 208 (56.67%) of the claws to be original and the remaining 159 (43.32%) claws to be regenerated. Additionally, I found that claw regeneration status and sex both influenced the claw strength of *F. virilis* (Fig 2.2, Table S2.1, Table S2.2). In males, the expected strength for an average-sized claw was 16.6 N for an original claw and 10.61 N for a regenerated claw. In females, the expected strength for an average-sized claw was 16.47 N for an original claw and 9.69 N for a regenerated claw. Additionally, I found that residual claw strength (strength for a given size) was influenced by claw regeneration status and sex (Fig 2.3, Table S2.3, Table S2.4). In both sexes, regenerated claws were weaker for a given size compared to original claws (Fig 2.3A). In males, the average residual strength was 2.50 N in original claws and -3.51 N in regenerated claws. In females, the average residual strength was 3.34 N in original claws and -4.07 N in regenerated claws. Thus, in both sexes, the strength for a given size is greater in original claws compared to regenerated claws. When analyzing how the relationship between closer claw muscle mass and claw strength was influenced by regeneration status and sex, I found that regenerated claws on average had lighter muscles compared to original claws in both males and females (Fig 2.4, Table S2.5,

Table S2.6). The average weight of a male's closer claw muscle was 2.12 g in original claws and 1.26 g in regenerated claws. In females, the average weight of the closer claw muscle was 1.36 g in original claws and 0.92 g in regenerated claws.

Discussion

Over 40% of claws included in the study were regenerated, which demonstrates the prevalence of regeneration in this species. Similarly, high rates of claw regeneration are commonly reported across Crustacea (Backwell et al., 2000; Bywater & Wilson, 2012). Despite being able to regenerate to lengths comparable to that of an original claw, I found that the maximal pinching strength of a regenerated claw is much less than the pinching force expected from an original claw, regardless of sex. Specifically, I found that for a given size, a regenerated *F. virilis* claw was on average 36% weaker in males and 40% weaker in females compared to the strength of an original claw. At intermediate claw sizes, a male with an original claw is expected to produce a pinching force of 16 N, whereas a regenerated claw of a similar size is capable of generating a pinching force of 10 N. Such differences in pinching strength should correlate to greater fighting ability in individuals with original claws. Prior work on *F. virilis* demonstrates that differences in claw strength between competitors influence the outcome of aggression (Graham, Padilla-Perez, et al., 2020). In *F. virilis*, competitors engage in repeated bouts of claw interlocking in which assessment of fighting ability occurs. If competition escalates past claw interlocking, both competitors will pinch each other until one competitor flees. Thus, because regenerated claws generate weaker pinching forces, crayfish with one or

two regenerated claws have a lesser fighting ability compared to a similar-sized crayfish with two original claws.

Two mechanisms likely underpin the weaker pinching forces in regenerated claws compared to original claws i.) regenerated claws have a smaller manus and thus less area for muscle tissue and ii.) regenerated claws do not develop enlarged calcified tubercles. Indeed, my findings support both of these mechanisms; I found that regenerated claws have much less muscle tissue compared to an original one. Furthermore, I also found that regenerated claws lack enlarged calcified tubercles (Fig 1). Interestingly, in the fiddler crab (*Uca pugilator*), the degree of tubercle regeneration depends on the body size at which claw autotomy occurs (McLain & Pratt, 2011). However, in *F. virilis*, no large tubercles were identified on the regenerated claws. Instead, small, calcified bumps (i.e. denticles) develop, which are unlikely to alter the mechanical advantage and thus pinching force of the claw. Tubercles located near the joint of the two fixed fingers of the claw enable pinching to occur close to the lever, which is the most biomechanically efficient location to pinch an opponent (Dennenmoser & Christy, 2013; O'Brien & Boisseau, 2018). Thus, in regenerated *F. virilis* claws, both the lack of tubercles and a reduction in manus area (and muscle mass) is consistent with previous work on regenerated claws in other crustacean species (Bywater et al., 2015; Dennenmoser & Christy, 2013; McLain & Pratt, 2011; Yasuda et al., 2014). Surprisingly, despite claw regeneration being conserved throughout crustaceans, the molecular bases of developing an original versus a regenerated claw is unknown. In lizards, differentially expressed microRNA's have been identified in regenerated tails and these microRNA's likely

influence the patterns of regeneration throughout this taxon (Hutchins, Eckalbar, Wolter, Mangone, & Kusumi, 2016). Because of the prevalence and fitness implications of regenerating a claw, similar molecular work should be pursued within crustaceans.

In several species of crustaceans, regenerated (large but weak) claws have been proposed to function as dishonest signals of strength during agonistic contests (Wilson & Angilletta, 2015). In *F. virilis*, I found that a regenerated claw was on average much weaker for a given size compared to an original claw. Because large but weak claws have been used as an index for the relative honesty of claws as signals of strength (Hughes, 2000; Wilson & Angilletta, 2015), my results suggest that regenerated claws may function as dishonest signals; however, I recommend caution about this interpretation. Recent work suggests that the spurious correlation between claw size and claw strength should be cautiously interpreted as evidence for dishonesty, and instead should only be used as evidence for *potential* dishonesty (Graham & Angilletta, 2020; Graham, Padilla-Perez, et al., 2020). Importantly, behavioral observations must be conducted to provide strong evidence for dishonest communication. In fact, previous research on *F. virilis* has found an unreliable correlation between claw size and claw strength, but claw regeneration was not accounted for in this study (Graham, Padilla-Perez, et al., 2020). However, when behavioral observations were conducted in this species, it was found that large but weak claws did not function as dishonest signals, because body size alone explained the variation in dominance, not claw size or claw strength. Although this previous work did not distinguish between original and regenerated claws, it suggests that regenerated claws do not function as dishonest signals in this species. To confirm the

hypothesis that regenerated claws do not function as dishonest signals in *F. virilis* (and potentially other crayfish species), the effect of claw regeneration on crayfish fighting behavior should be studied. Broadly, although my results demonstrate that regenerated *F. virilis* claws are weaker for a given size compare to original claws, I caution against the interpretation that these claws may function as dishonest signals.

As previously mentioned, past studies of crayfish communication and fighting behavior have not distinguished between regenerated claws and original claws. Because I have shown here that regenerated claws are weaker for a given size in *F. virilis*, I believe that claw regeneration may function as the mechanism that produces the weaker relationship between claw size and claw strength in other species that do engage in dishonest communication. For example, in the Australian slender crayfish (*Cherax dispar*), males, but not females have large but weak claws that function as dishonest signals of their strength. In *C. dispar*, the claws that are large but weak for their size may be regenerated; however, in *Cherax*, claw regeneration is not easily identifiable based on morphological features (Z. graham, personal observation). Although regeneration of claws occurs in all crayfish species, it is easier to identify regeneration in some species compared to others (Z. Graham, personal observation). To the best of my knowledge, I am unaware of a clear visual indicator that distinguishes between original and regenerated *Cherax* claws. In *F. virilis*, regenerated claws are easily identifiable based solely on visual characteristics, such as the longer pinching fingers relative to the inner margin of the manus as well as the presence/absence of tubercles (see Fig 2.1). To discern whether or not claw regeneration is the functional mechanism that produces dishonest signaling in *Cherax*

species, I suggest that careful observations of molting and regeneration processes will be essential.

Although crustacean claws are well studied for their function as weapons and signals during aggression, these claws are also multifunctional tools that are used in burrowing, foraging, and predator defense (Mariappan et al., 2000). Furthermore, claw autotomy and subsequent regeneration can influence the dynamics and outcomes of territorial aggression, and other functions of the claw are likely to be similarly affected by regeneration (reviewed in Juanes & Smith, 1995). For example, several crab species suffer from decreased foraging success with autotomized or regenerated claws (Brock & Smith, 1998; David Smith & Hines, 1991; F. Juanes & Hartwick, 1990; Tummon Flynn, Mellish, Pickering, & Quijón, 2015). Further, predators may preferentially predate upon individuals with autotomized or regenerated claws (Bildstein, McDowell, & Brisbin, 1989). Thus, claw regeneration may be an important factor in shaping the evolution of claw morphology based on predation rates within their environment. Future studies on crustaceans should quantify the relative influence of how predation and aggression influence rates of claw regeneration. In non-crustacean taxa, regeneration of appendages can similarly hinder an animal's performance (Maginnis, 2006). Male lizards (*Psammodromus algirus*) with regenerated tails have smaller home ranges and lesser access to females compared to males with normal tails (Salvador, Martin, & Lopez, 1995). Moreover, European garden spiders (*Araneus diadematus*) with regenerated legs will build webs that differ in size and shape compared to individuals with non-regenerated limbs which ultimately influence their ability to capture prey (Vollrath, 1987,

1990). Thus, in crayfish, claw regeneration may not only influence the outcome and dynamics of competition but may similarly influence how a crayfish is able to forage, burrow, or defend from predators. For example, it is unknown whether a crayfish with autotomized or regenerated claws can excavate a burrow. In primary burrowing crayfish that rely heavily on their burrows for refuge as well as mating, being unable to burrow would severely impact their fitness. Additionally, regenerated claws may be less efficient at deterring predators because of their weaker pinching strength. However, because large (but not necessarily strong) claws may be able to deter predators well enough, crayfish with regenerated claws may be able to successfully defend themselves from predation. Indeed, previous work has demonstrated that crayfish with larger claws are less susceptible to predation (Garvey, Stein, & Thomas, 1994), but it is unknown how claw regeneration influences the outcome of predation events.

Interestingly, although regenerated claws reach large sizes but do not contain large muscles, there are metabolic savings for wielding a regenerated claw (Bywater et al., 2014). Moreover, the increased metabolic cost of muscle tissue may explain why regenerated claws differ in form and function throughout crustaceans. Clearly, tradeoffs must exist when regenerating a claw, and the optimal strategy may be to quickly regenerate a large claw that maintains its function in some tasks (see above) as opposed to slowly regenerating a large and robust claw that matches the original claw. Thus, selection seems to favor quickly regenerating a large but weak claw as opposed to slowly regenerating a large but strong claw. Indeed, evidence suggests that female crustaceans avoid males with autotomized or partially regenerating claws, which supports the

hypothesis that quickly regenerating a large claw is more beneficial than slowly regenerating a claw that resembles the form and function of the original claw (Francis Juanes & Smith, 1995) . Further, this hypothesis is supported by work on the experimental autotomy and subsequent regeneration of claws in fiddler crabs; because larger individuals invest substantially into regenerating their claw while minimizing growth of the rest of their body (McLain & Pratt, 2011).

In conclusion, I provide the first evidence that regenerated claws in crayfish produce weaker pinching forces compare to original claws. Additionally, although I found that regenerated claws are weaker for a given size compared to original claws, I caution against interpreting this as evidence for dishonest signaling, because behavioral observations based on these data must be conducted. Together, I believe that future studies on the ecological and evolutionary impact of claw regeneration in crayfish are ripe for investigation, as crayfish are ideal organisms for such studies based on their aggressive nature and relatively easy of capture. Because there are clear differences in the morphology of regenerated and original crayfish claws, future work should be conducted to examine how the regenerated claws versus original claws may be used differently during aggression. In this way, because the morphology of weapons has presumably been selected to efficiently perform in species-specific fights (McCullough, Miller, & Emlen, 2016), crayfish with regenerated claws may use them differently in aggression compared with crayfish with original claws. For example, because regenerated claws are lighter than original claws, regenerated claws may be more likely to be used for claw strikes (a forward extension/jab of the claw). By contrast, because original claws are larger, more

robust, and stronger, an individual with original claws may be more likely to pinch their opponent as well as engage in claw interlocking behaviors. Lastly, whether or not regenerated claws in crayfish truly function as dishonest signals should receive further attention, both in previously studied species (*Cherax dispar*: Wilson et al., 2007; *Cherax destructor*: Walter et al., 2011; *Procambarus clarkii*: Malavé et al., 2017; *Faxonius palermi*: Robinson & Gifford, 2019) and unstudied species with divergent evolutionary histories such as primary burrowing species.

Fig 2.1. Images of an original (top) and a regenerated (bottom) claws of *Faxonius virilis*. Despite reaching similar lengths, there are clear visual differences between an original claw and a regenerated claw. Original claws are characterized by a robust manus, a relatively longer mesial margin of the palm, as well as enlarged tubercles residing in the inner margins of the claws pinching fingers. By contrast, regenerated claws have a reduced manus, a shorter mesial margin of the palm relative to pinching fingers, as well as no enlarged tubercles on the inner margins of their pinching fingers.

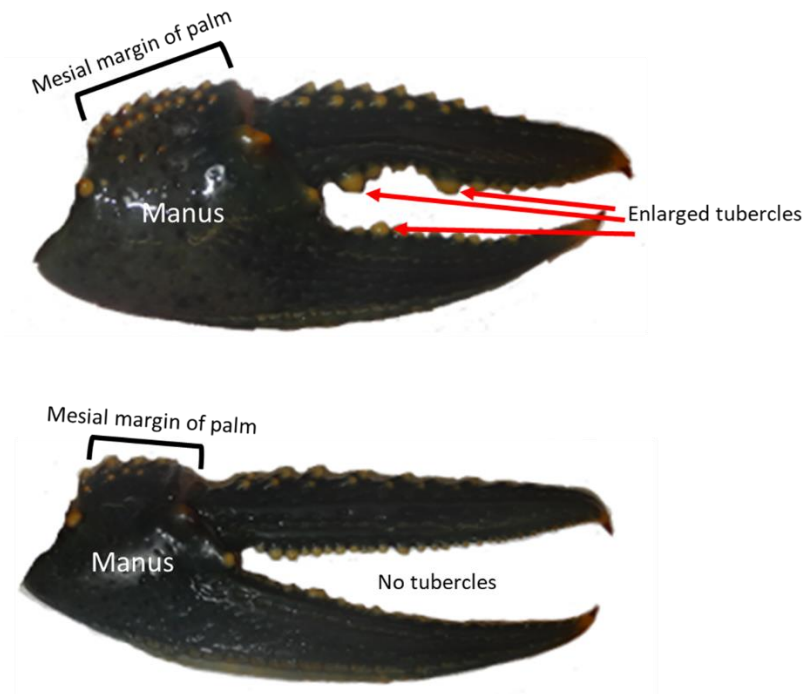


Fig 2.2. Relation between claw length and claw strength in (A) male and (B) female virile crayfish *Faxonius virilis*. Black and blue circles represent original claws and regenerated claws, respectively. Furthermore, the black and blue line represent the linear

relationship between claw length and claw strength in original claws and regenerated claws, respectively.

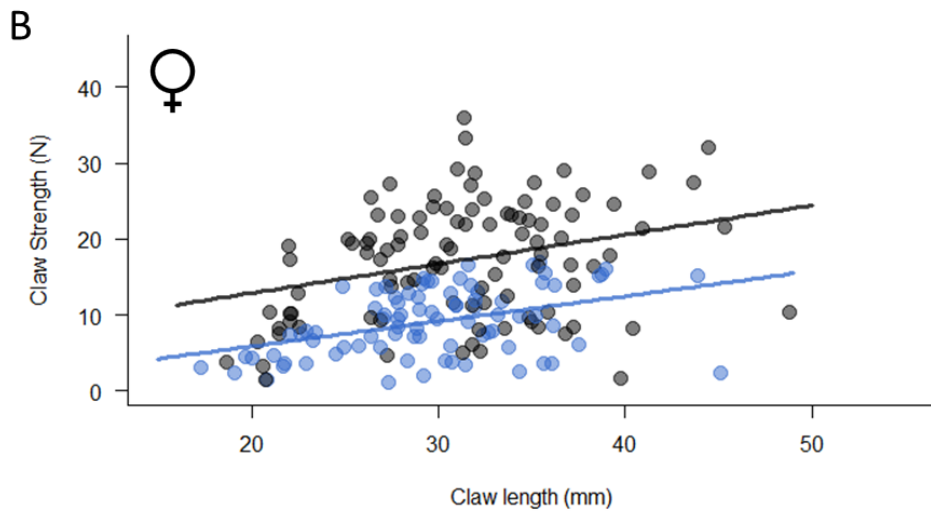
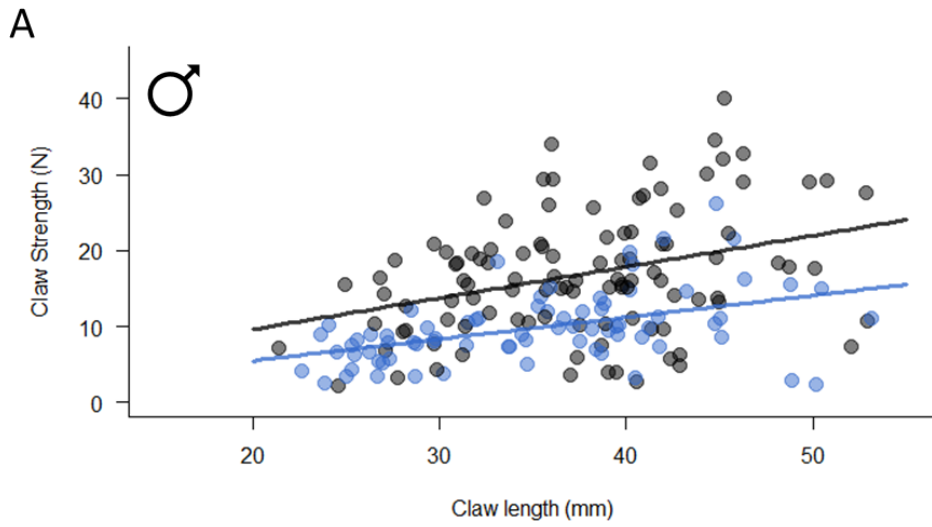


Fig 2.3. Relation between claw length and residual claw strength in (A) male and (B) female virile crayfish, *Faxonius virilis*. Black and blue circles represent data from original claws and regenerated claws, respectively. Furthermore, the black and blue line represent the linear relationship between claw length and claw strength in original claws and regenerated claws, respectively. Claws above the dotted line, with positive residual strength for their given size, represent claws that were strong for their size. Conversely, claws below the dotted line, with negative residual strength for their given size, represent claw that were weak for their size.

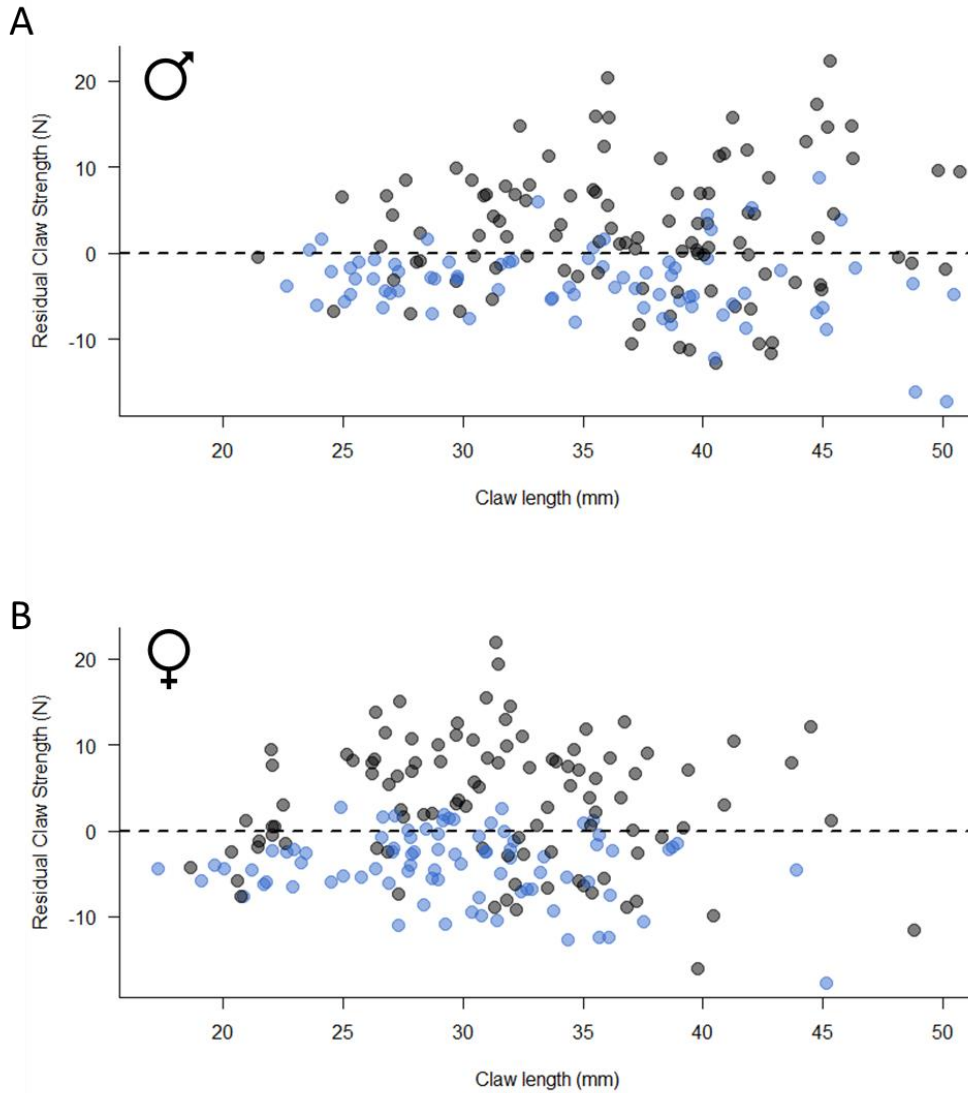
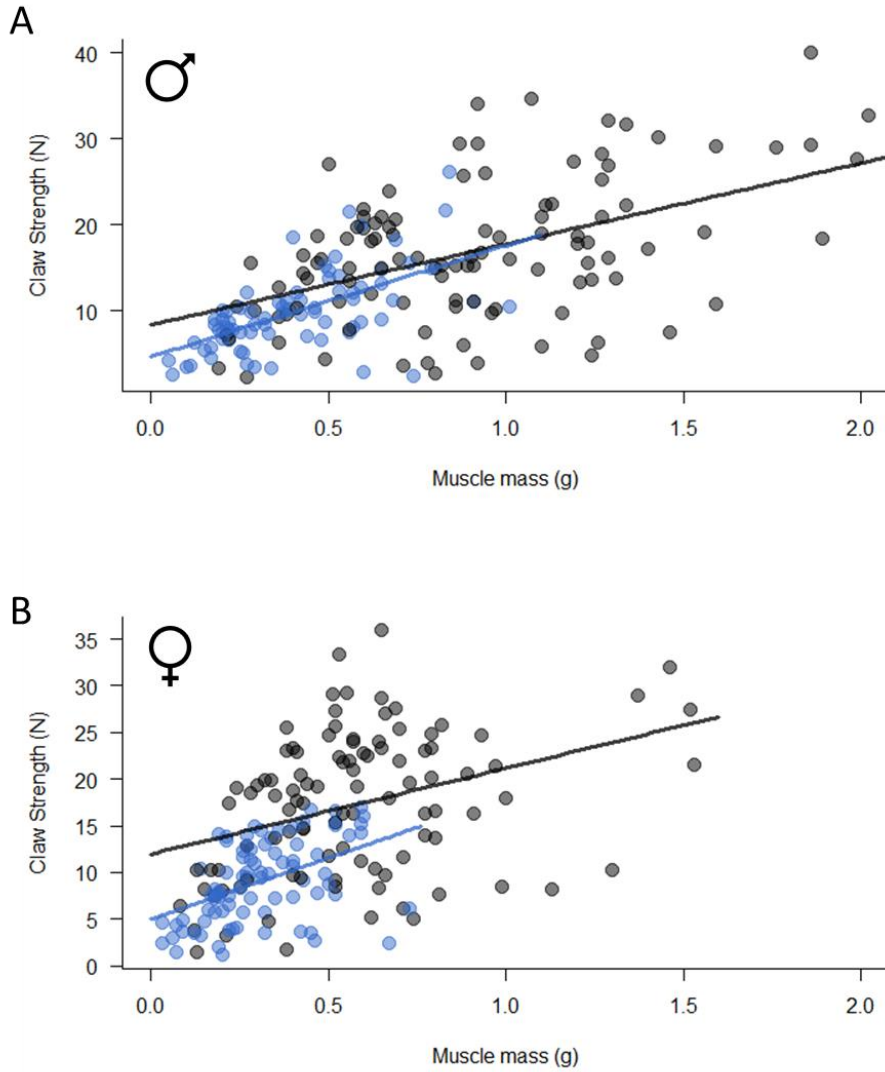


Fig 2.4. Relation between muscle mass and claw strength in male (A) and female (B) virile crayfish, *Faxonius virilis*. Black and blue circles represent data from original and regenerated claws, respectively. Furthermore, the black and blue line represent the linear relationship between claw length and claw strength in original and regenerated claws, respectively.



CHAPTER 3

VIRILE CRAYFISH ESCALATE AGGRESSION ACCORDING TO BODY SIZE INSTEAD OF WEAPON SIZE

(Published in *Animal Behaviour* 2020)

Abstract

Many crustaceans use their large claws to signal fighting ability during territorial contests. However, the size of a claw does not always reflect its strength or the bearer's ability to fight. If strength predicts the outcome of a fight, the mismatch between the size and strength of a claw creates dishonest signaling. Still, only a handful of species have been studied, leaving a gap in our knowledge of the taxonomic diversity and evolutionary history of dishonest signaling. To help fill this gap, I studied signaling and fighting between virile crayfish, *Faxonius virilis*. First, I measured the relationship between the size and strength of a claw in males and females. Then, I staged encounters between members of the same sex to determine how carapace length, claw size, and claw strength influence the probability of fighting and the probability of winning. Both males and females possessed claws whose size poorly predicted their strength, especially at larger sizes. Unlike other species, which escalate aggression based on claw size, virile crayfish escalated aggression based on relative body size. When fighting occurred, however, both body size and claw strength determined the winner. Likely, virile crayfish ignore claw size because body size provides a more reliable signal of dominance. My results stress the importance of observing behavioral dynamics in addition to functional relationships when studying the potential for dishonest signaling.

Introduction

Understanding whether animals signal honestly or dishonestly is a major aim of research on animal communication (Searcy & Nowicki, 2005). Early theoretical work casts doubt on the evolutionary stability of dishonest signaling (Enquist, 1985; Grafen, 1990). More recent models revealed that dishonest signaling can evolve in a variety of circumstances and can even become common in a population (Szalai & Számadó, 2009b; Számadó, 2000). Natural selection favors dishonest signals when individuals pay little cost to produce such signals (Adams & Mesterton-Gibbons, 1995) or a great cost to distinguish them from honest signals (Gardner & Morris, 1989). Recent studies of aggression have found that dishonest communication occurs in species where these conditions seem to be met (reviewed by Wilson & Angilletta, 2015).

Researchers have used signal residuals to identify traits potentially used for dishonest signaling (Hughes, 2000; J. Moore et al., 2009; Wilson & Angilletta, 2015). A signal residual comes from regressing the performance of an individual against the magnitude of its signal. In the context of aggression, animals often signal their ability to fight through the size of a weapon (e.g. antlers, teeth or claws). Presumably, the size of a weapon communicates its potential to inflict damage (Andersson, 1994; Emlen, 2008). However, when size poorly predict performance, large signal residuals represent the unexplained variation. A large negative residual reflects an individual with a much weaker weapon than expected for its size. For example, by examining signal residuals of big-clawed snapping shrimp, *Alpheus heterochaelis*, Hughes (2000) found that a small percentage of

males exaggerated their competitive ability by waving an open claw, despite being unable to produce substantial force with this weapon. Furthermore, the absence of large signal residuals indicates strong selection for honesty, often resulting in a signal that cannot be faked. For example, male collared lizards, *Crotaphytus collaris*, signal their ability to fight by displaying abductor muscles of the jaw (Husak et al., 2006). The size of collared lizards' muscle determines the strength of a bite when fighting (Husak et al., 2009, 2006; Lappin et al., 2006). Such honest signals reduce the chance of losing resources to an inferior competitor.

Crustaceans have been at the forefront of empirical research on dishonest signaling (Wilson & Angilletta, 2015). In many species, males use a pair of enlarged appendages, called chelae or claws, to deter competitors or attract mates (Callander et al., 2013; Dennenmoser & Christy, 2013; Mariappan et al., 2000; Wilson et al., 2007). For example, male fiddler crabs with larger claws are more likely to win territorial contests and attract mates (Shackleton et al., 2005). Interestingly, the size of a claw does not always reflect its strength, meaning that some individuals have large signal residuals (Backwell et al., 2000). The weakest claws, with negative signal residuals, tend to be ones that regenerated after an injury (Bywater et al., 2015, 2014). Although a strong claw helps to win fights, most encounters never escalate to physical combat. Therefore, a large but weak claw enables a fiddler crab to reap the benefit of a large claw without paying the cost of maintaining muscle (Bywater et al., 2014). Some crayfish also produce large but weak claws, presumably to signal their strength dishonestly (Angilletta & Wilson, 2012; Wilson et al., 2007, 2009). In crayfish, however, negative signal residuals are not

linked to regenerated claws. Moreover, both females and males can possess dishonest signals in some species. The poor relationships between claw size and claw strength in certain crustaceans offer an opportunity to explore the dynamics of dishonest communication.

Crustaceans provide some of the best evidence of dishonest signaling, but some results of past studies are difficult to interpret. Specifically, recent studies of crayfish relied on relationships between size and strength to quantify dishonesty (Malavé et al., 2017; Robinson & Gifford, 2019), without observing aggressive interactions. Even if claw size poorly reflects claw strength, however, the dynamics of aggression determines whether claw size actually serves as a signal. Furthermore, researchers studying *Cherax destructor* and *Cherax dispar* lumped multiple interactions to develop a single score of dominance (Walter et al., 2011; Wilson & Angilletta, 2015). This dominance score fails to capture the dynamics of aggression, because not all interactions escalate to physical combat. In some cases, claw size appears to have less influence on the outcome of aggression; for instance, males of *C. destructor* escalate aggression frequently enough that a large, weak claw might not effectively deter opponents (Walter et al., 2011). To resolve these issues, researchers must quantify how putative signals influence the sequence and extent of aggression in staged encounters. By accounting for the dynamics of aggression, we can better interpret whether traits effectively serve as dishonest signals of dominance.

I studied the dynamics of aggression in virile crayfish, *Faxonius virilis*, an aggressive species in which both sexes engage in territorial contests (Hale, Wilson, Loughman, & Henkanathgedara, 2016; James et al., 2016). *Faxonius virilis* has expanded its range through anthropogenic introductions and poses a serious threat to native species of crayfish. Therefore, knowledge of signaling and fighting behaviors of virile crayfish should help to determine how these crayfish will interact with native species. To date, most studies of dishonest signaling have revealed that males signal dishonestly, but females signal honestly. Based on this information, I expected the variation in residual strength of male crayfish to exceed that of female crayfish. For this variation in residual strength to qualify as dishonest communication, males must escalate aggression according to their relative claw size despite relative claw strength determining dominance when physical contact occurs. By contrast, if virile crayfish communicate honestly, males and females should possess claws whose size accurately predicts their strength. Furthermore, crayfish should escalate aggression according to signals that accurately predict the outcome of physical combat between competitors, whether these signals include claw size or not.

Methods

Collection and husbandry of animals

I captured 124 virile crayfish with baited traps at Saguaro Lake, Arizona, U.S.A. in June and July 2018. Upon capture, I transported them to a laboratory at Arizona State University. While in the laboratory, I housed each animal in an individual plastic aquarium (20 × 14 × 10 cm) filled with dechlorinated water. The water was dechlorinated

using a commercial dechlorinating agent. A 12:12 h light:dark cycle was in effect throughout the experiment. Because prior aggressive experience is known to alter outcomes of aggressive interactions in crayfish for 1–6 h (Bergman & Moore, 2003; Daws, Konzen, & Moore, 2002), I acclimated crayfish to laboratory conditions for a minimum of 4 days to eliminate any prior effects of fighting experience. All crayfish were intermolt and had fully intact left and right claws.

Claw size and claw strength measurements

I measured claw size with digital calipers from six measurements of the left and right claws of each crayfish (Bywater et al., 2008). The six measurements were width at heel, width at dactyl/manus joint, length of manus from heel to joint, width of pollex at dactyl joint, width of dactyl and length of pollex from tip to joint (Fig 3.1). For each claw, I converted the six measurements into principal components to obtain a single measurement that was an accurate representation of size. The first principal component (PC1) described more than 87% of the data from the six linear measurements. Therefore, I used the mean of the left and right claw PC1 scores as a measurement of claw size (see Table 3.1). I measured claw strength with a custom-built force transducer. When a crayfish applied force to the metal plates of the load cell with its claw, an amplifier unit output a voltage that was linearly proportional to pinching force. I measured the maximal strength of the left and right claws of each crayfish by introducing each claw to the force plates and recording the maximal strength of five pinches (Wilson et al., 2007). Importantly, *F. virilis* force measurements were repeatable across days (Fig 3.2). When calculating the repeatability of claw strength, I conducted a generalized linear mixed

model to determine the relationship between maximal strength recordings across multiple days with individual crayfish as a random factor. Additionally, I measured the carapace length of crayfish from the tip of the rostrum to the base of the abdomen. I used carapace length as an index of body size for the analyses.

Staged territorial contests

Prior to staged encounters, I randomly assigned each crayfish to be either a focal or opponent crayfish. Each focal crayfish engaged in at least two encounters with opponents, which allows me to control for individual variation during analysis. I staged 97 encounters between crayfish between pairs of males ($N = 43$) or pairs of females ($N = 54$). Encounters occurred in a glass aquarium ($35 \times 20 \times 15$ cm) filled with dechlorinated water and a removable screen divider. This divider prevented physical contact without preventing visual or chemical signaling between crayfish. I placed a pair of crayfish in the aquarium simultaneously and removed the divider after 5 min. Upon removing the divider, crayfish typically engaged in aggression within 5 min. Once aggression ensued, I observed the pair until a clear winner could be determined. The winner and loser were determined from chasing and fleeing, respectively (Wilson et al., 2007). Upon determining the winner, I stopped the fight to prevent injuries. Additionally, I categorized each encounter according to the final stage of escalation: (1) encounters that ended without physical contact and (2) encounters that ended after physical contact, such as tapping or grappling with claws. For my analyses, I considered any encounter that escalated to physical contact as a fight.

Statistical analysis

I modelled the factors influencing the probability that a crayfish escalated aggression to fighting and the probability of winning a fight. The independent variables comprised sex and the difference in body size, claw size and claw strength between competitors. These differences were calculated by subtracting the size or strength of the opponent's trait from the size or strength of the focal crayfish. In both analyses, I used a binomial distribution of error, in which fighting/winning and fleeing/losing were coded as 1 and 0, respectively. When modelling the probability of fighting, I controlled for variation among focal crayfish by including this factor as a random term in the model. I was unable to include a random term when modelling the probability of winning a fight because only a subset of encounters for each focal crayfish escalated to fighting. Therefore, I avoided pseudoreplication in this analysis by randomly selecting a single fight for each focal crayfish.

Following Burnham and Anderson (2002), I used multimodel averaging to estimate the most likely value of means. First, I fitted the most complex model to the data with the 'lme4' library of the R statistical package (Bates, 2010). Then, I fitted models that contained a subset of the terms in the full model. For each model, I calculated the Akaike information criterion (AICc) and the Akaike weight (i.e. the probability that the model described the data better than other models in the set). Finally, I used the parameters of each model and the Akaike weight to calculate a weighted average of each parameter. Collinearity was unlikely to bias the model-averaged parameters, given the weak correlation among carapace size, claw size and claw strength (all $r^2 < 0.3$). These model-

averaged parameters enable one to determine the expected probability for any combination of values for the fixed factors in the full model. This approach eliminates P values because all models (including the null model) contributed to the expected probabilities.

Results

The relationship between claw size and strength demonstrates that both males ($r^2 = 0.08$, $t_{42} = 1.88$, $P = 0.067$) and females ($r^2 = 0.04$, $df =$, $t_{78} = 1.84$, $P = 0.07$) have claws that are poor indicators of strength (Fig 3.3). Although the potential strength generated by a claw weakly increased as the size of the claw increased, so did the variation. For example, the strongest maximal force generated by a male crayfish was 31 N. However, male crayfish of a similar claw size produced maximal forces 3–10 times weaker (3.54–11.87 N).

Furthermore, despite the weak relationship between size and strength for males and females, a model of claw strength based on claw size was more likely than a null model (log likelihood = -340.63, $\Delta AICc = 7.74$). Moreover, the mean of residual strength was similar for males and females ($\beta = 0.50$, $F_{142} = 0.69$, $P = 0.41$; Fig. 3b). Importantly, the repeatability of strength produced by male and female individual claws shows that my claw strength measurements reflect true variation in force production ($r^2 = 0.70$, $t_{118} = 16.63$, $P < 0.001$) and not noise produced by motivation to pinch. An individual's maximal strength was reliable compared to the variation in force production for a given size. Therefore, virile crayfish gain little information about the potential strength of their opponents from their claw size and must engage in physical combat to gage their opponent's potential true strength.

Of the 97 staged encounters, 49 escalated to physical contact. During these encounters, the probability of fighting was only weakly related to relative claw size or sex (Table 3.2). Instead, a model based on relative body size (carapace length) was nine times more likely to predict the probability of fighting than any other model (Table 3.2, Fig 3.4). When an encounter escalated to fighting, both relative body size and relative strength influenced the probability of winning (Table 3.3, Fig 3.5). However, the effect size of relative body size was twice as great as the effect of claw strength.

Discussion

I found that males and females ignored claw size when assessing their opponent, and instead escalated aggression based on relative body size. That is, when the focal crayfish was larger, it was more likely to escalate aggression. But if the focal crayfish was smaller, it was less likely to escalate aggression and more likely to flee. When aggression escalated to physical contact, both body size and claw strength influenced the outcome, although body size was more than twice as important as claw strength. In other species, weapons such as claws often function as aggressive signals and usually determine whether fighting occurs (Andersson, 1994; Hardy & Briffa, 2013). Although these signals generally reflect the wielder's ability or quality, a crayfish can produce large but weak claws as a dishonest signal of strength (Wilson & Angilletta, 2015). For example, in *C. dispar*, males with the largest claws vary nearly 10-fold in their ability to generate force (Wilson et al., 2007). In *C. destructor*, both male and females possess claws whose size poorly predicts their strength (Walter et al., 2011). Although, in *C. destructor*, males are

more likely to escalate aggression to physical contact, prohibiting dishonest males from cheating opponents. Interestingly, males of *C. dispar* and *C. destructor* escalate aggression based on the relative size of their weapons, despite these weapons providing unreliable information regarding their underlying strength (Walter et al., 2011; Wilson et al., 2007). In contrast to these species, males of *F. virilis* largely ignored the size of their opponent's claws, instead escalating aggression based on body size. Although I do not know why virile crayfish behave differently than other species, several explanations seem plausible. These explanations focus on the cost and benefit of using claw size as a proxy for fighting ability (Adams & Mesterton-Gibbons, 1995).

Most likely, claw size in virile crayfish provides too little information about strength to elicit a response from competitors. As with *Cherax* spp., *F. virilis* exhibits large variation in strength for a given claw size, especially for crayfish with large claws (see Fig 3.2). Given the repeatability of claw strength (see Fig 3.1), the variation in claw strength most likely reflects variation in the quantity or quality of muscles rather than poor motivation of crayfish or imprecise measurements by researchers. If so, the size of a claw truly provides little information about its strength. Although all signals impart imperfect information, natural selection should favor receivers that ignore signals that convey too little information (Searcy & Nowicki, 2005). In such cases, the expected cost of heeding dishonest signals outweighs the benefit of heeding honest ones. If possible, receivers should use alternative cues that convey better information about the quality of an opponent (Edsman & Jonsson, 1996; P. Moore, 2007; Ueno & Nagayama, 2012). Because the relative size of a virile crayfish better predicted its chance of winning a fight,

I suspect that virile crayfish evolved to infer fighting ability from body size instead of claw size.

The fighting style of *F. virilis* could explain why these crayfish escalate aggression based on body size instead of claw size. *Cherax* crayfish species use their claws to grasp or push an opponent (Karplus, Sagi, Khalaila, & Barki, 2003). However, virile crayfish have larger and more robust claws than do *Cherax* species. Because the morphology of a weapon influences the style of fighting (McCullough, Miller, & Emlen, 2016; McCullough, Tobalske, & Emlen, 2014), virile crayfish might use different techniques than those used by *Cherax* species. A larger crayfish may physically manipulate and displace an opponent more easily (Nakata & Goshima, 2003; Pavey & Fielder, 1996). If virile crayfish focus more on grappling for position than on inflicting damage, the length or mass of the body might better explain variation in fighting success than the size or strength of the claw does. Furthermore, the fighting style probably affects the costs and benefits of aggression. Wilson and Angilletta (2015) argued that a minority of honest signalers could maintain a majority of dishonest signalers in a population, as long as crayfish pay a great cost when ignoring an honest signal of strength. This argument relies on the assumption that crayfish fight in a way that causes substantial damage to the loser. Yet, a style of fighting that relies on pushing instead of pinching probably causes less damage. Even if *F. virilis* does use a style similar to *Cherax* species, claw size would only be a useful signal of fighting ability if the frequency of honest signalers was sufficiently high. Therefore, modelling the costs and benefits of fighting along with the

frequencies of honest signalers and dishonest signalers should help to explain why some crayfish ignore weapon size when escalating aggression.

Previous instances of dishonest communication in crustaceans require a second examination. In the past, researchers have inferred the reliability of a signal from its relationship to the quality of the signaler. For aggressive signaling between crustaceans, researchers have focused on the relationship between the size and strength of a claw (Wilson & Angilletta, 2015). This approach assumes several conditions: (1) opponents respond to claw size, (2) the size of claw determines its strength and (3) the strength of a crayfish determines its fighting ability. In *F. virilis*, claw strength contributes less to fighting ability than body size does. Importantly, in the studies on *C. destructor* and *C. dispar*, researchers analyzed the reliability of signals and the outcome of fights. For this reason, these studies provided strong evidence that some crayfish signal dishonestly. However, in other studies, researchers concluded that crayfish communicate dishonestly based entirely on variation in size and strength, without observing aggressive behavior. In the recent study of western painted crayfish, *Faxonius palmeri longimanus*, Robinson and Gifford (2018) concluded that males, but not females, possess dishonest signals because muscle mass more accurately predicts force production in females (Robinson & Gifford, 2019). For a given claw size, claw strength of males varied much more than that of females (females: $r^2 = 0.43$; males $r^2 = 0.15$). Similarly, Malave and colleagues (Malavé et al., 2017) concluded that red swamp crayfish, *Procambarus clarkii*, possess dishonest signals based on the residual variation in claw strength (females: $r^2 = 0.52$; males: $r^2 = 0.15$). Although functional relationships potentially tell us about the reliability of

signaling, these relationships must be combined with behavioral observations to determine whether a trait serves as a signal and whether the signal predicts social dominance.

When studying aggression, one should consider that competitors rely on multiple signals as aggression escalates through a series of stages. For instance, male flower beetles (*Dicranocephalus wallichii bourgoini*) initially compare the length of their forelegs but shift to physical contact when this display fails to settle a contest. The length of a male's foreleg matters most in the early stage, whereas a male's body length matters more in the final stage. Similarly, horned weevils or snapping shrimp use different types of weapons at different stages of aggression (Eberhard & Garcia-c, 2000; Hughes, 2000). Previous studies of crayfish have ignored this dynamic nature of aggression, lumping a series of interactions between males into a single score of dominance (Seebacher & Wilson, 2007; Walter et al., 2011; Wilson et al., 2007). Had I not investigated separate stages of aggression, I might have concluded that some virile crayfish signal dishonestly based on the weak relationship between the size and strength of a claw. Moreover, if I had focused only the initial stage of aggression, I might have concluded that claws play no role in the outcome of a fight. By studying early and later stages independently, I discovered that body size and claw size enhance dominance, although only body size serves as a signal of dominance. Researchers must disentangle how each trait influences each stage of aggression, including the outcome of a contest, to understand whether these traits serve as honest signals.

Overall, I provide conflicting evidence regarding the generality of dishonest signaling in crustaceans. Just because a putative signal poorly reflects fighting ability does not mean that competitors signal dishonestly. Traits that operate as signals in some species will fail to do so in others. A trait will only serve as a signal when variation in the trait explains a sufficient amount of variation in fighting ability and when the cost of ignoring this information exceeds the cost of heeding a dishonest signal. Future studies should relate signaling behavior to the outcome of contests, manipulating the magnitude of a putative signal when possible (Dey, Dale, & Quinn, 2014; Ligon & McGraw, 2016; Tibbetts & Dale, 2004). I stress the importance of partitioning stages of contests experimentally or analytically to learn when different traits determine success at different stages of aggression. Although I report the first clear evidence of honest communication in crayfish, the broader evolutionary history of these signals is unknown. With over 650 species of crayfish spanning a diversity of ecological roles, comparative studies of aggressive signaling and fighting behavior are an obvious next step to address the generality of dishonest communication.

Table 3.1. Principal components loadings for claw size measurements of crayfish. The values presented are relative contributions of the six claw measurements to the variation explained by each principal component. PC1 values all having loadings in the same direction and best explain the variation in overall claw size.

Claw Measurement		PC1	PC2	PC3
1	Width at heel	0.430	0.104	0.192
2	Width at dactyl/manus joint	0.426	-0.072	0.379
3	Length of manus from heel to joint	0.413	0.155	0.530
4	Width of pollex at dactyl joint	0.406	0.158	-0.617
5	Width of dactyl	0.411	0.420	-0.345
6	Length of pollex from tip to joint	0.361	-0.871	-0.193
Eigenvalue		0.875	0.193	-0.180
% of variance		87.3	6.70	5.20
Cumulative		87.3	94.0	99.2

Table 3.2. The most likely models predicting the probability of engaging in aggression included the single predictor of body size difference. All models contained an intercept and error terms associated with the identity of the focal crayfish. For each model, the number of parameters (k), the corrected Akaike information criterion (AICc), Akaike weight (w), and the log likelihood (ll) are reported. Models were ranked according to their corrected Akaike information criterion (AICc).

model	k	Log-likelihood	AICc	Δ AICc	w
body size difference	3	-39.55	85.354	0.00	0.89
body size difference * sex	5	-39.48	89.629	4.276	0.10
claw size difference * claw strength difference	5	-43.57	97.800	12.446	0.00
claw size difference + claw strength difference	4	-45.72	99.879	14.525	0.00
claw size difference	3	-51.431	109.123	23.77	0.00
claw strength difference	3	-52.417	111.096	25.742	0.00
null	2	-59.60	123.336	37.982	0.00

Table 3.3. The most likely models predicting the probability of winning a contest included body size difference and strength difference. For each model, the number of parameters (k), the corrected Akaike information criterion (AICc), Akaike weight (w), and the log likelihood (ll) are reported. Models were ranked according to their corrected Akaike information criterion (AICc).

model	k	Log-likelihood	AICc	Δ AICc	w
body size difference + claw strength difference	3	-10.96	28.849	0.00	0.49
body size difference + claw strength difference + claw size difference	4	-10.54	30.679	1.83	0.19
body size difference + claw strength difference + sex	4	-10.88	31.353	2.504	0.14
body size difference	2	-14.27	32.978	4.129	0.06
body size difference + claw strength difference + claw size difference + sex	5	-10.502	33.504	4.655	0.04
body size difference + claw size difference	3	-13.96	34.839	5.99	0.02
body size difference + sex	3	-14.13	35.177	6.328	0.02
claw size difference + claw strength difference	3	-15.46	37.848	8.99	0.01
claw strength difference + sex	3	-18.286	43.495	14.646	0.00
null	1	-20.79	43.732	14.883	0.00
claw size difference + sex	3	-19.063	45.049	16.2	0.00

Fig 3.1. Diagram showing the six linear measurements to describe the size of each claw of the virile crayfish. The six measurements were as follows: 1) width at heel, 2) width at dactyl/manus joint, 3) length of manus from heel to joint, 4) width of pollex at dactyl joint, 5) width of dactyl, 6) and length of pollex from tip to joint.

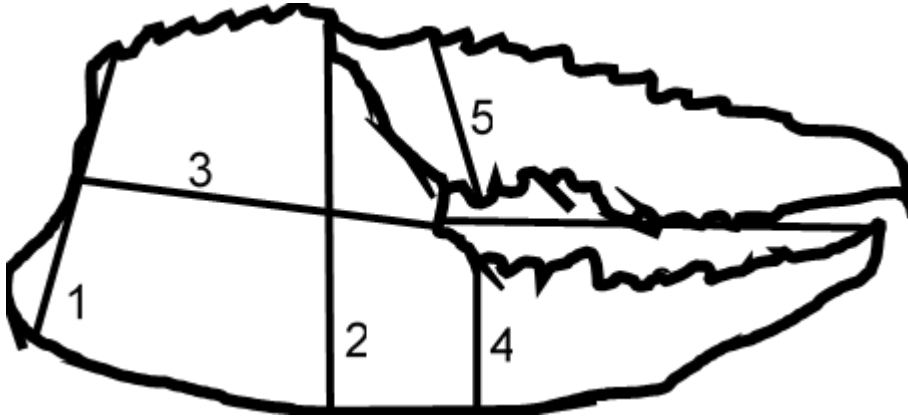


Fig 3.2. The strength of a claw in virile crayfish *Faxonius virilis* was repeatable between days. Maximal forces produced on day 1 and day 2 were highly correlated while controlling for the identity of each crayfish as a random factor in a generalized linear mixed model ($r^2 = 0.70$, $df = 118$, $t = 16.63$, $p < 0.001$). The dashed line serves as a reference for the equality of strength between days.

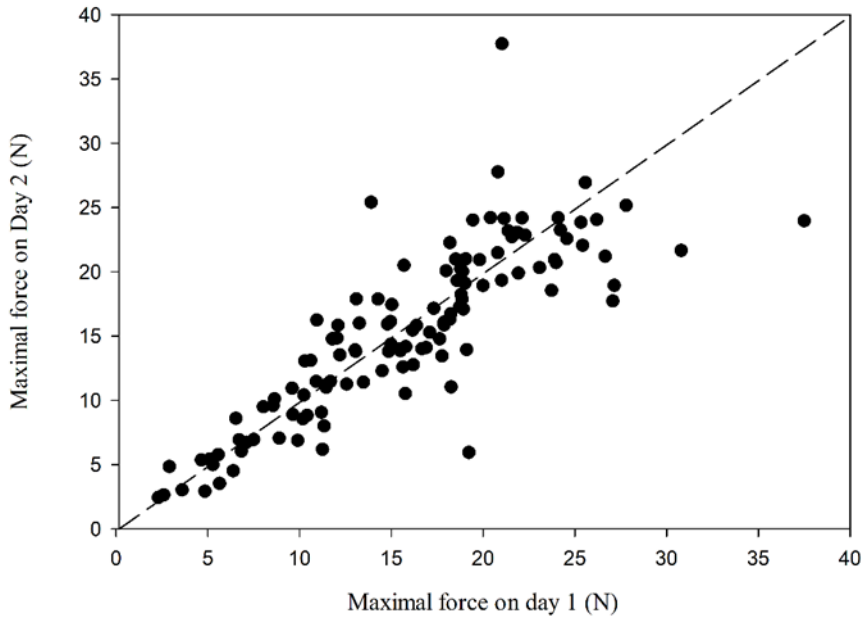


Fig 3.3. A and B) Claw size poorly predicted strength in virile crayfish *Faxonius virilis*, especially at larger sizes. Filled and unfilled circles represent data for males and females respectively. **A)** Claw size was characterized from the first principal component score (PC1) derived from six linear measurements from individuals' claws. **B)** Average claw size was calculated from the mean claw size (PC1) for each individual. Crayfish above the dotted line with positive residual strength for their given size represent crayfish that are strong for their size. Conversely, crayfish below the dotted line that have negative residual strength for their given size represent crayfish that are weak for their size.

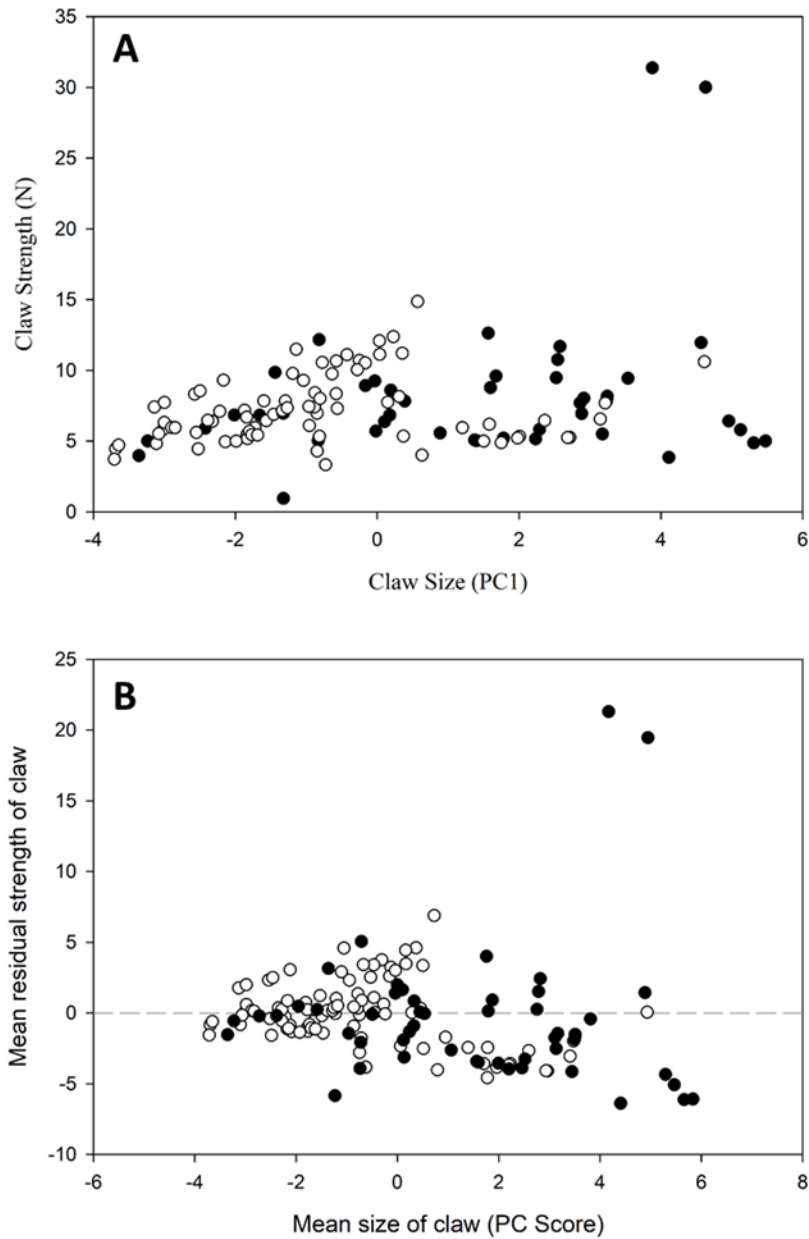


Fig 3.4. The probability that a crayfish escalates aggression to fighting depends on its carapace length relative to that of its opponent. Circles indicate the outcome of each encounter (0 or 1, where 1 represents fighting). The black line represents the fitted values of a statistical model that describes the data. The dotted line provides a reference for zero difference in carapace length.

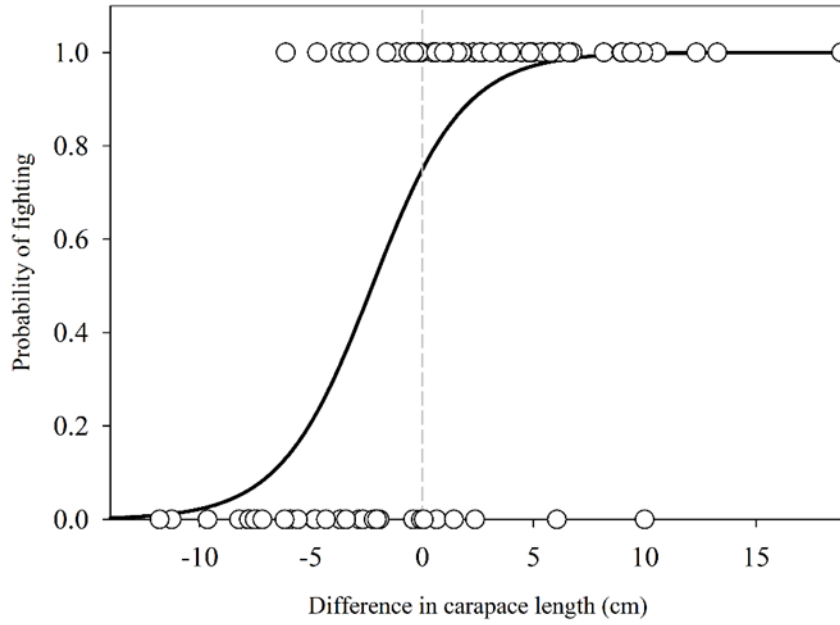
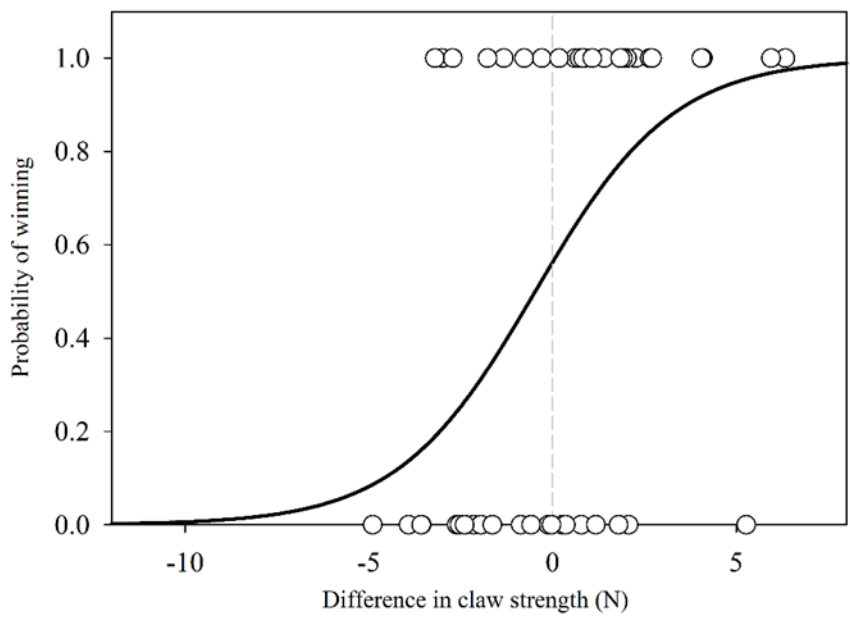
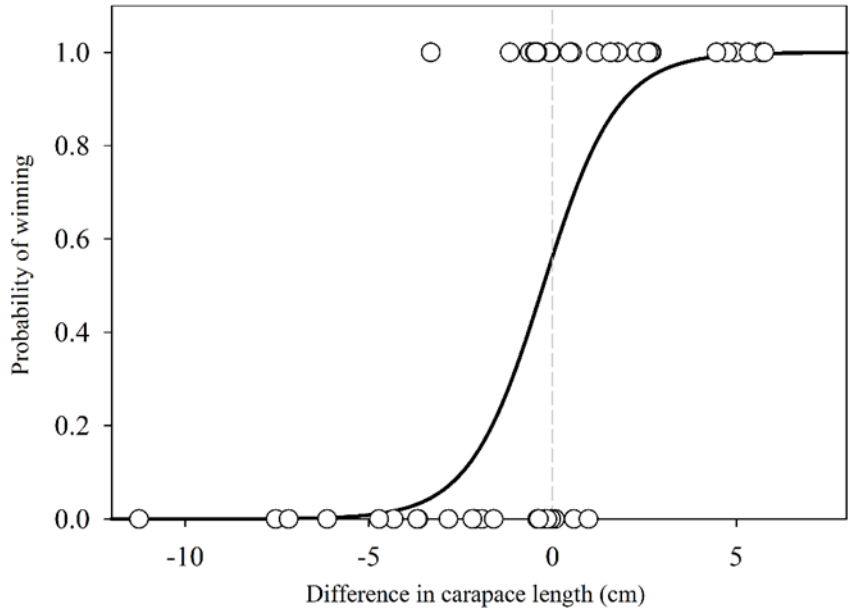


Fig 3.5. A) The probability that a crayfish wins a fight depends on its carapace length relative to that of its opponent. **B)** The probability that a crayfish escalates aggression to physical combat depended on its claw strength relative to that of its opponent. Circles indicate the outcome of each encounter (0 or 1, where 1 represents winning). The black line represents the fitted values of a statistical model that describes the data. The dotted line provides a reference for zero difference in carapace length or claw strength.



CHAPTER 4

CLAW SIZE PREDICTS DOMINANCE WITHIN AND BETWEEN INVASIVE SPECIES OF CRAYFISH

(Published in *Animal Behaviour* 2020)

Abstract

During aggression, animals signal their physical abilities to deter opponents from fighting. Competitors that have a coevolved system of communication can resolve the outcome of a fight without paying the cost of engaging in potentially injurious fighting. Invasive species complicate the dynamics of competition, because such species may not have a coevolved system of communication. For example, many species of crayfish have invaded freshwater ecosystems, where they compete with species never encountered before. I studied the traits used to escalate aggression within and between two species of invasive crayfish in Arizona, *Procambarus clarkii* and *Faxonius virilis*. Through staged encounters, I found that individuals of both species escalated aggression according to their relative claw size. Furthermore, claw size (instead of claw strength) determined the outcome of interactions that escalated to physical combat. Despite *P. clarkii* and *F. virilis* presumably not having a coevolved system of communication, claw size predicted the outcome of interspecific aggression. In crayfish and other species that have invasive potential, future investigations into intraspecific versus interspecific communication dynamics are warranted as they may shed light on the successes and failures of past and present species introductions.

Introduction

When defending resources, animals advertise their fighting ability to competitors to discourage costly fights (Hardy & Briffa, 2013). In most cases, aggression de-escalates without harm, because one of the competitors concedes the resource (Briffa & Sneddon, 2007; Logue et al., 2010). Competitors that have a coevolved system of communication can minimize the cost of aggression, because conflicts escalate only when the benefit of fighting outweighs the cost. In other words, de-escalation benefits the winner and the loser when the outcome of a fight can be determined prior to costly aggression. Animals communicate their fighting ability through a combination of morphological and behavioral signals (Hardy & Briffa, 2013). If low-quality individuals cannot pay the costs of producing signals of high quality (Briffa & Sneddon, 2007; Hurd, 1997), costly signals should convey honest information about the signaler's condition (Zahavi, 1975). For instance, male stalk-eyed flies advertise their quality to competitors through displays of their disproportionately large eyestalks (David et al., 1998; David, Bjorksten, Fowler, & Pomiankowski, 2000; Panhuis & Wilkinson, 1999). Because the overall size of male eyestalks affects their success in fights and mating, males with the largest eyestalks signal exceptional quality. In this case, an individual cannot signal deceptively because the signal's size is directly correlated to the quality being signaled. In other cases, an individual that signals deceptively will endure costly fighting without benefit (Szalai & Számádó, 2009a). Thus, natural selection maintains coevolved systems of communication in which honest information is conveyed for the benefit of signalers and receivers.

Because systems of communication require many generations to coevolve, invasive species might encounter competitors with whom they are unable to communicate with (Bradbury & Vehrencamp, 1998; Jaeger & Gergits, 1979). Species that compete regularly, such as two native species, have coevolved behaviors that minimize the time, energy and risk associated with their interactions (Searcy & Nowicki, 2005). However, introduced species may not recognize signals that have evolved in native species. Furthermore, more than one invasive species can occur within a single environment, where they impact the fitness of both native and invasive competitors (Chucholl, 2016). In fact, invasive species are often more aggressive than their native competitors, which might explain their successful invasion (Chapple, Simmonds, & Wong, 2012; Dalosto et al., 2015; Hudina, Hock, Žganec, & Lucić, 2012; Weis, 2010). Furthermore, an invasive species may behave more aggressively in its novel environment than in its native environment (Hudina, Hock, & Žganec, 2014; Juette, Cucherousset, & Cote, 2014). Thus, invasive species not only reduce the resources available to native species but also increases the frequency of aggression between competitors (Amarasekare, 2002; Carere & Gherardi, 2013; Moore, DiStefano, & Larson, 2013). Given the alarming rates at which invasive species have expanded their ranges in recent decades (Mooney & Cleland, 2001; Neubert & Parker, 2004), biologists should study aggressive communication between species that lack an evolutionary history of competition. In this way, the dynamics of interspecific aggression may shed light on the mechanisms in which invasive species are able to compete for resources in their novel, introduced environment.

Crayfish are a tractable system to study how signaling influences competition between invasive species, because crayfish have invaded freshwater systems around the world. Moreover, intraspecific signaling between crayfish has been a model system for understanding aggression and competition (Kubec, Kouba, & Buřič, 2018; P. Moore, 2007). Within their native range, crayfish defend space and other resources from members of the same or different species (Maiwald, Schulz, Šmietana, & Schulz, 2006; Nakata & Goshima, 2003; Sint & Füreder, 2010). To minimize the cost defending resources, crayfish chemically and visually signal their ability to fight (Bergman, Redman, Fero, Simon, & Moore, 2006; P. Moore, 2007). In some species, individuals decide whether to escalate aggression based on the relative size of their claws (Bywater et al., 2008; Walter et al., 2011). If competitors have claws of a similar size, the contest escalates to physical combat in which claws can inflict damage. In the few species studied thus far, individuals with stronger claws were more likely to win when aggression escalated to fighting (Walter et al., 2011; Wilson et al., 2007). However, strong crayfish often retreat from weak opponents that signal dishonestly by producing a large claw with weak muscles. This dishonest signaling works because the claw's exoskeleton does not allow competitors to assess the quality or strength of the muscles hidden underneath the exoskeleton (Wilson & Angilletta, 2015). A crayfish that produces large but weak claws can deter some opponents while saving the energy needed to maintain abundant muscle tissue (Bywater et al., 2008, 2014; Wilson et al., 2009).

Previous studies of dishonest signaling between crayfish have focused on intraspecific communication, but these studies raise questions about interspecific signaling between

invasive species. For example, when crayfish spread outside of their native ranges, how do their signals of fighting ability affect the outcome of competition with novel species of crayfish? Interspecific competition between crayfish has strongly influenced the decline of native and invasive species of crayfish (Hudina, Galic', Roessink, & Hock, 2011; Usio, Konishi, & Nakano, 2001; Westman, Savolainen, & Julkunen, 2002) and has contributed to the extinction of one species (Bouchard, 1977). Although competition between species of crayfish has been studied extensively, these studies have not focused on the mechanisms of aggression that lead to dominance between species. Here, I studied intra- and interspecific aggressive signaling between two invasive species of crayfish, the red swamp crayfish, *Procambarus clarkii*, and the virile crayfish, *Faxonius virilis*.

The virile crayfish has a native range throughout the Great Lakes region of the United States and the red swamp crayfish is native to the gulf coastal plain from Florida to Mexico. Both species were introduced outside of their native ranges (Dalosto et al., 2015; Figler, Finkelstein, Twum, & Peeke, 1995; Marks & Adams, 2016). In the United States, both species are co-occurring invaders in at least five separate states (iNaturalist.org, 2019). In Arizona specifically, both *F. virilis* and *P. clarkii* were introduced to control aquatic weeds and to sustain fish populations (Dean, 1969). Although both species were likely introduced in Arizona within the past 60 years, an exact date of introduction is unknown. Thus, competition between these two species is ripe for investigation because they are already in contact with each other in multiple locations. Importantly, both *P. clarkii* and *F. virilis* readily engage in territorial aggression in both laboratory and field environments, where they will often engage in repeated bouts of claw striking,

interlocking and grasping until a dominance hierarchy is formed. Furthermore, not all aggression between crayfish involves direct physical fighting, because many disputes can be resolved through assessment strategies, in which competitors can presumably determine the outcome of aggression through prefight assessment of signals, such as claw displays.

I conducted two experiments designed to understand dynamics of aggression between and within *F. virilis* and *P. clarkii*. First, I determined whether each species relies on claw size to decide whether to escalate aggression, as reported for other crayfish species (Wilson & Angilletta, 2015). Then, I quantified the relative roles of claw size and claw strength on the outcome of fights within and between species. If competition between *F. virilis* and *P. clarkii* is similar to competition between previously studied species, I predicted that claw size would determine whether or not aggression would escalate, but claw strength would determine the outcome of this aggression. By contrast, if these invasive species do not have a coevolved system of communication, they may not be able to use their claws as signals to resolve aggressive disputes. This study sheds light on the mechanisms of dominance between two species that have little evolutionary history of competition.

Methods

Collection and husbandry of animals

I collected 44 male *F. virilis* from Saguaro Lake, Arizona, whereas I collected 40 male *P. clarkii* from a pond outside of the Rio Salado Audobon Center in Phoenix, Arizona. Both

species occur in Saguaro Lake, where I collected *F. virilis*. However, I am are unaware of any reports of *F. virilis* being collected at the Audobon Center pond, where I collected *P. clarkii*. Although I collected each species from different locations, each species is likely to behave similarly throughout their introduced range because of genetic bottlenecks during their introduction to Arizona. Moreover, investigations of competitive interactions between species that have yet to come into contact is important because these species have the potential to compete throughout a newly shared range (Hale et al., 2016).

I collected all crayfish with baited traps, left overnight. Adults that were free of physical injuries were then transported to a research laboratory at Arizona State University. There, I housed each crayfish individually in plastic aquaria (20 × 14 cm and 10 cm high) with dechlorinated water. I used fluorescent lighting to maintain a 12:12 h light:dark photoperiod. All crayfish were acclimated to the laboratory conditions for a minimum of 4 days before engaging in staged encounters. Based on previous research (Daws et al., 2002), this period is sufficient to eliminate effects of prior aggression.

Claw size and claw strength measurements

To quantify mean claw size, I measured six dimensions of the left and right claws of each crayfish (Fi. 4.1). Because each crayfish has two claws, measurements from 168 claws were used in this analysis. To obtain a single measurement of claw size, I ran a principal component analysis (PCA) of these variables in the R software (v.2018, R Foundation for Statistical Computing, Vienna, Austria). The first component (PC1) described 89% of the variation in morphology within and between species and was as an overall

measurement of claw size (Table 4.1). In *P. clarkii*, but not *F. virilis*, as overall claw size (PC1) increases, the length of the manus disproportionately lengthens compared to the length of the other measurements. Thus, the second principal component described a difference in shape between the species (Fig 4.2b), primarily driven by the length of the manus (Table 4.1). Because the PC scores of left and right claws were highly correlated, I averaged the scores of both claws to get a single estimate of claw size for analyses of behavior.

To measure claw strength, I used a custom force transducer designed to estimate the forces applied to a set of metal plates. I measured the maximal strength of each claw by introducing crayfish to the force plates and allowing each claw to pinch the plates five times (Wilson et al., 2007). Importantly, claw force measurements are highly repeatable between days and conditions (Angilletta & Wilson, 2012; Bywater et al., 2008; Graham, Padilla-Perez, et al., 2020). To see whether claw size reliably signaled strength, I regressed the mean maximal force of a pair of claws onto the mean size. Because the variation in claw strength increased with increasing claw size, I compared the fit of models in which error was either an exponential or power function of claw size, rather than a constant (Zuur, 2013). I used the ‘nmlr’ library in R (Pinheiro et al., 2017) to fit linear models of size and strength. The most likely model, based on the corrected Akaike information criterion (AICc), was used to calculate the residual force of each claw. This residual force served as an index of dishonest signaling (Wilson & Angilletta, 2015; Wilson et al., 2007); positive or negative residuals denoted a dishonestly strong or weak claw, respectively.

Staged territorial encounters

Prior to staged encounters, I randomly assigned each crayfish to be either a focal or opponent crayfish. I conducted staged encounters in a glass aquarium (35 × 20 cm and 15 cm high), filled with dechlorinated water and 2 cm of gravel. A removable screen divided the aquarium in half. I introduced crayfish simultaneously to each half of the arena and allowed them to adjust to their surroundings for 5 min. Following this period, I removed the divider and observed their behavior until a clear winner and loser were determined. To prevent injuries, I removed crayfish from a staged encounter once a winner and loser had been established. Crayfish did not engage in staged encounters more than once during a 24 h period.

In my first experiment, to determine how the size and strength of claws influence the escalation of aggression, I examined the behavior of 10 focal crayfish of each species. Potential opponents were divided into quartiles based on mean claw size. For this experiment, I randomly paired each focal crayfish with an opponent from each of the four quartiles (including their own size quartile). This procedure ensured that every focal crayfish interacted with a range of opponents spanning the observed range of sizes. Given my interest in intraspecific and interspecific interactions, each focal crayfish was paired with four opponents of the same species and four opponents of the other species. This experimental design yielded 160 encounters, or 40 encounters for each type of interaction in each species. However, I included only 36 interactions between males of *P. clarkii* because one focal male died in between staged encounters. For each encounter, I

recorded whether the focal crayfish escalated aggression, either by fighting with its opponent or causing its opponent to flee. I determined that a crayfish escalated aggression if it chased away its opponent or if it had interlocked claws with its opponent. If the focal crayfish did not escalate aggression or fled from its opponent, it was determined to not have escalated aggression. Additionally, I determined whether a crayfish engaged in a fight with an opponent if I observed unrestrained use of their claws. In this way, crayfish that were determined to have escalated aggression or engaged in unrestrained use of their claws were given a score of 1, whereas crayfish that did not escalate aggression or fled were given a score of 0. No sustained physical combat was allowed to proceed, because this experiment was designed only to learn how crayfish decided to escalate aggression to the point of combat.

In my second experiment, to see how the size and strength of claws influenced the outcome of physical fighting, I staged encounters between crayfish of similar size. For this experiment, I matched crayfish according to the lengths of their cephalothoraxes, which were no more than 10% different in body length of each other. I matched 10 focal individuals of each species against two opponents of the same species. Additionally, 10 focal individuals of *F. virilis* were matched against two opponents of *P. clarkii*. This experimental design yielded data for 60 interactions. In this experiment, I was interested in the traits that predicted the outcome of physical fighting and dominance. Thus, winners and losers were determined based on observations of chasing and fleeing behaviour, respectively (Wilson et al., 2007). Crayfish that were determined to be winners were

given a score of 1, whereas crayfish that were determined to be losers were given a score of 0. Once a clear winner emerged, I stopped the fight to prevent injuries.

Statistical analysis

For my first experiment, I modelled the factors influencing the probability of escalating aggression. In the second experiment, I modelled the factors influencing the probability of winning a fight. In both cases, I fitted generalized linear mixed models to the data using the ‘lme4’ library of the R software (Bates, 2010). In each model, the independent variables were the difference in mean claw size between crayfish, the difference in mean claw strength, the residual strength of the focal crayfish and the species of the opponent. Furthermore, in both models, I included the identity of the focal crayfish as a random factor. Because each focal crayfish engaged in more than one encounter with unique opponents, I was able to use focal crayfish, but not opponent crayfish, as a random factor in the analyses. For both analyses, the residual error was modelled as a binomial distribution (0 or 1). Following Burnham and Anderson (2002), I used multimodel averaging to estimate the most likely values of means. First, I used the ‘MuMIn’ library (Bartón, 2018) to fit all possible models to the data. Then, I calculated the Akaike information criterion and Akaike weight of each model. Finally, I calculated the weighted average of each parameter including estimates from all models (Table S4.1, Table S4.2). The resulting values of parameters were used to calculate the most likely mean for each combination of factors. Importantly, collinearity was unlikely to bias the model-averaged parameters, given the weak correlations between carapace size, claw size and claw strength (all $r^2 < 0.5$). These model-averaged parameters enable one to determine the

expected probability for any combination of values for the fixed factors in the full model. This approach eliminates the need to interpret P values because all models (including the null model) contributed to the expected probabilities.

Results

The sizes and strengths of claws varied considerably within and between species. By comparing the PC scores of claw size between species (Fig 4.2), I revealed similar variation in overall size (PC1) in each species, although claws in the sample of *P. clarkii* were smaller than claws in the sample of *F. virilis*. Claws also differed in shape between the species, as shown by much lower and more variable scores for PC2 in *P. clarkii* relative to *F. virilis*. For both species, the relationship between size and maximal strength was best fitted with a linear model in which the variance increased exponentially with increasing size (Fig. 4.3a). In *F. virilis*, claw size was not correlated with claw strength ($\beta = 0.4475$, $N = 88$, $r^2 = 0.04$; Fig 4.3a, Fig 4.3b). By contrast, an individual of *P. clarkii* with small claws was very likely to be weak relative to an individual with large claws; still, considerable variation in strength existed among individuals with large claws ($\beta = 1.0791$, $N = 80$, $r^2 = 0.42$; Fig 4.3a, Fig4.3b).

In staged encounters, crayfish escalated aggression against opponents with claws of similar or smaller size (Fig 4.4). All likely models of aggression included effects of species or relative claw size (Table S4.1, Table S4.2). Relative claw strength was not included in any of the likely models. Based on model averaging, claw size was the best predictor of escalating aggression for intraspecific and interspecific contests (Fig 4.4).

When size-matched crayfish fought, claw size was again the primary factor contributing to the outcome (Fig 4.5). A model including relative claw size was twice as likely as a model that also included relative strength (Table S4.2, Table S4.3). Species was unlikely to affect the probability of winning or influence how other factors affected the probability of winning.

Discussion

During intraspecific competition, my results suggest both *P. clarkii* and *F. virilis* escalated aggression based on their relative claw size. Both species I studied were more likely to escalate aggression only when their claws were larger than their opponents' claws. Indeed, many crustaceans use claws not only as weapons during fights, but also as signals of their fighting ability (Chucholl, 2016; Lailvaux et al., 2009; Sneddon, Huntingford, Taylor, & Orr, 2000b; Wilson et al., 2009). Generally, the size of a weapon (e.g. claw, antler or horn) provides reliable information about the fighting ability of the owner (Searcy & Nowicki, 2005). Therefore, an opponent reduces its chance of incurring an injury by avoiding conflict with an individual that possesses a larger weapon (Rico-guevara & Hurme, 2018). Still, I was surprised that claw size, rather than claw strength, was more likely to determine the winner when aggression escalated to physical combat. Generally, when both the size and performance of an animal's weapon are both measured, the performance of the weapon is found to be a better predictor of fight outcome compared to the size of the weapon (Husak et al., 2009; Lappin et al., 2006). Prior to this work, the relative roles of size and strength had been studied in two species of crayfish. In slender crayfish, *Cherax dispar*, claw strength predicted dominance

between males and females better than claw size did (Bywater et al., 2008; Wilson et al., 2007). In a congeneric crayfish (*Cherax destructor*), claw strength predicted dominance in contests between males but not in contests between females (Walter et al., 2011). Surprisingly, my study found that claw size, not claw strength predicts physical fight success in a crustacean when both claw size and claw strength were evaluated.

I propose two explanations as to why weapon size might determine dominance, independently of weapon strength. First, large weapons may confer a direct biomechanical advantage during a fight. Indeed, weapon size is an important predictor of the outcome of territorial success in a variety of taxa (Emlen, 2008). In crayfish specifically, fights usually involve multiple bouts of pushing while interlocking claws, often ending with the one individual lifting or flipping its opponent (Moore, 2007). Crayfish with larger claws may be able to better manipulate other crayfish compared to smaller-clawed crayfish. Indeed, male crayfish with larger claws better coerce females to mate with them, supposedly because larger claws enable a male to manipulate the female into a mating position (Sneddon, 1990; Stein, 1975). In a similar way, male crayfish with larger claws may more easily manipulate and control their competitors during aggressive interactions; thus, making larger, but not stronger, claws beneficial during aggression. Second, the species that I investigated might simply fight differently than the species studied previously. The morphological weapons of animals are co-adapted with fighting behaviors (McCullough et al., 2016). If the fighting style of *P. clarkii* and *F. virilis* are different than species of *Cherax*, such as pushing more than pinching, I should expect claw size to predict dominance more reliably than claw strength. Although I did not

measure the frequency of pushing and pinching in mystudy, future studies should focus on behavior during fighting as well as the properties of weapons.

By studying the signaling systems of invasive species, I can infer the outcome of competition between species that do not share a common evolutionary history. Generally, competition between invasive crayfish should complicate our ability to predict dominance, because these species do not share a coevolutionary history and thus would not necessarily have compatible signals of aggression. However, I did not find evidence that interspecific signaling between two invasive species of crayfish differs from the signaling systems used within each species. Regardless of whether a crayfish was challenged by an opponent of the same species or a different species, relative claw size was the best predictor of whether the crayfish escalated aggression. Furthermore, claw size predicted success when fighting occurred between species, as well as within species. Despite not sharing a common evolutionary history, *F. virilis* and *P. clarkii* could de-escalate aggression through bouts of claw interlocking and prefight displays of claw size.

During staged encounters, individuals of *F. virilis* generally dominated individuals of *P. clarkii*, because the latter species tends to have smaller claws (Fig 4.2). Thus, the larger of these invasive species should predominate and pose a greater threat to native species. Interestingly, this rule of thumb may extend to other invasive species of crayfish. For example, the signal crayfish, *Pacifastacus leniusculus*, has spread throughout Europe, causing major declines in abundances of native crayfish (Harrison, Hoover, & Richardson, 2006; Machida & Akiyama, 2013; Nakata & Goshima, 2003; Westman et

al., 2002). Compared to the native species, the signal crayfish has disproportionately large claws for its body length (Nakata & Goshima, 2003; Westman et al., 2002). The large claws of the signal crayfish might have facilitated their successful invasion, because smaller species cannot acquire food and shelter when competing with the invader (Chucholl, 2016; Westman et al., 2002). Furthermore, signal crayfish possess conspicuous white patches on the dorsal surface of their claw's dactyl joint and red coloration on their claws ventral surface. Although these patches and markings may increase the visibility of the claw to competitors, I could not find direct evidence for this claim. However, other factors such as disease spread have been associated with the decline of native populations (Bubb, Thom, & Lucas, 2006; Chapple et al., 2012; Dunn, McClymont, Christmas, & Dunn, 2009; Gonçalves Loureiro, Silva Gentil Anastácio, Souty-Grosset, Araujo, & Pereira Almerão, 2015; Putra et al., 2018; Westman et al., 2002), all else being equal, I propose that crayfish with large claws for a given body size are likely to be better competitors, and thus invaders, compared to species with smaller claws.

The system of signaling in *F. virilis* and *P. clarkii* is a rare example of honest signaling in crustaceans. In other crayfish studied to date, the size of an individual's claw poorly predicts the strength of the claw, although relative claw size determines whether a crayfish will fight an opponent (Bywater et al., 2008; Walter et al., 2011; Wilson et al., 2007). If relative claw strength primarily determines which crayfish will dominate, some individuals can benefit by using large, weak claws to signal deceptively. In several species, deceptive signaling occurs commonly because ignoring an honest signal likely

carries a serious cost of injury (Wilson & Angilletta, 2015). Previously, researchers concluded that *P. clarkii* (Malavé et al., 2017) and a congener of *F. virilis* (Robinson & Gifford, 2019) signal dishonestly. However, these researchers studied the relationship between claw size and claw strength, without staging encounters to see whether claw strength contributed to dominance. To determine whether the poor relationship between claw size and claw strength leads to deceptive signaling, researchers must establish that relative claw strength determines the outcome of fighting, even though crayfish use relative claw size to decide whether to fight (Graham, Padilla-Perez, et al., 2020). If a species consists of individuals that signal deceptively, then this species would have an advantage when invading a freshwater system in which species signal honestly.

Furthermore, weak claws are relatively cheap to maintain, compared to claws filled with metabolically expensive muscle tissue (Bywater et al., 2015, 2014). Thus, species with large, weak claws may be able to better signal their dominance to competitors that communicate their visual signals of claw size. Additional studies of variation in the size and strength of invasive crayfish can provide evidence for this hypothesis. Furthermore, tests in other invasive species of crustaceans, such as crabs, should be conducted to further generalize these results.

As species invasions accelerate throughout the globe, biologists will benefit from research designed to assess the traits that predict dominance between invasive and native species (Gherardi, Aquiloni, Diéguez-Uribeondo, & Tricarico, 2011). Currently, wildlife managers lack knowledge of differences in competitive ability among species or, in some cases, do not know how to assess competitive ability directly. By assuming that systems

of aggressive communication work the same in all species, invasive or native, conservation efforts may be weakened. Because behavior and morphology determine a species' success following introduction (Holway & Suarez, 1999; Weis, 2010), we must first understand how individuals establish dominance within species to predict the outcome of competition between species. Crayfish are useful models for establishing research of this nature, because crayfish have long been model organisms for understanding chemical and visual communication (Kubec et al., 2018; P. Moore, 2007). If crayfish can interpret signals of dominant invaders and learn to avoid conflict, exclusions of subordinate species from their previous habitats may be less likely occur. These outcomes depend on the degree to which intraspecific and interspecific signaling follow similar rules, as they do in *F. virilis* and *P. clarkii*. Overall, I highlight the need for further research on the intersection of functional studies and animal communication.

Fig 4.1. Diagram showing the six linear measurements to describe the size of each claw. The six measurements were as follows: (1) width at heel, (2) width at dactyl/manus joint, (3) length of manus from heel to joint, (4) width of pollex at dactyl joint, (5) width of dactyl and (6) length of pollex from tip to joint.

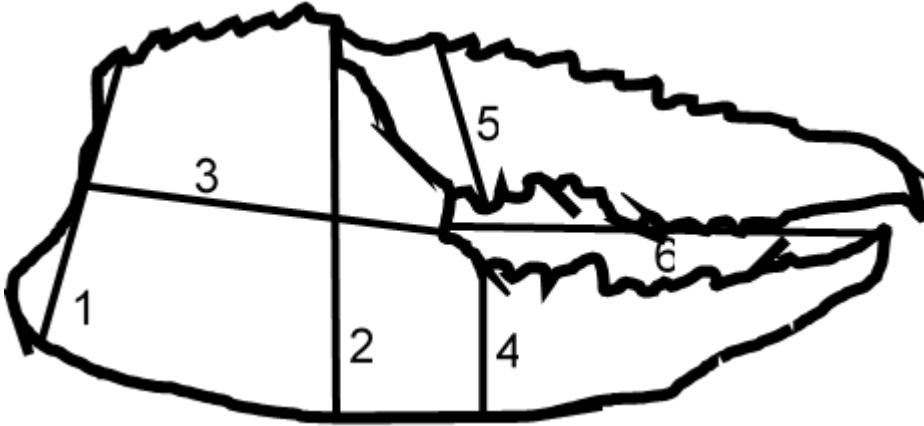


Fig 4.2. (A) Scatter plot of the first two measurements (PC1 and PC2) from the principal component analysis of crayfish claw size. Claw size and shape are highly variable across species, as demonstrated by the clustering of each species claw with no overlap. Closed and open circles represent individual claws from *Procambarus clarkii* and *Faxonius virilis*, respectively. (B) Traced outlines of the claws from *F. virilis* (top) and *P. clarkii* (bottom). The grey dashed line represents the length of the manus, which was the primary driver of PC2 between species.

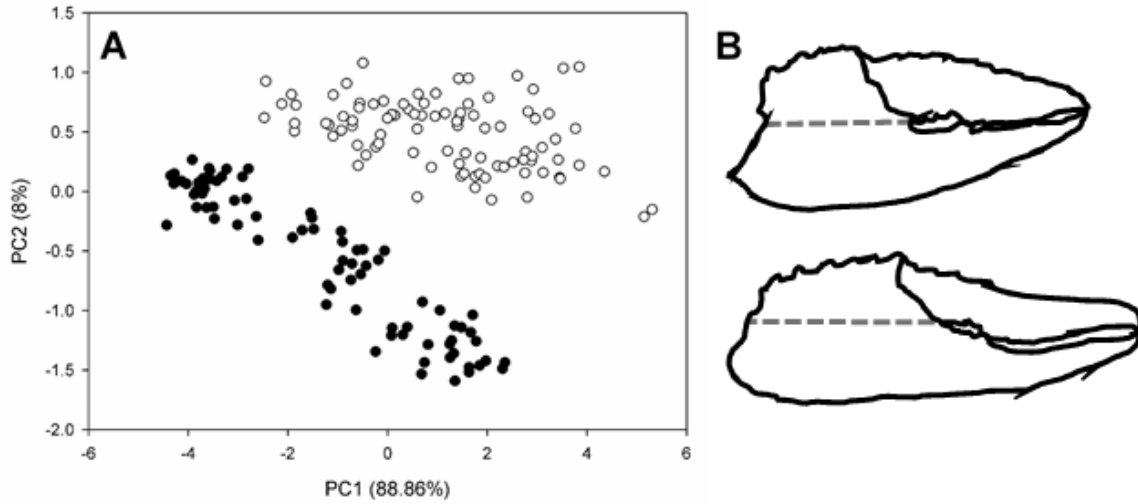


Fig 4.3. Mean and variance of claw strength with claw size for *Procambarus clarkia* (closed circles) and *Faxonius virilis* (open circles). (a) Claw size was characterized as the first principal component score (PC1) derived from six linear measurements from individuals' claws. The black and dotted lines represent the linear relationship for *Procambarus clarkii* and *Faxonius virilis*, respectively. (b) Average claw size was calculated from the mean claw size (PC1) for each individual. Crayfish above the dotted line, with positive residual strength for their given size, represent crayfish that were strong for their size. Conversely, crayfish below the dotted line, with negative residual strength for their given size, represent crayfish that were weak for their size.

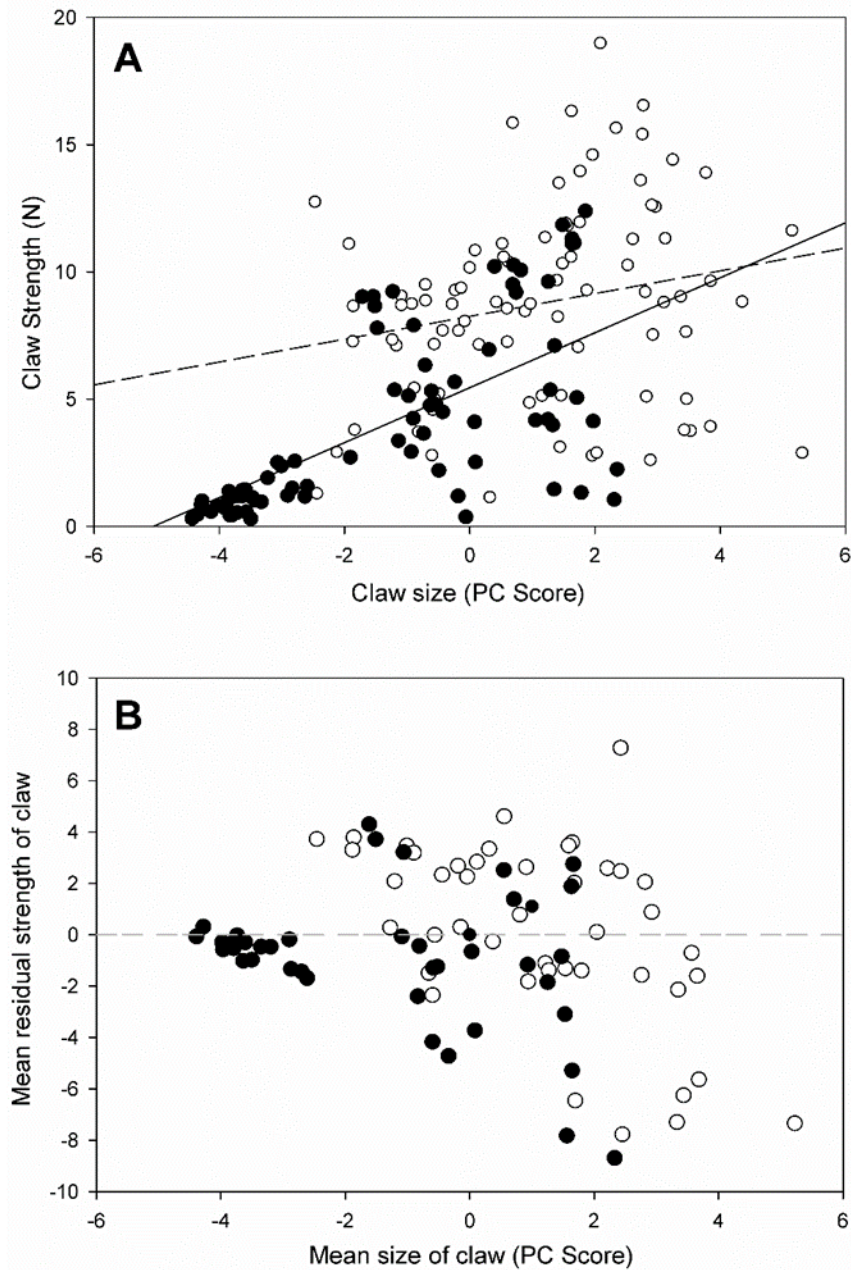


Fig 4.4. The probability of a focal (a) *Faxonius virilis* and (b) *Procambarus clarkii* engaging an opponent based on the difference in claw size and the opponent species. Predicted model fit from intraspecific (blue lines) and interspecific (red lines) encounters. Observed outcome of individuals in intraspecific (blue circles) and interspecific (red circles) encounters.

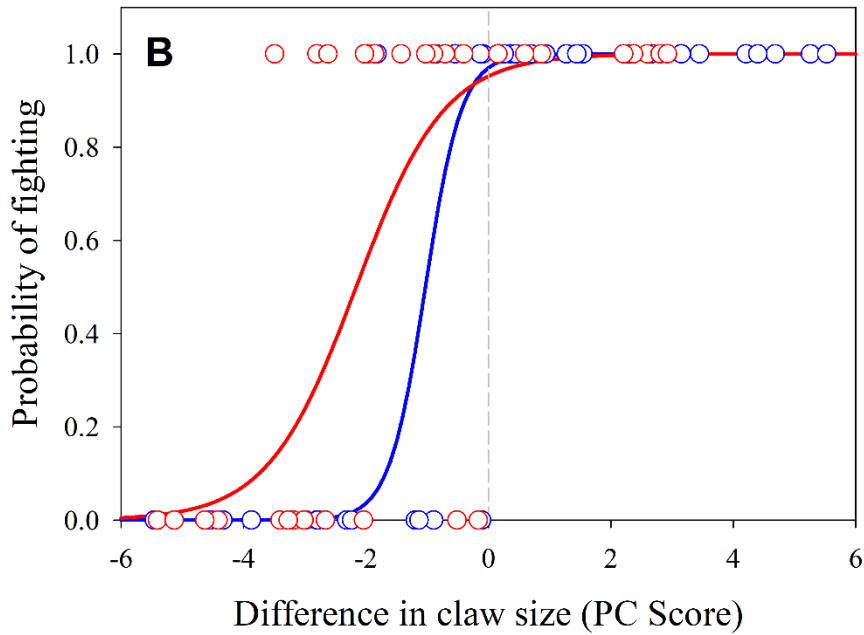
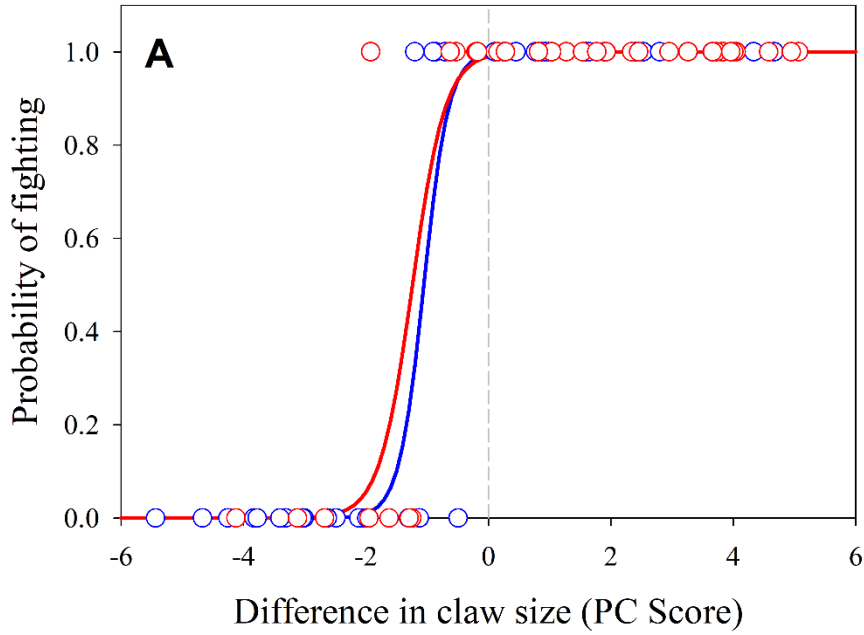
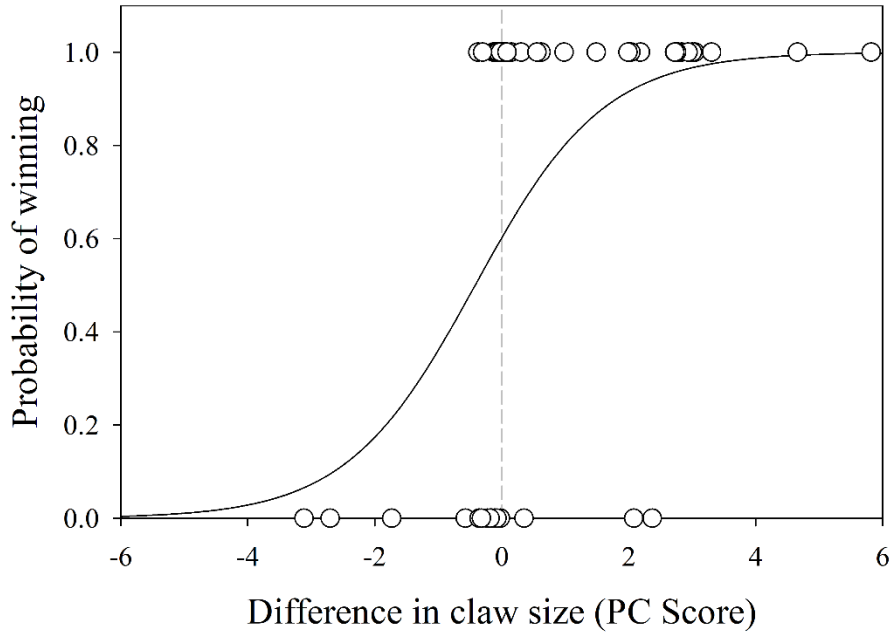


Fig 4.5. The probability of winning a fight based on the difference in claw size. When engaging in a fight, neither focal nor opponent species was predicted to be a likely model. Therefore, the black line indicates the predicted model fit from all size-matched fights. Circles indicate observed outcomes of fights.



CHAPTER 5

DISTINGUISHING DISHONEST SIGNALS FROM UNRELIABLE SIGNALS: A COMPARATIVE STUDY OF AGGRESSIVE COMMUNICATION IN CRAYFISH

Abstract

A major challenge in the study of animal communication is distinguishing whether animal signals convey honest or dishonest information. By understanding the honesty of a signal as a statistical property, dishonest signals are understood as when there is a breakdown in the correlation between a signal and some underlying quality. However, such variation might have resulted from inherent noise associated with developmental or evolutionary processes rather than dishonesty. Here, I separate functional variation in signaling systems from inherent biological noise by leveraging homologous structures that have evolved for separate functions—the claws of crayfish. Because the claws of burrowing crayfish are not aggressive signals, the size and strength of their claws should vary less than those of stream-dwelling crayfish, whose claws evolved as signals. Despite possessing claws that unreliably predict strength, I found no support for the hypothesis that claws function as dishonest signals in any of these species, because crayfish escalated aggression based on relative body size rather than claw size. My results highlight the shortcomings of prior methods of detecting dishonest signals and the advantages of comparative analysis for distinguishing dishonest signals from unreliable signals in systems of communication.

Introduction

Traditionally, biologists have thought that dishonest communication should be rare or absent among nonhuman animals (Maynard Smith & Harper, 1983; Searcy & Nowicki, 2005). This view emerged from theoretical models of how systems of communication evolve when senders and receivers have conflicting goals. According to these models, dishonest signals decline in frequency, because receivers benefit from ignoring signals that convey unreliable information (Adams & Mesterton-Gibbons, 1995; Enquist, 1985; Grafen, 1990; Számadó, 2000). However, the same models established that dishonest signals can persist in a population under special conditions: 1) when constraints prevent one from easily detecting unreliable information, and 2) when ignoring a reliable signal carries a great cost (Adams & Mesterton-Gibbons, 1995). These conditions can explain certain examples of dishonest signaling, such as when a recently molted mantis shrimp aggressively defends his burrow despite not being able to inflict damage; intruders cannot confirm that the mantis shrimp is unable to deal damage without potentially risking injury (Adams & Caldwell, 1990). If these conditions hold, the majority of signals in a population could be dishonest, especially when signalers benefit greatly from dishonesty (Botero, Pen, Komdeur, & Weissing, 2010; Searcy & Nowicki, 2005; Szalai & Számadó, 2009a; Számadó, 2008).

Experiments with crustaceans have provided compelling evidence of dishonest communication among non-human animals (Backwell et al., 2000; Bywater & Wilson, 2012; Callander et al., 2013). For example, the enlarged claw of a male fiddler crab functions as a weapon and a signal during territorial aggression (Dennenmoser & Christy,

2013). Typically, the size of a male's claw honestly signals its fighting ability, because claw size correlates with pinching strength (Bywater & Wilson, 2012; Lailvaux et al., 2009; McLain et al., 2010). Fiddler crabs decide whether to escalate aggression based on the relative size of their claws (Lailvaux et al., 2009). If fighting occurs, the strength of the claw predicts the winner (Lailvaux et al., 2009). However, some individuals lose their large claw to injuries. An injured fiddler crab will regenerate a claw of the original size, but this claw rarely matches the original strength because of the high metabolic cost of muscle tissue (Bywater et al., 2014; Lailvaux et al., 2009). Because other crabs cannot discern between an original claw and a regenerated claw (Callander et al., 2013; Perez & Backwell, 2019), a male with a large but weak regenerated claw dishonestly signals his ability to fight. Among populations of fiddler crabs, 10-45% of males possessed regenerated claws (Bywater & Wilson, 2012; Callander et al., 2013; McLain et al., 2010). Thus, dishonest signaling can be a common strategy to obtain resources without a fight. This conclusion has been further supported by subsequent studies of other species of crustaceans (Wilson & Angilletta, 2015), in which males also use large but weak claws as dishonest signals of strength (Bywater et al., 2008; Graham & Angilletta, 2020; Graham, Padilla-Perez, et al., 2020; Malavé et al., 2017; Robinson & Gifford, 2019; Walter et al., 2011; Wilson et al., 2009).

The initial evidence for dishonest signaling often comes from analyses of signal residuals (Bywater et al., 2008; Hughes, 2000; Wilson et al., 2009). A signal residual is calculated by regressing an index of an individual's quality onto the magnitude of its signal for a population of individuals. Positive signal residuals indicate that an individual has

exaggerated their quality, whereas negative signal residuals indicates that an individual has understated their quality. For example, in crustaceans, large residuals of claw strength indicate that claw size serves as an unreliable signal of fighting ability. Such signal residuals have been used to quantify the dishonesty of diverse forms of communication, including begging calls in chicks (Caro et al., 2016), courtship signals in flies (Funk & Tallamy, 2000), and territorial marks in dogs (McGuire, Olsen, Bemis, & Orantes, 2018). However, an unreliable signal of an individual's quality does not equate to a dishonest signal of that quality. Behavioral data are needed to establish that the putative dishonest signal enables an individual to deceive competitors (Graham, Padilla-Perez, et al., 2020). Yet such data have been collected in only a few cases with divergent results. In two species of Australian crayfish (*Cherax dispar* and *C. destructor*), weak individuals with large claws can successfully deter aggression from competitors; however, dishonest signalers are likely to lose due to their weak claws when aggression escalates to a fight (Wilson et al., 2007). In two species of North American crayfish (*Faxonius virilis* and *Procambarus clarkii*), however, competitors ignore claw size when escalating aggression (Graham & Angilletta, 2020; Graham, Padilla-Perez, et al., 2020). Importantly, residual variation in strength might also stem from genetic and environmental factors unrelated to signaling (Eldar & Elowitz, 2010; Richard & Yvert, 2014; Tsimring, 2014; Viney & Reece, 2013). And even if claw size has evolved as a signal of fighting ability, developmental noise or gene flow can increase variation in strength for a given size (Botero et al., 2010; Brumm, 2013; Tanner & Bee, 2019). Thus, large signal residuals represent a necessary but insufficient condition for establishing dishonest signaling in a population. Studies of signal residuals must be combined with studies of behavioral

responses to infer that the structures under investigation actually function as signals (Carazo & Font, 2013).

Here, I combined comparative and experimental methods to investigate the relationship between signal residuals and dishonest signaling in six species of freshwater crayfish. In each species, I studied the variation in size and strength of their claws—homologous structures that may signal the quality of an individual while also functioning as a weapon or tool. I contrasted signal residuals between species that occupy distinct ecological niches: burrowing species that use claws for digging versus stream-dwelling species that use claws for fighting. Burrowing crayfish make an excellent comparative control because they do not defend territories or exhibit ritualized fighting behavior (Dalosto, Palaoro, Costa, & Santos, 2013; Guiaşu, Saleh, Mozel, & Dunham, 2005); therefore, their claws play no role in aggressive signaling. Interestingly, the burrowing lifestyle has evolved independently several times in crayfish, demonstrating that burrowing and stream-dwelling crayfish are polyphyletic groups. In light of this fact, I expected burrowing crayfish to have less variation in claw size and claw strength than stream-dwelling crayfish have. Further, although both sexes engage in aggression, males fight more often and for longer compared to females (P. Moore, 2007). I also expected greater phenotypic variation among males than among females of stream-dwelling species, because males should benefit more from dishonest signals of aggression. To establish whether claw size and claw strength influence the outcome of aggression, I staged behavioral contests between stream-dwelling crayfish. If large claws deter competitors from escalating aggression, I can conclude that claw size (or a close correlate) serves as a

signal of fighting ability. If both stream-dwelling crayfish and burrowing crayfish have large residual variation in claw strength, I can conclude that signal residuals in stream-dwelling crayfish resulted from processes other than selection for dishonest signals. In this way, the combination of experimental and comparative analyses enables biologists to distinguish between unreliable signals and dishonest signals in a system of animal communication.

Methods

Study species

I studied six species of crayfish: three species described as primary burrowers (*Cambarus monongalensis*, *Cambarus dubius*, *Lacunicambarus thomai*), and three species described as tertiary burrowers (*Cambarus carinirostris*, *Cambarus robustus*, and *Faxonius obscurus*). The docile primary burrowers (hereafter referred to as burrowing crayfish) construct extensive burrows in which they spend most of their life (Hobbs, 1981). The aggressive tertiary burrowers (hereafter referred to as stream-dwelling crayfish) construct rudimentary burrows under refuge (Hobbs, 1981). Males and females of all six species possess enlarged frontal claws that perform distinct functions. Burrowing species do not defend or fight over burrows and lack ritualized behaviors associated with aggression (Dalosto et al., 2013; Guiaşu et al., 2005). By contrast, stream-dwelling crayfish possess enlarged claws used as weapons and potentially as signals of fighting ability (P. Moore, 2007; Robinson & Gifford, 2019; Wilson et al., 2007). In some stream-dwelling species, males produce large but weak claws that dishonestly signal their strength to competitors (Robinson & Gifford, 2019; Wilson & Angilletta, 2015). Variation in the size or

performance of claws within burrowing species serves as a reference to the degree of variation expected in stream-dwelling crayfish without adaptation of claws for signaling. Therefore, these two groups of species enable one to contrast the variation in a trait that has been selected as a signal and a trait that plays no role in signaling.

Collection and husbandry of animals

I collected 359 crayfish representing the six species throughout Pennsylvania and West Virginia, U.S.A from June 2019 to August 2019 (Table S5.1). All stream-dwelling species were either captured by hand or net. By contrast, burrowing species construct burrows in mud close to the water table. Thus, burrowing species were caught by flooding burrows with water and plunging the burrow with a closed fist. Usually, the resident crayfish surfaced to investigate the disturbance, enabling one to capture the animal. Often, multiple bouts of plunging were required to capture a burrowing crayfish. Because burrowing species are harder to locate and capture, I collected fewer individuals of burrowing species than I did for stream-dwelling species (see Table S5.1) For this study, I only collected adult crayfish during their intermolt period, such that all exoskeletons were hardened. Thus, I excluded any crayfish that showed signs of recent or current molting, such as a soft exoskeleton or cracks in the carapace. The sex of each crayfish was identified by the presence or absence of male gonopods.

Each crayfish was held in plastic container ($14 \times 9 \times 6$ cm) with 5 cm of dechlorinated tap water. Prior to any data collection, all crayfish were acclimated to laboratory conditions for two days to minimize any effect of nutritional state or social experience

(Bergman et al., 2003; Daws et al., 2002; Graham, Padilla-Perez, et al., 2020). While housed in the laboratory, crayfish were not fed and were kept on a 14:10 light day cycle. After data collection, each crayfish was returned to the location of capture.

Variation in the size and strength of claws

The claws of each crayfish were photographed on a background of white graph paper (mm²). Images were imported to ImageJ (Schneider, Rasband, & Eliceiri, 2012), a software package that allowed me to quantify six linear dimensions of each claw: 1) width at heel, 2) width at dactyl/manus joint, 3) length of manus from heel to joint, 4) width of pollex at dactyl joint, 5) width of dactyl, and 6) length of pollex (Fig S1). I converted the six dimensions to principal components, yielding a first principal component (PC1) describing more than 93.92% of the variation (Table S5.2). As in previous studies of crayfish (Bywater et al., 2008; Graham, Padilla-Perez, et al., 2020; Wilson et al., 2007, 2009), the scores of the first principal component were used as a measurement of claw size. To relate claw size to body size, I estimated body size as the length of the cephalothorax measured with digital calipers.

I measured the strength of each claw with a custom-built force transducer, consisting of two metal plates connected to a load cell and an amplifier. When held close to the plates, a crayfish used its claw to pinch the plates together. The amplifier generated a voltage that was transformed linearly to determine pinching force. I estimated maximal strength of each claw from the best of five consecutive pinches. Although studies have highlighted the pitfalls of measuring maximal performance (Losos, Creer, & Schulte, 2002), such

measurements have been highly repeatable for other species of crayfish (Graham, Padilla-Perez, et al., 2020; Wilson et al., 2009). To confirm this repeatability in my study, I re-measured maximal claw strength on two consecutive days for a subset of individuals of each species.

Analyses of claw strength

When analyzing the repeatability of claw strength, I conducted a generalized linear mixed model to determine the relationship between maximal strength recordings across consecutive days. Because I had data for both claws of each crayfish, I included the identity of crayfish as a random factor in the model. I also computed the intraclass correlation between measurements using the *irr* library in R (Gamer, 2010).

To quantify the residual variation in claw strength for a given claw size, I regressed the maximal claw strength onto the first principal component of claw size (scores of PC1). As in previous studies, I compared the fit of models in which the error was either an exponential, power, constant power, or fixed (Graham & Angilletta, 2020; Graham, Padilla-Perez, et al., 2020). To do so, I used the *nmle* library in R (Pinherio et al., 2017) to fit linear models of claw size and claw strength. The most likely model based on the corrected Akaike information criterion (AICc) was used to calculate the residual strengths of each claw.

Having estimated residual claw strengths, I then fit models with maximal claw strength as the dependent variable, and with a three-way interaction between claw size, lifestyle, and

sex as independent variables. Furthermore, I included a random intercept associated with individual crayfish. To test the hypothesis that the burrowing lifestyle would lead to reduced selection for claws with immense variation in strength, I compared models with and without lifestyle as a fixed factor. When testing my hypothesis about how sex, species, and lifestyle influence the variation in crayfish claw strength for a given size, I estimated the variance of claw strength for each combination of sex and lifestyle, to see whether adding these parameters provided a better fit to the data than a single variance for all groups. Based on my hypothesis, I expected that the most likely model would be one with a larger variance of claw strength for stream-dwelling crayfish than for burrowing crayfish. I also predicted a greater variance in claw strength for males than for females.

Social dominance in staged encounters

A subset of the males of each stream-dwelling species were used to stage encounters between competitors. Burrowing crayfish were not used during these trials because they neither behave aggressively nor defend territories (Dalosto et al., 2013; Guiaşu et al., 2005). Prior to staged encounters, I randomly selected half of the crayfish to be focal individuals and the other half to be opponents. Each focal individual experienced 2-4 staged encounters. Opponents were assigned randomly to fight with a focal crayfish with the constraint that no opponent could encounter the same focal crayfish more than once. At least 24 hours elapsed between the consecutive encounters for each focal individual. This procedure enabled me to collect a substantial amount of data from fights, while also controlling for any effects of prior experience (i.e., a winner or loser effect; see below).

In total, I staged 150 encounters: 40 encounters between males of *C. robustus*, 50 encounters between males of *C. carinirostris*, and 60 encounters between males of *F. obscurus*. Staged encounters were conducted using methods from previous studies (Graham & Angilletta, 2020; Graham, Padilla-Perez, et al., 2020). Encounters occurred in a glass aquarium (35 × 20 × 15 cm) filled with dechlorinated water and divided by a removable screen. The screen prevented physical contact between crayfish without preventing visual or chemical signaling. I placed a pair of crayfish in the aquarium simultaneously and removed the divider after 5 minutes. Prior to removing the divider, crayfish rarely moved throughout the arena and never displayed claws. Upon removing the divider, crayfish typically engaged in aggression within 5 minutes. Aggression typically consisted of multiple bouts of interlocking claws and grappling, with occasional pinching. Once aggression ensued, I observed the interaction until the winner chased and the loser fled (Wilson et al., 2007). Upon determining a winner, I stopped the trial to prevent injuries. Importantly, some contests were settled solely by displays of claws. Thus, I categorized each encounter according to its final stage of escalation: 1) encounters that ended without physical contact, and 2) encounters that ended after physical contact (e.g., tapping, grappling, or pinching with claws). For my analyses, I considered any encounter that escalated to physical contact as a fight. Collecting data on the final stage of aggression enabled me to disentangle factors promoting dominance prior to fighting and factors promoting dominance during fighting.

When testing my hypothesis about the traits that determine the outcome of territorial aggression in the stream-dwelling species, I modeled the factors influencing the probability of the focal crayfish engaging in aggression and the probability of winning a fight. I fit generalized linear mixed models to my data using the lme4 library of the R Statistical Package (Bates, 2010). In each case, the dependent variable was coded as a discrete outcome (0 or 1); therefore, I used a model with a binomial distribution of error. To control for the effects of collinearity in the fixed effects, I initially ran separate models of carapace size, claw size, and claw strength difference to see how these effects influenced the independent variables (probability of fighting or probability of winning). Because each focal crayfish engaged in more than one encounter with unique opponents, I used the identity of the focal crayfish as a random factor in my analysis. This allowed me to control for variation in behavior among focal crayfish and account for potential winner effects or loser effects (Graham & Angilletta, 2020; Graham, Padilla-Perez, et al., 2020). Additionally, a random slope of species was added to these models. The single term model with the lowest AIC value was then used for consequent analyses, described below. After determining the best model from the models comparing the fixed effects, I used multimodel averaging to estimate the most likely value of means. Then, I used the MuMIn library (Bartón, 2018) to fit all possible models to the data. After fitting all possible models, I calculated the Akaike information criterion and Akaike weight of each model, the latter variable being the probability that the model best describes the data. Finally, I calculated the weighted average of each parameter including estimates from all models. The resulting values of parameters were used to calculate the most likely mean for each combination of factors. This approach eliminates the need to interpret p values

because all models (including the null model) contributed to the most likely value of each mean.

Results

Both burrowing species and stream-dwelling species had substantial variation in claw strength, but the majority of this variation was unrelated to claw size (Table 5.1; Fig 5.1). Two factors rule out measurement error as a primary cause of residual variation in claw strength. First, claw strength was highly repeatable between consecutive days (Fig 5.2; Table S5.3). For 5 of the 6 species, the intraclass correlation of consecutive measurements ranged from 0.93-0.97; for the sixth species, *Cambarus monongalensis*, the correlation was 0.84. These high values of repeatability reflect the precision of my strength measurements. Second, the principal component score of claw size (PC1) correlated strongly with body length (Table S5.4), suggesting that my morphological measurements were also accurate. Surprisingly, burrowing crayfish had even greater residuals of claw strength than did stream-dwelling crayfish. (Fig 5.3). This difference was reflected in the standard deviations of strength in the linear model: 6.8 N and 6.6 N for males and females of burrowing crayfish, respectively, compared to 5.0 N and 6.1 N for males and females of stream-dwelling crayfish. Regarding the territorial contests in the stream-dwelling species, the difference in body length predicted the winner better than the difference in claw size did in fights that did not escalate to physical contact (Fig 5.4A, Table S5.5). Likewise, the same pattern was observed when I analyzed the outcome of contests that did escalate to fighting; the crayfish with a larger body length was more likely to win a fight (Fig 5.4B, Table S5.7). The identity of the species had a small effect

on the outcome of a contest, suggesting only subtle variation in aggressive behavior among species (Fig 5.4, Table S5.5, Table S5.7).

Discussion

Contrary to my prediction, I found that both stream-dwelling and burrowing species have substantial residual variation in the size and strength of their claws. In fact, the burrowing species have even greater variation in claw strength compared to stream-dwelling species. Importantly, the claw strength measurements I recorded were highly repeatable across days, which demonstrates that my measurements are truly precise recordings of maximal pinching strength and not variation in motivation. For these reasons, I believe that some biological process, as opposed to measurement error, underlies the extensive residual variation in claw strength that I observed.

In other signaling systems, residual variation in an index of quality has been interpreted as evidence of dishonesty (Mark Briffa, 2006; Caro et al., 2016; Funk & Tallamy, 2000; Hughes, 2000; J. Moore et al., 2009), but my data refute that interpretation. The greater residual variation in burrowing crayfish cannot be interpreted as evidence of dishonest signaling, because these crayfish do not engage in territorial or aggressive signaling. Unlike stream-dwelling crayfish, which interact aggressively, burrowing crayfish completely ignore one another when paired (Z. Graham, pers. obs.). Furthermore, I found equal rates of unreliability between the claws of males and the claws of females, although earlier studies have shown that dishonest signaling occurs more often in males (Robinson & Gifford, 2019; Wilson et al., 2007). Therefore, a large degree of residual variation, on

its own, should be considered poor evidence of dishonest signaling in a population. Moreover, stream-dwelling crayfish did not escalate aggression according to claw size. In staged contests, the probability of fighting was unrelated to the difference in claw size between crayfish. These patterns differ from those observed in studies of *Cherax* species of crayfish, in which a male with larger claws was more likely to chase away a competitor or win a fight (Seebacher & Wilson, 2007; Wilson et al., 2007); in those species, the relationship between claw size and social dominance lent credence to the view that claw size served as a signal of competitive ability.

Several factors other than dishonest signaling could account for the substantial variation in claw strength of crayfish. First, the claws might have been selected to function purely as weapons, with little or no signaling functions (McCullough et al., 2016). In this way, selection may not favor individuals with large and proportionately strong claws. Instead, traits other than claw size and claw strength may better predict fighting success, such as body length or mass (Graham, Padilla-Perez, et al., 2020; Ueno & Nagayama, 2012). Second, despite the different functions of claws in burrowing crayfish and claws of stream-dwelling crayfish, these groups share a developmental process that could create variation in strength for a given size. Crayfish must molt their exoskeleton to grow, with some individuals molting several times per year. The largest muscle of the claw undergoes substantial atrophy of muscle before molting (West, 1997). Although the exoskeleton of crayfish hardens quickly after a molt, a crayfish needs a longer period to regenerate atrophied muscles. Thus, crayfish must periodically regenerate muscles and cuticles of the claw (Chang, 1995). Because the strength of crustacean claws is likely

determined by the time since the last molt, a large difference in strength can occur between claws of similar size. In my study, I controlled for variation in strength due to molting by only including individuals in a hardened intermolt stage. Both burrowing crayfish and stream-dwelling crayfish molt regularly, potentially explaining the variation in claw strength that I observed. In species of crayfish that use claws as signals, such as *Cherax dispar*, molting may generate the variation in claw strength that enables dishonest signaling. In other species, such as the ones that I studied, the variation in claw strength may be too great for dishonest signaling to evolve; a signal must have a minimum degree of reliability to offer value to receivers. Interestingly, developmental and genetic constraints in the relationship between sexual dimorphism in the tradeoff between pre- and post-copulatory sexual traits is thought to have shaped the evolution of weaponry in male Coreid bugs (Miller, Joseph, Kilner, & Emberts, 2019). In a similar way, there may be underlying developmental constraints that create crayfish claws with variable strengths. These two explanations demonstrate that processes other than dishonest signaling can generate residual variation in claw strength. Similar developmental or evolutionary processes must be considered when determining the honesty of animal signals across taxa and signaling relationships.

My results underscore the need to distinguish unreliable signals from dishonest signals when studying animal communication. Dishonest signals have evolved to convey unreliable information through a breakdown in the correlation between the signal and the information being communicated (Searcy & Nowicki, 2005). By contrast, unreliable signals may arise from processes other than sexual selection of signals, such as

development noise or genetic drift (Botero et al., 2010; Brumm, 2013; Tanner & Bee, 2019). In other words, all dishonest signals are unreliable, but not all unreliable signals are dishonest signals. Although some crustaceans seem to signal strength dishonestly (Bywater et al., 2015; Bywater & Wilson, 2012; Wilson & Angilletta, 2015), the species that I studied simply had unreliable signals in the form of large but weak claws. As Carazo and Font (2013) emphasized, researchers have largely ignored the distinction between an unreliable signal and a dishonest signal. To promote future work in this area, I propose a framework that distinguishes among the processes generating variation in a signaling trait (Fig 5.5). A reliable signal emerges when natural selection creates a relationship between a trait's value and the bearer's quality; such a relationship could result from selection against extreme values of the trait (Fig 5.5A). Developmental noise or genetic drift can introduce residual variation around this relationship, leading to an unreliable signal of quality (Fig 5.5B). The reliability of the signal will depend on a balance between the benefits of dishonest signaling and the cost of social punishment (Fig 5.5C). As such, researchers should focus on experiments that reveal whether traits actually function as signals and how putative signals shape the outcome of competitive interactions, as I have done here with crayfish. At the same time, we can learn more about the relative contributions of stochastic processes and selective processes by comparing homologous traits that have diverged in function, such as the claws of stream-dwelling and the claws of burrowing crayfish.

Table 5.1. Results of covariation between claw size and claw strength for the six species included in my study. Across all species, the force measurement that I recorded exhibited substantial variation in strength for a given size; depicting by the low values of r^2 for each species. M= male. F= female. SD= stream-dweller, B= burrower.

Species	Lifestyle	Sex	r^2	p
<i>Cambarus carinirostris</i>	SD	M	0.26	< 0.001
		F	0.13	< 0.001
<i>Cambarus robustus</i>	SD	M	0.23	< 0.001
		F	0.15	< 0.002
<i>Faxonius obscurus</i>	SD	M	0.36	< 0.001
		F	0.30	< 0.001
<i>Cambarus monongalensis</i>	B	M	0.06	0.209
		F	0.26	< 0.001
<i>Cambarus dubius</i>	B	M	0.001	0.869
		F	0.27	< 0.001
<i>Lacunicambus thomai</i>	B	M	0.08	0.273
		F	0.19	0.014

Fig 5.1. Relation between claw size (PC1) and maximal claw strength in three (A) stream-dwelling and the (B) three burrowing species included in my study. Filled and unfilled circles represent data for males and females, respectively. Furthermore, the full black line and dotted black line represent the linear relationship between males and females, respectively. Species within the gray shaded area are the burrowing species, whereas the nonshaded areas represent the stream-dwelling species.

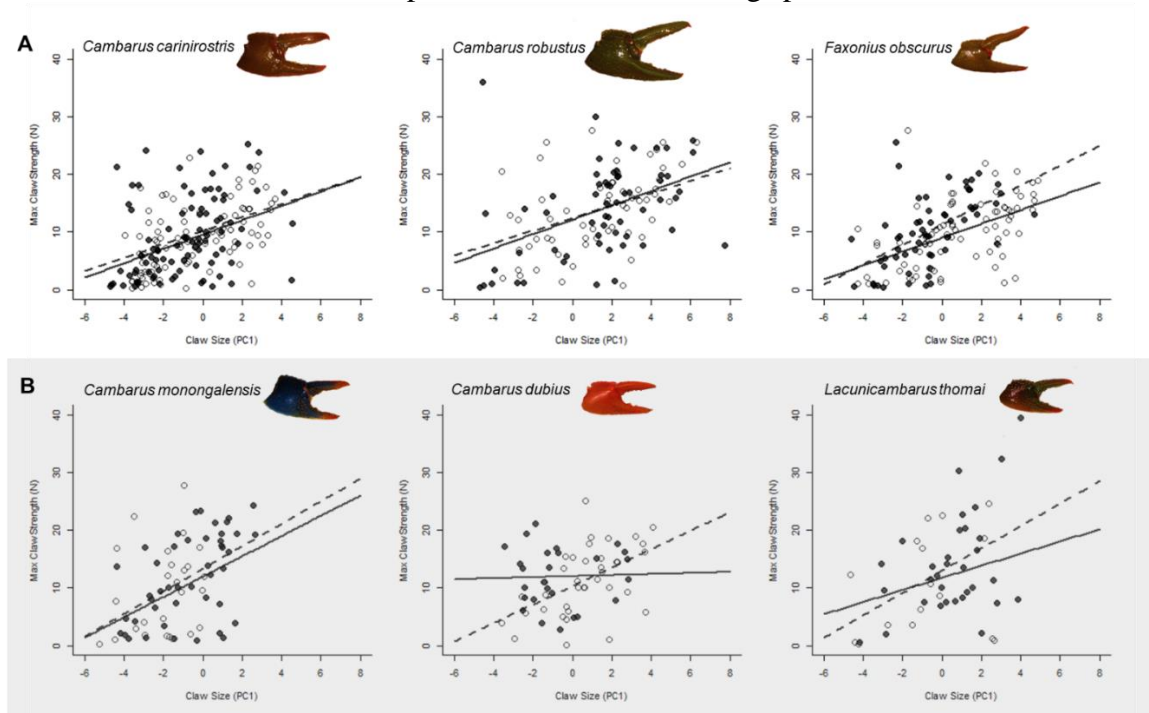


Fig 5.2. Relation between the strength of the claw in males and females of (A) the three stream-dwelling species and (B) the three burrowing species on day 1 and day 2 controlling for crayfish identity as a random factor in a generalized linear mixed model. The dashed line serves as a reference for the equality of strength between days. Species within the gray shaded area are the burrowing species, whereas the nonshaded areas represent the stream-dwelling species.

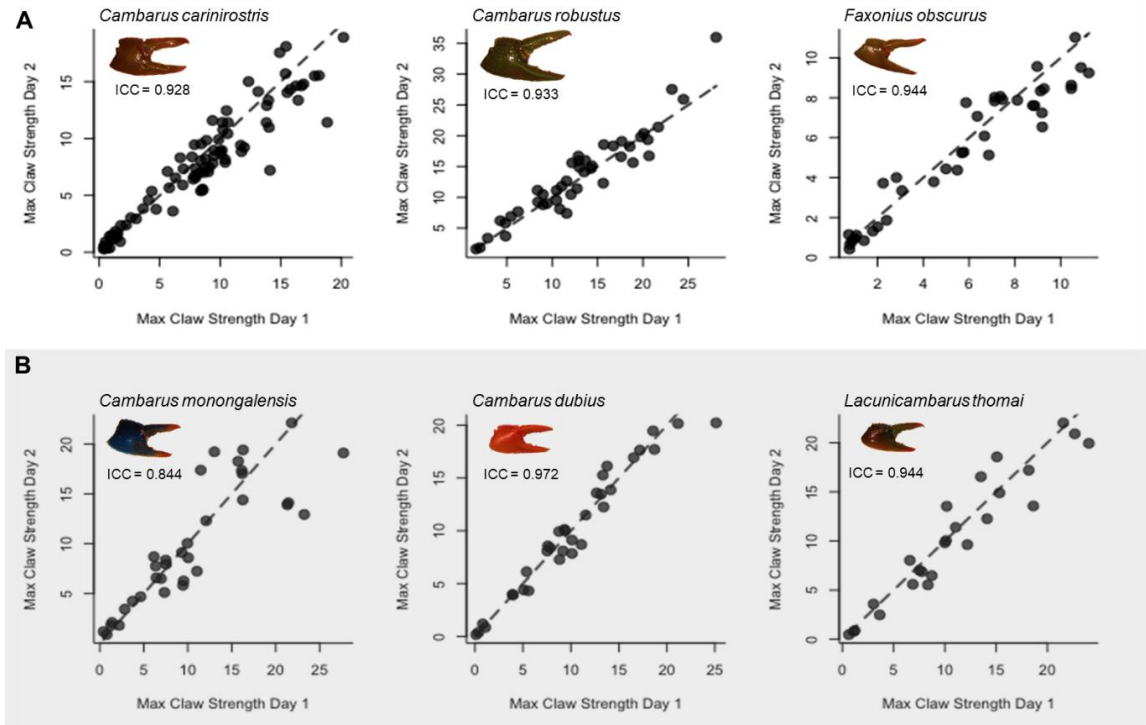


Fig 5.3. The residual maximal claw strength for (A) male and (B) females of the six species included in my study. Species within the gray shaded area are the burrowing species, whereas the nonshaded areas represent the stream-dwelling species. Contrary to my prediction, the residual variation in claw strength was greater in burrowing species. Error bars represent 95% confidence intervals around the mean residual strength for each species. The dashed references line depicts a residual strength value of zero.

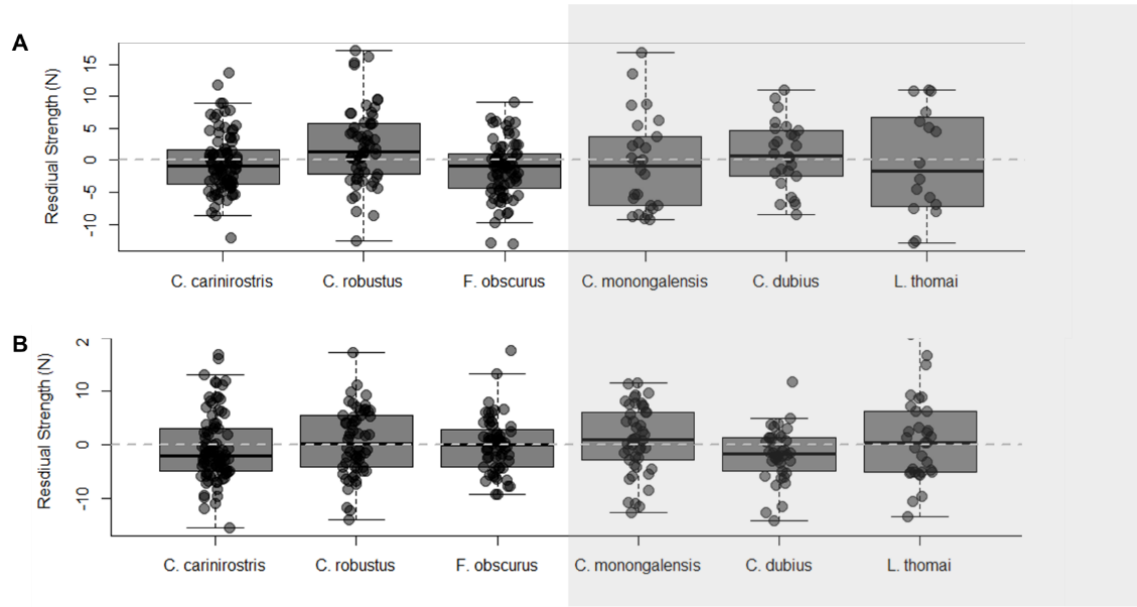


Fig 5.4. (A) Probability of escalating aggression to physical combat based on the difference in body size between opponents. (B) Probability of winning a fight based on the difference in body size between opponents. Circles indicate the outcome of each encounter (0 or 1, where 1 represents winning). The lines represent the fitted values of statistical model that describes my data. In both graphs, the red dots and lines represent the data from *C. carinistostris*. Blue dots and lines represent data *C. robustus*, and green dots and lines represent data from *F. obscurus* aggression. The dotted line provides a reference for zero difference in body size.

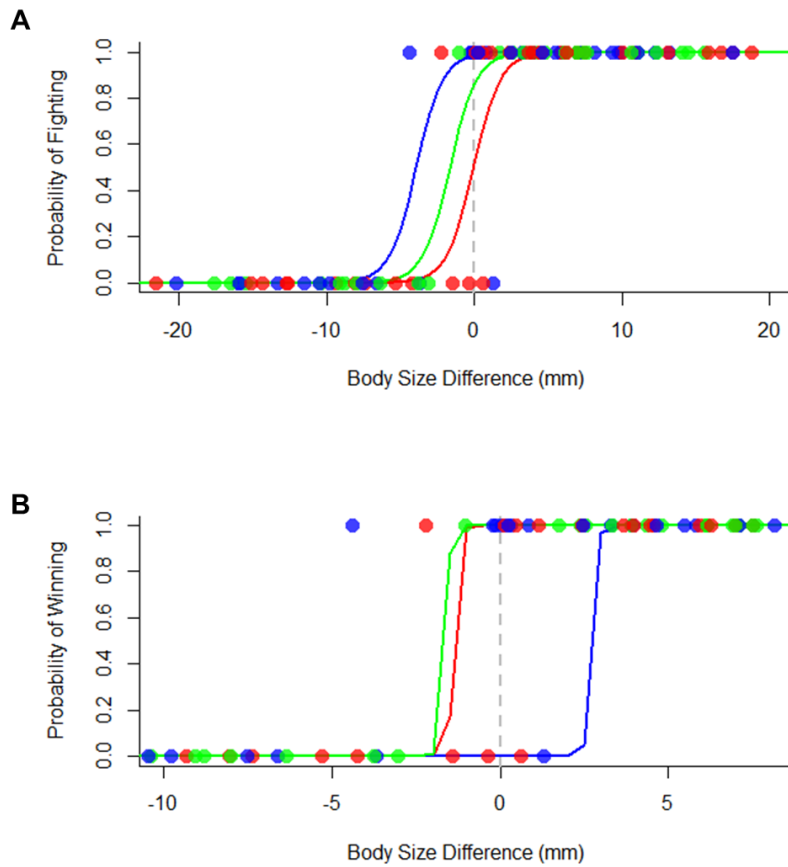
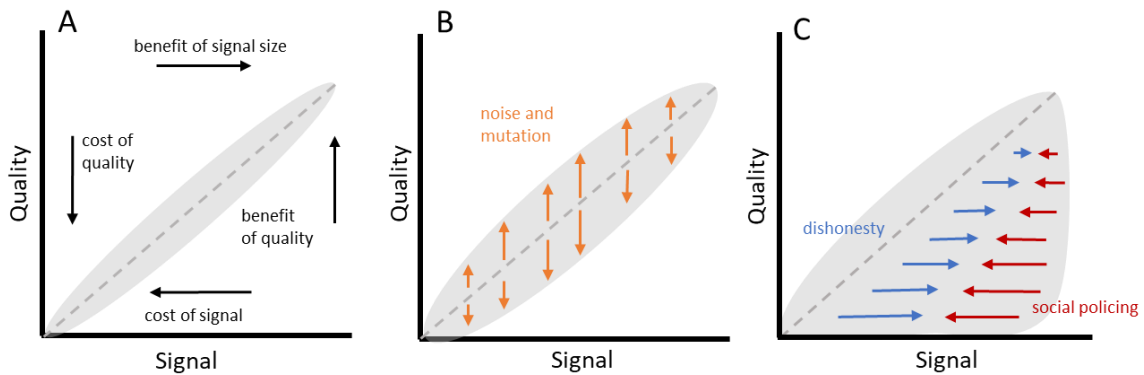


Fig 5.5. Graphical depiction of the covariation between a signal of quality and the actual quality of an individual. In all scenarios, the gray reference line depicts equal allocation of signal size and quality and the gray shaded area represents the morphological space that is being occupied. **A)** The covariation between a signal's size or magnitude and quality in which there is a predictable, reliable relationship between the signal and quality. In this scenario, there are costs and benefits for increasing or decreasing investment in the signal and the quality being signaled, indicated by the black arrows. **B)** The same covariation in **A)** with the addition of developmental noise and mutation (orange arrows), ultimately increasing the covariation between the signal and quality. **C)** In some scenarios, there may be selection for an unreliable (dishonest) relationship between the signal and quality (blue arrows), in which there is selection for large but poor performing signals. However, social policing of dishonest individuals (red arrows) is expected to reduce selection for dishonest signalers.



CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS

My dissertation highlights several issues with prior research on detecting and studying the reliability (or unreliability) of animal signals. Despite finding that claw size unreliably predicts claw strength in eight species of crayfish, I found little evidence that these unreliable claws function as dishonest signals. Thus, my results oppose those of previous researchers studying crayfish, which suggested that their large but weak claws function as dishonest signals. Rather, my results show the importance of conducting behavioral studies to demonstrate the function of a putative signal as an actual signal during behavioral interactions — something which has not been done for many putatively honest or dishonest signals. Furthermore, the results of my data chapters illustrate the importance of distinguishing between an unreliable signal selected for dishonesty and a signal (or non-signaling structure) that varies because of nonadaptive processes. Together, these results should have substantial repercussions on the field of honest communication by altering how biologists detect and study reliable (or unreliable) signals.

In my second chapter, I showed that regenerated *F. virilis* claws are on average 40% weaker for a given size compared to the strengths of original claws. Previous work on crustaceans would suggest that dishonest signaling may be present in males and females *F. virilis*, because crayfish with regenerated claws can signal to competitors as if they have a large and strong claw (Wilson & Angilletta, 2015). Thus, in isolation, the results of my second chapter suggest that regeneration in crayfish may result in dishonest

signaling, as it does in fiddler crabs (Wilson & Angilletta, 2015). Indeed, in several other species of crayfish (*Procambarus clarkii*, *Faxonius palermi longimanus*), researchers have used large but weak claws as evidence for dishonest signaling, although they did not control for claw regeneration (Malavé et al., 2017; Robinson & Gifford, 2019). For example, Robinson et al. (2017) found that the largest claws of males in *F. palmeri longimanus* generate forces ranging from 2.5 N to 10 N. Similarly, Malave et al (2018) found that the largest claws of males in *P. clarkii* generated forces ranging from 2 N to 12 N. Thus, it is likely that the large but weak claws in their sample were from regenerated claws, whereas the large but strong claws were original claws in both of these studies. Future studies should discern between original and regenerated claws when reporting data on pinching performance of crayfish claws to reveal whether or not regeneration is the functional mechanism that produces large but weak claws in other crayfish species. Further, despite not controlling for regeneration in their studies, no behavioral observations were conducted in the studies of the claws of *P. clarkii* or *F. palmeri longimanus*. Thus, reporting variation within the size and strength of a claw only serves as evidence for the *potential* for dishonest communication to occur, not that dishonest communication does occur (Graham & Angilletta, 2020). The issue of equating variation within a signaling system with dishonesty is not unique to studies of crustaceans, as similar methods have been used to assess the relative honesty of signals across Animalia (Carazo & Font, 2010, 2013; Caro et al., 2016). Importantly, to confirm the hypothesis that large but weak claws (whether they are regenerated or not) can lead to dishonest signaling, behavioral observations must be conducted to confirm the use of claw size as a signal and claw strength as the determining factor that influences dominance.

In Chapters 3 and 4, I studied the traits that best predict the outcome of territorial interactions between crayfish. Thus, I was able to determine whether large but weak claws function as dishonest signals in *F. virilis* and *P. clarkii*, as they do in other crustacean species. If large but weak claws function as dishonest signals, I predicted that crayfish would escalate aggression based on claw size (i.e. claw size is used as a signal) and that contests that escalated to direct physical combat would be determined by claw strength. In chapter 3, I found that both male and female *F. virilis* possessed substantial variation in the size and strength of their claws. Despite this, I found that these claws were not used as dishonest signals, because body size alone best predicted whether individuals achieved dominance within same-sex encounters. In chapter 4, I collected additional data on male-male communication and aggression within and between *F. virilis* and *P. clarkii*. Again, despite the males of both species possessing large but weak claws, I was unable to find support for these claws functioning as dishonest signals. Thus, despite all species and sexes in these studies having claws that unreliably predict their strength, the results of my studies suggest that none of them were used as dishonest signals of strength. These results demonstrate the importance of collecting behavioral data when studying dishonest signals; without behavioral data, my other results would have falsely supported the hypothesis that large but weak claws are dishonest signals of strength. Furthermore, Chapters 3 and 4 highlight several important areas of future research on crustacean dishonest communication. For example, we do not know why some species of crayfish (*C. dispar*, *C. destructor*) engage in dishonest communication, whereas others do not. Interestingly, differences in the species-specific fighting behavior

may explain this discrepancy. Both *C. dispar* and *C. destructor* exhibit a unique preliminary stage in their ritualized contests, in which competitors engage in repeated bouts of claw tapping. By contrast, *F. virilis* and *P. clarkii* do not engage in claw tapping but they engage in claw interlocking. In *Cherax*, but not other species, assessment of the claw size and claw strength may allow competitors to more accurately assess the size of their opponent's claws and therefore create the potential for individuals with large but weak claws to use their claws as dishonest signals.

In Chapter 5, I conducted a comparative study to distinguish between unreliable signals and dishonest signals. By leveraging the divergence in claw function in stream-dwelling crayfish species (which engage in aggression) and burrowing crayfish species (which do not engage in aggression), I demonstrated that, regardless of lifestyle (stream-dwelling vs. burrowing), there was substantial variation in the size and strength of claws in these species. Furthermore, even though the three stream-dwelling species wield claws that poorly correlated to claw strength, these claws were not used as dishonest signals.

Instead, body size alone predicted the outcome of aggression in all three species. Thus, inherent biological variation (such as the molting process) and not adaptive dishonest signaling may be generating the high variation in claw size-claw strength relationships observed in crustacean species (Richard & Yvert, 2014; Tsimring, 2014; Wiley, 2013).

This work is among the first to distinguish between unreliable and dishonest signals (Carazo & Font, 2010, 2013).

In summary, my dissertation has demonstrated that despite possessing claws that unreliably correlate to their claw strength, none of these species used their claws as dishonest signals. These results highlight several shortcomings in the prior research on detecting and studying dishonest signals in natural populations. Namely, researchers cannot simply rely on the spurious correlation between a putative signal and the quality that is presumably being signaled. My results are therefore not only applicable within the field of crustaceans, but the entire field of animal communication. Biologists must conduct behavioral observations to confirm the function of the putative signal as a signal. In my work, although it was assumed that claw displays are used during signaling interactions and that claw strength determines the outcome of aggression, my results demonstrate that this is not necessarily true. Overall, biologists' methods for detecting dishonest signals have impeded our understanding of how and why dishonest communication can evolve. Future research should continue to use quantitative and behavioral methods to determine and define dishonest signals. As such, work on crustaceans should be continued, both in crayfish and fiddler crabs. Ultimately, further investigations into dishonest signaling in crustaceans can provide biologists with a better understanding of how and why dishonest signals can evolve in natural populations.

REFERENCES

- Adams, E. S., & Caldwell, R. L. (1990). Deceptive communication in asymmetric fights of the stomatopod crustacean *Gonodactylus bredini*. *Anim. Behav.*, *39*, 706–716.
- Adams, E. S., & Mesterton-Gibbons, M. (1995). The Cost of Threat Displays and the Stability of Deceptive Communication. *Journal of Theoretical Biology*, *175*, 405–421. <https://doi.org/10.1006/jtbi.1995.0151>
- Amarasekare, P. (2002). Interference competition and species coexistence. *Proceedings of the Royal Society B: Biological Sciences*, *269*(1509), 2541–2550. <https://doi.org/10.1098/rspb.2002.2181>
- Andersson, M. B. (1994). *Sexual Selection*. Princeton University Press.
- Angilletta, M. J., & Wilson, R. S. (2012). Cryptic asymmetry: unreliable signals mask asymmetric performance of crayfish weapons. *Biology Letters*, *8*(4), 551–553. <https://doi.org/10.1098/rsbl.2012.0029>
- Backwell, P. R. Y., Christy, J. H., Telford, S. R., Jennions, M. D., & Passmore, N. I. (2000). Dishonest Signalling in a Fiddler Crab. *Proceedings of the Royal Society B*, (November 1999), 719–724.
- Bartón, K. (2018). MuMIn: Multi-Model Inference. R Packag version 1404. *CRANR-Project. Org/Package= MuMIn Bates*.
- Bates, D. M. (2010). lme4: Mixed-effects modeling with R.
- Bergman, D. A., Kozlowski, C. P., McIntyre, J. C., Huber, R., Daws, A. G., & Moore, P. A. (2003). Temporal dynamics and communication of winner-effects in the crayfish, *orconectes rusticus*. *Behaviour*, 805–825.
- Bergman, D. A., & Moore, P. A. (2003). Field Observations of Intraspecific Agonistic Behavior of Two Crayfish Species , *Orconectes rusticus* and *Orconectes virilis* , in Different Habitats. *Biological Bulletin*, (August), 26–35.
- Bergman, D. A., Redman, C. N., Fero, K. C., Simon, J. L., & Moore, P. A. (2006). The impacts of flow on chemical communication strategies and fight dynamics of crayfish. *Marine and Freshwater Behaviour and Physiology*, *39*(4), 245–258. <https://doi.org/10.1080/10236240600980608>
- Bildstein, K. L., McDowell, S. G., & Brisbin, I. L. (1989). Consequences of sexual dimorphism in sand fiddler crabs, *Uca pugilator*: differential vulnerability to avian predation. *Animal Behaviour*, *37*(PART 1), 140–152. [https://doi.org/10.1016/0003-3472\(89\)90014-6](https://doi.org/10.1016/0003-3472(89)90014-6)
- Botero, C. A., Pen, I., Komdeur, J., & Weissing, F. J. (2010). The evolution of individual variation in communication strategies. *Evolution*, *64*(11), 3123–3133. <https://doi.org/10.1111/j.1558-5646.2010.01065.x>
- Bouchard, R. W. (1977). Distribution, systematic status and ecological notes on five

poorly known species of crayfishes in western North America (Decapoda: Astacidae and Cambaridae). *Freshwater Crayfish*, 3, 409–423.

- Bradbury, J. W., & Vehrencamp, S. L. (1998). *Principles of animal communication*. Retrieved from <https://www.sinauer.com/media/wysiwyg/tocs/PrinciplesAnimalCommunication2.pdf>
- Briffa, M., & Sneddon, L. U. (2007). Physiological constraints on contest behaviour. *Functional Ecology*, 21(4), 627–637. <https://doi.org/10.1111/j.1365-2435.2006.01188.x>
- Briffa, Mark. (2006). Signal residuals during shell fighting in hermit crabs: Can costly signals be used deceptively? *Behavioral Ecology*, 17(3), 510–514. <https://doi.org/10.1093/beheco/arj059>
- Brock, R. E., & Smith, L. D. (1998). Recovery of claw size and function following autotomy in *Cancer productus* (Decapoda: Brachyura). *Biological Bulletin*, 194(1), 53–62. <https://doi.org/10.2307/1542513>
- Brumm, H. (2013). *Animal communication and noise*. Springer Science & Business Media.
- Bubb, D. H., Thom, T. J., & Lucas, M. C. (2006). Movement, dispersal and refuge use of co-occurring introduced and native crayfish. *Freshwater Biology*, 51(7), 1359–1368. <https://doi.org/10.1111/j.1365-2427.2006.01578.x>
- Burnham, K. P., & Anderson, D. R. (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer.
- Bywater, C., Angilletta, M., & Wilson, R. (2008). Weapon size is a reliable indicator of strength and social dominance in female slender crayfish (*Cherax dispar*). *Functional Ecology*, 22(2), 311–316. <https://doi.org/10.1111/j.1365-2435.2008.01379.x>
- Bywater, C., Seebacher, F., & Wilson, R. S. (2015). Building a dishonest signal: the functional basis of unreliable signals of strength in males of the two-toned fiddler crab, *Uca vomeris*. *Journal of Experimental Biology*, 218(19), 3077–3082. <https://doi.org/10.1242/jeb.120857>
- Bywater, C., White, C., & Wilson, R. (2014). Metabolic incentives for dishonest signals of strength in the fiddler crab *Uca vomeris*. *The Journal of Experimental Biology*, 217(Pt 16), 2848–2850. <https://doi.org/10.1242/jeb.099390>
- Bywater, C., & Wilson, R. (2012). Is honesty the best policy? Testing signal reliability in fiddler crabs when receiver-dependent costs are high. *Functional Ecology*, 26(4), 804–811. <https://doi.org/10.1111/j.1365-2435.2012.02002.x>
- Callander, S., Kahn, A. T., Maricic, T., Jennions, M. D., & Backwell, P. R. Y. (2013). Weapons or mating signals? Claw shape and mate choice in a fiddler crab. *Behavioral Ecology and Sociobiology*, 67, 1163–1167.

<https://doi.org/10.1007/s00265-013-1541-6>

- Candaten, A., Possenti, A. G., Mainardi, Á. A., Carvalho, M., & Palaoro, A. V. (2020). Fighting scars : heavier gladiator frogs bear more injuries than lighter frogs, (Kluge 1981).
- Carazo, P., & Font, E. (2010). Putting information back into biological communication, *23*, 661–669. <https://doi.org/10.1111/j.1420-9101.2010.01944.x>
- Carazo, P., & Font, E. (2013). “Communication breakdown”: the evolution of signal unreliability and deception. *Animal Behaviour*, *87*, 17–22. <https://doi.org/10.1016/j.anbehav.2013.10.027>
- Carere, C., & Gherardi, F. (2013). Animal personalities matter for biological invasions. *Trends in Ecology and Evolution*, *28*(1), 5–6. <https://doi.org/10.1016/j.tree.2012.10.006>
- Caro, S. M., West, S. A., & Griffin, A. S. (2016). Sibling conflict and dishonest signaling in birds. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(48), 13803–13808. <https://doi.org/10.1073/pnas.1606378113>
- Chang, E. S. (1995). Physiological and biochemical changes during the molt cycle in decapod crustaceans: an overview. *Journal of Experimental Marine Biology and Ecology*, *193*(1–2), 1–14. [https://doi.org/10.1016/0022-0981\(95\)00106-9](https://doi.org/10.1016/0022-0981(95)00106-9)
- Chapple, D. G., Simmonds, S. M., & Wong, B. B. M. (2012). Can behavioral and personality traits influence the success of unintentional species introductions? *Trends in Ecology and Evolution*, *27*(1), 57–64. <https://doi.org/10.1016/j.tree.2011.09.010>
- Chucholl, C. (2016). The bad and the super-bad: prioritising the threat of six invasive alien to three imperilled native crayfishes. *Biological Invasions*, *18*(7), 1967–1988. <https://doi.org/10.1007/s10530-016-1141-2>
- Dalosto, M. M., Palaoro, A. V., Souty-Grosset, C., Bueno, S. L. de S., Loureiro, T. G., Almerão, M. P., ... Santos, S. (2015). One step ahead of the enemy: investigating aggressive interactions between invasive and native crayfish before the contact in nature. *Biological Invasions*, *17*(12), 3503–3515. <https://doi.org/10.1007/s10530-015-0974-4>
- Dalosto, M. M., Palaoro, A. V., Costa, J. R., & Santos, S. (2013). Aggressiveness and life underground : the case of burrowing crayfish, *150*, 3–22. <https://doi.org/10.1163/1568539X-00003034>
- David, P., Hingle, A., Greig, D., Rutherford, A., Pomiankowski, A., & Fowler, K. (1998). Male sexual ornament size but not asymmetry reflects condition in stalk-eyed flies. *Proceedings of the Royal Society B: Biological Sciences*, *265*(1411), 2211–2216. <https://doi.org/10.1098/rspb.1998.0561>
- David, Patrice, Bjorksten, T., Fowler, K., & Pomiankowski, A. (2000). Condition-dependent signalling of genetic variation in stalk-eyed flies. *Nature*, *406*(6792),

186–188. <https://doi.org/10.1038/35018079>

- David Smith, L., & Hines, A. H. (1991). The effect of cheliped loss on blue crab *Callinectes sapidus* Rathbun foraging rate on soft-shell clams *Mya arenaria* L. *Journal of Experimental Marine Biology and Ecology*, *151*(2), 245–256. [https://doi.org/10.1016/0022-0981\(91\)90127-I](https://doi.org/10.1016/0022-0981(91)90127-I)
- Daws, A. G., Konzen, K., & Moore, P. A. (2002). Previous Experiences Alter the Outcome of Aggressive Interactions Between Males in the Crayfish, *Procambarus Clarkii*. *Marine and Freshwater Behaviour and Physiology*, *35*(3), 139–148. <https://doi.org/10.1080/1023624021000014725>
- Dean, J. L. (1969). Biology of the crayfish *Orconectes causeyi* and its use for control of aquatic weeds in trout lakes. *US Fish and Wildlife Service*, *24*.
- Dennenmoser, S., & Christy, J. H. (2013). The design of a beautiful weapon: Compensation for opposing sexual selection on a trait with two functions. *Evolution*, *67*(4), 1181–1188. <https://doi.org/10.1111/evo.12018>
- Dey, C. J., Dale, J., & Quinn, J. S. (2014). Manipulating the appearance of a badge of status causes changes in true badge expression. *Proceedings. Biological Sciences / The Royal Society*, *281*(1775), 20132680. <https://doi.org/10.1098/rspb.2013.2680>
- Dunn, J. C., McClymont, H. E., Christmas, M., & Dunn, A. M. (2009). Competition and parasitism in the native White Clawed Crayfish *Austropotamobius pallipes* and the invasive Signal Crayfish *Pacifastacus leniusculus* in the UK. *Biological Invasions*, *11*(2), 315–324. <https://doi.org/10.1007/s10530-008-9249-7>
- Eberhard, W. G., & Garcia-c, J. M. (2000). Ritual jousting by horned *Parisoschoenus expositus* weevils (Coleoptera, Curculionidae, Baridinae). *Psyche*, (103), 55–85.
- Edsman, L., & Jonsson, A. (1996). The effect of size, antennal injury, ownership and ownership duration on fighting success in male signal crayfish *Pacifastacus leniusculus* (Dana). *Nordic Journal of Freshwater Research*, *72*(February), 80–87. Retrieved from [http://www.google.com/search?client=safari&rls=en-us&q=The+effect+of+size,+antennal+injury,+ownership+and+ownership+duration+on+fighting+success+in+male+signal+crayfish+Pacifastacus+leniusculus+\(Dana\)&ie=UTF-8&oe=UTF-8%5Cnpapers3://publication/uuid/F83C2C](http://www.google.com/search?client=safari&rls=en-us&q=The+effect+of+size,+antennal+injury,+ownership+and+ownership+duration+on+fighting+success+in+male+signal+crayfish+Pacifastacus+leniusculus+(Dana)&ie=UTF-8&oe=UTF-8%5Cnpapers3://publication/uuid/F83C2C)
- Eldar, A., & Elowitz, M. B. (2010). Functional roles for noise in genetic circuits. *Nature*, *467*(7312), 167–173. <https://doi.org/10.1038/nature09326>
- Embets, Z., Escalante, I., & Bateman, P. W. (2019). The ecology and evolution of autotomy. *Biological Reviews*, *1*. <https://doi.org/10.1111/brv.12539>
- Embets, Z., St. Mary, C. M., Herrington, T. J., & Miller, C. W. (2018). Males missing their sexually selected weapon have decreased fighting ability and mating success in a competitive environment. *Behavioral Ecology and Sociobiology*, *72*(5). <https://doi.org/10.1007/s00265-018-2494-6>
- Embets, Z., St Mary, C. M., & Miller, C. W. (2016). Coreidae (Insecta: Hemiptera)

- Limb Loss and Autotomy. *Annals of the Entomological Society of America*, 109(5), 678–683. <https://doi.org/10.1093/aesa/saw037>
- Emlen, D. J. (2008). The Evolution of Animal Weapons. *Annual Review of Ecology, Evolution, and Systematics*, 39(1), 387–413. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173502>
- Enquist, M. (1985). Communication during aggressive interactions with particular reference to variation in choice of behaviour. *Animal Behaviour*, 33(4), 1152–1161.
- Figler, M. H., Finkelstein, J. E., Twum, M., & Peeke, H. V. S. (1995). Intruding male red swamp crayfish, *Procambarus clarkii*, immediately dominate members of established communities of smaller, mixed-sex conspecifics. *Aggressive Behavior*, 21(3), 225–236. [https://doi.org/10.1002/1098-2337\(1995\)21:3<225::AID-AB2480210305>3.0.CO;2-#](https://doi.org/10.1002/1098-2337(1995)21:3<225::AID-AB2480210305>3.0.CO;2-#)
- Funk, D. H., & Tallamy, D. W. (2000). Courtship role reversal and deceptive signals in the long-tailed dance fly, *Rhamphomyia longicauda*. *Animal Behaviour*, 59(2), 411–421. <https://doi.org/10.1006/anbe.1999.1310>
- Gamer, M. (2010). irr: Various coefficients of interrater reliability and agreement. [Http://Cran.r-Project.Org/Web/Packages/Irr/Irr](http://Cran.r-Project.Org/Web/Packages/Irr/Irr).
- Gardner, R., & Morris, M. R. (1989). The evolution of bluffing in animal contests: an ESS approach. *Journal of Theoretical Biology*, 137(2), 235–243. [https://doi.org/10.1016/S0022-5193\(89\)80209-7](https://doi.org/10.1016/S0022-5193(89)80209-7)
- Garvey, J. E., Stein, R. A., & Thomas, H. M. (1994). Assessing how fish predation and interspecific prey competition influence a crayfish assemblage. *Ecology*, 75(2), 532–547. <https://doi.org/10.2307/1939556>
- Gherardi, F., Aquiloni, L., Diéguez-Urbeondo, J., & Tricarico, E. (2011). Managing invasive crayfish: Is there a hope? *Aquatic Sciences*, 73(2), 185–200. <https://doi.org/10.1007/s00027-011-0181-z>
- Gonçalves Loureiro, T., Silva Gentil Anastácio, P. M., Souty-Grosset, C., Araujo, P. B., & Pereira Almerão, M. (2015). Red swamp crayfish: biology, ecology and invasion - an overview. *Nauplius*, 23(1), 1–19.
- Grafen, A. (1990). Biological signals as handicaps. *Journal of Theoretical Biology*, 144, 517–546. Retrieved from Grafen1990.pdf
- Graham, Z. A., & Angilletta, M. J. (2020). Claw size predicts dominance within and between invasive species of crayfish. *Animal Behaviour*, 166, 153–161. <https://doi.org/10.1016/j.anbehav.2020.06.021>
- Graham, Z. A., Garde, E., Heide-Jørgensen, M. P., & Palaoro, A. V. (2020). The longer the better: evidence that narwhal tusks are sexually selected. *Biology Letters*, 16(3), 20190950. <https://doi.org/10.1098/rsbl.2019.0950>
- Graham, Z. A., Padilla-Perez, D. J., & Angilletta, M. J. (2020). Virile crayfish escalate

aggression according to body size instead of weapon size. *Animal Behaviour*, 163(May), 9–15. <https://doi.org/10.1016/j.anbehav.2020.02.014>

- Green, P. a, & Patek, S. N. (2015). Contests with deadly weapons : telson sparring in mantis shrimp (Stomatopoda). *Biology Letters*, 11, 20150558. <https://doi.org/10.1098/rsbl.2015.0558>
- Guiaşu, R. C., Saleh, N., Mozel, E., & Dunham, D. W. (2005). Low aggression in juvenile burrowing crayfish, *Fallicambarus fodiens* (Cottle, 1863) (Decapoda, Cambaridae). *Crustaceana*, 78(4), 421–428. <https://doi.org/10.1163/1568540054473503>
- Hale, P., Wilson, J., Loughman, Z., & Henkanaththegedara, S. (2016). Potential impacts of invasive crayfish on native crayfish: Insights from laboratory experiments. *Aquatic Invasions*, 11(4), 451–458. <https://doi.org/10.3391/ai.2016.11.4.09>
- Hall, M. D., McLaren, L., Brooks, R. C., & Lailvaux, S. P. (2010). Interactions among performance capacities predict male combat outcomes in the field cricket. *Functional Ecology*, 24(1), 159–164. <https://doi.org/10.1111/j.1365-2435.2009.01611.x>
- Hardy, I. C. W., & Briffa, M. (2013). *Animal Contests*. Cambridge University Press.
- Harrison, M. L., Hoover, T. M., & Richardson, J. S. (2006). Agonistic behaviours and movement in the signal crayfish, *Pacifastacus leniusculus*: can dominance interactions influence crayfish size-class distributions in streams? *Canadian Journal of Zoology*, 84, 1495–1504. <https://doi.org/10.1139/z06-141>
- Hobbs, H. H. (1981). The Crayfishes of Georgia. *Smithsonian Contributions to Zoology*, (318), 1–549. <https://doi.org/10.5479/si.00810282.318>
- Holway, D. A., & Suarez, A. V. (1999). Animal behavior: an essential component of invasion biology. *Trends in Ecology & Evolution*, 14(8), 328–330. [https://doi.org/10.1016/S0169-5347\(99\)01636-5](https://doi.org/10.1016/S0169-5347(99)01636-5)
- Hopkins, P. M., & Das, S. (2015). Regeneration in Crustaceans. *Physiology*, 4, 168–198.
- Hudina, S., Galic´, N., Roessink, I., & Hock, K. (2011). Competitive interactions between co-occurring invaders : identifying asymmetries between two invasive crayfish species. *Biological Invasions*, 13, 1791–1803. <https://doi.org/10.1007/s10530-010-9933-2>
- Hudina, S., Hock, K., & Žganec, K. (2014). The role of aggression in range expansion and biological invasions. *Current Zoology*, 60(3), 401–409. <https://doi.org/10.1093/czoolo/60.3.401>
- Hudina, S., Hock, K., Žganec, K., & Lucić, A. (2012). Changes in population characteristics and structure of the signal crayfish at the edge of its invasive range in a European river. *Annales de Limnologie*, 48(1), 3–11. <https://doi.org/10.1051/limn/2011051>

- Hughes, M. (2000). Deception with honest signals: signal residuals and signal function in snapping shrimp. *Behavioral Ecology*, *11*(6), 614–623.
- Hurd, P. L. (1997). Is signalling of fighting ability costlier for weaker individuals? *Journal of Theoretical Biology*, *184*(1), 83–88.
<https://doi.org/10.1006/jtbi.1996.0246>
- Husak, J. F., Lappin, A. K., & Bussche, R. A. V. A. N. D. E. N. (2009). The fitness advantage of a high-performance weapon. *Biological Journal of the Linnean Society*, *96*(4), 840–845.
- Husak, J. F., Lappin, K. A., Fox, S. F., & Lemos-Espinal, J. A. (2006). Bite-Force Performance Predicts Dominance in Male Venerable Collared Lizards (*Crotaphytus antiquus*). *Copeia*, *2*, 301–306. [https://doi.org/10.1643/0045-8511\(2006\)6](https://doi.org/10.1643/0045-8511(2006)6)
- Hutchins, E. D., Eckalbar, W. L., Wolter, J. M., Mangone, M., & Kusumi, K. (2016). Differential expression of conserved and novel microRNAs during tail regeneration in the lizard *Anolis carolinensis*. *BMC Genomics*, *17*(1), 1–11.
<https://doi.org/10.1186/s12864-016-2640-3>
- Jaeger, R. G., & Gergits, W. F. (1979). Intra- and interspecific communication in salamanders through chemical signals on the substrate. *Animal Behaviour*, *27*, 150–156. [https://doi.org/10.1016/0003-3472\(79\)90134-9](https://doi.org/10.1016/0003-3472(79)90134-9)
- James, J., Thomas, J. R., Ellis, A., Young, K. A., England, J., & Cable, J. (2016). Over-invasion in a freshwater ecosystem: Newly introduced virile crayfish (*Orconectes virilis*) outcompete established invasive signal crayfish (*Pacifastacus leniusculus*). *Marine and Freshwater Behaviour and Physiology*, *49*(1), 9–18.
<https://doi.org/10.1080/10236244.2015.1109181>
- Juanes, F., & Hartwick, E. B. (1990). Prey size selection in Dungeness crabs: the effect of claw damage. *Ecology*, *71*(2), 744–758. <https://doi.org/10.2307/1940327>
- Juanes, Francis, & Smith, L. D. (1995). The ecological consequences of limb damage and loss in decapod crustaceans- a review and prospectus, *193*, 197–223.
- Juette, T., Cucherousset, J., & Cote, J. (2014). Animal personality and the ecological impacts of freshwater non-native species. *Current Zoology*, *60*(3), 417–427.
 Retrieved from <https://academic.oup.com/cz/article-abstract/60/3/417/1935255>
- Karplus, I., Sagi, A., Khalaila, I., & Barki, A. (2003). The soft red patch of the Australian freshwater crayfish *Cherax quadricarinatus* von Martens: a review and prospects for future research. *Journal of Zoology*, *259*(2003), 375–379.
<https://doi.org/10.1017/S0952836902003369>
- Kubec, J., Kouba, A., & Buřič, M. (2018). Communication, behaviour, and decision making in crayfish: A review. *Zoologischer Anzeiger*.
<https://doi.org/10.1016/j.jcz.2018.10.009>
- Lailvaux, S. P., Herrel, A., VanHooydonck, B., Meyers, J. J., & Irschick, D. J. (2004). Performance capacity, fighting tactics and the evolution of life-stage male morphs in

- the green anole lizard (*Anolis carolinensis*). *Proceedings of the Royal Society B: Biological Sciences*, 271(1556), 2501–2508. <https://doi.org/10.1098/rspb.2004.2891>
- Lailvaux, S. P., Reaney, L. T., & Backwell, P. R. Y. (2009). Dishonest signalling of fighting ability and multiple performance traits in the fiddler crab *Uca mjoebergi*. *Functional Ecology*, 23(2), 359–366. <https://doi.org/10.1111/j.1365-2435.2008.01501.x>
- Lappin, a K., Brandt, Y., Husak, J. F., Macedonia, J. M., & Kemp, D. J. (2006). Gaping displays reveal and amplify a mechanically based index of weapon performance. *The American Naturalist*, 168(1), 100–113. <https://doi.org/10.1086/505161>
- Ligon, R. A., & McGraw, K. J. (2016). Social costs enforce honesty of a dynamic signal of motivation. *Proceedings of the Royal Society B-Biological Sciences*, 283.
- Logue, D. M., Abiola, I. O., Rains, D., Bailey, N. W., Zuk, M., & Cade, W. H. (2010). Does signalling mitigate the cost of agonistic interactions? A test in a cricket that has lost its song. *Proceedings of the Royal Society B: Biological Sciences*, 277(1693), 2571–2575. <https://doi.org/10.1098/rspb.2010.0421>
- Losos, J. B., Creer, D. A., & Schulte, J. A. (2002). Cautionary comments on the measurement of maximum locomotor capabilities. *Journal of Zoology*, 258(1), 57–61. <https://doi.org/10.1017/S0952836902001206>
- Machida, Y., & Akiyama, Y. B. (2013). Impacts of invasive crayfish (*Pacifastacus leniusculus*) on endangered freshwater pearl mussels (*Margaritifera laevis* and *M. togakushiensis*) in Japan. *Hydrobiologia*, 720(1), 145–151. <https://doi.org/10.1007/s10750-013-1665-8>
- Maginnis, T. L. (2006). The costs of autotomy and regeneration in animals: A review and framework for future research. *Behavioral Ecology*, 17(5), 857–872. <https://doi.org/10.1093/beheco/arl010>
- Maiwald, T., Schulz, H. K., Śmietana, P., & Schulz, R. (2006). Aggressive interactions and interspecific competition between the indigenous crayfish *Astacus astacus* (Linne) and the Non-indigenous *Orconectes limosus* (Rafinesque). *Freshwater Crayfish*, 15(January), 203–211.
- Malavé, B. M., Styga, J. M., & Clotfelter, E. D. (2017). Size, shape, and sex-dependent variation in force production by crayfish chelae. *Journal of Morphology*, 279(3), 1–7. <https://doi.org/10.1002/jmor.20773>
- Mariappan, P., Balasundaram, C., & Schmitz, B. (2000). Decapod crustacean chelipeds: An overview. *Journal of Biosciences*, 25(3), 301–313. <https://doi.org/10.1007/BF02703939>
- Marks, J. C., & Adams, K. J. (2016). Population response of the invasive crayfish *Orconectes virilis* (Hagen, 1870) (Decapoda: Astacoidea: Cambaridae) to restoration: what are the consequences of changes in predatory regulation and physical habitat in Fossil Creek, Arizona, USA? *Journal of Crustacean Biology*,

36(5), 597–606. <https://doi.org/10.1163/1937240X-00002471>

- Maynard Smith, J., & Harper, D. (1983). *Animal Signals*. Oxford University Press.
- McCullough, E. L., Miller, C. W., & Emlen, D. J. (2016). Why Sexually Selected Weapons Are Not Ornaments. *Trends in Ecology & Evolution*, 31(10), 742–751. <https://doi.org/10.1016/j.tree.2016.07.004>
- McCullough, E. L., Miller, C. W., & Emlen, D. J. (2016). Why Sexually Selected Weapons Are Not Ornaments. *Trends in Ecology and Evolution*, 31(10), 742–751. <https://doi.org/10.1016/j.tree.2016.07.004>
- McCullough, E. L., Tobalske, B. W., & Emlen, D. J. (2014). Structural adaptations to diverse fighting styles in sexually selected weapons. *Proceedings of the National Academy of Sciences*, 111(40), 14484–14488. <https://doi.org/10.1073/pnas.1409585111>
- McGuire, B., Olsen, B., Bemis, K. E., & Orantes, D. (2018). Urine marking in male domestic dogs: honest or dishonest? *Journal of Zoology*, 1–8. <https://doi.org/10.1111/jzo.12603>
- McLain, D. K., McBrayer, L. D., Pratt, A. E., & Moore, S. (2010). Performance capacity of fiddler crab males with regenerated versus original claws and success by claw type in territorial contests. *Ethology Ecology & Evolution*, 22(1), 37–49. <https://doi.org/10.1080/03949370903515950>
- McLain, D. K., & Pratt, A. E. (2011). Body and claw size at autotomy affect the morphology of regenerated claws of the sand fiddler crab, *UCA pugilator*. *Journal of Crustacean Biology*, 31(1), 1–8. <https://doi.org/10.1651/10-3298.1>
- Miller, C. W., Joseph, P. N., Kilner, R. M., & Emberts, Z. (2019). A weapons-testes trade-off in males is amplified in female traits. *Proceedings of the Royal Society B: Biological Sciences*, 286(1908). <https://doi.org/10.1098/rspb.2019.0906>
- Mooney, H. A., & Cleland, E. E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences*, 98(10), 5446–5451. Retrieved from www.pnas.org/cgi/doi/10.1073/pnas.091093398
- Moore, J., Obbard, D., Reuter, C., West, S., & Cook, J. (2009). Male morphology and dishonest signalling in a fig wasp. *Animal Behaviour*, 78(1), 147–153. <https://doi.org/10.1016/j.anbehav.2009.04.006>
- Moore, M. J., DiStefano, R. J., & Larson, E. R. (2013). An assessment of life-history studies for USA and Canadian crayfishes: identifying biases and knowledge gaps to improve conservation and management. *Freshwater Science*, 32(4), 1276–1287. <https://doi.org/10.1899/12-158.1>
- Moore, P. (2007). Agonistic behavior in freshwater crayfish: the influence of intrinsic and extrinsic factors on aggressive behavior and dominance. *Evolutionary Ecology of Social and Sexual Systems: Crustaceans as Model Organisms*, 90–114. <https://doi.org/10.1093/acprof:oso/9780195179927.003.0005>

- Nakata, K., & Goshima, S. (2003). Competition for Shelter of Preferred Sizes between the Native Crayfish Species *Cambaroides Japonicus* and the Alien Crayfish Species *Pacifastacus Leniusculus* in Japan in Relation to Prior Residence, Sex Difference, and Body Size. *Journal of Crustacean Biology*, 23(4), 897–907. <https://doi.org/10.1651/C-2411>
- Neubert, M. G., & Parker, I. M. (2004). Projecting rates of spread for invasive species. *Risk Analysis*, 24(4), 817–831. <https://doi.org/10.1111/j.0272-4332.2004.00481.x>
- O'Brien, D. M., & Boisseau, R. P. (2018). Overcoming mechanical adversity in extreme hindleg weapons. *Plos One*, 13(11), e0206997. <https://doi.org/10.1371/journal.pone.0206997>
- Palaoro, A. V., & Briffa, M. (2017). Weaponry and defenses in fighting animals: How allometry can alter predictions from contest theory. *Behavioral Ecology*, 28(1), 328–336. <https://doi.org/10.1093/beheco/arw163>
- Panhuis, T. M., & Wilkinson, G. S. (1999). Exaggerated male eye span influences contest outcome in stalk-eyed flies (Diopsidae). *Behavioral Ecology and Sociobiology*, 46, 221–227.
- Pavey, C. R., & Fielder, D. R. (1996). The influence of size differential on agonistic behaviour in the freshwater crayfish, *Cherax cuspidatus* (Decapoda: Parastacidae). *Journal of Zoology*, 238(3), 445. <https://doi.org/10.1111/j.1469-7998.1996.tb05405.x>
- Perez, D. M., & Backwell, P. R. Y. (2019). Selection for conspicuous visual signals in a fiddler crab. *Behavioral Ecology and Sociobiology*, 73(5). <https://doi.org/10.1007/s00265-019-2670-3>
- Pinheiro, J., Bates, D., Debroy, S., Sarkar, D., Heisterkamp, S., Van Willigen, B., & Maintainer, B. (2017). nlme: Linear and nonlinear mixed effects models. *R Package Version 3.1*.
- Pinto, N. S., Palaoro, A. V., & Peixoto, P. E. C. (2019). All by myself? Meta-analysis of animal contests shows stronger support for self than for mutual assessment models. *Biological Reviews*, 55, brv.12509. <https://doi.org/10.1111/brv.12509>
- Putra, M. D., Bláha, M., Wardiatno, Y., Krisanti, M., Yonvitner, Jerikho, R., ... Patoka, J. (2018). *Procambarus clarkii* (Girard, 1852) and crayfish plague as new threats for biodiversity in Indonesia. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 28(6), 1434–1440. <https://doi.org/10.1002/aqc.2970>
- Rendall, D., Owren, M. J., & Ryan, M. J. (2009). What do animal signals mean? THE ROLE OF INFORMATION IN COMMUNICATION THEORY. *Animal Behaviour*, 78, 233–240. <https://doi.org/10.1016/j.anbehav.2009.06.007>
- Richard, M., & Yvert, G. (2014). How does evolution tune biological noise? *Frontiers in Genetics*, 5(OCT), 1–8. <https://doi.org/10.3389/fgene.2014.00374>
- Rico-guevara, A., & Hurme, K. J. (2018). Introsexually selected weapons. *Biol. Rev.*

<https://doi.org/10.1111/brv.12436>

- Robinson, C. D., & Gifford, M. E. (2019). Sexual dimorphism in performance and muscle allocation in the western painted crayfish *Faxonius palmeri longimanus* (Faxon, 1898) (Decapoda: Astacidea: Cambaridae). *Journal of Crustacean Biology*. <https://doi.org/10.1093/jcbiol/ruz005>
- Rosenberg, M. S. (2002). Fiddler crab claw shape variation: a geometric morphometric analysis across the genus. *New York*, 75(2), 147–162. <https://doi.org/10.1046/j.1095-8312.2002.00012.x>
- Salvador, A., Martin, J., & Lopez, P. (1995). Tail loss reduces home range size and access to females in male lizards, *Psammmodromus algirus*. *Behavioral Ecology*, 6, 382–387.
- Schenk, S. C., & Wainwright, P. C. (2001). Dimorphism and the functional basis of claw strength in six brachyuran crabs. *Journal of Zoology*, 255(1), 105–119. <https://doi.org/10.1017/S0952836901001157>
- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675.
- Science. (2013). Intraspecific Deception by Bluffing : A Defense Strategy of Newly Molted Stomatopods (Arthropoda : Crustacea) Author (s): Rick Steger and Roy L . Caldwell Source : Science , New Series , Vol . 221 , No . 4610 (Aug . 5 , 1983), pp . 558-560 Published, 221(4610), 558–560.
- Searcy, Wi., & Nowicki, S. (2005). *The Evolution of Animal Communication: Reliability and Deception in Signaling Systems*. Princeton University Press. <https://doi.org/10.1007/s13398-014-0173-7.2>
- Seebacher, F., & Wilson, R. S. (2007). Individual recognition in crayfish (*Cherax dispar*): the roles of strength and experience in deciding aggressive encounters. *Biology Letters*, 3(5), 471–474. <https://doi.org/10.1098/rsbl.2007.0289>
- Shackleton, M. A., Jennions, M. D., & Hunt, J. (2005). Fighting success and attractiveness as predictors of male mating success in the black field cricket, *Teleogryllus commodus*: The effectiveness of no-choice tests. *Behavioral Ecology and Sociobiology*, 58(1), 1–8. <https://doi.org/10.1007/s00265-004-0907-1>
- Sint, D., & Füreder, L. (2010). Interspecific competition between two endangered European freshwater decapods, the stone crayfish (*Austropotamobius torrentium*) and white-clawed crayfish (*Austropotamobius pallipes*). *Freshwater Crayfish*, 17, 213–219.
- Skinner, D. M. (1985). Molting and regeneration. *The Biology of Crustacea*, 9, 43–146.
- Smith, L. D. (1990). Patterns of limb loss in the blue crab, *Callinectes sapidus* Rathbun, and the effects of autotomy on growth. *Bulletin of Marine Science*, 46(1), 23–36.
- Sneddon. (1990). Determinants of Male Mating Success in the Temperate Crayfish

- Orconectes Rusticus: Chela Size and Sperm Competition. *Behaviour*, 115, 100–113.
- Sneddon, L. U., Huntingford, F. a., Taylor, a. C., & Orr, J. F. (2000a). Weapon strength and competitive success in the fights of shore crabs (*Carcinus maenas*). *Journal of Zoology*, 250(3), 397–403. <https://doi.org/10.1017/S0952836900003125>
- Sneddon, L. U., Huntingford, F. A., Taylor, A. C., & Orr, J. F. (2000b). Weapon strength and competitive success in the fights of shore crabs (*Carcinus maenas*). *Journal of Zoology*, 250(3), 397–403. <https://doi.org/10.1111/j.1469-7998.2000.tb00783.x>
- Stein, R. A. (1975). Sexual dimorphism in crayfish chelae: functional significance linked to reproductive activities. *Canadian Journal of Zoology*, 54(2), 220–227.
- Szalai, F., & Számadó. (2009a). Honest and cheating strategies in a simple model of aggressive communication. *Animal Behaviour*, 78(4), 949–959. <https://doi.org/10.1016/j.anbehav.2009.06.025>
- Szalai, F., & Számadó, S. (2009b). Honest and cheating strategies in a simple model of aggressive communication. *Animal Behaviour*, 78(4), 949–959. <https://doi.org/10.1016/j.anbehav.2009.06.025>
- Számadó, S. (2000). Cheating as a mixed strategy in a simple model of aggressive communication. *ANIMAL BEHAVIOUR*, 59, 221–230. Retrieved from <http://www.idealibrary.com>
- Számadó, S. (2008). How threat displays work : species-specific fighting techniques , weaponry and proximity risk, (1990), 1455–1463. <https://doi.org/10.1016/j.anbehav.2008.07.010>
- Tanner, J. C., & Bee, M. A. (2019). Within-individual variation in sexual displays: signal or noise? *Behavioral Ecology*, 30(1), 80–91. <https://doi.org/10.1093/beheco/ary165>
- Taylor, P. W., & Jackson, R. R. (2003). Interacting effects of size and prior injury in jumping spider conflicts. *Animal Behaviour*, 65(4), 787–794. <https://doi.org/10.1006/anbe.2003.2104>
- Tibbetts, E. a, & Dale, J. (2004). A socially enforced signal of quality in a paper wasp. *Nature*, 432(7014), 218–222. <https://doi.org/10.1038/nature02949>
- Tsimring, L. S. (2014). Noise in biology. *Reports on Progress in Physics*, 77(2). <https://doi.org/10.1088/0034-4885/77/2/026601>
- Tummon Flynn, P. S., Mellish, C. L., Pickering, T. R., & Quijón, P. A. (2015). Effects of claw autotomy on green crab (*Carcinus maenas*) feeding rates. *Journal of Sea Research*, 103, 113–119. <https://doi.org/10.1016/j.seares.2015.07.002>
- Ueno, R., & Nagayama, T. (2012). Interlocking of chelae is a key factor for dominance hierarchy formation in crayfish. *Journal of Experimental Biology*, 215(16), 2841–2848. <https://doi.org/10.1242/jeb.072520>
- Umbers, K. D. L., Tatarnic, N. J., Holwell, G. I., & Herberstein, M. E. (2012). Ferocious

Fighting between Male Grasshoppers. *PLoS ONE*, 7(11), 1–5.
<https://doi.org/10.1371/journal.pone.0049600>

- Usio, N., Konishi, M., & Nakano, S. (2001). Species displacement between an introduced and a ‘vulnerable’ crayfish: the role of aggressive interactions and shelter competition. *Biological Invasions*, 3(2), 179–185.
- Vieira, M. C., & Peixoto, P. E. C. (2013). Winners and losers: a meta-analysis of functional determinants of fighting ability in arthropod contests. *Functional Ecology*, (27), 305–313. <https://doi.org/10.1111/1365-2435.12051>
- Viney, M., & Reece, S. E. (2013). Adaptive noise. *Proceedings of the Royal Society B: Biological Sciences*, 280(1767), 3. <https://doi.org/10.1098/rspb.2013.1104>
- Vollrath, F. (1987). Altered geometry of webs in spiders with Regenerated Legs. *Nature*, (328), 16–17.
- Vollrath, F. (1990). Leg regeneration in web spiders and its implications for orb weaver phylogeny. *Bulletin of the British Arachnological Society*, 8, 177–184. Retrieved from <http://agris.fao.org/agris-search/search.do?recordID=US201301743144>
- Walter, G. M., van Uitregt, V. O., & Wilson, R. S. (2011). Social control of unreliable signals of strength in male but not female crayfish, *Cherax destructor*. *Journal of Experimental Biology*, 214(19), 3294–3299. <https://doi.org/10.1242/jeb.056754>
- Weis, J. S. (2010). The role of behavior in the success of invasive crustaceans. *Marine and Freshwater Behaviour and Physiology*, 43(2), 83–98.
<https://doi.org/10.1080/10236244.2010.480838>
- West, J. M. (1997). *Ultrastructural and Contractile Activation Properties of Crustacean Muscle Fibres Over the Moulting Cycle*. *Biochem. Physiol* (Vol. 117). Retrieved from https://ac.els-cdn.com/S0305049196003306/1-s2.0-S0305049196003306-main.pdf?_tid=2fe284da-7f36-46b4-a01b-6de7448b5691&acdnat=1549041070_c0fe6ccff19b51e6d22dd09bb24a1685
- Westman, K., Savolainen, R., & Julkunen, M. (2002). Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a small, enclosed Finnish lake: A 30-year study. *Ecography*, 25(1), 53–73.
<https://doi.org/10.1034/j.1600-0587.2002.250107.x>
- Wiley, R. H. (2013). Signal Detection, Noise, and the Evolution of Communication. In *Animal communication and noise*. https://doi.org/10.1007/978-3-642-41494-7_2
- Wilson, R. S., & Angilletta, M. J. (2015). *Dishonest Signalling during aggressive interactions: theory and empirical evidence* (Vol. 53).
<https://doi.org/10.1017/CBO9781107415324.004>
- Wilson, R. S., Angilletta, M. J., James, R. S., Navas, C., & Seebacher, F. (2007). Dishonest signals of strength in male slender crayfish (*Cherax dispar*) during agonistic encounters. *The American Naturalist*, 170(2), 284–291.
<https://doi.org/10.1086/519399>

- Wilson, R. S., James, R. S., Bywater, C., & Seebacher, F. (2009). Costs and benefits of increased weapon size differ between sexes of the slender crayfish, *Cherax dispar*. *The Journal of Experimental Biology*, 212(Pt 6), 853–858.
<https://doi.org/10.1242/jeb.024547>
- Yasuda, C. I., Matsuo, K., & Wada, S. (2014). Rapid regeneration of the major cheliped in relation to its function in male-male contests in the hermit crab *Pagurus middendorffii*. *Plankton and Benthos Research*, 9(2), 122–131.
<https://doi.org/10.3800/pbr.9.122>
- Zahavi, A. (1975). *Mate Selection-A Selection for a Handicap*. *Journal of Theoretical Biology* (Vol. 53). Retrieved from https://ac.els-cdn.com/0022519375901113/1-s2.0-0022519375901113-main.pdf?_tid=e022f9b2-e3f0-4278-9b81-fa49dca26787&acdnat=1549035028_63dc8e2cbb479b802f30755bfe3404e4
- Zuur. (2013). *Mixed Effects Models and Extensions in Ecology with R*. *Journal of Chemical Information and Modeling* (Vol. 53).
<https://doi.org/10.1017/CBO9781107415324.004>

APPENDIX A
SUPPLEMENTAL MATERIAL

Table S2.1. The most likely models predicting the maximal claw pinching strength

Model	df	Log likelihood	AICc	ΔAICc	w
claw length + regeneration status + sex + regeneration*sex	7	-1151.24	2316.79	0.00	0.54
claw length + regeneration status + sex	6	-1153.19	2318.61	1.82	0.22
claw length + regeneration status + sex + claw length*regeneration status + regeneration*sex	8	-1152.02	2320.44	3.65	0.09
claw length + regeneration status + sex + claw length*sex + regeneration*sex	8	-1152.27	2320.94	4.14	0.07
claw length + regeneration status + sex + claw length*sex	7	-1154.07	2322.45	5.66	0.03
claw length + regeneration status	5	-1156.61	2323.39	6.60	0.02
claw length + regeneration status + sex + claw length*regeneration status	7	-1154.63	2323.57	6.78	0.02
claw length + regeneration status + sex + claw length*sex + claw length*regeneration status + regeneration*sex	9	-1153.10	2324.70	7.91	0.01
null	3	-1225.86	2457.79	140.99	0.00

All models contained an intercept and error terms associated with the identity of the focal crayfish. For each model, the degrees of freedom (*df*), the corrected Akaike information

criterion (AICc), Akaike weight (w) and the log likelihood are reported. Models were ranked according to their corrected AICc.

Table S2.2. Coefficients and standard errors for the model predicting the maximal claw pinching strength, based on full model averaging. Coefficients are listed based on absolute value.

<i>Independent variable</i>	<i>Coefficient</i>	<i>SE</i>
regeneration status	-6.15	1.52
intercept	4.75	2.29
sex	-2.23	1.951
regeneration status * sex	1.12	1.24
claw length	0.38	0.07
claw length * regeneration status	-0.01	0.04
claw length * sex	-0.008	0.05
claw length * regeneration status * sex	-0.0001	0.007

Table S2.3. The most likely models predicting the residual claw pinching strength

Model	<i>df</i>	Log likelihood	AICc	ΔAICc	<i>w</i>
regeneration status + sex + regeneration status * sex	6	-1149.76	2311.74	0.00	0.45
regeneration status	4	-1152.40	2312.91	1.17	0.25
regeneration status + sex	5	-1151.65	2313.46	1.71	0.19
claw length + regeneration status + sex + regeneration status * sex	7	-1151.12	2316.55	4.71	0.04
claw length + regeneration status	5	-1153.82	2317.81	6.06	0.02
claw length + regeneration status + sex	6	-1152.99	2318.22	6.48	0.02
claw length + regeneration status + sex + claw length * regeneration status + regeneration status * sex	8	-1151.94	2320.28	8.53	0.01
null	3	-1200.84	2407.75	96.01	0.00

All models contained an intercept and error terms associated with the identity of the focal crayfish. For each model, the degrees of freedom (*df*), the corrected Akaike information criterion (AICc), Akaike weight (*w*) and the log likelihood are reported. Models were ranked according to their corrected AICc.

Table S2.4. Coefficients and standard errors for the model predicting the residual claw pinching strength, based on full model averaging. Coefficients are listed based on absolute value.

<i>Independent variable</i>	<i>Coefficient</i>	<i>SE</i>
regeneration status	-6.20	0.86
intercept	2.89	1.06
regeneration status * sex	0.75	1.09
sex	-0.43	0.96
claw length	< 0.01	0.02
claw length * regeneration status	< 0.001	0.01
claw length * sex	< 0.001	0.01
claw length * sex * regeneration status	< 0.001	< 0.01

Table S2.5. The most likely models predicting the closer claw muscle mass for individual claws

Model	df	Log likelihood	AICc	ΔAICc	W
Muscle weight + regeneration status + sex + muscle weight*regeneration status + muscle weight*sex + regeneration status*sex + muscle weight*regeneration status*sex	10	-1122.72	2266.06	0.00	0.72
Muscle weight + regeneration status + sex + muscle weight*regeneration status + muscle weight*sex + regeneration status*sex	9	-1125.24	2268.99	2.93	0.17
Muscle weight + regeneration status + sex + muscle weight*regeneration status + regeneration status*sex	8	-1127.10	2270.60	4.54	0.07
Muscle weight + regeneration status + sex + muscle weight*regeneration status + muscle weight*sex +	8	-1128.40	2273.20	7.14	0.02
Muscle weight + regeneration status + sex + regeneration status*sex + muscle weight*regeneration status*sex	8	-1128.87	2274.15	8.09	0.01

Model	<i>df</i>	Log	AICc	ΔAICc	<i>W</i>
		likelihood			
Muscle weight + regeneration status + sex + muscle weight*regeneration status*sex	7	-1130.72	2275.76	9.70	0.01
null	3	-1225.86	2457.79	191.72	0.00

All models contained an intercept and error terms associated with the identity of the focal crayfish. For each model, the degrees of freedom (*df*), the corrected Akaike information criterion (AICc), Akaike weight (*w*) and the log likelihood are reported. Models were ranked according to their corrected AICc.

Table S2.6. Coefficients and standard errors for the model predicting the closer claw muscle mass, based on full model averaging. Coefficients are listed based on absolute value.

<i>Independent variable</i>	<i>Coefficient</i>	<i>SE</i>
intercept	10.9150	1.3210
muscle weight	10.2494	1.9693
<i>regeneration status</i>	-6.1232	1.5520
<i>muscle weight*regeneration status</i>	4.9328	3.5753
<i>sex</i>	-3.0733	1.9233
<i>regeneration status*sex</i>	2.9772	2.1389
<i>muscle weight*regeneration status*sex</i>	-0.7779	4.1351
<i>muscle weight*sex</i>	-0.7155	2.3874

Table S4.1. Principal components loadings for claw size measurements of virile crayfish, *Faxonius virilis*, and red swamp crayfish, *Procambarus clarkii*. The values presented are relative contributions of the six claw measurements to the variation explained by each principal component. PC1 values all had loadings in the same direction and described 88.9% of the variation in morphology within and between species. Therefore, I used PC1 as a single measurement to reflect overall claw size. PC2 primarily explained variation in claw shape, driven by measurement of the length of the manus from heel to joint.

Claw measurement	Description	PC1	PC2	PC3
1	Width at heel	0.426	0.096	0.285
2	Width at dactyl/manus joint	0.429	0.057	0.244
3	Length of manus from heel to joint	0.331	-0.930	0.027
4	Width of pollex at dactyl joint	0.421	0.173	-0.378
5	Width of dactyl	0.416	0.275	0.501
6	Length of pollex (tip to joint)	0.417	0.131	-0.68
Eigenvalue		5.33	0.48	0.11
% Variance		88.9	8.00	1.81
Cumulative		88.9	96.9	98.71

Table S4.2. The most likely model of contest escalation behaviour included the interaction between claw size difference and opponent species. All models contained an intercept and error terms associated with the identity of the focal crayfish. For each model, the number of parameters (k), the corrected Akaike information criterion (AICc), Akaike weight (w) and the log likelihood (LL) are reported. Models were ranked according to their corrected Akaike information criterion (AICc).

Model	k	LL	AICc	Δ AICc	w
Claw size difference*species	9	-28.64	76.52	0.00	0.37
Claw size difference + species	6	-32.13	76.82	0.30	0.32
Claw size difference	3	-35.66	77.48	0.96	0.23
Claw size difference + residual strength	5	-34.68	79.75	3.23	0.07
Strength difference	3	74.28	154.72	78.20	0.00
Species	5	-83.06	176.53	-100.01	0.00
Null	2	-90.25	184.57	108.606	0.00

Table S4.3. The most likely model of winning a fight included claw size difference alone. For each model, the number of parameters (k), the corrected Akaike information criterion (AICc), Akaike weight (w) and the log likelihood (LL) are reported. Models were ranked according to their corrected Akaike information criterion (AICc).

Model	k	LL	AICc	Δ AICc	w
Claw size difference	2	-30.07	64.35	0.00	0.43
Claw size difference + strength difference	3	-29.61	65.65	1.30	0.22
Species + claw size difference	4	-28.75	66.23	1.88	0.17
Species*claw size difference	6	-26.92	67.42	3.07	0.09
Claw size difference*strength difference	4	-29.59	67.90	3.55	0.07
Strength difference	2	-33.83	71.86	7.51	0.01
Null	1	-37.46	76.99	12.64	0.00
Species	3	-35.37	77.18	12.83	0.00

Table S5.1. Total sample size, number of males, number of females and burrowing status each individual species included in this study. SD= stream-dweller, B= burrower.

Species	N (males, females)	Lifestyle
<i>Cambarus carinirostris</i>	114 (57,57)	SD
<i>Cambarus robustus</i>	61 (30,31)	SD
<i>Faxonius obscurus</i>	82 (47,35)	SD
<i>Cambarus monongalensis</i>	40 (17,23)	B
<i>Cambarus dubius</i>	34 (14,20)	B
<i>Lacunicambus thomai</i>	28 (17,11)	B

Table S5.2. Principal components loadings for claw size measurements of crayfish. The values presented are relative contributions of the six claw measurements to the variation explained by each principal component. PC1 values all having loadings in the same direction and best explain the variation in overall claw size.

Claw Measurement		PC1	PC2	PC3
1	Width at heel	0.411	0.312	0.134
2	Width at dactyl/manus joint	0.418	0.158	0.012
3	Length of manus from heel to joint	0.403	0.515	-0.518
4	Width of pollex at dactyl joint	0.407	-0.414	0.429
5	Width of dactyl	0.413	0.069	0.470
6	Length of pollex from tip to joint	0.396	-0.651	-0.556
Eigenvalue		0.729	-0.542	-0.135
% of variance		93.92	3.45	1.11
Cumulative		93.92	97.37	98.48

Table S5.3. Results of the repeatability of claw strength data for male and female crayfish across consecutive days for the six species include in my study. Across all species, the force measurement recorded on the first day highly correlated to the force measurement recorded on the second day; depicting by the high values of r^2 for each species. SD= stream-dweller, B= burrower.

Species	Lifestyle	r^2	P
<i>Cambarus carinirostris</i>	SD	0.89	< 0.001
<i>Cambarus robustus</i>	SD	0.89	< 0.001
<i>Faxonius obscurus</i>	SD	0.91	< 0.001
<i>Cambarus monongalensis</i>	B	0.74	< 0.001
<i>Cambarus dubius</i>	B	0.95	< 0.001
<i>Lacunicambus thomai</i>	B	0.91	< 0.001

Table S5.4. Results of covariation between body size and claw size for the six species include in my study. M= male. F= female. SD= stream-dweller, B= burrower.

Species	Lifestyle	Sex	r^2	p
<i>Cambarus carinirostris</i>	SD	M	0.78	< 0.001
		F	0.83	< 0.001
<i>Cambarus robustus</i>	SD	M	0.90	< 0.001
		F	0.94	< 0.001
<i>Faxonius obscurus</i>	SD	M	0.69	< 0.001
		F	0.86	< 0.001
<i>Cambarus monongalensis</i>	B	M	0.81	< 0.001
		F	0.70	< 0.001
<i>Cambarus dubius</i>	B	M	0.645	< 0.001
		F	0.38	< 0.001
<i>Lacunicambus thomai</i>	B	M	0.96	< 0.001
		F	0.55	< 0.001

Table S5.5. The most likely models predicting the probability of engaging in aggression

Model	<i>df</i>	Log likelihood	AICc	ΔAICc	<i>w</i>
body size difference	3	-12.87	32.00	0.00	0.65
body size difference + species	5	-11.27	33.19	1.20	0.35
null	2	-50.57	105.27	73.28	0.00
species	4	-50.38	109.19	77.19	0.00

All models contained an intercept and error terms associated with the identity of the focal crayfish. For each model, the degrees of freedom (*df*), the corrected Akaike information criterion (AICc), Akaike weight (*w*) and the log likelihood are reported. Models were ranked according to their corrected AICc.

Table S5.6. Coefficients and standard errors for the model of probability of winning a fight with an opponent crayfish, based on full model averaging.

<i>Independent variable</i>	<i>Coefficient</i>	<i>SE</i>
intercept	1.2475	1.1971
body size difference	1.0331	0.3196
<i>C. robustus</i>	4.2587	3.1332
<i>F. obscurus</i>	1.7607	1.9610

Table S5.7. The most likely models predicting the probability of winning a fight

Model	<i>df</i>	Log likelihood	AICc	ΔAICc	<i>w</i>
body size difference + species	5	--12.65	36.84	0.00	0.50
body size difference	3	-15.69	37.97	1.13	0.28
body size difference + claw size difference	4	-14.91	38.83	1.99	0.18
body size difference + claw size difference + species	6	-14.01	42.24	5.40	0.03
claw size difference	3	-22.21	51.00	14.17	0.00
claw size difference + species	5	-21.38	54.30	17.46	0.00
null	2	-31.17	66.62	29.79	0.00
species	4	-29.74	68.49	31.65	0.00

All models contained an intercept and error terms associated with the identity of the focal crayfish. For each model, the degrees of freedom (*df*), the corrected Akaike information criterion (AICc), Akaike weight (*w*) and the log likelihood are reported. Models were ranked according to their corrected AICc.

Table S5.8. Coefficients and standard errors for the model of probability of winning a fight with an opponent crayfish, based on full model averaging.

<i>Independent variable</i>	<i>Coefficient</i>	<i>SE</i>
intercept	8.1567	10.7999
body size difference	7.7786	5.1074
<i>C. robustus</i>	-25.8976	26.6746
<i>F. obscurus</i>	1.3855	6.9467
claw size difference	0.6765	2.0474

Figure S5.1. Diagram showing the six linear measurements to describe the size of each claw of the virile crayfish. The six measurements were as follows: 1) width at heel, 2) width at dactyl/manus joint, 3) length of manus from heel to joint, 4) width of pollex at dactyl joint, 5) width of dactyl, 6) and length of pollex from tip to joint.

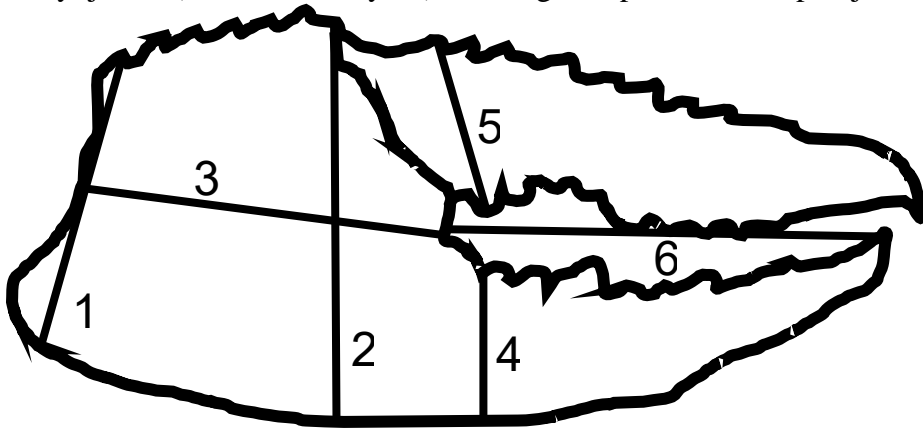


Figure S5.2. Scatter plot of the first two measurements (PC1 and PC2) from the Principal Component Analysis of crayfish claw size. Claw sizes are variable across species, demonstrated by the clustering of individual's species claws. Circles and triangles represent individual claws from male and female species, respectively. Species are represented by different colors.

