HOW DOES ALTERATION OF CHEMICAL INFORMATION AFFECT ASSESSMENT IN MALE AND FEMALE CRAYFISH, ORCONECTES RUSTICUS?

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ABSTRACT

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This dissertation builds on our limited understanding of female agonism through the lens of assessment and the information driving these behavioral strategies. While empirical studies have demonstrated differences in winning probabilities and contest duration for male and female intrasexual contests, we know very little about the rules dictating differences in behavioral strategies and information used in these contests (Rosvall, 2011). The work outlined in this dissertation fills these gaps in knowledge by examining (1) sex-based differences in behavioral response to a signal within agonistic contests, (2) the effect signal removal on assessment in intersexual contests, and (3) sex-based differences in population level assessment strategies under differing social and environmental contexts.

I used crayfish as my model organism due to their well-studied aggressive behaviors and the propensity of both sexes to engage in inter- and intrasexual interactions. Because of their heavy reliance on chemical information across many aspects of their behavior, I first examined differences in male and female crayfish contest strategies in light of differential use of and response to chemical stimuli. Chemical information (i.e. urine) release and subsequent behavioral changes were quantified across male and female inter- and intrasexual contests, revealing that the behavioral response to this urine release differed based on opponent sex and relative size (Wofford et al., 2017). In line with previous studies, mixed sex contests seemingly followed a different set of rules and contest resolution than male or female same sex contests.

The second aim of this dissertation further examined these differences by blocking chemosensory ability in mixed sex contests. Intersexual contest duration was significantly impacted by the sex of the lesioned opponent. Specifically, male lesioned contests were
relatively unaffected by impaired chemosensation while female lesioned contests showed significant shifts in duration. Furthermore, the assessment strategy in place differed based on which sex had access to chemical information. In the final aim, I explored sex-based assessment differences due to alteration of social and environmental context. Artificial populations that varied in sex composition (male or female population), strength ratio (weak or strong skewed populations), and resource availability (i.e. resource value) demonstrated significant variation in assessment strategies in place. These findings confirm previous work that (1) suggests assessment fluidity across environmental and social contexts and (2) posits that the rules dictating male and female contest strategies significantly differ.
According to the Uncertainty Principle, there is much in life that we cannot control. Since we cannot control the universe, we do nothing. Since we cannot control the universe, we can also do everything, just for the fun of it. The earth moves, but we do not feel its motion.

We do nothing, but we do it all.

-Qiguang Zhao
This dissertation is dedicated to my family: Bill, Janet, and Catherine Wofford.

Thank you for believing in me, even when I did not believe in myself.
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CHAPTER I: GENERAL INTRODUCTION

While direct physical interactions (i.e. fighting) can convey access to vital resources, the time and energy investment necessary to engage in these behaviors necessitates some type of strategy to assess when costs have outweighed the potential benefits (Parker, 1974). For example, using information about oneself and/or one’s opponent to dictate contest retreat can prevent persistence against a superior opponent or contest termination when the probability of success is high. The behavioral decision to retreat from a contest, termed an assessment strategy, is dependent on several variables, including social and environmental contexts (Arnott and Elwood, 2008; Tibbets et al., 2013), previous experiences (Fawcett and Johnstone, 2010; Garcia et al., 2012), and information availability (Oliveira et al., 1998; Rillich et al., 2007; Chen and Fernald, 2011). Due to nuanced differences in life history and reproductive investment for male and female organisms, current theory posits that sexual conflict and varying selective pressures should drive differentiation in male and female agonism and assessment (Clutton-Brock and Huchard, 2013b). While empirical studies have demonstrated differences in winning probabilities and contest duration for male and female intrasexual contests, we know very little about distinctions in the rules dictating behavioral strategies and information use (Rosvall, 2011).

The last few decades have seen a surge in empirical studies of female intrasexual agonism; however, our knowledge of ultimate and proximate drivers behind physical female competition is still lacking. Females of some species demonstrate a similar propensity for aggression compared to their male counterparts (Bro-Jørgensen, 2002; Watson and Simmons, 2010; Cain and Langmore, 2015), and, in some cases, female aggression can significantly alter male behavior and fitness (Persons and Uetz, 2005; Stoltz et al., 2008; Kralj-Fišer et al., 2012). Studies probing the evolutionary drivers/selective pressures behind contest behavior have
revealed nuanced differences for males and females (Rosvall, 2011). While studies on male aggression and fighting behavior center around mate attraction or obtaining resources used to attract females, female based studies have found that females not only fight for their own resource provision and survival but also for offspring provision and, in some species, offspring protection (Wolff, 1985; Crespi 1990; Wittig and Boesch, 2003; Öst et al., 2007; Watson and Simmons, 2010). Evidence also suggests that males and females utilize similar signals during contest behavior but perhaps use them in different ways. For example, female song has been found to be a widespread and ancestral trait across bird species (Odom et al., 2014), and is commonly used in aggressive interactions (Vondrasek, 2006; Cain et al., 2015; Krieg and Getty, 2016). However, there are nuanced differences in male and female song (Yamaguchi, 1998; Hahn et al., 2015) and evidence suggests that males and females use aggressive singing bouts under different contexts (Vondrasek, 2006). Mounting evidence suggests that, because the sexes differ in selective pressures and information driving agonistic behavior, males and females are likely to differ in the rules of engagement used to determine contest persistence (Rosvall, 2011).

Empirical and theoretical work has demonstrated that assessment differs based on a number of factors including previous contest experience (Fawcett and Johnstone, 2010; Garcia et al., 2012), resource availability (Arnott and Elwood, 2008; Tibbets, 2013), and even the stage of contest (Hsu et al., 2008). A growing number of studies are also finding that strategies for contest resolution appear to differ between males and females (Draud et al., 2004; Briffa and Dallaway, 2007; Elias et al., 2010; Wofford et al., 2015). Some suggest that these differences lie in the motivation driving contest engagement. For instance, studies in wasps and jumping spiders suggest that female contest strategies are driven more by information about the value of a resource (RV) while male assessment is dictated by opponent resource holding potential (RHP)
(Goubault et al., 2007; Elias et al., 2010; Dunn et al., 2015). Others suggest that rules for conspecific engagement simply differ for males and females. Female jumping spiders appear to have no general ritual to their agonism while males do. Consequently, females of this species tend to have higher instances of injury and death (Elias et al., 2010). Similarly, in intersexual contests, male hermit crabs (*Pagurus bernhardus*) are more likely to win/evict a female conspecific, but the female hermit crabs fight harder as evidenced by higher frequencies of shell rapping behavior (Briffa and Dallaway, 2007). Unfortunately, only a handful of studies have begun probing female assessment strategies and information use in contests; fewer have made across-sex comparisons or examined how sex-based distinctions affect male-female interactions.

The work outlined in this dissertation begins to fill this gap in knowledge via exploration of differences in assessment strategies for male and female individuals as well as the consequences of these differences. Specifically, the focus of this dissertation revolves around better understanding the role that differential information use plays in male and female contest persistence (i.e. assessment) and how these differences influence population-level interactions. The following aims will address (1) sex-based differences in behavioral response to a well-documented signal within agonistic contests, (2) the effect of the removal of that signal on assessment in intersexual contests, and (3) differences in population-level assessment strategies for males and females based on various social and environmental contexts.

The studies outlined in this dissertation utilized crayfish to accomplish these aims as these organisms make ideal models for the study of contest strategies. Their previous establishment as a model for aggression and agonistic interactions provides a literature base of a well-documented, stereotyped behavioral repertoire (reviewed in Moore, 2007). This makes behavioral effects of manipulations easy to track. Furthermore, several decapod crustacean
species (including crayfish) have been established as models for the role of chemical signals in contest outcome and behavior (Bergman & Moore, 2005a; Zeil & Hemmi, 2006; Aggio & Derby, 2011; Breithaupt, 2011; Mowles & Briffa, 2012). Specifically, experimental manipulation of chemical information exchange during dyadic contests has demonstrated significant effects on status recognition (Zulandt-Schneider et al., 2001; Shabani et al., 2009), winner effects (Bergman et al., 2003; Johnson and Atema, 2005), and pre-copulatory fighting bouts (Bushman and Atema, 1997; Simon and Moore, 2007; Berry and Breithaupt, 2010). Many of these studies have implicated a singular chemical signal (urine) as the primary vehicle for this information (Zulan
dt-Schneider et al., 2001; Breithaupt and Eger, 2002; Bergman et al., 2005; Aggio and Derby, 2011). However, evidence suggests that some aspect of this chemical signal differs for male and female individuals (Atema and Cowan, 1986; Fadool et al., 1989; Stebbing et al., 2003; Belanger and Moore, 2006). Finally, due to large population densities (Mather and Stein, 1993; Perry et al., 1997; Nystrom, 2002; Huber and Davis, 2007), and propensity for agonistic behaviors (Bovbjerg, 1953; Moore, 2007), both male and female crayfish frequently engage in inter- and intrasexual fights for resources (Bergman and Moore, 2003; Martin III and Moore, 2007; Fero and Moore, 2014).

Due to the reliance on chemical information in almost all aspects of crayfish behavior, Aim 1 focused on the differential role of chemical communication for male and female assessment. Specifically, this project addressed whether male and female crayfish demonstrate differences in frequency of urine release as well as their behavioral response to this chemical signal. Urine has been shown to significantly impact crayfish contest behavior and outcome (Breithaupt and Eger, 2002; Bergman et al., 2005; Simon and Moore, 2007). Therefore, I hypothesized that this chemical information would play an integral role in crayfish assessment.
Based on previous evidence showing male and female crayfish are likely utilizing different rules for contest resolution (Wofford et al., 2015), I also hypothesized males and females would differ in their behavioral reaction to urine release across intra- and intersexual contests.

If urine release plays a significant role in contest behavior, and if this role is sex-dependent, then removal of chemical information should have differential impacts on male and female behavior. Aim 2 of this dissertation further examined the role of chemical information in contest assessment by blocking male and female chemosensory ability in mixed sex contests. I hypothesized that males and females would demonstrate significantly different behavior when chemical information was unavailable due to previous findings that the sexes respond differently to urine release.

In the final aim (Aim 3), I explored the ecologically relevant impact of sex-based assessment differences via the alteration of social and environmental context across male and female populations. These populations were varied based on sex composition (all male or all female population), strength ratio (weak or strong-skewed populations), and resource availability (i.e. resource value) to test previously developed theoretical models suggesting assessment fluidity across contexts (Mesterton-Gibbons and Heap, 2014). I hypothesized that the nuanced differences seen previously in male and female contests would manifest in population-level variation in behavior. Specifically, I expected significant differences in assessment for males and females in the high resource value treatments as previous work has suggested that female contest assessment is likely driven by RPV rather than RHP (Draud et al., 2004; Elias et al., 2010; Dunn et al., 2015).
CHAPTER II: INFORMATION DEPENDS ON CONTEXT: BEHAVIORAL RESPONSE TO CHEMICAL SIGNALS DEPENDS ON SEX AND SIZE IN CRAYFISH CONTESTS

Introduction

Animals gather information from a fluctuating environment to make decisions directly tied to survival and reproduction (Dusenberry, 1992; Dall et al., 2005). For species that engage in agonistic contests over resources, the social and physical status of individuals within the local population can be an important source of information dictating contest persistence. While intraspecific contests can provide access to resources, contests are costly, and engaging with superior opponents is disadvantageous. Utilizing information about oneself or an opponent can provide important information that can directly affect contest engagement, escalation, and resolution (Arnott & Elwood, 2009). These decisions, which can influence contest behavior based on a fluctuating cost to benefit ratios, are known as assessment strategies (Parker, 1974; Arnott & Elwood, 2008; Arnott & Elwood, 2009).

Broadly, assessment strategies fall along a spectrum ranging from self to mutual assessment, based on the type of information available and deemed to be in use to dictate contest escalation or retreat (Fawcett & Mowles, 2013; Mesterton-Gibbons & Heap, 2014). Self-assessment strategies are associated with information regarding “self” only (i.e. energetic reserves, fighting ability) and a mutual assessment strategy hinges on the ability of individuals engaged in a contest to ascertain relative fighting ability between oneself and an opponent (Taylor & Elwood, 2003; Arnott & Elwood, 2009). All animals are expected to have some amount of information about one’s own resource holding potential (RHP), or overall fighting

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ability, but the distinction between self and mutual assessment appears to be determined by the type of information available regarding potential opponents (Mesterton-Gibbons & Heap, 2014). General “public” information about potential contestants is approximate and more likely to facilitate a self-assessment type strategy. This type of information is gathered through prior experience or an approximated probability of where one’s RHP falls within the population distribution (Fawcett & Johnstone, 2010; Mesterton-Gibbons & Heap, 2014). In other words, how likely is an individual to win or lose a contest based on the proportion of previous contests won or lost or the proportion of conspecifics stronger than that individual in the population (Mesterton-Gibbons & Heap, 2014)? Gathering specific (and perhaps more reliable) information directly from a particular opponent would lend to a relative comparison of RHP and thus a mutual assessment type strategy (Enquist et al., 1990; Mesterton-Gibbons & Heap, 2014). To gain this type of information about an opponent, individuals are likely to exchange signals via displays or direct physical interactions (Mesterton-Gibbons & Heap, 2014). Because there is a positive selective pressure to both the sender and receiver to exchange the information efficiently (Otte, 1974), this information exchange can provide contestants with potentially more reliable information about the costs of engaging an opponent.

Exchange of information in animal contests can mitigate costs before, during, and after physical contact. Pre-contest exchanges take the form of cue assessment or signal displays and likely convey information about social status of the potential opponents (Arnott & Elwood, 2009). For example, coloration and visual displays in grey triggerfish (Cleveland & Lavalli, 2010), vocalizations (bellows) in bison (Wyman et al., 2012), or chemical badges in Iberian rock lizards (Martin et al., 2007) appear to tie directly to dominance status and influence the likelihood of engaging conspecifics carrying these badges/exchanging this information. Post
contest displays are also gaining more attention as a means to convey post-fight status (i.e. dominance or submission). Post-victory stridulations in mangrove crabs (Chen et al., 2014) and submissive electric chirps in electric fish (Batista et al., 2012) are used to deter further physical contact and reduce costs after the contest has been “settled.” Once engaged in a contest, information can be directly tied to one’s own fighting capacity or some relative measure of competition with the opponent (Arnott & Elwood, 2009).

Several decapod crustacean species (e.g. fiddler crabs, hermit crabs, lobsters, crayfish) have been established as models for the role signals play in agonistic contest outcome and behavior (Bergman & Moore, 2005a; Zeil & Hemmi, 2006; Aggio & Derby, 2011; Breithaupt, 2011; Mowles & Briffa, 2012). Diverse visual and tactile displays, mostly relating to the chelae, have been implicated in pre/post contest displays and assessment strategies (Zeil & Hemmi, 2006; Mowles & Briffa, 2012). However, fully aquatic species have demonstrated a heavy reliance on chemical information in most aspects of their behavior, particularly contests (Hay, 2009; Aggio & Derby, 2011; Breithaupt, 2011). Specifically, experimental manipulation of chemical information exchange during dyadic contests has demonstrated significant effects on status recognition (Zulandt-Schneider et al., 2001; Shabani et al., 2009), winner effects (Bergman et al., 2005; Johnson & Atema, 2005), and pre-copulatory fighting bouts (Bushman & Atema, 1997; Simon & Moore, 2007; Berry & Breithaupt, 2010) in various lobster and crayfish species. The bulk of these studies have implicated urine as the primary vehicle for this chemical information. From a metabolic point of view, urine is used for osmoregulation (Vogt, 2002). Yet, musculature and hormonal glands surrounding the nephropores (urine release sites) allow for modulation of content (i.e. chemical composition) as they appear to play a role in pheromone production and are likely linked to the controlled release of chemical products into
the urine (Bushman & Atema, 1996). Previous research has shown that urine output is significantly increased during contest behavior (Breithaupt & Eger, 2002), the absence of urine alters contest outcomes (Zulandt-Schneider et al., 2001; Bergman et al., 2003), and urine can influence behavior in the absence of physical interaction (Bergman & Moore, 2005b). Taken together, we can conclude that crustacean urine is a potent chemical signal that drives contest outcomes and decisions.

However, numerous studies have demonstrated that male and female crustaceans use this information differently. Due to large population densities (Mather & Stein, 1993; Perry et al., 1997; Nystrom, 2002; Davis & Huber, 2007) and propensity for agonistic behaviors (Bovbjerg, 1953; Moore, 2007), both male and female crayfish frequently engage in inter- and intrasexual fights for resources (Bergman & Moore, 2003; Martin III & Moore, 2007; Fero & Moore, 2014). Males and females are capable of chemical recognition of the other sex (Stebbing et al., 2003; Belanger & Moore, 2006), and they exhibit differing contest assessment strategies (Wofford et al., 2015). This led us to hypothesize that males and females respond differently to urine release during contests.

This study aimed to establish first the status of urine as a chemical signal in crayfish contests. Specifically, the release of this chemical stimulus by a sender should elicit significant behavioral changes in a receiver immediately after release. While we know that urine can determine contest outcome and dynamics, we sought to examine fine scale temporal dynamics (i.e. within contest decisions) surrounding a release event. Furthermore, we wanted to determine if the behavioral changes caused by urine release were context dependent. Specifically, do factors that change the social context of an agonistic interaction (i.e. sex of the opponents, body size of the opponents) significantly alter behavioral reaction to the release of urine during a
contest? We used a previously developed method (Breithaupt & Eger, 2002) to visualize and quantify urine release. Behavioral changes were measured before and after a release event to determine if information exchange had taken place. We staged contests in which dyads varied in sex and opponent size to examine these hypothesized behavioral changes under different ecologically relevant social contexts.

**Methods**

**Animals**

Male and female crayfish, *Orconectes rusticus*, were collected using a kick seine method from the Portage River near Bowling Green, Ohio (Wood County, 41°21′42″N, 83°35′28″W). All crayfish were measured, using calipers, for chelae length (males: mean ± SEM = 2.40 ± 0.06 cm; females: mean ± SEM = 2.58 ± 0.23 cm) and post orbital carapace length (males: mean ± SEM = 2.48 ± 0.04 cm; females: mean ± SEM = 2.63 ± 0.03 cm). Crayfish with intact walking legs, chelae, and sensory appendages were housed in a recirculating system within an environmental chamber held at a constant temperature (23 °C) and light/dark cycle (12L: 12D). All crayfish were visually and mechanically isolated for a minimum of seven days to eliminate effects of prior social history developed in the field (Karavanich & Atema, 1998; Zulandt-Schneider et al., 2001). Crayfish were fed an ad lib diet of commercial rabbit food pellets three times a week. Each animal was used only once in these trials, and were frozen after a trial as required by collection permit protocols. An animal was secured in an individual plastic container (7.5cm x 7.5cm x 7.5cm) and placed in a commercial freezer (approximately -15°C) until movement ceased (~10 to 15 minutes). All crayfish were in non-reproductive form. Males were considered non-reproductive if their stylets were cornified and non-bifurcated while females were considered non-reproductive if glair, a white substance used for egg adherence, was absent.
from the ventral portion of the telson and the base of the walking legs (McLay & van den Brink, 2016).

**Experimental Design**

The purpose of this experiment was to quantify a chemical signal and examine its use during agonistic assessment of crayfish contests. Chemical visualization was possible via the injection of a fluorescent dye (explained below). The presence of the chemical signal was correlated with the behavioral dynamics of fighting in same-sex and mixed-sex contests. Thus, we had a fully factorial 3 x 2 design with the first factor (sex of opponents) having three conditions (male only, female only, and mixed sex) and the second factor (combatant size) having two conditions (size matched and size different). Within each of these sex condition treatments, there were two size conditions in which opponents were either size matched (SM) or size different (SD). Crayfish were matched based on carapace length. Size matched animals were selected such that carapace length of the two contestants was within 10 percent. Beyond a 10 percent carapace length difference, larger animals begin winning significantly more often than chance (Pavey & Fielder, 1996; Daws et al., 2002). Consequently, size different animals were randomly paired to achieve carapace length differences between approximately 11 and 30 percent (range: 11.11 % - 33.33 %; mean ± SEM: 17.57 ± 0.55 %). We decided to cap size differences at 30 percent to achieve more realistic pairings (Bergman & Moore, 2003; Fawcett & Mowles, 2013). In mixed sex contests, the size different treatment included equal trial numbers in which males were larger and in which females were larger.

**Injection Protocol**

Both contestants used per trial were injected with a 0.05% sodium fluorescein (Sigma: F-6377, Lot 103H3412; Sigma-Aldrich, St. Louis, MO, USA) solution dissolved in Van Harreveld crayfish saline (Van Harreveld, 1936). All animals were subjected to injections at 0.01 ml/gram
of body mass using a 1 mL syringe affixed with a 26 gauge (0.45 mm x 13 mm) needle. This protocol was based on techniques first developed by Breithaupt and Eger (2002) and modified by Bergman et al. (2005) and Simon and Moore (2007). The injection site was located on the dorsal surface of the carapace. The needle was inserted approximately 2 mm at the injection site, and the syringe contents were then slowly injected into the body cavity. The needle was quickly removed and a small dot of Loctite® gel control super glue was placed over the injection site, followed by a small strip of black electrical tape. Super glue was used to quickly adhere the tape to the animal to stop haemolymph loss. Super glue was favoured over the wax method used by Breithaupt and Eger (2002) to reduce weight additions to the animal, which could potentially skew perceived RHP. Tape strips did not exceed 0.25 square cm and thus only covered a small section of the animal’s carapace. Additionally, *O. rusticus* are primarily dark brown and green in colour, making the black tape inconspicuous; aggressive behaviors did not appear altered based on the presence of the tape patch. Animals were allowed a minimum 1 hour recovery period before being used in a trial and were monitored for signs of distress.

**Fight Arena**

All trials were conducted in a dark room using a specialized arena constructed of “2 x 4” blocks of wood (4.44 cm x 9.52 cm) painted black to reduce reflection of light (Figure 1). A 21 L aquarium (40.6 cm x 20.3 cm x 25.4 cm) was affixed with two opaque Plexiglas® (ePlastics, San Diego, CA, USA) inserts to reduce tank dimensions (22.9 cm x 20.3 cm x 25.4 cm). This reduction increased the probability of agonistic behavior and ensured that the entire arena was in view of the side camera (defined below). The aquarium was filled with 7.1 L of pre-conditioned tap water and suspended on top of two “2 x 4” wood pieces in order to allow for filming from underneath. Both cameras used were Sony HDR-CX405 9.2 megapixel cameras (Sony
Electronic Inc., Novi, MI, USA). The side camera was positioned on a tripod separate from the arena structure, approximately 20 cm from the side of the aquarium. The bottom camera was affixed to a black “2 x 4” suspended approximately 48 cm from the bottom of the aquarium. Both cameras were set to low lux to account for the lighting conditions. The focus and exposure were adjusted manually before each trial to achieve the clearest picture possible. Five 60 Watt black lights were affixed to the top and sides of the wooden structure to facilitate lighting of the tank and visualization of the fluorescein-laced urine. While this arrangement utilized more (and greater wattage) black lights than the methods used in Bergman et al. (2005) and Simon and Moore (2007), those experiments also used two Kodak Ekta Graphic IIIA slide projectors (with ~300 Watt bulbs) with no obvious changes to fighting behavior. Furthermore, the crayfish contests in this study demonstrated the same behavioral repertoires performed under varied lighting conditions (Zulandt-Schneider et al., 2001; Bergman et al., 2005; Simon & Moore, 2007) consistent with behaviors expected from a well-established crayfish fight ethogram (Bergman & Moore, 2003).

**Fight Protocol**

Crayfish were placed into the arena and were visually and physically isolated for a 15 minute period to allow them to acclimate to the trial conditions (Bergman et al., 2005; Simon & Moore, 2007). Trials lasted for 20 minutes, and the arena was drained and rinsed between each trial. The room was darkened and the black lights were turned on prior to crayfish retrieval and placement in the test arena. Lighting conditions were then held constant across acclimation and trial periods to minimize behavioral differences. Crayfish were not blinded for use in these trials. Previous studies (Bergman et al., 2005; Simon & Moore, 2007) and preliminary trials
(unpublished data) have demonstrated that social behaviors are largely unaffected by the visual presence of fluorescein.

**Analysis**

Only trials in which one or both crayfish demonstrated visible urine release during physical fighting behavior (i.e. opponent to opponent contact) were considered for analysis. We obtained 92 trials in which physical contact classified as fighting behavior between opponents occurred. Of these trials, 68.5 percent (63 trials) demonstrated visible urine release by one or both opponents. In order to keep trial numbers as similar as possible across treatment types, a female size matched (SM) trial in which urine release was visible during an interaction was randomly chosen to be excluded from analysis. This left us with 62 total trials that met the release criteria.

Videos obtained from the side view camera were the primary source of data for analysis. Videos obtained from the bottom view camera were used to corroborate or supplement incomplete data from the side camera videos. Videos were scored manually by an observer blind to treatment type and included the determination of a winner and loser for each contest, identification of intensity levels throughout the contest for each opponent, and quantification of urine release events. Contest winners and losers were determined by retreat behaviors (Moore, 2007). Specifically, losers were determined as those that retreated from the interaction via backwards walking or a rapid retreat (i.e. tail flip). The contest was considered over when the two individuals were separated by more than two body lengths for at least 15 seconds. The intensity levels recorded were determined using a modified version of previously developed ethograms (Table 2: Bergman et al., 2005; Simon & Moore, 2007). Urine release events were recorded alongside the changes in intensity levels for both contestants. A release event was
determined to have occurred when fluorescein was ejected from the nephropores. Behavioral changes and urine release events were recorded for both individuals (i.e. senders and receivers) in each trial. Senders were defined as the animal that released urine at a given time point and receivers were defined as the animal that did not release at that time point. Therefore, the sender and receiver title was assigned for each release event.

Linear mixed models (LMM) followed by analysis of deviance tables using Type II Wald Chi Square tests (Zuur et al., 2009) were used to determine the effect of opponent sex and size on behavior pre and post urine release in the lme4 package (Bates et al., 2015) in R statistical software (version 3.3.0) (R Development Core Team, 2016). Models were constructed using sex treatment (male only, female only, or mixed sex dyads), size treatment (size matched or size different dyads), and time (pre or post release) as fixed effects and trial number and release number as nested random effects. Differences of least squares means (‘difflsmeans’) from the lmerTest package (Kuznetsova et al., 2016) in R was used as a post hoc test to discern which factors were responsible for significant differences within significant main effects detected by the ANOVAs.

Preliminary mixed model analyses revealed that sender and receiver behavior did not significantly differ after urine release events. Therefore, we chose to focus only on sender behavior for the remainder of our analyses. Because the number of release events per contest was inconsistent across trials, we chose to analyse only the first three release events that occurred in each trial, leaving us with 78 total observations (i.e. release events) (Table 1).

An Excel macro was used to compile the appropriate response variables (described below) obtained from manual video scoring and used in the LMM. Mean behavioral intensity and mean shifts in behavioral intensity were calculated at the time of the urine release event as
well as 2 seconds, 5 seconds, and 10 seconds pre and post release. The 10 to 1 (overall behavioral intensity change over 10 seconds) measure was calculated by subtracting the intensity 10 seconds before the release event from the intensity at the time of the release event (pre 10 to 1) or by subtracting the intensity at the release event from the intensity 10 seconds after the release event (post 10 to 1). Intensity levels recorded were also classified into non-contact phase, non-escalated contact phase, or escalated phase (Table 2). The proportion of time spent at each of those intensity levels was calculated pre and post release. Finally, the sequence of behaviors was created by turning the numerical intensities into string values, denoting the numerical sequence of behaviors that took place leading up to and following a release event. The behavioral sequence was not considered in the LMM, but these values were later averaged across all contests types to create graphical representations of intensity changes over time.

We performed a test for collinearity (Zuur et al., 2009) and found a significant, positive correlation between the mean behavioral intensity at 2 seconds, 5 seconds, and 10 seconds. Consequently, we chose to analyse mean behavioral intensity at the 5 second time point only. The best-fit model for our response variables included the interaction of all three fixed effects (sex treatment, size treatment, and time) and the inclusion of both random effects (trial and release number). Release number was nested under trial number. We determined the best-fit model using the Akaike Information Criterion (AIC) value for each model.

Results

Qualitative Analysis

The release plots (averaged across all treatments) revealed a characteristic increase in agonistic intensity leading up to the release event and then an equally characteristic drop in behavioral intensity (behavioral de-escalation) following a urine release event (Figure 2A).
Release plots separated by sex treatments (Figure 2B) and relative opponent size (Figure 2C) demonstrated differences in behavioral intensity changes that were subsequently verified by statistical analyses.

**LMM Analyses**

**Effect of Time**

Time played a significant role in several response variables (Table 3). The change in intensity level over ten seconds (i.e. 10 to 1) differed significantly pre and post release ($X^2 = 21.93$, df = 1, $p < 0.001$). Intensity level increased prior to urine release (positive 10 to 1 value) and decreased after the urine release event (negative 10 to 1 value). There was also a significant time effect on the second to second behavioral intensity shifts occurring at the 5 second time point ($X^2 = 5.96$, df = 1, $p < 0.05$) and at the 10 second time point ($X^2 = 29.40$, df = 1, $p < 0.001$). In both cases intensity shifted from lower to higher levels pre-release (positive values), but shifted from higher to lower intensities (negative values) post release.

**Effect of Sex**

Sex of the opponents significantly influenced the proportion of time spent in the non-contact phase behaviors ($X^2 = 6.36$, df = 2, $p < 0.05$). Specifically, female same sex dyads spent a greater proportion of time in the non-contact phase compared to male same sex contests and mixed sex contests (Least Squares Means: $t = 2.73$, df = 27.2, $p < 0.05$).

**Interaction Effects**

There was a significant interaction effect of time and sex treatment on behavioral intensity shifts at the 5 second time point ($X^2 = 9.06$; df = 2; $p < 0.05$). Mixed sex contests exhibited a significant difference in intensity shifts pre and post release (Least Squares Means: $t = -3.85$, $p < 0.001$). Specifically, there were positive behavioral intensity shifts pre-release and
negative shifts post release. Male and female same sex contests did not demonstrate a significant difference in behavioral shifts pre and post release. However, mixed sex contests also demonstrated significantly greater shifts pre-release (i.e. positive values) than female same sex contests (i.e. negative values) (Least Squares Means: \( t = -2.11, p < 0.05 \)).

The proportion of time spent in the non-escalated contact phase was the only response variable impacted by the interaction of all three fixed effects; however, this difference was only marginally significant (\( X^2 = 5.82, \text{df} = 2, p = 0.05 \); Table 3). The post hoc did not reveal any significant differences.

**Discussion**

We found that the presence of a chemical signal (urine) induced a stereotypical de-escalation of aggressive behavior in crayfish contests, regardless of the sex or relative size of the contestants (Figures 2 A-C). Behavioral intensity shifts (e.g. 10 to 1 data, shifts at 5 and 10 seconds) demonstrated significant changes from lower to higher intensity levels pre-release and decreases to lower intensity levels after a release event. These data indicate an escalation phase occurs prior to urine release and de-escalation occurs after release. This is consistent with our expectation that urine is an important driver in contest dynamics in crayfish contests.

We also found that the sex and relative size of the opponents play a significant role in the magnitude of change elicited by this chemical signal. The sex of the opponents dictated the intensity of behavior and the magnitude and direction of intensity changes seen due to a release event. For instance, while mixed sex contests followed the pre-release escalation and post release de-escalation trend we would expect, female and male same sex contests did not demonstrate an obvious change. These data suggest that urine potentially sends a different message in same and mixed sex contests, and supports previous studies that suggest differences in contest resolution
for same sex and mixed sex contests in crayfish (Wofford et al., 2015). The role of opponent size requires a more complex explanation. While the post hoc test did not reveal any significant differences in the proportion of time spent at non-escalated contact behaviors, some general trends were present. Size matched and size different dyads only seemed to play a pronounced role in mixed sex contests. Regardless of opponent size, females showed little change in the proportion of time spent at non-escalated contact behaviors. Males exhibited consistent increases in proportion of non-escalated contact behaviors performed post release, but this trend was also persistent across size matched and size different trials. However, mixed sex contests experienced opposite directional changes pre and post release, and these trends were dependent on relative opponent size (Figure 3). Relative opponent size not only altered the proportion of time spent at certain intensities, these trends were also dependent on the sex of the opponent. These results support the long-standing hypothesis that urine functions as a signal in crayfish contests and our hypothesis that the social information contained within is dependent on the sex and relative size of the opponents.

Signals and cues play an integral role in contest behavior by reducing the chances of escalation and potential injury (Bradbury & Vehrencamp, 1998; Arnott & Elwood, 2009). Specifically, information exchanged during physical contact can convey information about energetic state (of oneself or an opponent) or intent to retreat (i.e. Scheel et al., 2016). This could then elicit de-escalation in aggressive behaviors or retreat from a contest. These anticipated behavioral changes (i.e. de-escalation) were seen in both senders and receivers and were consistent across treatment types, suggesting that urine release conveys information pertinent to contest resolution in crayfish. This information could be driving assessment strategies in use for
crayfish under different social contexts that could alter dominance relationships and, consequently, hierarchy establishment.

The sex of the opponents is one social context in which we consistently find assessment based differences. Many studies have attributed sex based assessment differences to disparate information sources driving contest persistence and escalation. Some hypotheses assert differences in RHP based signals (i.e. weaponry strength versus visual or vocal displays) as well as the role of perceived resource value (PRV) in contest resolution. Male contests tend to be heavily tied to RHP of the opponents while female contest resolution appears to rely on the PRV, especially in reproductive periods (Draud et al., 2004; Elias et al., 2010). However, in our system non-reproductive crayfish demonstrate limited sexual dimorphism (McLay & van den Brink, 2016), and non-reproductive males and females vie for the same resources (e.g. food and shelters) throughout the season (Martin III & Moore, 2010; Fero & Moore, 2014), likely diminishing (but not negating) both RHP and RV asymmetries. This limited asymmetry coupled with previous work and the data presented here suggest that deviation in chemical information is likely an important driver in sex-based assessment differences in crayfish.

Although crayfish urine release is a means of homeostatic regulation between the hypertonic haemolymph and the hypotonic external environment, amino acids have been found in urine excretions as well as higher than normal glucose levels under stressful conditions (Vogt, 2002). Consequently, the chemical composition of this signal can change depending on various physiological and dietary factors. Given the metabolic usage of urine, the chemical composition of the urine signal could relay information about one’s internal state. Nutritional status (i.e. starvation; Schirf et al., 1987), environmental stressors (i.e. hypoxia, desiccation; Buckup et al., 2008), and exposure to toxicants (Torreblanca et al., 1992) all significantly influence chemical
composition of crayfish haemolymph and tissues, which can contribute to differential composition of urine and, consequently, the “message” conveyed by urine release. The sex of the individual is another variable that likely contributes to the chemical composition of crayfish urine released during contests. We know that crayfish have the ability to discriminate sex (among other things) based on odour alone (Stebbing et al., 2003; Bergman & Moore, 2005b; Belanger & Moore, 2006), and the release of urine during a contest elicits varied behavioral changes based on sex. Furthermore, mounting evidence suggests that males and females behave and perhaps communicate differently when engaged in mixed sex contests (Simon & Moore, 2007; Martin III & Moore, 2010; Wofford et al., 2015). Altogether, there is the implication that urine is conveying different information based on the sex of the individuals engaged in the contest and consequently differentially influencing contest resolution for males and females.

Size differential of the opponents also played a role in behavioral changes caused by urine release. This was unsurprising as body size is an important and ubiquitous RHP proxy known to influence assessment and contest outcome (Arnott & Elwood, 2009). However, the role of relative size was less prevalent than (and modulated by) the sex of the opponents. Only mixed sex contests seemed to be affected by the size of the opponent in terms of reaction to a chemical signal. Based on the crayfish ethogram (Table 2), non-escalated contact behaviors could be classified as “exploratory” behaviors. These types of behaviors (i.e. initial touch, closed claw boxing) are likely used as low cost information collection phases (Bruski & Dunham, 1987; Seebacher & Wilson, 2007; Hsu et al., 2008; Percival & Moore, 2010). In mixed sex contests, we see a trend suggesting that different sized opponents decrease the prevalence of these exploration behaviors post urine release. However, size matched opponents increase these behaviors post release. Because chemical information is almost certainly acting in concert with other modalities
(i.e. visual or tactile information; Bruski & Dunham, 1987; Smith & Dunham, 1990; Callaghan et al., 2012), these data suggest that urine might serve as a climactic or initial signal depending on what other types of information are readily available. For example, in size asymmetrical contests visual information is available to distinguish aspects of opponent RHP once the interaction begins. However, for contests in which opponents are the same size, tactile or chemical information likely plays a much larger role. These differences in information availability might explain why we see a drop in exploratory based behaviors after urine release in size different contests (climactic information) and a rise in exploratory based behaviors in size matched contests (initial information).

Perhaps the most intriguing finding was what was not significant. There was no significant difference between sender or receiver behavior upon urine release. This suggests that chemical information is possibly acting as a source of information for both the sender and the receiver. Previous work suggests that urine released during crayfish contests contains various neurochemicals or hormones that can be used as information about dominance or individual identification (Bergman & Moore, 2005a; Moore & Bergman, 2005). For example, serotonin and its metabolites play significant roles in crustacean aggression and are hypothesized to be excreted in urine (Huber et al., 1997). The injection of serotonin has consistently shown significant effects on crayfish aggression and contest outcome (Edwards & Kravitz, 1997; Huber & Delago, 1998), and in vivo studies of amine metabolism in crayfish and lobsters have shown the excretion of a metabolically expensive serotonin conjugate, serotonin-O-sulfate, in urine (Huber et al., 1997). The presence of serotonin and the sulfate conjugate in urine has been hypothesized as a source of cheat-proof information in a contest. Specifically, greater relative
concentrations of serotonin-\(O\)-sulfate might carry the message of a socially or energetically dominant individual (Huber et al., 1997).

Recent findings suggest that both male and female crayfish are actually using a form of self-assessment during dyadic contests (Wofford et al., 2015), leading one to hypothesize that any chemical information about internal state is available to the sender as well as a receiver. Accordingly, crayfish anatomy facilitates simultaneous chemically mediated opponent and auto-communication. Urine is released from nephropores, which are located directly beneath antennules, the primary chemosensory organs (Vogt, 2002). Meaning, crayfish can potentially obtain self-referent information about their internal hormonal or energetic state (i.e. serotonin or metabolic by-product levels). Potential high stress situations (e.g. an escalated contest or attempted predation event) could also influence internal levels of serotonin or other biogenic amines and their relative concentrations in urine excretion. Similarly, handling stress (i.e. injections) could artificially elevate these levels, influencing contest dynamics surrounding a urine release event. However, the authors are confident the hour long recovery period and the fact that both opponents underwent the injection protocol negated behavioral effects of any differences in relative amine concentrations due to stress. If urine is providing self-referent information and is acting as an external “checks and balances” system for an individual, it would pay for the opponent to eavesdrop on this information. Indeed, the lack of significant difference between sender and receiver behavior in this study demonstrates that both contestants are gaining information from urine, presenting the opportunity for assessment strategy switching or a mixed strategy in crayfish.

Recent work has called for a reorganization of the way in which we think about assessment strategies, specifically, in terms of the information utilized by contestants.
Understandably, the spectrum of assessment strategies ranging between self and mutual assessment have previously been associated with a gradient of cognitive abilities (Elwood & Arnott, 2012; Fawcett & Mowles, 2013). Mutual assessment strategies are argued to be reserved for more cognitively complex organisms (Elwood & Arnott, 2012; Fawcett & Mowles, 2013). Fawcett and Mowles (2013) argue that “comparative decision making” required for mutual assessment is already ubiquitous across non-contest related contexts (e.g. mate selection, resource preference) and is prevalent in cognitively “simple” organisms (Dussutour et al., 2010; Egge et al., 2011). Furthermore, theoretical (Fawcett & Johnstone, 2010; Mesterton-Gibbons & Heap, 2014) and empirical work (Hsu et al., 2008; Garcia et al., 2012) suggest that assessment strategies vary across populations, individuals, and even throughout different stages of a single contest.

Crayfish provide us with a model system for studying the spectrum of assessment strategies possible, as their populations are a conglomeration of different sized animals of both sexes constantly vying for resources (Bergman & Moore, 2003; Martin III & Moore, 2007) or hierarchy establishment for access to resources (Fero & Moore, 2008; Fero & Moore, 2014). Because males and females have inherently different costs and resource needs (Trivers, 1972), we would also expect them to have different choice paradigms when engaging in contests. Therefore, it is not unreasonable to assume that males and females are using different information, especially during the reproductive season when RHP and RPV asymmetries can become quite large between the sexes (Martin III & Moore, 2010). However, as in the previous assessment study by Wofford et al. (2015), non-reproductive male and female same sex contests seem to behave similarly in terms of urine release and behavioral response (Figures 5). The breakdown of these trends in mixed sex contests suggests that males and females are using different
information to make within-contest decisions, but this only becomes apparent in mixed sex contests. Perhaps the differential information usage is based on a source other than urine (i.e. mechanical information from chelae boxing) (Gherardi, 2002). Alternatively, the message encoded in the urine may be different for males and females. Considering that the sexes differ in chelae morphology (Stein, 1976; Gherardi, 2002) and, hypothetically, in chemical signature (Bushman & Atema, 1997; Stebbing et al., 2003; Belanger & Moore, 2006) either of these explanations is plausible.
CHAPTER III: LOSS OF CHEMICAL INFORMATION IN INTERSEXUAL CONTESTS DIFFERENTIALLY IMPACTS MALE AND FEMALE CRAYFISH, ORCONECTES RUSTICUS

Introduction

Organisms are in constant competition with conspecifics for access to resources that are tied to fitness, and in many species this competition can escalate to physical contests (Parker, 1974). The traditionally held view is that males perform the vast majority of these fighting behaviors as they engage with other males to obtain resources vital to individual survival, resources needed to attract female mates, or over mating opportunities with females (Clutton-Brock and Huchard, 2013b). This unisex focus has resulted in decades of work intricately outlining ultimate (Hunt et al., 2009) and proximate (Wingfield et al., 1990; Goessmann et al., 2000; Oliveira et al., 2009) drivers of male intrasexual competition, as well as the rules that govern contest escalation and persistence (Enquist et al., 1990; Schnell et al., 2015). However, while males are historically assumed to be the more combative sex, a vast body of work has demonstrated that females of many species engage in agonistic competition for direct access to resources (Ueda and Kidokoro, 2002; Elias et al., 2010), as a form of parental care (Figler et al., 2001; Goubault et al., 2007), and to establish dominance hierarchies (Clutton-Brock and Huchard, 2013a; Correa et al., 2013).

Female intrasexual agonism has been established in species ranging from parasitoid wasps (Goubault and Decuigniere, 2012) to humans (Schuster, 1983; Campbell, 2013) covering behaviors from indirect or covert aggression (Lagerspetz et al., 1988; Bjorkqvist et al., 1994) to mortal combat (Dunn et al., 2015). However, while females demonstrate comparable levels of aggression and propensity for agonistic behavior, female and male intrasexual contests differ in
the selective pressures driving their prevalence and intensity (Rosvall, 2011). Females, like males, compete for access to resources to maximize their own survival; however female dominance in dyadic and population level interactions can also convey direct (and indirect) benefits to offspring. For example, more aggressive or dominant females can gain access to higher quality food patches or reproductive resources (Witig and Boesch, 2003, Watson and Simmons, 2010) or better protected areas within a herd or social group (Öst et al., 2007). Increased instances of aggression can also deter other females (or males) from infanticide (Wolff, 1985, Crespi, 1990). Sex role reversal and dynamic operational sex ratios can also produce populations in which females compete for access to mates such as in some fish species (Kvarnemo et al., 1995; Forsgren et al., 2004). Moreover, in some cases of mate competition, females may engage in intrasexual competition for access to high quality males or males with high quality resources (Forsgren, 1997; Wolfenbarger, 1998). Higher quality mates can provide direct benefits (e.g. nuptial gifts, high quality territory) or indirect benefits (i.e. good genes, influencing post-copulatory female investment) to the female and her offspring (Thornhill, 1983; Wedell, 1996; Schuett et al., 2011). Altogether, female agonism significantly impacts fecundity (in addition to individual survival) in ways that male contests commonly do not.

However, while agonistic contests can convey access to vital resources, fighting behavior comes with inherent time and energy costs that must be weighed against the benefits of fighting (Arnott and Elwood, 2009). Potential opponents can significantly differ in variables affecting resource holding power (RHP) such as body size or weaponry, resource value (RV), or previous experience. All of these variables can affect the dynamics and outcome of a contest (Hurd, 2006; Arnott and Elwood, 2009). Therefore, information about one’s own RHP (Fawcett and Johnstone, 2010; Percival and Moore, 2010), resource availability (RPV) (Goubault et al., 2007),
or potential opponents (Mesterton-Gibbons, 1999; Pratt et al., 2003) can assist in evolutionarily beneficial decisions (e.g. engage only in fights where potential benefits outweigh the potential costs). Studies of male intrasexual contests suggest that this strategy varies across species, but new theoretical models and empirical evidence suggest that the strategy in use is quite fluid, and dictated by the social or environmental context surrounding a contest (i.e. age, previous experience, RHP, RPV) (Hsu et al., 2008; Fawcett and Johnstone, 2010; Garcia et al., 2012; Mesterton-Gibbons and Heap, 2014).

Because female and male intrasexual contests are under varied selection pressures, the rules dictating contest resolution and the strategies in place to make evolutionarily beneficial decisions are predicted to differ as well (Rosvall, 2011). A growing body of work on male and female contest assessment has found evidence for these differences and suggests that sex is an important factor in the fluidity of assessment strategies (Draud et al., 2004; Briffa and Dallaway, 2007; Elias et al., 2010). Some have suggested that the source of information guiding decisions about contest escalation or retreat drives these differences for males and females. While RHP tends to be the primary predictor of male contest intensity and success (Bee et al., 1999; Morrell et al., 2005; Briffa, 2008; Sacchi et al., 2009; Copeland et al., 2011), RV tends to be more important for females (Goubault et al., 2007; Elias et al., 2010). Others hypothesize that the rules of engagement simply differ for the sexes. For instance, male and female cardinals (Cardinalis cardinalis) both engage in aggressive singing bouts against real and simulated territorial intrusions; however, female aggressive song rates differ based on seasonal and social context while male singing does not (Vondrasek, 2006). In jumping spiders (Phiddipus clarus), females demonstrate less ritualized fighting than males which results in a higher probability of risky behaviors for female intrasexual contests (Elias et al., 2010).
Although we have only begun to understand female intrasexual competition and contest resolution, we know even less about how male and female assessment differences impact intersexual contests between conspecifics. Pre-copulatory conflict is not uncommon (Arnqvist, 1992; Arnqvist and Rowe, 1995; Olsson, 1995; Jormalainen and Merilaita, 1995; Villanelli and Gherardi, 1998), and some species engage in non-copulatory intersexual aggression for resources or offspring protection (Yoshino and Goshima, 2002; Draud et al., 2004; Briffa and Dallaway, 2007; Fero and Moore, 2014). Variation in selective pressures driving contest engagement for the sexes, as well as evidence suggesting differentiation in the rules dictating contest retreat, presents a ripe opportunity for the exploration of assessment differences based on the sex of one’s opponent. The few studies that have explored sex-based differentiation in contest strategies in intersexual contests have found that males and females differ in contest strategies and that these differences impact fight outcome (Draud et al., 2004; Briffa and Dallaway, 2007; Wofford et al., 2015).

Crayfish provide an ideal model for exploring differentiation in male and female contest resolution as both sexes readily engage in intra- and intersexual contests throughout the reproductive and non-reproductive seasons (Simon and Moore, 2007; Martin III and Moore, 2010). While female contests are commonly shorter and less intense than male contests (Bruski and Dunham, 1987; Walter et al., 2011), male and female intrasexual contests appear to be following the same approximate rules for contest resolution (Wofford et al., 2015). However, this similarity dissolves in intersexual bouts between crayfish, suggesting nuanced differences in the strategy or information in use by males and females. Previous work has revealed that these differences are likely driven by disparate responses to a chemical signal (i.e. urine) released during contests (Wofford et al., 2017). There is a large body of evidence that chemical
information, specifically urine, plays a significant role in crayfish contests. Urine is released significantly more often during aggressive interactions compared to other behaviors (i.e. foraging, habitat exploration) (Breithaupt and Eger, 2002) and the manipulation or exclusion of urine significantly impacts contest dynamics and outcome (Zulandt-Schneider et al., 2001; Bergman et al., 2003; Berry and Breithaupt, 2010). Furthermore, physiological evidence suggests that hormones and hormone metabolites are likely released in the urine, conveying crucial information about internal state to oneself or an opponent (Huber et al., 1997; Breithaupt, 2011).

Agonistic urine release occurs across intra and intersexual crayfish contests and plays a significant role in contest dynamics (Breithaupt and Eger, 2002; Bergman et al., 2005). However, the role of urine in dictating these dynamics appears to be mediated by the sex of the opponents engaged in a contest (Wofford et al., 2017). While the frequency of urine release does not differ for males and females, behavioral response to urine release differs across sex treatments (i.e. male and female intrasexual contests, intersexual contests). The following study aims to further examine the differential role of this chemical information for males and females in intersexual contests of the crayfish Orconectes rusticus. We hypothesized that removing the ability to sense chemical information would have significantly different impacts on male and female aggressive behavior and contest resolution in intersexual contests.

Methods

Animals

Male and female crayfish, Orconectes rusticus, were collected using minnow traps from the Carp Lake River near Carp Lake, Michigan, USA (Carp Lake Township: 45°40’48.0”N, 84°48’51.2”W) or via hand nets in Maple Bay of Burt Lake near Brutus, Michigan, USA (Burt Township: 45°29’14.2”N, 84°42’24.5”W). All crayfish were measured, using calipers,
chelae length (males: mean ± SEM = 2.59 ± 0.27 cm; females: mean ± SEM = 2.14 ± 0.04 cm)
and post orbital carapace length (males: mean ± SEM = 2.47 ± 0.03 cm; females: mean ± SEM =
2.53 ± 0.03 cm). Crayfish with intact walking legs, chelae, and sensory appendages were housed
in a flow through system in the Lakeside Laboratory at the University of Michigan Biological
Station (Pellston, Michigan, USA). Unfiltered water from the adjacent Douglas Lake was
pumped into a 330.2 cm by 61.0 cm by 45.7 cm trough where animals were housed individu-
ally. Temperature of the water ranged from 17.7 °C to 27.6 °C throughout the summer and the light :
dark cycle ranged from 15.5 hours light : 8.5 hours dark (June) to 13.3 hours light : 10.7 hours
dark (August). All crayfish were visually and mechanically isolated for a minimum of seven
days in 1.2 L plastic containers to eliminate effects of prior social history developed in the field
(Karavanich and Atema, 1998; Zulandt-Schneider et al., 2001). Crayfish were fed an ad lib diet
d of commercial rabbit food pellets twice a week and were used only once in these trials. Due to
collection protocols, all animals were frozen immediately following a trial. All crayfish were in
non-reproductive form. Non-reproductive form was determined by examining the morphology of
the male copulatory stylets and confirming the absence of the egg adhering substance (i.e. glair)
on the female tail and walking legs (Holdich, 2002).

Experimental Design

The purpose of this experiment was to elicit the role of a chemical signal used during
agonistic fights in which individuals had impaired sensory capabilities. Sensory input was
reduced by choosing one contestant at random to undergo chemical lesions (described below).
We lesioned only one contestant per trial in order to effectively parse out the effects of impaired
sensory abilities on male and female contestants. We also injected individuals with a fluorescent
dye (described below) to aid in visualization of the use of the chemical signal of interest (i.e.
Thus, we had a fully factorial 2 x 2 design with the first factor (sex of lesioned contestant) having two conditions (male or female) and the second factor (contestant size) having two conditions (size matched and size different) (Table 4). Within the size different condition, we considered contests in which the female was the larger contestant and in which the male was the larger contestant.

Lesion Protocol

To examine the effects of reduced chemical communication proficiency, the primary chemosensory organs (the paired antennules) were lesioned using a salt water lesion technique. This method is based on similar techniques previously used in lobsters (Beglane et al., 1997) in which chemoreceptors are damaged while mechanoreceptors are left intact. Crayfish were restrained dorsal surface up, oriented downward, on a 22.9 by 27.9 cm piece of Plexiglas using 2.0 mL pipette tips and rubber bands (Figure 4). The antennules were placed in an enclosed pipette tip (0.5 mL) filled with 50 ppt saltwater. After a period of 120 minutes, the animal’s antennules were then placed in a pipette tip containing deionized water for 10 minutes, effectively lysing the cells due to rapid changes in osmotic pressure. This technique has been shown to effectively limit chemosensory abilities while leaving mechanoreceptors intact and functioning in crayfish (Kraus-Epley et al., 2015). Lesioning was performed 24 to 48 hours before fight trials in order to allow recovery and to ensure the animals survived the process. Previous studies in spiny lobsters have shown that damaged chemoreceptors do not exhibit significant olfactory unit regeneration until 20 to 40 days post-damage (Harrison et al., 2003; Harrison et al., 2004). Handling controls were performed on contestants not chosen for lesioning. Handling controls underwent the same restraint process except their antennules were soaked in only deionized water for the full 130 minutes.
**Injection Protocol**

Both animals in each contest were injected with a 0.05% sodium fluorescein (Sigma: F-6377, Lot 103H3412; Sigma-Aldrich, St. Louis, MO, USA) solution dissolved in Van Harreveld crayfish saline (Van Harreveld, 1936). All animals were subjected to injections at 0.01 ml/gram of body mass using a 1 mL syringe affixed with a 26 gauge (0.45 mm x 13 mm) needle. This protocol was based on techniques first developed by Breithaupt and Eger (Breithaupt and Eger, 2002) and modified by Bergman et al. (Bergman et al., 2005) and Simon and Moore (Simon and Moore, 2007). The injection site was located on the dorsal surface of the carapace. The needle was inserted through the carapace at a depth of approximately 2 mm. The syringe contents were then slowly injected into the body cavity. The needle was quickly removed and a small dot of Loctite® gel control super glue was placed over the injection site followed by a small strip of black electrical tape. Animals were allowed a minimum 1 hour recovery period before being used in a trial (Wofford et al., 2017). During this time, animals were monitored for signs of distress (i.e. irregular movement or inability to move appendages).

**Fight Arena**

All trials were conducted in a dark room using a specialized arena (Figure 1). A 21 L aquarium (40.6 cm x 20.3 cm x 25.4 cm) was affixed with two black Plexiglas® (ePlastics, San Diego, CA, USA) inserts to reduce tank dimensions (22.9 cm x 20.3 cm x 25.4 cm) in order to increase the probability of agonistic behavior and to ensure that the entire arena was in view of the camera. The aquarium was filled with 7.1 L of water from Douglas Lake that had been filtered using an Aqua-Tech® aquarium filtration system for a minimum of 24 hours. The aquarium was placed on a dark surface and the sides were covered using black fabric to reduce glare and assist in visualization of urine release. A Sony HDR-CX405 9.2 megapixel camera
(Sony Electronic Inc., Novi, MI, USA) was positioned on a tripod approximately 20 cm from the side of the aquarium. The camera was set to low lux to account for the lighting conditions, and the focus and exposure were adjusted manually before each trial to achieve the clearest picture possible. Three 60 Watt black lights were used to facilitate lighting of the tank and visualization of the fluorescein-laced urine.

**Fight Protocol**

The fight arena set up described above was assembled prior to crayfish introduction. Crayfish were placed into the arena and were visually and physically isolated by an opaque barrier for a 15 minute period to allow them to acclimate to the trial conditions (Bergman et al., 2005; Simon and Moore, 2007). Lighting and arena conditions were identical in the acclimation and trial periods. At the conclusion of the acclimation period, the camera was set to record, the partition separating the contestants was removed, and individuals were allowed to interact. Trials lasted for 20 minutes after which the contestants were removed and the arena was drained and rinsed before the next trial. Trials were analyzed to determine fight outcome, fight dynamics, and behavioral responses to release events.

**Data Analysis**

Each trial was analyzed to determine the duration of the first bout, the winner and loser of that bout and the duration of escalated and non-escalated behaviors during the bout. The first bout was defined as the first interaction in which both individuals engaged in chelae to chelae contact for greater than 10 seconds. The loser of the first bout was defined as the individual that retreated the greatest number of times within the defined bout period. If neither opponent retreated or both retreated an equal number of times, no clear winner or loser was declared and the trial was removed from analysis. Escalated and non-escalated behaviors were defined based
on a pre-established fight ethogram (Table 2). Non-escalated behaviors were defined as “low cost”, minimal contact behaviors (Intensity Levels 3-6) while escalated behaviors were defined as “high cost” behaviors that involved increased probability of injury to the opponents (Intensity Levels 7-10). These data parameters were also assessed on non-lesioned contests under the same injection and fighting conditions performed in a previous study (Wofford et al., 2017). Urine release events were also identified throughout contests, regardless of the bout in which they occurred. A release event was determined to have occurred when fluorescein laced urine was ejected from the nephropores. Because of a low release frequency in this project, behavioral data surrounding release events were not analyzed.

A two-way ANOVA followed by Tukey HSD post hocs were performed in the base package of R statistical software (version 3.3.0) (R Development Core Team, 2016). We used these analyses to determine the role of size treatment (size matched or different size contestants) and lesion treatment (no lesion, female-lesioned, or male-lesioned) on the first bout duration. A separate two-way ANOVA was also utilized on size different contests to discern the role of opponent sex (larger female or larger male) and lesion treatment. The same analyses were used for non-escalated and escalated phase durations; however, only male and female lesion data were available for phase duration analyses. We utilized individual chi square tests on each contest classification to determine if there were significant differences in the proportion of male and female wins. A Tukey Multiple Comparisons of Proportions test was used to determine the individual and interactive effects of size and lesion treatments on the proportion of wins by males across treatments.

Regression analyses were also performed in the base package of R statistical software and were used to evaluate the assessment strategy hypothesized to be in place across treatment
types (e.g. Taylor and Elwood, 2003; Arnott and Elwood, 2009) by comparing our results to expected outcomes seen in Table 5. RHP proxies (i.e. smaller and larger contestant body size and % asymmetry between the two contestants) were regressed against total bout duration and non-escalated and escalated phase duration to ascertain what type of strategy was likely in place for male and female-lesioned contests. One outlier was removed from the male-lesioned dataset during these analyses. This outlier was determined via model validation methods (e.g. Cook’s Distance) outlined in Zuur et al. (2009). Using the same model validation methods, we examined residual normality and consequently applied data transformation methods to achieve the best conformation to QQ residual plots possible. Total bout duration was natural log transformed, non-escalated phase duration was log transformed, and escalated phase duration was square root transformed. These transformations were consistent across male and female-lesioned data.

Results

Contest Outcome

Regardless of lesion and size treatment, males won significantly more contests than females overall ($\chi^2 = 5.63$, df = 1, $p < 0.05$). Within lesion treatments, only contests in which the male was lesioned produced a significant difference in winning proportion; males won significantly more often than females ($\chi^2 = 5.77$, df = 1, $p < 0.05$). The same was true of size matched contests ($\chi^2 = 8.65$, df = 1, $p < 0.01$).

The interaction of size and lesion treatment also significantly impacted winning proportions. Males won significantly more often than females in size matched contests in which neither opponent was lesioned ($\chi^2 = 4.00$, df =1, $p < 0.05$) and in which only the male was lesioned ($\chi^2 = 4.76$, df = 1, $p < 0.05$). Males also won significantly more often than females in size different contests in which only the male opponent was lesioned ($\chi^2 = 14.22$, df = 1, $p <$
Finally, we isolated size different contests to examine the role of sex of the larger individual. We found that males won significantly more often when they were the larger opponent when the male was lesioned ($\chi^2 = 5.44$, df = 1, $p < 0.05$). Larger males also won more often in contests in which females were lesioned (male wins: 9, female wins: 0); however, we were unable to run a chi square test since the females did not win any contests.

A chi square analysis coupled with the Tukey multiple comparisons of proportions test revealed that the proportion of male wins differed significantly across treatments ($\chi^2 = 31.85$, df = 18, $p < 0.05$). Within size different contests, the proportion of male wins significantly differed as a factor of lesion treatment and sex of the larger individual. Specifically, in contests in which neither individual was lesioned and in which only the female was lesioned, males had greater winning proportions when they were the larger sex (no lesion: $Q_{(\text{inf},\text{df}=18,0.05)} = 5.02$, $p < 0.05$; female lesion: $Q_{(\text{inf},\text{df}=18,0.05)} = 7.43$, $p < 0.05$). Although the same comparison was not significant in male-lesioned contests ($Q_{(\text{inf},\text{df}=18,0.05)} = 4.89$, $p > 0.05$), males did exhibit higher winning proportions when they were the larger sex in size different contests.

**Bout Duration**

Size treatment individually did not have a significant effect on first bout duration ($F_{(1,108)} = 0.42$, $p > 0.05$). However, there was a marginally significant effect of lesion treatment ($F_{(2,108)} = 2.41$, $p = 0.09$). Female-lesioned contests tended to have longer bout durations compared to contests in which neither contestant was lesioned (Tukey HSD: $p = 0.09$). There was no interactive effect of size and lesion treatment on bout duration ($F_{(2,180)} = 1.72$, $p > 0.05$).

To further elucidate any differences due to the sex, we also examined the individual and interactive effects of the sex of the larger individual and lesion treatment on first bout durations in only the size different trials ($N = 67$). There was a significant effect of the sex of the larger
individual ($F_{(1,61)} = 4.25, p < 0.05$). First bout durations were significantly longer when the female was the larger individual in size different contests (Tukey HSD: $p < 0.05$). Lesion treatment also significantly influenced first bout duration in size different contests ($F_{(2,61)} = 4.49, p < 0.05$). Contests in which females were lesioned lasted significantly longer than male-lesioned contests (Tukey HSD: $p < 0.05$) and contests in which neither contestant was lesioned (Tukey HSD: $p = 0.05$). There was also an interactive effect of larger individual sex and lesion treatment ($F_{(2,61)} = 4.65, p < 0.05$) (Figure 5). Female-lesioned contests significantly differed in bout duration based on the sex of the larger opponent (Tukey HSD: $p < 0.05$). However, male-lesioned bout duration and non-lesioned bout duration were uninfluenced by the sex of the larger opponent (Tukey HSD $> 0.05$). Furthermore, in contests in which the female was larger, female-lesioned contests lasted significantly longer than male-lesioned contests (Tukey HSD: $p < 0.01$) but did not differ compared to non-lesioned contests (Tukey HSD: $p > 0.05$). Contests in which the male was the larger opponent were unaltered due to lesion type (Tukey HSD $p > 0.05$).

Phase Duration

The interaction of size and lesion treatments significantly impacted the duration of the non-escalated phase duration of contests in which individuals were lesioned ($F_{(1,73)} = 4.20, p < 0.05$). A Tukey post hoc test revealed one difference that approached significance. In contests in which the individuals were different sizes, female-lesioned contests were more likely to have longer non-escalated phase durations than male-lesioned contests (Tukey HSD: $p = 0.09$). Neither size treatment, lesion treatment, nor the interaction of the two terms significantly affected escalated phase duration; however, lesion treatment did approach significance ($F_{(1,73)} = 2.75, p = 0.10$).
To further elucidate any differences due to sex, we examined the individual and interactive effects of the sex of the larger individual and lesion treatment on phase durations in only the size different trials. Lesion treatment had a significant effect on the non-escalated phase duration in size different trials ($F_{(1,41)} = 7.74, p < 0.01$). Contests in which the female was lesioned had significantly longer non-escalated phase durations than contests in which the male was lesioned (Tukey HSD: $p < 0.01$). There was also an interactive effect of lesion treatment and sex of the larger individual on non-escalated phase duration in size different contests ($F_{(1,41)} = 5.85, p < 0.05$) (Figure 6A). In contests in which the female was the larger individual, non-escalated phase durations were significantly longer in female-lesioned contests compared to male-lesioned contests (Tukey HSD: $p < 0.01$).

Escalated phase duration in size different contests was significantly impacted by lesion treatment ($F_{(1,41)} = 6.18, p < 0.05$). Female-lesioned contests demonstrated significantly longer escalated phase durations than male-lesioned contests (Tukey HSD: $p < 0.05$). Sex of the larger individual also demonstrated a marginal effect on escalated phase duration ($F_{(1,41)} = 3.87, p = 0.06$). Escalated phase duration was longer in contests in which the female was the larger contestant (Tukey HSD: $p = 0.06$). The interaction of lesion treatment and sex of the larger individual also significantly impacted escalated phase duration ($F_{(1,41)} = 9.98, p < 0.01$) (Figure 6B). In contests in which the female was the larger individual, female-lesioned contests had significantly longer escalated phase durations than male-lesioned contests (Tukey HSD: $p < 0.01$). Furthermore, in female-lesioned contests, contests with larger females had significantly longer escalated phase durations than contests in which the male was larger (Tukey HSD: $p < 0.01$).
Regression Analyses

Regressions of contests in the female-lesioned treatment revealed a marginally significant, positive relationship between the carapace size of the larger contestant and bout duration (slope = 0.99, p = 0.07), non-escalated phase duration (slope = 0.46, p = 0.09), and escalated phase duration (slope = 4.34, p = 0.09) (Table 6). No other regressions were found to be significant; these data are not consistent with any type of assessment strategy (Table 5; Taylor and Elwood, 2003; Arnott and Elwood, 2009).

Regression of contests in the male-lesioned treatment revealed a significant, positive relationship between the carapace size of the smaller contestant and non-escalated phase duration (slope = 0.45, p = 0.05). Furthermore, the regression between smaller opponent carapace size and total bout duration demonstrated a positive slope that approached significance (slope = 0.63, p = 0.18) (Table 6). There were also significant, negative regressions between percent asymmetry in contestant carapace size and total bout duration (slope = -0.05, p < 0.01), non-escalated phase duration (slope = -0.02, p < 0.05), and escalated phase duration (slope = -0.16, p < 0.05) (Table 6). No significant relationships were present for larger contestant carapace size, although total bout duration and escalated phase duration demonstrated positive slopes (Table 6). Total bout duration and non-escalated phase duration correlations are consistent with the expectations for a self-assessment type strategy (Table 5; Taylor and Elwood, 2003; Arnott and Elwood, 2009). Escalated phase correlations are not consistent with any assessment strategy.

Discussion

This study supports previous findings that male and female crayfish are using chemical information differently during agonistic contests. Intervertebral contests were largely unaffected by male access to chemical information, especially if the male was the larger opponent (Figures 2, 3). Furthermore, males were more likely to win in the contests in which they were larger.
Together, these findings suggest a threshold for size over which males will be dominant to females regardless of chemical information availability. Alternatively, intersexual contest dynamics were significantly altered due to female opponent size and female access to chemical information (Figures 2, 3). Female-lesioned contests lasted significantly longer if the female was the larger opponent, and contests with larger female contestants lasted significantly longer in female-lesioned (compared to male-lesioned) contests. Furthermore, females and males had roughly equal proportion of wins in these contest types. This suggests that larger females have an enhanced probability of winning an intersexual contest but that these contests are longer and more intense, especially when females do not have chemical information.

Previous studies that have blocked access to chemical information in male crayfish contests have found significantly longer durations and increased fighting intensities (Zulandt-Schneider et al., 2001) as well as altered win probabilities (Bergman et al., 2003). Consequently, chemical stimuli have been hypothesized to convey important social status information in crayfish contests. Our data demonstrated these expected outcomes in female-lesioned contests; however, male-lesioned contests were shorter in duration than female-lesioned contests and did not significantly differ from non-lesioned contests (Figure 5). Because males tend to overpower and dominate size matched and smaller females (Martin III and Moore, 2010; Fero and Moore, 2014) perhaps chemical information about status is less important for males in intersexual contests, especially if the female’s ability to sense a male opponent is intact. Chemical cues play a strong role in sex recognition (Acquistapace et al., 2002; Stebbing et al., 2003; Aquiloni and Gherardi, 2010); perhaps the female, when multi-modal information is available, can determine opponent sex, sense that she is fighting a male, and adopt submissive behaviors, effectively ending the contest. If this scenario were true, it would explain why males are still significantly
more likely to win intersexual contests overall even though male-lesioned contests were consistent in bout and phase duration.

Because the information dictating contest duration appears to differ for males and females, we would also expect differentiation in the assessment strategy used to determine retreat behaviors. Previous assessment work in crayfish found strong evidence for self-assessment in male and female intrasexual contests but no reliable evidence for any type of strategy in mixed sex contests (Wofford et al., 2015). The authors hypothesized that, while the strategy might be similar in nature for males and females, the information utilized to resolve contests fundamentally differs for each sex. This difference then manifests as a mix of strategies in intersexual contests that we are unable to untangle with current measurements of assessment. The current study further supports this hypothesis that males and females are utilizing similar strategies but different information to make decisions about contest resolution. Intersexual contests demonstrated strong evidence for a self-assessment strategy when the males were lesioned; however, female-lesioned contests did not appear to follow any pre-classified strategy for assessment (Table 5).

Agonistic studies have asserted that chemical stimuli are the primary information drivers in crayfish contest behavior and hierarchy establishment (Zulandt-Schneider et al., 1999; Zulandt-Schneider et al., 2001; Bergman et al., 2005; Moore and Bergman, 2005; Berry and Breithaupt, 2010; Breithaupt, 2011; Callaghan et al., 2012). However, the bulk of these studies assumed that chemical information, notably urine release, was used as a means of mutual assessment or as a means to send information about one’s own physical state to an opponent. The finding that crayfish are likely using self- rather than mutual assessment (Wofford et al., 2015; this study) suggests that chemical information may play a different role in contest dynamics than
previously thought. For instance, urine release may be primarily utilized as a self-mediated checks and balances system (e.g. energy reserves or metabolite build up) (Huber et al., 1997; Breithaupt, 2011) rather than an opponent directed signal.

Generally, the response to urine release in crayfish contests is to rapidly de-escalate behavioral intensity; however, the rate and degree of de-escalation differs based on sex and relative size of the opponent. Specifically, mixed sex contests demonstrated more robust intensity changes after a urine release event than either male or female intrasexual contests (Wofford et al., 2017). While chemical information is undoubtedly an important driver in crayfish contest outcome and dynamics, visual and mechanical stimuli also play significant roles in individual recognition and mate selection as well as dominance establishment (Aquistapace et al., 2002; Aquiloni and Gherardi, 2010; Callaghan et al., 2012). Findings that crayfish, like many other organisms, rely on multi-modal information presents the opportunity for informational “hierarchies” based on social and environmental information. For example, crayfish appear to utilize visual information quite readily (Bruski and Dunham, 1987; Aquistapace et al., 2002; Correia et al., 2007; Aquiloni and Gherardi, 2010), but low light conditions can push crayfish to rely on chemical rather than visual information (Bruski and Dunham, 1987; Bouwma and Hazlett, 2001). Furthermore, studies that systematically block one or more modality have found that while lack of chemical information does alter behavior, visual and mechanical information also play important roles (Callaghan et al., 2012). This suggests that the primary modality used to make behavioral decisions can shift based on environmental conditions and the social information that is readily available. Perhaps the reason that males and females are differentially impacted by the lack of chemical information is that the sexes have different informational
hierarchies. Chemical information plays a role in contest dynamics for both males and females, but perhaps females are more significantly impacted by its loss than males.

Just as females compete in agonistic interactions in much the same way as males, females and males of some species also utilize similar social and agonistic signals (Jenssen et al. 2000; Riebel, 2003). However, just as in fighting behavior, the intensity and use of these signals differs slightly between males and females. Female lizards (*Anolis carolinensis*) utilize dewlap extensions and head bobbing techniques in intrasexual displays, similar to those seen male contests (Jenssen et al., 2000). However, while males and females have the same repertoire of aggressive signals, there are sexual dimorphisms in the signal structure (e.g. females had smaller dewlap area) and the context under which the signals are used (Jenssen et al., 2000). Similarly, several species of passerine song birds have been found to demonstrate robust rates of female singing during aggressive interactions, notably for territorial defense (Vondrasek, 2006; Cain and Langmore, 2015). In northern cardinals (*Cardinalis cardinalis*) male and female birds exhibit very similar song repertoires, but differ significantly in its use. Specifically, males do not show significant differences in song rates across the breeding season whereas female song rates are significantly influenced by a variety of social factors (e.g. pair bonding, territorial intrusion) (Vondrasek, 2006). Likewise, male and female crayfish exhibit the same agonistic behaviors and signal release repertoire in agonistic contests. However, the behavioral reaction to this signal release differs across sex (Wofford et al, In Press), suggesting that the signal itself maybe similar but the social context surrounding that signal influences males and females in different ways.

**Conclusions and Future Directions**

Our collective understanding of the fundamental differences in female and male agonistic behavior continues to grow, but is still incomplete. While we have a much better understanding
of the underlying selective pressures that drive some of these differences, the distinctions in the rules dictating these contests and the complexity of the signals that contribute to contest resolution in males and females are still vastly unknown. This study contributes to the growing number of studies that have found that males and females significantly differ in the rules that dictate contest escalation and resolution and the importance of different signal modalities in these decisions. Future studies, in crayfish and other species, should continue to tease apart the role that signal structure and social context play in male and female contest resolution.
CHAPTER IV: ASSESSMENT STRATEGIES DIFFER FOR MALES AND FEMALES ACROSS VARIED ENVIRONMENT AND SOCIAL CONTEXTS

Introduction

Organisms are in constant competition with conspecifics for access to resources and this competition can manifest as physical interactions (i.e. fighting behavior) (Maynard Smith and Price, 1973). However, while fighting can be a means to obtain vital resources, these behaviors are energetically costly, take time from other behaviors (e.g. mating, foraging), and come with the risk of injury (Maynard Smith and Price, 1973; Maynard Smith, 1974; Parker, 1974). Subsequently, animals have ritualistic behavioral mechanisms in place to trigger escalation or retreat from a contest when these costs have outweighed the potential benefit of obtaining a given resource (Parker, 1974). Since the withdrawal from a fight is concomitant with loss of resources, the decision to retreat needs to be based on critical information gathered before or during the interaction. The assessment strategy, which uses the information gathered, that underlies the behavioral decision to withdraw is determined by the type of information hypothesized to determine that retreat point.

Many assessment-based studies over the last decade have grouped these strategies into broad categories (i.e. self- and mutual assessment), and have subsequently focused on determining which strategy was in use for particular species. However, recent years have seen a significant shift in theoretical and empirical studies focused on understanding assessment. Many of these studies have introduced the idea of a spectrum or continuum based model of assessment rather than a strict dichotomy (Arnott and Elwood, 2009; Fawcett and Mowles, 2013, Mesterton-Gibbons and Heap, 2014). Further, a wealth of evidence has revealed significant variability and fluidity of assessment strategies even within a single agonistic encounter (Hsu et al., 2008). Sex
(Draud et al., 2004; Elias et al., 2010; Wofford et al., 2015), age (Fawcett & Johnstone, 2010), and previous experience (i.e. winner/loser effects) (Garca et al., 2012; Hsu et al., 2014) are all variables that have been empirically or theoretically demonstrated to influence assessment. Likewise, the availability of information about potential opponents, costliness of information exchange, and the value of the resource or the benefit obtained from a contest have been suggested as key determinants that have not been fully incorporated into our understanding of assessment strategies (Oliveira et al., 1998; Giraldeau et al., 2002; Mohamad et al., 2010; Mesterton-Gibbons and Heap, 2014).

One way in which animals’ behavioral decisions are influenced during a contest is by direct or indirect measures of resource holding power (RHP). Components of RHP such as body size, signal quality, energy reserves, or shelter residency can all influence the likelihood of contest engagement and subsequent contest retreat (Briffa and Elwood, 2004; Reby et al., 2005; Rudin and Briffa, 2012; Järvisto et al., 2013; Chibucos et al., 2015). Generally, individuals are hypothesized to have some approximation of their own RHP compared to potential opponents via estimation of the RHP distribution throughout the population or through direct, previous interactions with a given opponent (Kasumovic et al., 2010; Fawcett and Mowles, 2013; Mesterton-Gibbons and Heap, 2014). This information can help an individual to assess potential RHP asymmetries and avoid potentially costly interactions. Whether a contestant is relying on more general (i.e. population approximations) or specific (i.e. signals/cues form an opponent) information to make contest decisions influences where they fall along the assessment continuum (Mesterton-Gibbons and Heap, 2014). Subsequently, many environment and social variables within a population can shift which types of information might be more readily available and/or
costly to obtain, potentially sliding a given individual along the assessment continuum, effectively altering assessment.

Models exist (Mesterton-Gibbons and Heap 2014) to test this concept of variable assessment across a fluctuating landscape of the costs and benefits and different types of information (general or specific) about given opponents. Subsequently, individuals’ placement on this landscape was indicative of whether self- or mutual assessment strategies were more likely to be at play for the population (Figure 7). The authors used a modified hawk-dove game to examine the effects of alteration of RHP and resource distribution on assessment strategies within a population. They used the outcome of these games to assign behavioral strategies to individuals using a three dimensional vector based on how much to invest in specific information (i.e. exchanging signals and cues about RHP with another individual) and engaging in a physical interaction (i.e. playing hawk) upon perceiving the opponent as weak or strong. The authors also developed a particular evolutionary stable strategy (ESS) for each population based on whether pure self-assessment or some form of mutual assessment was in use (Figure 7).

We chose to adopt the game designed by Mesterton-Gibbons and Heap (2014) with our current biological model (crayfish) to test the hypotheses formulated by their model. Specifically, their model predicted that strong and weak individuals in a population would differ or switch between self- and mutual assessment based on the costliness of a contest, the resource availability, and the relative strength of the rest of the population. Costly contests (e.g. high rate of escalation, high RV) should see weak individuals avoiding fight behavior altogether whereas strong individuals will be more likely to engage in mutual assessment to avoid costly interactions (Figure 7). Meaning, as fights become more costly and/or the stakes become higher, strong-skewed populations should exhibit mutual assessment while weak-skewed populations would
likely demonstrate either self-assessment or no strategy at all. In relatively inexpensive contests (e.g. low chance of injury, low RV), all individuals (weak or strong) benefit from contest engagement as the cost to benefit ratio becomes low. Weaker individuals will likely demonstrate mutual assessment in order to avoid fights that they likely will not win while strong individuals are likely to exhibit self-assessment or no strategy as they will win encounters regardless of whether they can gather information about a potential opponent or not (Figure 7).

Previous work in crayfish assessment has demonstrated that both male and female crayfish are using a form of self-assessment in intrasexual contests (Wofford et al., 2015). However, in intersexual or mixed sex contests, any discernible strategy disappears. Subsequent studies have also shown that this difference for males and females is likely driven by differential information sources utilized to make assessment based decisions and that removal of a sensory modality can alter the assessment strategy in use for crayfish (Wofford and Moore, in Prep). These findings suggest that assessment strategies in crayfish populations under different environmental (i.e. resource availability) and social (i.e. RHP proportion in the population) contexts could significantly differ. Thus, we chose to test male and female crayfish populations for their placement on the assessment landscape introduced by Mesterton-Gibbons and Heap (2014) by manipulating the RHP composition and resource availability within a mesocosm scale experiment. We subsequently analyzed the assessment strategy in place for these population treatments to discern any differentiation due to sex, RHP distribution of the population, and perceived resource value for the individuals.

Methods

Animals

Male and female crayfish, *Orconectes rusticus*, were collected using a kick seine method
from the Portage River near Bowling Green, Ohio (Wood County, 41°21’42”N, 83°35’28”W).

All individuals were measured, using calipers, for chelae length (males: mean ± SEM = 3.04 ± 0.06 cm; females: mean ± SEM = 2.48 ± 0.04 cm) and post orbital carapace length (males: mean ± SEM = 2.47 ± 0.04 cm; females: mean ± SEM = 2.58 ± 0.04 cm). Animals with intact walking legs, chelae, and sensory appendages were housed in a recirculating system within an environmental chamber held at a constant temperature (23 - 25 °C) and light/dark cycle (12L: 12D). All crayfish were visually and mechanically isolated for a minimum of seven days to eliminate effects of prior social history developed in the field (Karavanich & Atema, 1998; Zulandt-Schneider et al., 2001). While in isolation, individuals were fed an ad lib diet of commercial rabbit food pellets three times a week; however, feeding was suspended for 7-10 days prior to a trial to increase motivation for competition over a food resource.

Some animals were used across multiple trials; however, individuals were given at least one week of recovery between trial appearances and were not used in the same treatment set twice. Trials were conducted between November 2016 and February 2017. Consequently, all crayfish were in reproductive form. Males were considered reproductive if their stylets were uncornified and bifurcated while females were considered reproductive if glair, a white substance used for egg adherence, was present on the ventral portion of the telson and the base of the walking legs (McLay & van den Brink, 2016).

Experimental Design

This project was designed to provide empirical evidence of Mesterton-Gibbon and Heap’s (2014) model positing fluid assessment strategies under varying contexts. Consequently, experiments followed a fully factorial 2 by 2 by 2 design with each factor (sex, strength ratio, resource value) having two conditions (Table 7). The sex factor had two conditions: all male or
all female population. Strength ratio conditions consisted of “weak skewed” or “strong skewed”
populations. The population of five individuals was composed of four weak individuals and one
strong individual (weak skewed) or vice versa (strong skewed). Strength was defined by body
size (i.e. carapace and chelae length) for consistency. Previous studies in crayfish agonism have
demonstrated that body and chelae length serve as strong measures of an individual’s resource
holding potential (RHP) as larger individuals or individuals with larger chelae (i.e. weaponry)
are more likely to win contests and secure resources (Schroeder and Huber, 2001; Bywater et al.,
2008). Many other factors might determine strength (i.e. previous contest experiences, age,
chelae pinch force), but these variables are also highly correlated with overall body and chelae
size (Pavey and Fielder, 1996). To achieve the appropriate differences in size necessary to
delineate strong and weak individuals, we used animals that ranged between a 20% to 30%
difference in body length. Thus, a strong-skewed population had four individuals that were size
matched (within 10% carapace and chelae length) that were also 20% to 30% larger than a single
weak individual. A weak-skewed population had four size matched individuals that were 20% to
30 % smaller than a single strong individual. The final factor was resource value, which was
determined by the availability of a resource (specifically food and shelter) to the population. A
high resource value treatment meant that resource availability was low while a low resource
value treatment indicated high resource availability relative to the population size (Fero &
Moore, 2014). We used a shelter coupled with a small food source (detailed below) as the
resource unit. The number of resource units available was manipulated to create different
resource value conditions. A high resource value environment was one that had few resources
relative to the number of individuals in the population (i.e. one resource for five individuals) and
a low resource value environment was one that had a one to one resource to individual ratio.
Animals were starved for one week before a trial to ensure food-based motivation. Trials were conducted in large, open-spaced arena with no shelter other than the resource units to ensure shelter-seeking behavior (Bergman and Moore, 2003; Martin III and Moore, 2007).

All trials were staged in 227 L tanks (dimensions: 80.00 cm x 80.00 cm x 35.56 cm) to allow for adequate movement and interaction between five individuals. Trials were video-taped from above for 12 hours using a Swann SWPro 530 cam security camera and SVAT 4 Channel DVR. All trials ran throughout the night as crayfish are primarily nocturnal (Gherardi, 2002). Tanks were drained and rinsed with tap water between each trial.

Tanks

Two trial tanks were housed in an environmental chamber held at a constant temperature of 24 - 25 °C. A false bottom was created for each tank using plastic egg crating and window screening in order to ensure consistent substrate height and to allow for easy cleaning after trials. A 13 mm layer of gravel was spread evenly across the tank to create the substrate. Tanks were filled with 146 L of pre-conditioned tap water at least one hour prior to trials to reduce water temperature fluctuations by trial time. Each tank was affixed with two air lines (in opposite corners) sheathed in 2 inch diameter PVC pipe halves (length: 22.86 cm) to ensure adequate oxygen levels throughout the trial. Shelters were added to the tank approximately 10 minutes before trial time and were arranged in pentagonal shape around the tank and given assigned positions (Figure 8). This shape ensured consistent spacing from the center of the tank to each shelter (~29.2 cm) as well as spacing between each shelter (~34.3 cm).

Resource Units

Resource units were composed of a paired shelter and food source. Shelters have been demonstrated to be of high value to crayfish in previous lab-based (Alonso and Martinez, 2006;
Fero and Moore, 2014) and field-based studies (Bergman and Moore, 2003; Martin III and Moore, 2007), likely due to their use in avoiding predation. Shelters consisted of a 3 inch diameter PVC pipe half affixed to a 15.24 cm by 15.24 cm piece of Plexiglas with waterproof silicone. Plastic egg crating was affixed to the back of the shelter to ensure only one entrance (facing the center of the tank). A 1.3 cm by 1.3 cm piece of Velcro was super glued to the front left corner of the shelter’s Plexiglas platform for food source adherence. The food source was a food cap (2.5 cm diameter x 1.2 cm depth) filled with 1.5 mL of fish gelatin. Fish gelatin was made by mixing 600 mL of boiling water, four packets of Knox flavorless gelatin, and 46 grams of canned sardines in a blender. The mixture was then immediately added to the plastic food caps using a 1 mL plastic disposable pipette. Food caps were allowed to cool for approximately 2 hours before being added to a refrigerator to solidify. After 24 hours, each food cap was covered with a strip of Parafilm and returned to the refrigerator. Food caps were used within one week. Crayfish readily consume fish gelatin as a food source, and have been shown to preferentially navigate to it as a food resource (Keller et al., 2001; Kraus-Epley and Moore, 2002). Prior to being filled with fish gelatin, each food cap was affixed with a 1.3 cm by 1.3 cm strip of Velcro, assigned a unique identifying number, and weighed to create a database of cap weights. Fish gelatin filled food caps were weighed immediately before use in a trial and within 10 minutes of removal from a trial to estimate food consumption. High resource value trials had only one shelter/food resource located at the α position while low resource value trials had five shelter/food resources located at each of the five corresponding resource positions (Figure 8).

Analysis

Videos were used to determine the number of interactions throughout each trial, the duration of each interaction, and the winner/loser of those interactions. For initial data analyses,
only the first 50 interactions were logged. Within these time points, any interactions that were unresolved, involved more than two individuals, or lasted less than 5 seconds were excluded. Winner/loser information was subsequently paired with contestants’ carapace length. This allowed us to run regression analyses for determination of assessment strategies (i.e. RHP measures versus duration). Regression analyses were performed in Origin Pro 8 and were used to evaluate the assessment strategy hypothesized to be in place across treatment types (e.g. Taylor and Elwood, 2003; Arnott and Elwood, 2009). The regressions obtained from our analyses were compared to the expected outcomes for different assessment strategies seen in Table 5. The results of these comparisons were used to compare assessment strategies across treatments.

Amount of food resources consumed during the trial was also calculated to provide a rough approximation of resource use across treatment types. Food consumption was calculated as a proportion using the mass of food remaining and food mass prior to resource introduction to a trial. Mass of the food cap container and gelatin mass gained due to water absorption were accounted for during these calculations.

Food consumption and interaction duration were both analyzed using a mixed model analysis in R statistical software (version 3.3.0) (R Development Core Team, 2016). Linear mixed models (LMM) followed by Type III analysis of deviance tables (Zuur et al., 2009) with Satterthwaite approximations were used to determine the effect of sex, strength ratio, and resource value on food consumption and contest duration. Models were constructed using sex of the individual in the trial (male or female), the strength ratio of the trial (strong skewed or weak skewed), and resource value (low or high) as fixed effects. Tank number and trial number were entered as (non-nested) random effects. LMMs were run using the lme4 package (Bates et al., 2015), and differences of least squares means (‘difflsmeans’) from the lmerTest package
(Kuznetsova et al., 2016) was used as a post hoc test to discern which factors were responsible for significant differences within significant main effects detected by the ANOVAs. Four duration data points were deleted as outliers in the data conditioning phase (Table 8), and all duration data were log transformed.

**Results**

**Resource Consumption**

Resource value was the only fixed effect that significantly impacted resource consumption ($F_{1,28} = 48.56, p < 0.001$). High RV treatments had higher proportion of food consumption than low RV treatments ($t = 6.97, p < 0.001$). However, strength ratio did have a marginal effect on resource consumption ($F_{1,27} = 2.60, p = 0.12$). Strong-skewed populations had slightly higher consumption proportions than weak-skewed populations ($t = 1.61, p = 0.1$).

**Contest Duration**

Contest duration was not significantly affected by any fixed effects or their interactions. However, sex treatment ($F_{1,7} = 3.34, p = 0.11$) as well as the interaction between sex and RV treatment ($F_{1,7} = 2.77, p = 0.14$) and sex and strength ratio treatments ($F_{1,7} = 5.06, p = 0.06$) demonstrated marginal effects. Male populations tended to have longer contest durations than female populations ($t = -1.83, p = 0.1$). Within female populations, low RV treatments had longer contest durations than high RV treatments ($t = -2.22, p = 0.06$) and strong-skewed populations had longer contest durations than weak-skewed populations ($t = 2.35, p = 0.05$). Across sex treatments, males in high RV treatments had longer contest durations than females in high RV treatments ($t = -2.54, p = 0.05$), and males in weak-skewed populations had longer contest durations than females in weak-skewed populations ($t = -3.06, p <0.05$).
**Regression Analyses**

**Single Term Effects**

Low RV populations exhibited evidence for a self-assessment strategy while high RV populations did not exhibit relationships consistent with any strategy (Table 9). Strong-skewed populations demonstrated significant positive relationships for loser and winner RHP and a significant negative relationship for percent asymmetry, indicative of a self-assessment strategy (Table 5, Table 9). However, while weak-skewed populations showed a strong positive relationship between loser RHP and duration, the lack of relationships between winner RHP and percent asymmetry and duration were not indicative of any type of assessment strategy (Table 9). Female populations demonstrated strong evidence for self-assessment (Table 5, Table 9); male populations did not demonstrate evidence indicative of any assessment strategy.

**Interactive Effects**

Within female populations, strong skewed treatments and high RV treatments both demonstrated evidence for self-assessment (Table 10). Weak skewed treatments and low RV treatments comprised of entirely female individuals did not demonstrate evidence of any assessment strategy. Within male populations, low RV treatments exhibited relationships consistent with self-assessment (Table 5, Table 10). However, neither high RV treatments nor weak or strong skewed treatments demonstrated evidence of any discernible strategy.

In low RV treatments, strong-skewed populations exhibited strong evidence for a self-assessment type strategy. Weak-skewed populations demonstrated a significant negative relationship between winner RHP and duration which is indicative of cumulative or mutual assessment strategies (Table 5, Table 10). However, the non-significant relationship between percent asymmetry and duration is slightly inconsistent with these strategy predictions (Table
Neither strong nor weak-skewed populations within high RV treatments demonstrated significant relationships readily indicative of any type of strategy.

Female, strong-skewed populations under the high RV treatment demonstrated strong evidence for self-assessment. The same type of population under a low RV treatment could be interpreted as using self-assessment, although the relationships are not entirely consistent with expectations (Table 5, Table 10). The same could be said for female, weak-skewed populations under high and low RV treatments (Table 10).

Male, strong-skewed populations under the low RV treatment demonstrated strong evidence for self-assessment while those under the high RV treatment were somewhat consistent with self-assessment (Table 5, Table 10). Alternatively, male weak-skewed populations did not readily demonstrate evidence consistent with any type of strategy regardless of RV treatment (Table 10).

Discussion

These preliminary data illustrate two important findings. First, this study provides empirical support for the varied assessment model built by Mesterton-Gibbons and Heap (2014). We found that high RV treatments did not demonstrate a clear strategy for contest resolution regardless of whether the population was weak or strong skewed (Table 10). Within low RV treatments, strong-skewed populations demonstrated strong evidence for self-assessment while weak-skewed populations demonstrated evidence for cumulative or mutual assessment (Table 10). These data provide preliminary support for this model and the subsequent hypotheses that assessment strategies in use should change across social and environmental contexts.

Mesterton-Gibbon and Heap’s (2014) model predictions asserted that, in costly contests (e.g. high cost of engagement relative to the benefits of winning), populations would, overall,
adopt a “careful” evolutionary stable strategy (ESS). Meaning, weak individuals would likely avoid fighting altogether while stronger individuals would benefit from aggression against weaker opponents (Mesterton-Gibbons and Heap, 2014). Therefore, under low RV conditions, strong-skewed populations would be more likely to display the predictions above (strong individuals utilize mutual assessment while weak individuals engage in self-assessment or no assessment). However, weak-skewed populations would demonstrate either this strategy or the “daring” ESS (described below) depending on relative RV. We found strong evidence for a self-assessment strategy (Table 10) in low RV, strong-skewed populations. This partially aligns with the model’s predictions for this particular treatment type. However, as we have not yet parsed out specific strategies in use for only strong or only weak individuals in these population treatments, we are currently unable to more precisely align our data with model predictions. Furthermore, we found preliminary evidence for a mutual or cumulative assessment type strategy (Arnott & Elwood, 2009) in low RV, weak-skewed populations as a consequence of a significantly negative relationship between winner size and contest duration. Whether this result conforms to model predictions is currently unknown since low RV, weak-skewed populations are predicted to be found at the intersection of careful and daring ESS strategies (Mesterton-Gibbons and Heap, 2014), resulting in an amalgamation of possible strategies, and because we have not yet parsed out strategies unique to only strong or weak individuals within a population.

In less costly contests, (e.g. low cost of engagement relative to the benefits of winning) Mesterton-Gibbons and Heap (2014) predicted that populations would adopt either a “daring” ESS in which all individuals would engage in aggressive interactions, with strong individuals persisting unconditionally and weak individuals only engaging other weak individuals, or a “conventional” ESS in which strong individuals would play hawk (always fight) and weak
individuals would play dove (always retreat). In conventional ESS populations, the model predicted neither self nor mutual assessment type strategies as no information would be gathered prior to a contest; individuals would utilize pre-determined strategies (i.e. hawk/dove). In high RV, strong-skewed populations, the model predicted the presence of the conventional ESS (no assessment) and in high RV, weak-skewed populations, the model predicted the daring ESS (strong individuals use self-assessment and weak individuals use mutual assessment). We found partial support for this assumption as weak-skewed populations under high RV treatments exhibited evidence consistent with either self- or mutual assessment (strong positive relationships between loser size and contest duration). However, strong-skewed populations did exhibit one significant relationship (winner size versus duration), that could be consistent with a self-assessment type strategy. This finding does not readily align with model predictions.

Second, in line with previous work, we found that males and females differ in contest duration and demonstrate nuanced differences in assessment strategy, especially under varied contexts. Male populations tended to engage in longer interactions than female populations. This is consistent with other studies that have found that males engage in longer, more intense fights more often than females, especially reproductive individuals (Martin III and Moore, 2010), which were also used in this study. However, our data also imply context dependence in this difference as males and females showed differentiation in duration due to both RV and strength ratio treatments. Resource availability does not appear to play a role in male contests as male populations did not differ in contest duration across high and low RV treatments. However, female contest duration was significantly affected by RV treatment. Females in low RV populations had higher contest durations than those in high RV populations. Males were also largely unaffected by the strength ratio present in the population; contest durations were
consistent across strong and weak-skewed populations. Females, however, demonstrated significantly longer contest durations in strong-skewed populations than weak-skewed populations.

Males and females also varied in the type of assessment strategy in use across environmental and social contexts. Overall, female contests demonstrated strong evidence for self-assessment while male regressions did not align with expectations for any strategy (Table 5, Table 10). This is at odds with previous findings in dyadic interactions that found that male and female crayfish both use self-assessment in same sex interactions (Wofford et al., 2015). There was also differentiation in the type of strategy in use for male and female populations across RV and strength ratio treatments, further suggesting that male and female crayfish utilize different rules for contest persistence under different contexts. Interestingly, when we examined male and female populations, we also saw further deviations from model predictions.

Males in strong-skewed populations under low RV treatments demonstrated strong evidence for self-assessment which partially aligns with model predictions; however, female populations in the same treatment could not be readily identified as using either self- or mutual assessment (Table 10). Evidence for cumulative or mutual assessment in weak-skewed populations at low RV treatments disappeared when we examined the contribution of sex. Female populations demonstrated relationships consistent with either self- or mutual assessment, but male contests were not readily indicative of any type of assessment strategy. Males in strong-skewed populations under high RV treatments also did not readily indicate conformity to any particular strategy which aligns with model predictions of the no assessment/conventional ESS for this population type. However, female populations demonstrated preliminary evidence for a self-assessment type strategy in use (Table 10). These relationships were reversed for males and
females in high RV treatments in weak-skewed populations. Females did not readily conform to any strategy type while males indicated preliminary evidence consistent with a self-assessment strategy.

Altogether, these initial analyses indicate (1) preliminary support for a model for assessment strategy predictions that incorporates resource availability and population-wide RHP, (2) the need to incorporate sex-based differences (directly or indirectly) into future models, and (3) crayfish serve as a valuable animal model to test these hypotheses.
CHAPTER V: SUMMARY AND GENERAL CONCLUSIONS

The field of animal behavior has been presented with two frontiers for a better understanding of factors underpinning physical competition. First, our historical focus on male intraspecific agonism has left a dearth of understanding of female agonism. Additionally, information on the ultimate and proximate explanations for its presence and differentiation from males is lacking. Mounting evidence suggests that not only do females differ from males in the selection pressures driving fighting behavior but also in the physiological, neurological, and communicatory mechanisms that dictate intensity and resolution (Draud et al., 2004; Elias et al., 2010; Rosvall, 2011). Second, theoretical/modelling studies that address the factors underlying fluidity in assessment strategies need empirical evidence to support or alter these hypotheses. Increasing numbers of assessment studies suggest a robust array of strategies that vary based on social and environmental contexts, including the sex of the individuals engaged in contests (Oliveira et al., 1998; Giraldeau et al., 2002; Mohamad et al., 2010; Mesterton-Gibbons and Heap, 2014). Better understanding the interaction of variables that influence behavioral strategies can have wide implications from conservation (Aionso et al, 2008; Scales et al., 2011) to human ethology (Lagerspetz et al., 1988; Benenson, 2013). The studies outlined in this dissertation are found at the interface of these two frontiers and contribute to our growing knowledge of female contest strategies and the context-dependent nature of assessment.

Previous studies in crayfish agonism demonstrated the tendency of females to engage in contest behavior as often as males. However, these same studies also revealed nuanced differences in the outcomes, dynamics, and information driving males and females in these interactions (Figler et al., 2001; Simon and Moore, 2007; Martin III and Moore, 2010; Wofford et al., 2015). In mixed sex population studies, males out-compete females for resources (Fero and
Moore, 2014); however, brooding females have also demonstrated significant maternal effects, displacing males readily from shelters (Figler et al., 2001). Others found that the dynamics of intra-and intersexual competition differ, especially under reproductive and non-reproductive contexts (Simon and Moore, 2007; Martin III and Moore, 2010). All of this led to the proposal that the differences seen in male and female contests may be due to variations in behavioral strategies dictating contest persistence. A subsequent study found that both sexes rely on the same strategy in intrasexual contests (Wofford et al., 2015). However, the same study also revealed the disintegration of any obvious strategy in mixed sex contests, suggesting that the underlying rules or information dictating these strategies fundamentally differ for male and female crayfish.

The first project of this research demonstrated that chemical information (specifically, urine release) plays a role in differences in behavioral strategies for male and female crayfish during a contest. While urine release frequency and reaction to release (i.e. intensity de-escalation) were consistent across intra- and intersexual dyads, the magnitude of this change was context dependent. The sex of the opponents dictated behavioral intensity as well as the magnitude and direction of intensity changes seen after a release event. Furthermore, the interaction of relative opponent size and the sex of the opponents appeared to affect the proportion of time spent in non-escalated contact behaviors in mixed sex contests. These results support the long-standing hypothesis that urine functions as a signal in crayfish contests and our hypothesis that the social information contained within is dependent on the sex and relative size of the opponents.

Just as we found that males and females reacted differently to the presence of a chemical signal, we found that they were differentially impacted by the absence of chemical information.
The second project found that alterations to mixed sex interactions due to limited information availability were contingent on the sex of the lesioned opponent. Intersexual contests were largely unaffected by male access to chemical information, especially if the male was the larger opponent. However, intersexual contest dynamics were significantly altered due to female opponent size and female access to chemical information. This further supports the findings of project one that the information driving contest resolution is not only dependent on the sex of the opponent but also on relative opponent size and the interaction of these two variables.

Furthermore, the research contained in the second project supports the hypothesis that males and females are utilizing similar strategies but different information to make decisions about contest resolution. Intersexual contests demonstrated strong evidence for a self-assessment strategy when the males were lesioned; however, female-lesioned contests did not appear to follow any pre-classified strategy for assessment.

The first and second projects collectively revealed that (1) male and female crayfish differ in strategy and information use for contest resolution and (2) manipulating variables (i.e. information availability) in dyadic interactions can significantly impact these strategies. Specifically, urine release induced a stereotypic response across contest treatments, which aligns with previous findings (Breithaupt and Eger, 2002). However, the nuanced differences in reaction to urine release across sex and size treatments support hypotheses that males and females differ in the chemical information they release (Ameyaw-Akumfi and Hazlett, 1975; Stebbing et al., 2003; Belanger and Moore, 2006; Simon and Moore, 2007) or, at least, differ in their reaction to chemical information during a contest. Project two provided significant support for the second hypothesis. Differential availability of chemical information for males and females resulted in variation in intersexual contest duration and resolution. Together, these
results demonstrate that males and females gather contest-relevant information from varied sources and suggest that the “hierarchy of relevant contest resolution” might differ for the sexes. Therefore, the focus of project three was to examine how changes in environmental and social context and, consequently, changes to population-relevant information, differentially impact male and female contest resolution. Specifically, predictions from assessment models were tested by altering the strength ratio (RHP), resource value (RV), and sex of crayfish populations under experimental conditions. Preliminary data illustrate two important findings. First, project three provides empirical support for the assessment model built by Mesterton-Gibbons and Heap (2014). Assessment strategies differed across RV and strength ratio treatments, in part, matching model predictions. Second, in line with previous work, males and females differed in contest duration and assessment strategy, especially under different contexts. If further data collection and analysis continue to support model expectations, this could have significant impacts on behavioral strategy predictions across varied environmental and social conditions.

Intraspecific competition for resources is ubiquitous and agonistic interactions are common across animal species. These physical interactions can influence anything from individual position within a single group/herd/flock (Öst et al., 2007) to sexual and natural selection (Maynard Smith and Price, 1973; Parker, 1974; Clutton-Brock and Huchard, 2013b). Likewise, the intrinsic and extrinsic variables playing a role in these interactions are equally diverse and widespread. Information about oneself, such as energy reserves, previous contest performance, or RHP, interplay with relative and specific information about potential opponents, the state of the environment, and more to influence behavioral decisions (Oliveira et al., 1998; Giraldeau et al., 2002; Fawcett and Johnstone, 2010; Mohamad et al., 2010; Fawcett and Mowles, 2013; Mesterton-Gibbons and Heap, 2014; Berthelot et al., 2017). Different
combinations of these variables necessitate different strategies to successfully gain beneficial resources while mitigating the time and energy costs inherent in obtaining them. Consequently, when these variables change, strategies must change with them.

As stated previously, the inherent differences in male and female life histories, physiology, and reproductive effort manifest in significant differences in behavior. This is especially true in agonistic interactions. The list of species that engage in female intraspecific agonism continues to grow and the bulk of these studies have determined that males and females differ in contest related behaviors (Bro-Jørgensen, 2002; Draud et al., 2004; Vondrasek, 2006; Elias et al., 2010; Watson and Simmons, 2010; Cain and Langmore, 2015; Wofford et al., 2015). Furthermore, inherent differences in males and females present unique combinations of the aforementioned variables that interact to influence contest resolution strategies. Meaning, males and females should fundamentally differ in the strategies driving contest escalation and persistence. Limited evidence, including that found in this dissertation, support this hypothesis.
REFERENCES


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Walter, G.M., van Uitregt, V.O. & Wilson, R.S. (2011). Social control of unreliable signals of


Figure 1: Schematic of fight arena set up. The drawing on the left shows the view into the test arena used in Chapters II and III from the side-view camera. The drawing on the right shows the set up from the side, rotated 90 degrees. Black lights were placed around the top of the structure to illuminate fluorescein release.
Figure 2A

Figure 2B
Figure 2: Behavioral changes pre and post urine release. Release plots demonstrate qualitative changes in behavioral changes over time in relation to a urine release event. 2A shows changes in behavior at each second averaged across all release events (N = 78). 2B shows differences in behavioral changes between different sex conditions [male-male (N = 21), female-female (N = 24), male-female (N = 33)]. 2C shows differences in behavioral changes between size matched (N = 42) and size different (N = 36) conditions. Trial numbers included in these graphs differ from those loaded into the LMM (Table 1) because pre/post behaviors are represented as a single unit per trial on this graph and because all release events were represented on these graphs rather than just the first three release events.
Figure 3: Fixed effects interactive effects on non-escalated contact phase. Bar plot exhibits significant interactive effect of all three fixed variables on non-escalated contact phase ($X^2 = 7.86, \text{df} = 2, p < 0.05$). Female same sex contests showed little to no change in non-escalated contact behaviors post release, regardless of size treatment. Males showed a trend towards increased proportion of non-escalated contact behaviors post release for both size matched and size different contests. Changes for these behaviors post release in mixed sex contests were dependent on the size treatment.
**Figure 4: Lesion apparatus.** Figure outlines apparatus used for the lesion technique outlined in the methods of Chapter III. An individual was restrained via rubber bands and 2 mL pipette tips to a Plexiglas board. The crayfish was oriented downward such that the primary chemosensory organs (i.e. antennules) were fully submerged in a 0.5 mL pipette tip holding either 50 ppt saltwater, deionized water, or lake water, depending on treatment type. Lesioned animals were subjected to 50 ppt saltwater for 120 minutes followed by 10 minutes of deionized water. Sham-lesioned animals were subjected to a full 130 minutes of lake water. Lake water was applied to animals throughout the process to prevent desiccation.
Figure 5: Bout duration in size different contests. Figure shows the interactive effect of the sex of the larger individual and lesion treatment on total bout duration in size different contests ($F_{(2,61)} = 4.65, p < 0.05$). In female-lesioned contests, bouts were significantly longer when the female was the larger individual (Tukey HSD: $p < 0.05$). In contests in which the female was larger, female-lesioned contests lasted significantly longer than male-lesioned contests (Tukey HSD: $p < 0.01$).
**Figure 6: Phase duration in size different contests.** Figure shows non-escalated and escalated phase duration in size different contests. Figure 6A demonstrates the interactive effect of the sex of the larger individual and lesion treatment (within male and female-lesioned contests) on non-escalated phase duration ($F_{(1,41)} = 5.85$, $p < 0.05$). In contests in which the female was the larger individual, non-escalated phase durations were significantly longer in female-lesioned contests compared to male-lesioned contests (Tukey HSD: $p < 0.01$). Figure 6B shows the interactive effect of the larger individual sex and lesion treatment (within male and female-lesioned contests) on escalated phase duration ($F_{(1,41)} = 9.98$, $p < 0.01$). In contests in which the female was the larger individual, female-lesioned contests had significantly longer escalated phase durations than male-lesioned contests (Tukey HSD: $p < 0.01$). Female-lesioned contests had significantly longer escalated phase durations when females were the larger contestant (Tukey HSD: $p < 0.01$).
Figure 7: Model predictions. Figure (adapted from Mesterton-Gibbons and Heap, 2014) illustrates expectations for assessment strategies in use across varying RV and population-wide RHP distribution. Light gray triangle denotes high cost contests, in which the majority of the population demonstrates a “careful” strategy (i.e. weak individuals always retreat from/avoid contests, strong opponents only engage with weaker opponents). Weak individuals will likely utilize no strategy while strong opponents will utilize mutual assessment. Dark gray triangle denotes low cost contests, and the majority of the population demonstrates a “daring” strategy (i.e. strong individuals fight unconditionally, weak opponents engage only with other weak opponents). In this region, weaker individuals are expected to engage in mutual assessment while strong individuals utilize no assessment or self-assessment. White portion demonstrates a region of “conventional” strategies where strong opponents always persist and weak opponents always retreat.
**Figure 8: Tank configuration.** Figure illustrates resource unit configuration in a low RV (i.e. high resource availability) treatment. Shelter resources (α – ε) were configured such that all shelter entrances faced the center of the tank and each shelter was equally spaced from the tank’s center (~29.2 cm) and adjacent shelters (~34.3 cm). Each shelter resource was paired with a fish gelatin food cap, denoted in this figure by the small black circles. High resource value (i.e. low resource availability) treatments had only one resource unit, located at the α position.
Table 1: Trial numbers for Chapter II. Table represents (1) the number of trials per treatment in which crayfish engaged in physical contact and one or both individuals released (“Trials Meeting Release Criteria”) and (2) the number of observations per treatment loaded into the LMM analysis (“Release Events Loaded into LMM”).

<table>
<thead>
<tr>
<th>Sex Treatment</th>
<th>Size Treatment</th>
<th>Release Events Loaded into LMM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male versus Male</td>
<td>Size Matched</td>
<td>N = 15</td>
</tr>
<tr>
<td></td>
<td>Size Different</td>
<td>N = 6</td>
</tr>
<tr>
<td>Female versus Female</td>
<td>Size Matched</td>
<td>N = 9</td>
</tr>
<tr>
<td></td>
<td>Size Different</td>
<td>N = 15</td>
</tr>
<tr>
<td>Male versus Female</td>
<td>Size Matched</td>
<td>N = 18</td>
</tr>
<tr>
<td></td>
<td>Size Different</td>
<td>N = 15</td>
</tr>
</tbody>
</table>
**Table 2: Extended ethogram.** Table represents an extended ethogram used in this study which was based on a previously developed and utilized ethogram (Bergman et al., 2005; Simon and Moore, 2007). The numerical intensity levels represented in the middle column were used to determine behavioral intensities utilized for analyses. We also broadly classified these intensities into three separate phases (left column).

<table>
<thead>
<tr>
<th>Classification</th>
<th>Intensity Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-contact Phase</td>
<td>-2</td>
<td>Tailflip away from opponent</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>Walking backwards away from opponent, no tail flip</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>Ignore opponent with no response or threat display</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Approach without a threat display</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Approach with threat display using meral spread</td>
</tr>
<tr>
<td>Non-escalated Contact Phase</td>
<td>3</td>
<td>Touching opponent with open or closed claws; no forceful movement</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Antennal whipping</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Forcefully pushing opponent away with one closed claw</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Initial claw use by boxing or pushing with closed claws</td>
</tr>
<tr>
<td>Escalated Contact Phase</td>
<td>7</td>
<td>Active claw use by boxing or pushing with open claws</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Active grabbing of opponent’s claws and other appendages</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Unrestrained grabbing and tearing; attempting to rip or tear opponent’s claws or appendages</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Inversion of one or both contestants with locked claws and unrestrained grabbing and tearing</td>
</tr>
</tbody>
</table>
**Table 3: LMM analyses.** Table represents significant findings from the LMM analyses. Table shows response variables significantly impacted, fixed effects responsible for changes, and reports from post-hoc ANOVAs.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Fixed Effects</th>
<th>$\chi^2$</th>
<th>Degrees of Freedom</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of Non-Contact Behaviours</td>
<td>Sex Treatment</td>
<td>6.35</td>
<td>2</td>
<td>0.04</td>
</tr>
<tr>
<td>Proportion of Non-Escalated Contact Behaviours</td>
<td>Sex Treatment * Size Treatment * Time</td>
<td>5.82</td>
<td>2</td>
<td>0.05</td>
</tr>
<tr>
<td>Behavioural Intensity Overall Change (10 to 1)</td>
<td>Time</td>
<td>21.93</td>
<td>1</td>
<td>2.82 X 10^{-6}</td>
</tr>
<tr>
<td>Mean Behavioural Intensity Shift (5 Seconds)</td>
<td>Time</td>
<td>5.96</td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean Behavioural Intensity Shift (10 Seconds)</td>
<td>Sex Treatment * Time</td>
<td>9.06</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>29.40</td>
<td>1</td>
<td>5.90 X 10^{-8}</td>
</tr>
</tbody>
</table>
Table 4: Trial numbers for Chapter III. Table conveying treatment sample sizes. Size matched contests were composed of one female and one male opponent with no greater than a 10% difference in carapace length. Size different contests were composed of one female and one male opponent in which one (randomly chosen) contestant had a carapace length at least 10% larger than the opponent. Lesion treatment was based on the sex of the lesioned individual. Only one contestant was lesioned during a trial. Similar data for non-lesioned animals from a previous study were also included for bout duration comparisons (Wofford et al., 2017).

<table>
<thead>
<tr>
<th></th>
<th>Size Matched Contests</th>
<th>Size Different Contests (Female Larger)</th>
<th>Size Different Contests (Male Larger)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Lesioned</td>
<td>N = 17</td>
<td>N = 12</td>
<td>N = 12</td>
</tr>
<tr>
<td>Male Lesioned</td>
<td>N = 15</td>
<td>N = 11</td>
<td>N = 10</td>
</tr>
<tr>
<td>No lesion</td>
<td>N = 15</td>
<td>N = 12</td>
<td>N = 10</td>
</tr>
</tbody>
</table>
**Table 5: Expected assessment regressions.** Expected regression relationships for assessment strategies. Table indicates the slope direction and strength of relationship indicative of self-, cumulative, or mutual assessment strategies. Adapted from Arnott and Elwood (2009) and Taylor and Elwood (2003).

<table>
<thead>
<tr>
<th></th>
<th>Pure self-assessment</th>
<th>Cumulative assessment</th>
<th>Mutual assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Smaller or Loser RHP vs. Duration</strong></td>
<td>Strong, positive</td>
<td>Strong, positive</td>
<td>Strong, positive</td>
</tr>
<tr>
<td><strong>Larger or Winner RHP vs. Duration</strong></td>
<td>Weak, positive (if present)</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>RHP Asymmetry vs. Duration</strong></td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Table 6: Regression analyses for Chapter III. Reports for regression analyses run to determine the assessment strategy in place across lesion treatments (i.e. male and female-lesioned contests). Table includes slope, adjusted $R^2$ values, and p values obtained from linear regression in R statistical software (R Development Core Team, 2016).Italicized values denote a $p \leq 0.1$. Bold values denote a $p \leq 0.05$. Within male-lesioned contests, non-escalated phase duration regressions are indicative of a self-assessment type strategy (Table 5). Total bout duration in male contests also demonstrates evidence in line with self-assessment; escalated phase duration regressions do not readily align with any assessment strategy outlined in Table 5. Bout and phase and duration within female-lesioned contests were also not indicative of any assessment strategy outlined in Table 5.

<table>
<thead>
<tr>
<th></th>
<th>Male lesioned contests</th>
<th>Female lesioned contests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$, slope</td>
<td>p</td>
</tr>
<tr>
<td><strong>Bout duration vs.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smaller RHP</td>
<td>0.02, 0.63</td>
<td>0.18</td>
</tr>
<tr>
<td>Larger RHP</td>
<td>-0.03, -0.05</td>
<td>0.91</td>
</tr>
<tr>
<td>% Size Asymmetry</td>
<td><strong>0.16, -0.05</strong></td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td><strong>Non-escalated phase duration vs.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smaller RHP</td>
<td><strong>0.08, 0.45</strong></td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>Larger RHP</td>
<td>-0.01, 0.20</td>
<td>0.42</td>
</tr>
<tr>
<td>% Size Asymmetry</td>
<td><strong>0.11, -0.02</strong></td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td><strong>Escalated phase duration vs.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smaller RHP</td>
<td>0.01, 2.22</td>
<td>0.25</td>
</tr>
<tr>
<td>Larger RHP</td>
<td>-0.03, 0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>% Size Asymmetry</td>
<td><strong>0.10, -0.16</strong></td>
<td><strong>0.03</strong></td>
</tr>
</tbody>
</table>
Table 7: **Trial numbers for Chapter IV.** Table conveys treatment sample sizes for the population assessment project. This study had a fully factorial 2 by 2 by 2 design with population sex, population strength ratio, and resource availability as our variables of interest. Strength ratio was titrated by altering the relative numbers of “strong” RHP individuals (larger carapace length) and “weak” RHP individuals (smaller carapace length) in the treatment population. Resource availability was altered via the number of resource units found in each treatment population. Low RV treatments had high resource availability (1:1 resource to animal ratio) while high RV treatments had low resource availability (1:5 resource to animal ratio).

<table>
<thead>
<tr>
<th></th>
<th>Female Population</th>
<th></th>
<th>Male Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low RV (5 Resource Units)</td>
<td>High RV (1 Resource Unit)</td>
<td>Low RV (5 Resource Units)</td>
</tr>
<tr>
<td><strong>Weak skewed</strong></td>
<td>Collected: (N = 5)</td>
<td>Analyzed: (N = 2)</td>
<td>Collected: (N = 4)</td>
</tr>
<tr>
<td>(4 weak : 1 strong)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Strong skewed</strong></td>
<td>Collected: (N = 5)</td>
<td>Analyzed: (N = 2)</td>
<td>Collected: (N = 5)</td>
</tr>
<tr>
<td>(4 strong : 1 weak)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 8: Outliers removed during analyses. The data conditioning phase for contest duration analyses revealed four outliers that were subsequently removed from the analyses. The following table denotes the treatment, trial number, interaction number, and duration of the data points removed.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trial Number</th>
<th>Interaction Number</th>
<th>Duration (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>17</td>
<td>28</td>
<td>1205</td>
</tr>
<tr>
<td>High RV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong Skewed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>4</td>
<td>618</td>
</tr>
<tr>
<td>Low RV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak Skewed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25</td>
<td>26</td>
<td>855</td>
</tr>
<tr>
<td>Low RV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong Skewed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25</td>
<td>30</td>
<td>492</td>
</tr>
<tr>
<td>Low RV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong Skewed</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 9: Regression analyses for single terms in Chapter IV. Reports for regression analyses run to determine the assessment strategy in place across treatment groups (i.e. sex treatment, strength ratio treatment, RV treatment). Table includes slope, adjusted R² values, and p values obtained from linear regression in R statistical software (R Development Core Team, 2016). Italicized values denote a p ≤ 0.1. Bold values denote a p ≤ 0.05.

<table>
<thead>
<tr>
<th>RV Treatment</th>
<th>R², Slope</th>
<th>p</th>
<th>R², Slope</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High RV</td>
<td></td>
<td>Low RV</td>
</tr>
<tr>
<td>Loser RHP</td>
<td>0.00, 0.07</td>
<td>0.21</td>
<td>0.10, 0.43</td>
<td>1.22 x 10⁻⁸</td>
</tr>
<tr>
<td>Winner RHP</td>
<td>0.01, 0.09</td>
<td>0.10</td>
<td>0.00, -0.03</td>
<td>0.60</td>
</tr>
<tr>
<td>% Asymmetry</td>
<td>0.00, 0.00</td>
<td>0.93</td>
<td>0.01, -3.00 x 10⁻³</td>
<td>0.03</td>
</tr>
<tr>
<td>Strength Ratio Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Strong Skewed</td>
<td></td>
<td>Weak Skewed</td>
</tr>
<tr>
<td>Loser RHP</td>
<td>0.03, 0.21</td>
<td>2.00 x 10⁻³</td>
<td>0.05, 0.36</td>
<td>3.30 x 10⁻⁵</td>
</tr>
<tr>
<td>Winner RHP</td>
<td>0.01, 0.16</td>
<td>0.06</td>
<td>5.58 x 10⁻⁵, -0.05</td>
<td>0.31</td>
</tr>
<tr>
<td>% Asymmetry</td>
<td>0.01, 0.00</td>
<td>0.06</td>
<td>0.00, -1.28 x 10⁻⁴</td>
<td>0.94</td>
</tr>
<tr>
<td>Sex Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td></td>
<td>Female</td>
</tr>
<tr>
<td>Loser RHP</td>
<td>0.00, 0.08</td>
<td>0.36</td>
<td>0.10, 0.34</td>
<td>8.71 x 10⁻¹⁰</td>
</tr>
<tr>
<td>Winner RHP</td>
<td>0.00, 0.02</td>
<td>0.79</td>
<td>0.00, 0.09</td>
<td>0.10</td>
</tr>
<tr>
<td>% Asymmetry</td>
<td>0.00, 0.00</td>
<td>0.93</td>
<td>0.01, -2.99 x 10⁻³</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Table 10: Regression analyses for interaction terms in Chapter IV. Reports for regression analyses run to determine the assessment strategy in place across treatment group interactions. Table includes slope, adjusted $R^2$ values, and $p$ values obtained from linear regression in R statistical software (R Development Core Team, 2016). Italicized values denote a $p \leq 0.1$. Bold values denote a $p \leq 0.05$.

<table>
<thead>
<tr>
<th>RV Treatment * Sex Treatment</th>
<th>Strength Ratio Treatment * Sex Treatment</th>
<th>RV Treatment* Strength Ratio Treatment (Males Only)</th>
<th>Sex Treatment * RV Treatment* Strength Ratio Treatment (Males Only)</th>
<th>Sex Treatment * RV Treatment* Strength Ratio Treatment (Females Only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loser RHP</td>
<td>0.04, 0.20</td>
<td>0.00, 0.05</td>
<td>0.00, 0.17</td>
<td>0.03, -0.01</td>
</tr>
<tr>
<td>Winner RHP</td>
<td>0.00, 0.08</td>
<td>0.00, 0.09</td>
<td>0.00, 0.01</td>
<td>0.00, 0.00</td>
</tr>
<tr>
<td>% Asymmetry</td>
<td>0.03, -4.79x10^-3</td>
<td>0.03</td>
<td>0.02, 0.01</td>
<td>0.02, -0.01</td>
</tr>
<tr>
<td>High RV * Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low RV * Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strength Ratio</td>
<td>0.061, 0.31</td>
<td>0.02, 0.25</td>
<td>0.07, 0.36</td>
<td>0.02, -0.01</td>
</tr>
<tr>
<td>Treatment * Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winner RHP</td>
<td>0.00, 0.05</td>
<td>0.00, 0.00</td>
<td>0.00, 0.00</td>
<td>0.00, 0.00</td>
</tr>
<tr>
<td>% Asymmetry</td>
<td>0.03, -0.01</td>
<td>0.03</td>
<td>0.02, 0.01</td>
<td>0.02, -0.01</td>
</tr>
<tr>
<td>Strong Skewed * Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak Skewed * Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High RV * Strong Skewed</td>
<td>0.03, 0.43</td>
<td>0.03</td>
<td>0.07, 0.36</td>
<td>0.02, -0.01</td>
</tr>
<tr>
<td>High RV * Weak Skewed</td>
<td>0.00, 0.05</td>
<td>0.00, 0.00</td>
<td>0.00, 0.00</td>
<td>0.00, 0.00</td>
</tr>
<tr>
<td>Male * High RV* Strong Skewed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male * Low RV* Strong Skewed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loser RHP</td>
<td>0.00, -0.17</td>
<td>0.05, 0.57</td>
<td>0.15, 0.85</td>
<td>0.01, 0.50</td>
</tr>
<tr>
<td>Winner RHP</td>
<td>0.06, 0.55</td>
<td>0.01, 0.20</td>
<td>0.03, 0.62</td>
<td>0.03, -0.19</td>
</tr>
<tr>
<td>% Asymmetry</td>
<td>0.02, 0.01</td>
<td>0.01, 0.01</td>
<td>0.03, 0.62</td>
<td>-0.01, 0.00</td>
</tr>
<tr>
<td>Male * High RV* Weak Skewed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female * High RV* Strong Skewed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female * Low RV* Strong Skewed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loser RHP</td>
<td>0.03, 0.22</td>
<td>0.03, 0.39</td>
<td>0.05, 0.35</td>
<td>0.21, 0.68</td>
</tr>
<tr>
<td>Winner RHP</td>
<td>0.00, 0.16</td>
<td>-0.01, 0.00</td>
<td>-0.01, 0.02</td>
<td>-0.01, -0.06</td>
</tr>
<tr>
<td>% Asymmetry</td>
<td>0.06, -0.01</td>
<td>0.00, 0.00</td>
<td>0.00, 0.00</td>
<td>0.00, 0.00</td>
</tr>
</tbody>
</table>