Male Dominance and Sexual Selection in the Crayfish
Orconectes quinebaugensis

By Amy Warren

A Thesis

Submitted to the Faculty

of the

WORCESTER POLYTECHNIC INSTITUTE

in partial fulfillment of the requirements for the

Degree of Master of Science

In

Biology & Biotechnology

May 2009

ABSTRACT

In many taxa, social structures are mediated by agonistic interactions and the formation of dominance hierarchies. In crayfish, dominance hierarchies may have evolved as a result sexual selection, allowing dominant males greater access to females, thereby increasing their reproductive success. This work tests the hypothesis that high male investment in dominance interactions may have evolved as a result of intra- and/or inter-sexual selection pressures by testing specific predictions in two parts: first, that reproductive males would invest more in agonistic interactions than reproductive females or non-reproductive members of both sexes; and second, that females would prefer odors of dominant males over subordinates, and that dominant males would be either more efficient at mating or be able to mate longer than subordinates. Investment in agonistic interactions was examined in intrasexual pairs of male and female crayfish in both the reproductive and non-reproductive season. As predicted, reproductive males invested more in agonistic interactions overall than reproductive females, while there was no significant difference in investment by non-reproductive males or females. However, no significant difference was found in agonistic investment between reproductive males and non-reproductive males. These data indicate that investment in agonism differs by sex and by reproductive status, and may indicate that dominance interactions are under sexual selection in males. Alternatively, this differential investment may be explained by seasonal changes in the individual costs and benefits of agonism, or by depressed investment by reproductive females. Female odor preference was tested using a y-maze containing control and male treated water. For tests of male mating, time spent in each of three stages of mating was recorded for male-female pairs. Of these tests, the only significant trend produced was that dominant males spent more time associated with the female during and after copulation than subordinates. This may indicate an advantage in fertilization success for males through decreased sperm competition. A pilot study was also conducted testing the predictions that females mated to dominant males invest more in offspring than those mated to subordinates and that such offspring have greater survivability, but no significant conclusions could be drawn from these data.

ACKNOWLEDGEMENTS

I would firstly like to thank my advisor, Dr. Lauren Mathews, for all of her support and guidance throughout the research process that culminated in this thesis. She has gone above and beyond in helping me to complete and learn from this work, both as an advisor and as a sounding board for ideas during my struggles, and I greatly appreciate the time and effort she has put in to make this experience possible for me. This experience has proved invaluable to me, and I could not have done it without her. I thank Dr. Jelle Atema for continually supporting and challenging me in my research, and for making repeated treks out to my committee meetings to provide unique insight into the development of my projects. I thank Dr. Elizabeth Ryder for her consistent and invaluable assistance with data analysis and statistical quandaries, and for her general good humor concerning the multiple crises I have presented her with. I have learned more about statistics in my work on this project than I learned in the whole of my undergraduate career. I thank Dr. Michael Buckholt for his continuing effort and interest in my project, for his generous donations of time and materials, and particularly for hours spent assisting in collections when we absolutely needed "just 10 more". Each of these people comprise my graduate advisory committee, and I would like to thank them all more than I have been able to express here for their constant support and insight, and for making this experience both enjoyable and educational for me. I will carry what I have learned from them throughout my continuing career.

I also extend my gratitude to Carol Butler and Eileen Dagostino, administrative assistants to the Biology and Biotechnology department, for all of their help in making the logistics of my research run smoothly. I would also like to thank the numerous undergraduates who have assisted me at various points throughout my research; Laura Saltzman, Larissa Amendola, Eric Greenway, Kari Ricker, Robert Stanford, Whitney Thurrott, Timothy Mulhern, and Maggie Becker. Research can be a daunting task to undertake with only one pair of hands, and both their assistance and company was greatly appreciated throughout the duration of my thesis work. Lastly, I would like to thank my friends and family for all of their support, for putting up my nervous breakdowns when it seems nothing was working right, and for always believing in me.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

OVERVIEW

In many taxa, dominance hierarchies are established through agonistic interactions. In many cases, success in such interactions leads to increased access to resources such as food, shelter, and mates (Moore, 2007). It has been suggested that males compete primarily for mates, with all other competitions necessary in so far as they contribute to this ultimate goal (Trivers, 1972). Therefore, sexual selection may influence the evolution of male dominance if this trait is at least partially heritable and confers reproductive advantages to one or both sexes. The work presented here examines the possible role of sexual selection in dominance interactions of the crayfish *O. quinebaugensis* and seeks to elucidate selection pressures that may shape the dominance trait from one generation to the next.

Darwin defined sexual selection as a combination of two components: intrasexual selection, in which members of one sex compete among themselves for access to members of the opposite sex, and intersexual selection, in which members of one sex select between members of the opposite sex based on some set of characteristics (Darwin, 1871; Trivers, 1972). Due to differential initial investment in gamete production, it is typically males that compete for access to females and females that select for male traits (Trivers, 1972). As a result, traits conferring advantages to males in competition, such as large antlers in caribou (Barrette & Vandal, 1985), are subject to intrasexual selection pressures while traits that augment male attractiveness, such as the elaborate coloration seen in peacock tails (Petrie & Williams, 1993), are subject to intersexual selection. Some traits confer advantages in both of these aspects, and have likely evolved under both types of selection (Berglund et al., 1996). Dominance may confer advantages in competition among males (Wong & Candolin, 2005; Trivers, 1972), and females of many taxa have been shown to prefer males that are successful in agonistic contests (reviewed in Berglund et al., 1996). Given this, intra- and/or inter-sexual selection are likely to act on dominance if dominance a) is at least partially heritable, and b) confers advantages for reproductive success to one or both sexes.

Agonistic interactions involved in the establishment of dominance hierarchies are often intense and pose a considerable risk of potential injury to the individuals participating. Optimality theory states that individuals should maximize their own ratio of benefits to costs for a given behavior (Maynard Smith, 1978), and so it stands to reason that possessing high dominance status confers some advantage on those willing to take the risk to achieve it. Due to the disparity in investment in initial gamete production, male reproductive success typically depends on the number of mates successfully fertilized, with relative success increasing with each successive

mate (Trivers, 1972). Investing heavily in dominance interactions may therefore be an adaptive advantage to males, if by becoming more dominant an individual increases his likelihood of obtaining mates, whether through increasing his monopolization of mates or through becoming more likely to be selected as a mate by receptive females. Dominant males have been shown to have a mating advantage over subordinates in other taxa (cockroaches [*Nauphoeta cinerea*; Breed et al., 1980], elephant seals [*Mirounga angustirostris*, Cox & LeBoeuf, 1977], vervet monkeys [*Cercopithecus aethiops sabaeus*; Raleigh & McGuire, 1989]). In crayfish, it has been shown that duration and intensity of agonistic interactions increase relative to the perceived value and availability of a resource (Bergman & Moore, 2003; Stocker & Huber, 2001). Males may therefore be likely to risk involvement in potentially costly dominance interactions, particularly in the mating season when these benefits are most likely to occur.

Unlike males, female reproductive success typically depends not on mate number, but on the quality of mates obtained and the subsequent quality and number of offspring produced (Trivers, 1972). Females may therefore be less likely to invest as heavily in dominance interactions than males during the breeding season, and may instead devote energy to aspects of reproduction such as egg production or mate searching, which may ultimately serve to increase their own reproductive success (Bernardo, 1996). However, females may still achieve fitness benefits by selecting mates of high dominance if mating with a dominant male increases her own access to resources (such as food, shelter, or preferred breeding territory) or indirect benefits she receives (good genes, sexy sons, higher offspring survivorship) (Kokko et al., 2003; Jennions & Petrie, 2000; Wong & Candolin, 2005). Male crayfish are known to spend considerable time in agonistic interactions and the establishment of dominance hierarchies. These interactions tend to be highly stereotyped in progression and employ both visual displays (Bruski & Dunham 1987) and the exchange of chemosensory information (Bergman et al., 2005; Zulandt Schneider et al., 2001; Breithaupt & Eger, 2002), . This makes them an ideal system to study dominance interactions. Crayfish of *O. quinebaugensis* also provide a good system in which to study sexual selection, as the disparity between male and female parental investment is great in this species (males provide no parental care beyond initial gamete investment), which may lead to selection for choosy females and, in turn, for strong male competition (Trivers, 1972).

Differential investment in dominance interactions by sex and reproductive status may indicate that these interactions are under sexual selection pressures. Similarly, female preferences for dominant males would indicate that dominance may be under intersexual selection. Many studies to date have focused on social influences on dominance interactions, and have considered both males and females to compete for similar access to resources. In contrast, few have

examined the possible influence sexual selection may have on such behavior. If male dominance is under sexual selection in this species, there must be some reproductive benefit present to balance the potentially risky investment required to achieve and maintain such status.   I hypothesize that male dominance is positively correlated with reproductive success through either or both intra- and inter-sexual selection in the crayfish *O. quinebaugensis*, under the assumption that dominance is at least partially heritable in this species. Chapter 1 of this work examines whether or not investment in agonistic interactions differs between sex and season. I specifically tested the prediction that reproductively active males invest more in agonistic interactions than both reproductively active females and non-reproductive forms of both sexes.   This chapter is currently in press for publication in the Journal of Crustacean Biology. Chapter 2 of this work then examines potential benefits conferred through intrasexual and intersexual selection throughout the reproductive process. Specifically, I tested the predictions that females prefer the odors of dominant males over subordinates and that dominant males are either more efficient at mating or are able to mate longer than subordinates. This chapter also describes a pilot study examining two post-copulatory effects of dominance: (1) do females invest more in clutches sired by more dominant males, and (2) do offspring sired by dominant males survive better to independence than offspring sired by subordinates.

REFERENCES

Barrette, C. & Vandal, D. 1985. Social rank, dominance, antler size, and access to food in snow-bound wild woodland caribou. Behaviour. 97: 118-146

Berglund, A., A. Bisazza, and A. Pilastro. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. Biological Journal of the Linnean Society 58: 385–399.

Bergman, D. A., and P. A. Moore. 2003. Field observations of intraspecific agonistic behavior of two crayfish species, *Orconectes rusticus* and *Orconectes virilis*, in different habitats. Biological Bulletin 205: 26–35.

Bergman, D., Martin, A., Moore, P. 2005. Control of information flow through the influence of mechanical and chemical signals during agonistic encounters by the crayfish, Orconectes rusticus. Animal Behaviour. 70: 485-496.

Bernardo, J. 1996. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence, and interpretations. American Zoologist. 36: 216-236.

Breed, M. D., S. K. Smith, and B. G. Gall. 1980. Behavioral strategies during inter-male agonistic interactions in a cockroach. Animal Behaviour 28: 1063-1069.

Breithaupt, T. & Eger, P. 2002. Urine makes the difference: chemical communication in fighting crayfish made visible. Journal of Experimental Biology. 205: 1221-1231.

Bruski, C. & Dunham, D. 1987. The importance of vision in agonistic communication of the crayfish *Orconectes rusticus*, I: an analysis of bout dynamics. Behaviour. 63: 83-107.

Cox, C. R., and B. J. LeBoeuf. 1977. Female incitation of male competition: a mechanism in sexual selection. American Naturalist 111: 317-335.

Darwin, C. 1871. The descent of man, and selection in relation to sex. London: John Murray.

Jennions, M. and M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. Biological Reviews. 75: 21-64.

Kokko, H., R. Brooks, M. Jennions, J. Morley. 2003. The evolution of mate choice and mating biases. Proceedings of the Royal Society London B. 270: 653-664.

Moore, P. A. 2007. Agonistic behavior in freshwater crayfish – the influence of intrinsic and extrinsic factors on aggressive encounters and dominance, pp. 90-114. In E. J. Duffy and M. Thiel (eds.), Evolutionary ecology of social and sexual systems – crustaceans as model organisms. Harvard University Press, New York.

Petrie, M. & Williams, A. 1993. Peahens lay more eggs for peacocks with larger trains. Proceedings of the Royal Society London B. 251: 127-131.

Raleigh, M. J. and M. T. McGuire. 1989. Female influences on male dominance acquisition in captive vervet monkeys, *Cercopithecus aethiops sabaeus*. Animal Behaviour 38: 59-67.

Stocker, A. M. and R. Huber. 2001. Fighting strategies in crayfish *Orconectes rusticus* (Decapoda, Cambaridae) differ with hunger state and the presence of food cues. Ethology 107: 727-736.

Trivers, R. L. 1972. Parental investment and sexual selection, pp. 139-179. In B. Campbell (ed.), Sexual selection and the descent of man. Aldine, Chicago.

Wong, B. & Candolin, U. 2005. How is female mate choice affected by male competition? Biological Reviews. 80: 559-571.

Zulandt Schneider, R. A., R. Huber, and P. A. Moore. 2001. Individual and status recognition in the crayfish, *Orconectes rusticus*: the effects of urine release on fight dynamics. Behaviour 138: 137–153.

CHAPTER 1:

AGONISTIC INTERACTIONS DIFFER BY SEX AND SEASON IN THE CRAYFISH

ORCONECTES QUINEBAUGENSIS

Amy H. Warren, Laura Saltzman, Michael A. Buckholt, and Lauren M. Mathews

Department of Biology, Worcester Polytechnic Institute, Worcester, MA, 01609, USA

ABSTRACT

In many taxa, social structures are mediated by agonistic interactions and the formation of dominance hierarchies. In crayfish, dominance hierarchies may have evolved as a result of sexual selection, allowing dominant males greater access to females. We examine strategies of investment in agonistic behaviors for males and females of the crayfish *Orconectes quinebaugensis* in both the reproductive and non-reproductive seasons. We hypothesized that reproductive males would invest more in agonistic behaviors than reproductive females and non-reproductive crayfish. We tested this hypothesis in the laboratory with 4 treatment groups: males and females in the autumn reproductive season and males and females in the summer non-reproductive season, with each group subdivided by size to control for size effects. As predicted, reproductive males spent significantly more time in agonistic behaviors and had significantly more fights reaching maximum intensity than reproductive females, while there was no significant difference in the time spent in agonistic interactions by non-reproductive males or females. We did find that small females in the summer had significantly fewer fights reaching maximum intensity than either males or large females in the summer. However, there was no significant difference in time spent in agonistic interactions or proportion of fights reaching maximum intensity between reproductive males and non-reproductive males, as was predicted by our hypothesis. We did observe a significant effect of size for both males and females in the non-reproductive season, with larger animals spending more time in agonistic behaviors, and with large females having a more fights at maximum intensity than small females; this difference was not recapitulated in the reproductive season. These data indicate that investment in agonism differs by sex and by reproductive status, and the differential investment by sex in reproductive animals may indicate that dominance interactions are under sexual selection in males. However, high investment in agonism by both males and females in the non-reproductive season is not consistent with our hypothesis. Alternatively, the differential investment in agonism by reproductive males and females could be explained by seasonal changes in the individual costs and benefits of agonism, or by depressed investment by reproductive females.

Key words: agonism, dominance, sexual selection, crayfish

INTRODUCTION

Agonistic interactions have been a subject of interest in many taxa, and dominance hierarchy formation is a particularly intriguing result of these interactions in many of these cases. Animals that participate in repeated agonistic interactions in a specific area form dominance hierarchies that allow for the control of resources within the population. Individuals that obtain high social status within a dominance hierarchy may receive increased access to resources. For example, success in agonistic interactions has been shown to lead to increased reproductive success in vervet monkeys (*Cercopithecus aethiops sabaeus*; Raleigh & McGuire, 1989) and cockroaches (*Nauphoeta cinerea;* Breed et al., 1980), and preferential access to food resources in rainbow trout (*Oncorhynchus mykiss*; Johnsson, 1997), and spotted hyenas (*Crocuta crocuta*; Tilson & Hamilton, 1984; reviewed in Zulandt Schneider et al., 2001). When high social status leads to increased access to resources, this may result in fitness benefits for individuals that are socially dominant (Moore, 2007). Conversely, defense of a dominance status in a population can have high costs to an individual's fitness, largely through injuries and/or energy expended during agonistic interactions, and maintenance of a high dominance status requires good physical condition to participate in repeated agonistic interactions. When the fitness costs and benefits of engaging in dominance interactions with conspecifics are potentially large, strategies for engaging in these interactions should be under strong selection, such that they maximize benefits while reducing costs (Maynard Smith, 1978). In addition, the relative costs and benefits of investing in agonistic interactions are likely to change throughout an individual's lifetime, both with changes in individual condition and with ecological and life history changes. For example, the potential rewards of high dominance status may vary depending on the reproductive status of individuals in a population or with the availability of food or shelter.

When dominance status has effects on an individual's access to mates, investments in agonistic interactions are likely to be under inter- and/or intra-sexual selection. Female selectivity in mating with dominant males has been shown in the three-spined stickleback (*Gasterosteus aculeatus*), where females selectively mate with males whose vibrant coloring reflects success in agonistic encounters (Candolin, 1999), and in guppies (*Poecilia reticulate*), where success in agonistic encounters enhances the reproductive success, through female choice, of previously unattractive males (Kodric-Brown, 1992). Dominant males have been shown to monopolize available females in the European bitterling (*Rhodeus sericeus*) regardless of female preference, who subsequently have limited control over the paternity of their offspring (Reichard et al., 2005). Particularly when there is a strong differential investment in offspring between

males and females, the lower-investing sex (usually males; Trivers, 1972) may receive relatively greater benefits through high social status.

Females may also have reasons for investing in dominance interactions that, like males, are tied to the reproductive advantages they may receive. However, while parental investment theory predicts that males may experience large fitness benefits through multiple matings, females are predicted to benefit little through polyandry (Trivers, 1972), and thus the fitness benefits of investments in agonism may differ by sex. Female investments in agonism may instead be related to defense of offspring or of resources required by offspring. It has been shown that the presence of eggs or juveniles accompanies increased aggression in female crayfish (Figler et al., 1995b), and that this tendency towards aggressive behavior is increased above that of both non-maternal females and reproductively mature males (Moore, 2007). Consistent with the offspring defense hypothesis (Figler et al., 2001), these behaviors may lead to increased protection, and thus survival, for offspring during the maternal season.

Under these conditions, males and females are likely to evolve quite different strategies for investing in agonistic interactions with conspecifics, and when the chance for matings is constrained in time, this differential investment should be similarly limited in time. For example, males may be willing to invest more in agonism than females, particularly when the chance for mating benefits is high, such as during a mating season, but when the chance for matings decreases during a non-mating season, males may decrease their investment in agonism. Furthermore, in some taxa, the potential benefits of high social status may encompass multiple resources, such as increased access to both food and mates, resulting in a dynamic system in which the benefits of agonism change predictably (e.g., seasonally) or unpredictably (e.g., with changing ecological conditions).

We use freshwater crayfish (*Orconectes quinebaugensis*) to test hypotheses about individual investment in agonistic interactions. Crayfish are good models for such an investigation, because, like some other crustaceans, they are structured by dominance hierarchies maintained by visual displays and fighting involving the large and potentially dangerous chelae, and through chemosensory communication (Moore and Bergman, 2005). In addition, temperate crayfish populations in North America are structured by strong seasonality in life history changes (Hamr, 2002). Crayfish growth and molting occurs largely during the summer, after which males undergo a molt into the Form I breeding morphology. Mating occurs in the fall, and polygamy in one or both sexes has been reported in a number of species (reviewed in Galeotti et al., 2007) and, in *Orconectes placidus*, multiple paternity of single broods is apparently common (Walker et al., 2002). Females store sperm throughout the winter, which they use to fertilize eggs in the spring.

Females provide all of the parental care of the young, including brooding developing embryos and caring for newly hatched juveniles for weeks. Such sharply differential parental investment would lead to strong selection on males for mate competition through dominance hierarchies, and possibly on females for mate selectivity.

Many factors have been shown to affect aggression in crayfish. The relative importance of certain resources, such as food or mating opportunities, can vary with a number of intrinsic and extrinsic factors, such as sex (Figler et al., 2001), age (Schroeder and Huber, 2001), reproductive season (Figler et al., 2005), resource availability (Stocker and Huber, 2001) and shelter presence (Bergman and Moore, 2003), such that competition for limited resources may vary in time, and aggression levels may change accordingly. For example, a dominant individual may be able to monopolize food resources during the summer, when growth is critical for both sexes in preparation for the fall breeding season. Monopolizing available food can lead to increased growth of the individual and improved fitness (Fero et al., 2007). Previous studies of crayfish aggression have largely focused on the mechanisms by which dominance is established, such as the role of chemical communication via urine (reviewed in Moore and Bergman, 2005), effect of previous experiences on subsequent behavior of individuals (Daws et al., 2002; Bergman et al., 2003), effects of hunger state (Stocker and Huber, 2001), and the trade-off between predation risk and mating opportunities (Pecor, 2006). Less focus has been placed on the function of dominance, such as the role of sexual selection on agonistic behaviors relating to dominance (but see Aquiloni et al., 2008; Fero et al., 2007). Here, we report the results of our experiments to gain insight into how individuals of *O. quinebaugensis* invest in agonistic interactions as an indirect measurement of investment in dominance. Specifically, we predicted that, during the mating season, males (in the Form I breeding morphology) would invest more in intrasexual conflicts than would sexually mature females. Furthermore, we compared mating season interactions with interactions during the non-mating season, when both males and females were apparently non-reproductive. We predicted that non-reproductive males and females would show reduced aggression relative to Form I males, and that non-reproductive males and females would show similar levels of aggression.

MATERIALS AND METHODS

Collection and maintenance of animals

Crayfish were collected from the Quinebaug River in Sturbridge, MA in Aug - Oct 2007 for the reproductive group (80 total), and from the Quinebaug and Mill Rivers (in Blackstone,

MA) in April – June 2008 for the non-reproductive season (58 and 28 total, respectively). In the latter case, because crayfish molt in the summer, we were unable to obtain enough inter-molt individuals from the Quinebaug River site for adequate sample size. Members of this species have a mating season from approx. Oct. – Jan (personal observation). In both seasons, male reproductive status was assessed by the morphology of the gonopods; in the fall, all males were in Form I and in the summer, all males were in Form II. Female reproductive status was assessed by the development of glair glands; in the fall, all females had fully developed glair glands, indicating sexual maturity; in the summer, all females had no glair gland development and were not brooding embryos or juveniles. In addition, in the fall collections, all females were collected by early September and housed individually in the laboratory, and were likely to be unmated. Therefore, we had four treatments groups (Fig. 1).

Crayfish were housed at the laboratory at Worcester Polytechnic Institute (WPI) in closed, re-circulating freshwater systems with biological, mechanical and UV filtration. All crayfish were held for at least 2 weeks before use in a trial. Each crayfish was housed individually in a 4 L plastic tank with a clay pot for shelter, such that, during the holding period, individuals were in chemical contact with other crayfish but never in physical contact. Crayfish were fed 3 times per week on an alternating diet of commercial shrimp pellets and frozen broccoli. Water temperature and light conditions mimicked those occurring naturally. For both autumn and summer collections, crayfish were isolated both physically and chemically for one week prior to use in aggression trials, since in some species, chemical signaling has been shown to affect agonistic behaviors (Bergman & Moore, 2005). During this time, each animal received a 50% water change on each day that it received food. Any crayfish molting within 2 weeks before or after use in trials was excluded from the dataset, as were any trials in which one opponent died within 6 days after use. Only a single trial from the large reproductive male group was so excluded after the allotted time.

Experimental design

Carapace length and the length of the right chela were measured for all animals collected. All animals that were missing >1 walking leg, or had clearly asymmetrical chelae, were excluded. The experimental design was constrained by subsequent use of male crayfish in the Form I category in additional trials for another experiment (not reported herein), which required that they be randomly paired with males within a larger group comprising 36 crayfish. Therefore, for each of the 4 groups, crayfish were divided roughly into small and large size categories based on the approximate median carapace length, and, for each size category, we calculated the mean

carapace and right chela lengths. Any individuals not within +/- 10% of the mean carapace length and within +/- 15% of the mean right chela length for each group were excluded from the group. A random number generator (www.random.org) was used to generate a random sequence of unique numbers between 1 and the total number of crayfish in each trial group. Crayfish assigned the first two numbers were paired as opponents, followed by the next two, etc., until all were matched. Each crayfish was used only once in this experiment.

Data collection

Paired opponents were placed in 30.5-cm width x 40.6-cm length x 14.6-cm depth plastic tanks filled with fresh, filtered tap water; within the tanks, opponents were separated with transparent dividers and were allowed to acclimate for 2 min. Dividers were then lifted and agonistic interactions were video-recorded for 10 min. At the conclusion of each trial, both animals were returned to their holding tanks. All tanks were thoroughly rinsed and refilled with fresh, filtered water between each trial. Scoring of video footage was carried out by a blind reviewer. Videos were scored for time spent in physical agonistic interactions, denoted "grapple", and defined as boxing, striking, grasping, or pushing with the chelae by one or both crayfish; and for occurrence of behaviors indicating various intensity levels of aggressive interaction (Table 1). Total time spent in "grapple" behavior and the proportion of fights reaching maximum intensity were compared between treatments as measures of overall agonistic investment.

Data analysis

We used t-tests to compare the mean sizes between seasons separately for males and females; we compared both the overall mean and the mean for each of the two size groups, for a total of 6 comparisons, and evaluated them with a Bonferroni correction of $\alpha=0.0083$. The aggression data were examined for normality of residuals with the Shapiro-Wilks W test and for equal variance with Levene's Test of Equality of Error Variances before further analyses were run. In both cases, we considered each of the 8 size-by-treatment groups separately. Variances of error within all comparisons were found to be equally distributed. Assumptions of residual normality were violated in 3 of 8 size-by-treatment groups examined. However, ANOVA analysis has shown to be robust to moderate deviations from residual normality (Hays, 1994; Kirk, 1995; Winer et al., 1991), and was therefore considered appropriate for the analysis of the data. Four separate univariate analyses of covariance (ANCOVA) were conducted, each with 2 fixed factors (sex or season and size group), and with total time spent in agonistic behavior as the

dependent variable. We first included the difference in carapace length between crayfish in each trial pair as a covariate, but this covariate was removed from all analyses because it had no significant effect. Therefore, we ran analyses of variance (ANOVA) with the two factors (sex or season and size group) and an interaction term for the two factors; the interaction term was removed from the final model when P>0.05 for the interaction.   For all analyses of the agonistic interactions, statistical significance was assessed with a Bonferroni correction for multiple comparisons; each of the 4 treatment groups was used in two comparisons (one with the fixed effect of season, and one with the fixed effect of sex), so we set α=0.0125.

The conflict intensity data were analyzed using the Fisher's exact test.   Comparisons were first made between small and large groups within each treatment, and those comparisons producing non-significant differences were pooled into a single treatment group. Comparisons were then made across sex and season with resultant groups to test biologically relevant relationships in accordance with our hypotheses. Bonferroni correction was used to adjust α based on the number of comparisons made with each data set. The maximum number of comparisons for any data set was used as an overall correction factor, resulting in α=0.0125. All analyses were run in SPSS v. 14.0.

RESULTS

Size groups within each sex were initially examined for differences in overall size between fall and summer collections. There was no significant difference in the overall size of females between the fall (n=34, x=35.203mm) and the summer (n=28, x=34.993mm, t=0.219, DF=60, p=0.827), or for females in either the small (fall: n=11, x=30.572mm; summer: n=11, x=31.046mm, t=-0.555, DF=20, p=0.585) or large (fall: n=23, x=37.417mm; summer: n=17, x=37.547mm, t=-0.214, DF=38, p=0.832) size classes between seasons. There was a significant difference in the overall size of males between the fall (n=34, x=35.247mm) and the summer (n=42, x=33.136mm, t=2.867, DF=74, p=0.005), but not for either the small (fall: n=14, x=32.000mm; summer: n=24, x=30.746mm, t=2.376, DF=36, p=0.023) or the large (fall: n=20, x=37.520mm; summer: n=18, x=36.322mm, t=2.275, DF=36, p=0.029) groups separately.

 In comparing time spent in total agonistic behavior, both treatment and size showed significant effects in different comparisons (Table 2, Fig. 1). Males (n=17) and females (n=22) in the reproductive season showed a significant effect of sex on total time spent in agonistic behaviors, with males spending more time in such behaviors than females, and no significant effect of size on total agonistic behavior in these two groups. There was no significant difference

in total time spent in agonistic behaviors between males (n=21) and females (n=19) in the non-reproductive season. However, both males and females for this comparison showed a significant effect of size, with larger animals spending significantly more time in agonistic behaviors than smaller animals. The comparisons between reproductive and non-reproductive individuals showed no significant differences in total time spent in agonistic behavior based on either season or size for either sex. However, for females, the interaction between reproductive state and size had a considerable effect; it was retained in the model even though it was not significant at the Bonferroni corrected $\alpha=0.0125$.

We found similar effects of size and treatment when we compared the proportion of fights that reached the maximum intensity level (Figure 2). In all but the one case (females in the summer, non-reproductive season, p=0.018), there was no significant difference in proportion of trials reaching maximum intensity between small and large size groups (reproductive males p=0.153, reproductive females p=0.364, non-reproductive males p=0.367). Consequently, large and small groups were pooled for the latter three treatments. As with duration of agonistic interactions, significantly more fights between reproductive males reached maximum intensity than fights between reproductive females (p=0.001). No significant difference was found in the proportion reaching maximum intensity between non-reproductive males and large non-reproductive females in the summer (p=0.721); however, significantly more fights between non-reproductive males reached maximum intensity than between small non-reproductive females (p=0.003). Similarly, significantly more trials between large non-reproductive females reached maximum intensity than trials between reproductive females (p=0.020), while there was no significant difference in intensity between small non-reproductive females and reproductive females (p=1.000). There was no significant difference in proportion of trials reaching maximum intensity between males in the reproductive and non-reproductive seasons (p=0.743).

DISCUSSION

There are many factors, both intrinsic (body and chelae size, sex, reproductive state, previous social experience, motivational state, neurochemistry) and extrinsic (environmental communication factors, perceived resource value) that contribute to the amount of time and risk individuals are willing to invest in agonistic interactions (Moore, 2007). Our data indicate that in the fall reproductive season, males of *O. quinebaugensis* spent significantly more time in agonistic behaviors, and participated in a higher proportion of fights at maximum intensity, than females as we predicted. This may indicate that males respond to sexual selection by investing

more energy in agonism, if it can be shown that dominant males gain some reproductive advantage over subordinates.

If dominance is indeed related to increased fitness for males, it would be expected that more time, and therefore greater risk, would be invested during the reproductive season when this resource is most valuable. It has been generally perceived that individuals should invest more in risky agonistic behavior when the value of a given resource is high (Enquist & Leimar, 1987). This has been shown specifically in Orconectid crayfish, where both the intensity and duration of agonistic encounters is correlated with the value of the resource under contention and its availability to those involved (Bergman & Moore, 2003; Stocker & Huber, 2001; reviewed in Moore, 2007). For example, maternal females (those bearing eggs or juveniles) may be much more aggressive than non-maternal females (Figler et al., 1995b) in the spring when juvenile quality and survival contributes directly to female fitness. In our experiment, however, there was no significant difference in time invested in agonism or proportion of fights reaching maximum intensity between reproductive and non-reproductive males. Particularly in the large size classes for the duration analysis, Form II males spent as much time in aggression as Form I males. Likewise, large non-reproductive females spent more time in aggression and had a higher proportion of fights at maximum intensity than did fall females. Though this difference was significant in relation to fight intensity, it was not significant for time spent in agonistic behavior, probably as a result of a strong size effect in the non-reproductive females. In sum, while the behavior of the reproductive males and females is consistent with our hypothesis that sexual selection drives investment in agonism, the data on non-reproductive crayfish do not. We propose two alternative explanations for this observation.

First, the summer season constitutes a major growth period for both juvenile and adult crayfish of *O. quinebaugensis*; such seasonal patterns of molting and growth are common in temperate crayfish (Reynolds, 2002). Under these conditions, resources such as food and shelter may increase in their value to both males and females. Monopolizing food resources can lead to increased growth and improved individual fitness (Fero et al., 2007), and size has often been shown to correlate positively with fight outcomes in crayfish (e.g. Pavey & Fielder, 1996; Daws et al., 2002; Schroeder & Huber, 2001; Figler et al., 1995a; Klocker & Strayer, 2004; reviewed in Moore, 2007) and with fecundity in females (Rubolini et al., 2006; Reynolds, 2002), potentially leading to reproductive advantages later in the life cycle for both sexes. Shelters may also be a resource of equal value that would warrant increased agonistic investment in both sexes, since frequent molting also increases the proportion of time a crayfish is more vulnerable to predation (Hamr, 2002). Therefore, in the summer, males and females may invest similarly in agonism

because the most valuable resources are equally important to both sexes. In the fall, the relative importance of resources may shift in response to slowing growth and cooling water temperatures, and investment in competition over mates rather than food or shelter is reflected in a sex-biased investment in agonism.

Alternatively, females during the reproductive season may derive greater benefit from abstaining from this increased agonistic investment in favor of other energetic needs. It has been suggested for males that investment in agonistic interactions must be in competition with investment in other components relating to fitness, such as mate attraction or parental care (Qvarnström, 1997; Griffith & Sheldon, 2001), and one would expect similar trade-offs in energy expenditure to apply to females (e.g. Wetzel, 2002; Bernardo, 1996). Female primary reproductive effort during this time consists of egg formation (Galeotti et al., 2006), which is likely energetically costly. Females may accrue greater fitness benefits by investing heavily in egg production over fighting for access to mates or food (since female reproductive success is limited by the number of eggs she produces and the quality and survivorship of her offspring (Trivers, 1972). Egg size has been suggested to be a critical determinant in offspring fitness (Bernardo, 1996), and investing energy in fights with conspecifics may limit a female's ability to produce large eggs or clutches.

Mate searching and discrimination may also carry energetic costs for females (Kokko et al., 2002). Investment in agonism in the reproductive season may also detract from time and energy females could spend in selection of, and mating with desirable, high quality males, which may ultimately affect the quality of their offspring (Kokko et al., 2002; Berglund et al., 1996). In a species such as *O. quinebaugensis*, where females store sperm and delay fertilization until months after mating has occurred (Hamr, 2002), females may also gain indirect benefits from allotting time and energy to acquiring multiple matings (Jennions & Petrie, 2000). Any injuries incurred from increased aggressive behavior during the reproductive season might also preclude females from being able to mate with a desired male, or to successfully mate at all.

In the spring, when a female's investment in offspring is in the form of embryos and partially independent juveniles, females may benefit through increased aggression if this allows them to maintain residence in a shelter, presumably as a contributing component of offspring defense (Figler et al., 2001). Moore (2007) found in his review of the relevant literature that maternal females were more aggressive than both non-maternal females and reproductive males, and Figler et al. (2001) suggested that this was due in part to internal state, as even maternal females with offspring removed showed increased shelter defense over non-maternal females in comparing previous studies. Together, this suggests that female investment in agonistic

interactions may be under some form of sexual selection, but in a different direction than would be expected for males.

We also report conflicting data on the effects of size on agonistic behavior. In the summer, there was a significant effect of size group for both males and females based on fight duration, with larger crayfish of both sexes spending more time in agonistic behaviors than smaller crayfish. In the summer, there was also an effect of size on intensity of aggression between females, with large females having a higher proportion of fights reaching maximum intensity than small females. It is generally accepted that size is a major factor that typically predicts outcome and aggressive state in crayfish interactions (e.g. Pavey & Fielder, 1996; Daws et al., 2002; Klocker & Strayer, 2004; Figler et al., 1995a; Schroeder & Huber, 2001; reviewed in Moore, 2007), and it has been suggested that small and large crayfish may adopt different fighting strategies based on the value of the resource and the risk to the individual (Schroeder & Huber, 2001). Larger, more dominant individuals potentially risk less in engaging in agonistic behavior, since it is more likely that they will be successful, particularly when paired with a smaller opponent. However, we observed no similar size effects in the mating season, when both large and small individuals of both sexes invested similarly in fighting.

While the explanation for this observation is unclear, we note that in the non-reproductive collection of animals, some (perhaps many) of the individuals of both sexes in the smaller size group may never have undergone a period of reproduction, while individuals in the larger size groups are more likely to have reproduced in the previous year. This may account for the significant difference seen in the proportion of fights reaching maximum intensity between reproductive females in the fall and large non-reproductive females in the summer, while the comparison between reproductive females in the fall and small non-reproductive females in the summer was non-significant. Maternal females of other crayfish species have been shown to have heightened aggression relative to both reproductive males and non-maternal females (Figler et al, 1995b), and this has been suggested to be partially due to a change in internal state (Figler et al., 2001). It is therefore possible that the large females in the summer may have had some residual internal effect related to a recently dispersed clutch of juveniles, whereas small females would be less likely to have had a recent clutch (though the recent maternal state of most females in the summer group was unknown).

Overall, our data show that individual investments in agonism by individuals of O. *quinebaugensis* vary by season, sex, and size. However, we specifically hypothesized that dominance interactions are a sexually selected behavior, and predicted that reproductive males would show elevated aggression relative to both reproductive females and non-reproductive

crayfish. Our data provide only partial support for this hypothesis: agonistic interactions in *O. quinebaugensis* may be regulated by a complex and dynamic set of individual costs and benefits. Additional experimentation is needed to elucidate the role of environmental, life history, and other factors on the agonistic behavior of this species.

REFERENCES

Berglund, A., A. Bisazza, and A. Pilastro. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. Biological Journal of the Linnean Society 58: 385–399.

Bergman, D. A., C. Kozlowski, J. C. McIntyre, R. Huber, A. G. Daws, and P. A. Moore. 2003. Temporal dynamics and communication of winner-effects in the crayfish, *Orconectes rusticus*. Behavior 140: 805-825.

Bergman, D., and P. Moore. 2005. Prolonged exposure to social odours alters subsequent social interactions in crayfish *Orconectes rusticus*. Animal Behaviour 70: 311-318.

Bergman, D. A., and P. A. Moore. 2003. Field observations of intraspecific agonistic behavior of two crayfish species, *Orconectes rusticus* and *Orconectes virilis*, in different habitats. Biological Bulletin 205: 26–35.

Bernardo, J. 1996. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. American Zoologist. 36: 216-236.

Breed, M. D., S. K. Smith, and B. G. Gall. 1980. Behavioral strategies during inter-male agonistic interactions in a cockroach. Animal Behaviour 28: 1063-1069.

Bruski, C. & Dunham, D. 1987. The importance of vision in agonistic communication of the crayfish Orconectes rusticus, I: an analysis of bout dynamics. Behaviour. 63: 83-107.

Candolin, U. 1999. Male-male competition facilitates female choice in sticklebacks. Proceedings of the Royal Society of London B. 266: 785-789.

Cox, C. R., and B. J. LeBoeuf. 1977. Female incitation of male competition: a mechanism in sexual selection. American Naturalist 111: 317-335.

Daws, A. G., J. Grills, K. Konzen, and P. A. Moore. 2002. Previous experiences alter the outcome of aggressive interactions between males in the crayfish, *Procambarus clarkii*. Marine and Freshwater Behaviour and Physiology 35: 139-148.

Enquist, M. & Leimar, O. 1987. Evolution of fighting behaviour: The effect of variation in resource value. Journal of Theoretical Biology. 127: 187-205.

Fero, K., J. S. Simon, V. Jourdie, and P. A. Moore. 2007. Consequences of social dominance on crayfish resource use. Behaviour 144: 61-82.

Figler, M. H., J. E. Finkelstein, M. Twum, and H. V. S. Peeke. 1995a. Intruding male red swamp crayfish, *Procambarus clarkii*, immediately dominate members of established communities of smaller, mixed-sex conspecifics. Aggressive Behavior 21: 225-236.

Figler, M. H., M. Twum, J. E. Finkelstein, and H. V. S. Peeke. 1995b. Maternal aggression in red swamp crayfish (*Procambarus clarkii*, Girard): the relation between reproductive status and outcome of aggressive encounters with male and female conspecifics. Behaviour 132: 107-125.

Figler, M. H., G. S. Blank, and H. V. S. Peeke. 2005. Shelter competition between resident male red swamp crayfish *Procambarus clarkii* Girard and conspecific intruders varying by sex and reproductive status. Marine and Freshwater Behaviour and Physiology 38: 237-248.

Figler, M. H., G. S. Blank, and H. V. S. Peeke. 2001. Maternal territoriality as an offspring defense strategy in red swamp crayfish *Procambarus clarkii*, Girard. Aggressive Behavior 27: 391-403.

Galeotti, P., D. Rubolini, G. Fea, D. Ghia, P. Nardi, F. Gherardi, M. Fasola. 2006. Female freshwater crayfish adjust egg and clutch size in relation to multiple male traits. Proceedings of the Royal Society London B. 273: 1105-1110.

Galeotti, P., F. Pupin, D. Rubolini, R. Sacchi, P. A. Nardi, and M. Fasola. 2007. Effects of female mating status on copulation behavior and sperm expenditure in the freshwater crayfish *Austropotamobius italicus*. Behavioral Ecology and Sociobiology 61:711–718.

Griffith, S. and B. Sheldon. 2001. Phenotypic plasticity in the expression of sexually selected traits: neglected components of variation. Animal Behaviour. 61: 987-993.

Guiasu, R. C. and D. W. Dunham. 1998. Inter-form agonistic contests in male crayfishes, *Cambarus robustus* (Decapoda, Cambaridae). Invertebrate Biology 117: 144-154.

Hamr, P. 2002. *Orconectes*, pp. 585-608. In D. M. Holdich, (ed.), Biology of freshwater crayfish. Blackwell Science, Oxford.

Hays, W. L. 1994. Statistics. Harcourt Brace, Fort Worth, TX.

Jennions, M. and M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. Biol. Rev. 75: 21-64.

Johnsson, J. I. 1997. Individual recognition affects aggression and dominance relations in rainbow trout, *Oncorhynchus mykiss*. Ethology 103: 267-282.

Kirk, R.E. 1995. Experimental Design: Procedures for the Behavioral Sciences. Brooks/Cole Publishing Company, Pacific Grove, CA.

Klocker, C. A., and D. L. Strayer. 2004. Interactions among invasive crayfish (*Orconectes rusticus*), a native crayfish (*Orconectes limosus*), and native bivalves (Sphaeriidae and Unionidae). Northeastern Naturalist 11: 167-178.

Kodric-Brown*, A.* 1992. Male dominance can enhance mating success in guppies. Animal Behaviour 44: 165-167.

Kokko, H., R. Brooks, M. Jennions, J. Morley. 2003. The evolution of mate choice and mating biases. Proceedings of the Royal Society London B. 270: 653-664.

Maynard Smith, J. 1978. Optimization theory in evolution. Annual Review of Ecology and Systematics 9: 31-56.

Moore, P. A. 2007. Agonistic behavior in freshwater crayfish – the influence of intrinsic and extrinsic factors on aggressive encounters and dominance, pp. 90-114. In E. J. Duffy and M. Thiel (eds.), Evolutionary ecology of social and sexual systems – crustaceans as model organisms. Harvard University Press, New York.

Moore, P. A., and D. A. Bergman. 2005. The smell of success and failure: The role of intrinsic and extrinsic chemical signals on the social behavior of crayfish. Integrative and Comparative Biology 45: 650-657.

Pavey, C. R. and D. R. Fielder. 1996. The influence of size differential on agonistic behavior in the freshwater crayfish, *Cherax cuspidatas* (Decapoda: Parastacidae). Journal of Zoology 238: 445-457.

Pecor, K. W. 2006. The mate pursuit-predation avoidance tradeoff in the virile crayfish, *Orconectes virilis.* Marine and Freshwater Behavior and Physiology 39: 229-233.

Qvarnström, A. 1997. Experimentally increased badge size increases male competition and reduces male parental care in the collared flycatcher. Proceedings of the Royal Society London B. 264: 1225-1231.

Qvarnström, A., and E. Forsgren. 1998. Should females prefer dominant males? Trends in Ecology and Evolution 13: 498-501.

Raleigh, M. J. and M. T. McGuire. 1989. Female influences on male dominance acquisition in captive vervet monkeys, *Cercopithecus aethiops sabaeus*. Animal Behaviour 38: 59-67.

Reichard, M., J. Bryja, M. Ondračková, M. Dávidová, P. Kaniewska, and C. Smith. 2005. Sexual selection for male dominance reduces opportunities for female mate choice in the European bitterling (*Rhodeus sericeus*). Molecular Ecology 14: 1533-1542.

Reynolds, J. D. 2002. Growth and reproduction, pp. 152-191. In, D. M. Holdich, (ed.), Biology of freshwater crayfish. Blackwell Science, Oxford.

 Rubolini, D., P. Galeotti, G. Ferrari, M. Spairani, F.Bernini, and M. Fasola. 2006. Sperm allocation in relation to male traits, female size, and copulation behavior in a freshwater crayfish species. Behavioral Ecology and Sociobiology 60: 212-219.

Schroeder, L., and R. Huber. 2001. Fight strategies differ with size and allometric growth of claws in crayfish, *Orconectes rusticus*. Behavior 138: 1437-1449.

Stocker, A. M. and R. Huber. 2001. Fighting strategies in crayfish *Orconectes rusticus* (Decapoda, Cambaridae) differ with hunger state and the presence of food cues. Ethology 107: 727-736.

Tilson, R. H. and W. J. Hamilton III. 1984. Social dominance and feeding patterns of spotted hyenas. Animal Behaviour 32: 715-724.

Trivers, R. L. 1972. Parental investment and sexual selection, pp. 139-179. In B. Campbell (ed.), Sexual selection and the descent of man. Aldine, Chicago.

Walker, D., B. A. Porter, and J. C. Avise. 2002. Genetic parentage assessment in the crayfish *Orconectes placidus*, a high-fecundity invertebrate with extended maternal brood care. Molecular Ecology 11: 2115-2122.

Wetzel, J. 2002. Form alteration of adult female crayfishes of the genus Orconectes (Decapoda: Cambaridae). American Midland Naturalist. 147: 326-337.

Winer, B. J., D. R. Brown, and K. M. Michels. 1991. Statistical principles in experimental design. McGraw-Hill, New York.

Zulandt-Schneider, R. A., R. Huber, and P. A. Moore. 2001. Individual and status recognition in the crayfish, *Orconectes rusticus*: the effects of urine release on fight dynamics. Behaviour 138: 137–153.

TABLES & FIGURES

Table 1. Ethogram of definitive behaviors used to approximate fight intensity levels. All behaviors were scored for both crayfish as a pair. Ethogram adapted from Bruski & Dunham (1987) for use in the current investigation.

Intensity Level	Description
0	No interaction
1	Approach - approach within one body length of opponent resulting in opponent's retreat in any direction
2	Lunge/Threat - rapid approach of opponent with chelae raised in threat display
3	Grapple - boxing, striking, grasping, or pushing with the chelae by one or both crayfish
4	Overturn - crayfish turned on side or back by opponent while being grasped with opponent's chelae

Table 2. Results of ANOVAs with fixed effects of season (e.g., reproductive males compared to non-reproductive males) or sex (e.g., reproductive males compared with reproductive females). In all comparisons, size was included as a factor; the interaction term was non-significant (p>0.05) in all cases except the comparison of reproductive females with non-reproductive females, and was removed from analyses in which it was non-significant.

Contrast	factor 1 (size)		factor 2 (season or sex)		factor 1*factor2	
	F	p	F	p	F	p
Between seasons:						
1. Reproductive males vs. non-reproductive males	0.468	0.498	1.174	0.286	—	—
2. Reproductive females vs. non-reproductive females	4.820	0.034	2.207	0.146	4.027	0.052
Between sexes:						
3. Reproductive males vs. reproductive females	0.065	0.801	9.071	0.005*	—	—
4. Non-reproductive males vs. non-reproductive females	7.381	0.010*	0.025	0.876	—	—

*indicates statistical significance at α=0.0125.

.
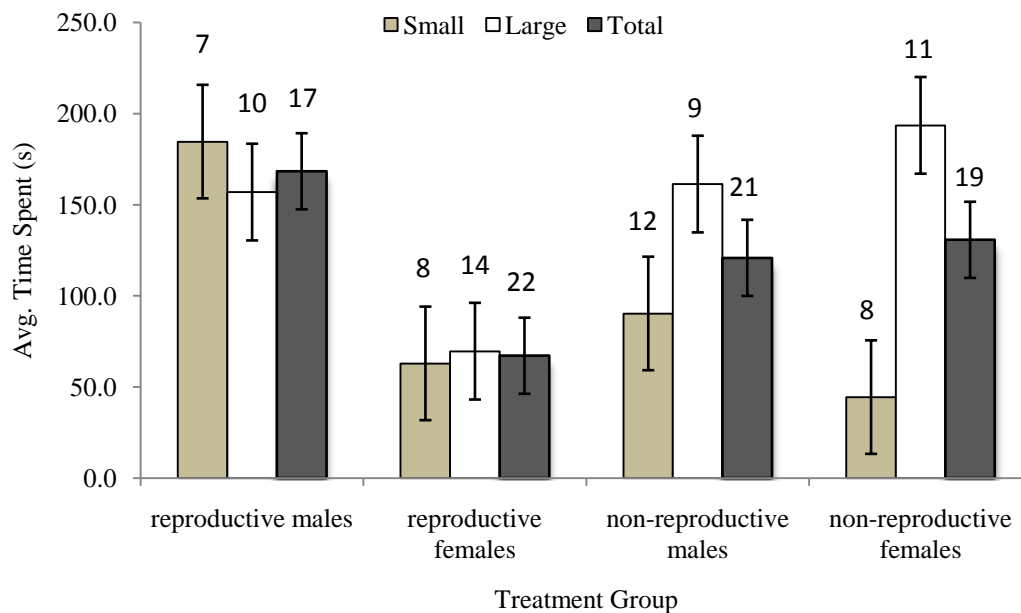
Figure 1. Mean time spent in physical agonistic behavior ("grapple") for each treatment group, shown for large and small groups separately and combined. Error bars represent standard error, and numbers above each bar indicate sample sizes (each replicate represents one pair of crayfish).
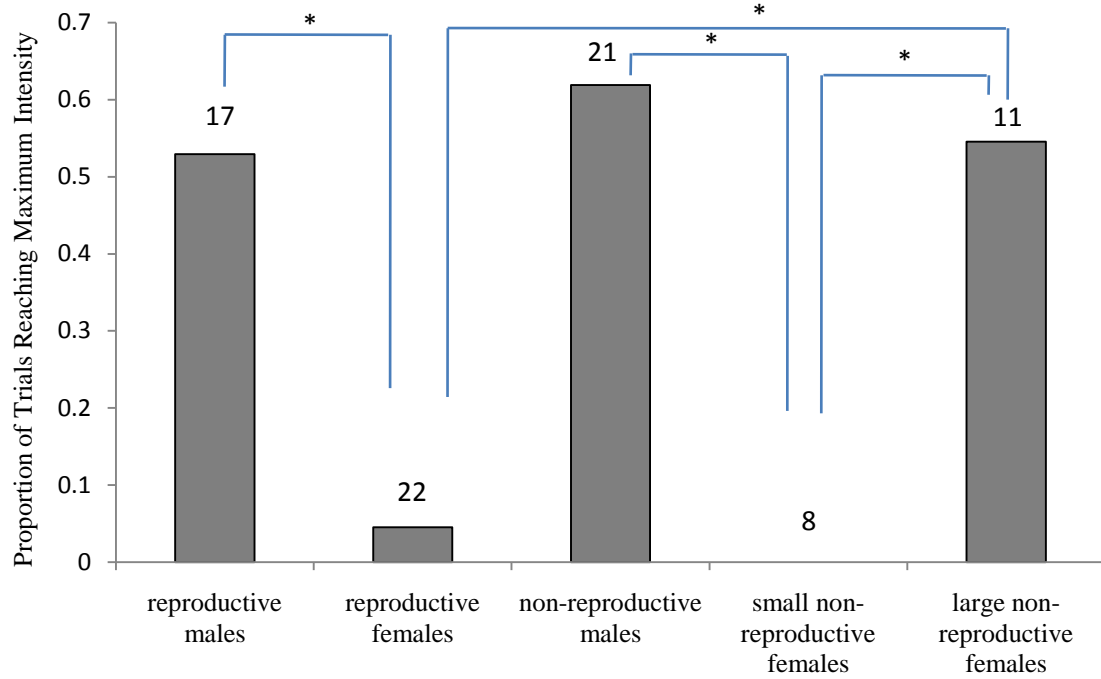
Figure 2. Proportion of trials reaching maximum intensity level for groups after pooling. Numbers above each bar indicate sample sizes (each replicate represents one pair of crayfish). Asterisks denote significant relationships at α=0.0125.

CHAPTER 2:

EXAMINATION OF THE ROLE OF DOMINANCE IN INTRA- AND INTER-SEXUAL SELECTION IN THE CRAYFISH *ORCONECTES QUINEBAUGENSIS*

Amy H. Warren

Department of Biology, Worcester Polytechnic Institute, Worcester, MA, 01609, USA

ABSTRACT

In many taxa, dominance hierarchies are established through agonistic interactions. In many cases, success in such interactions leads to increased access to resources such as food, shelter, and mates. It has been shown that dominant males gain a mating advantage over subordinates in a variety of species, and therefore, dominance may play a part in or be influenced by either or both intra- and inter-sexual selection if this trait is at least partially heritable and confers reproductive advantages to one or both sexes. In the previous chapter, I reported that dominance interactions differ by sex and season and therefore may be under sexual selection pressure. In this chapter, I examine the role of dominance in both intra- and inter-sexual selection to elucidate what factors, if any, may influence the development of this trait from one generation to the next. I conducted experiments to test two predictions: that receptive females prefer odors produced by dominant over subordinate males, and that dominant males gain an advantage in mating through either being more efficient at mating or being able to mate longer than subordinates. I also conducted a pilot study to test the prediction that females invest more in offspring sired by dominant males, and that these offspring show greater survivability than juveniles sired by subordinates. I found that, contrary to my hypothesis, females showed no preference for odors of either dominant or subordinate males. Similarly, I found no significant relationship between pre-copulatory mating behavior and male dominance score. However, once the correct copulatory position had been achieved, dominant males spent significantly more time associated with females than subordinates, resulting in longer matings. This may in turn lead to increased fertilization success for dominant males. Lastly, no significant correlation between male dominance score and any of several egg characteristics or juvenile survivability was found. Though I found little support for my predictions, sexual selection may still influence male dominance. The benefits examined here are a few of many possible reproductive benefits that may be accrued through dominance. Further experimentation is needed to gain a more complete picture of how sexual selection may influence behavior related to such traits in complex social systems such as this.

INTRODUCTION

The formation and inclusion of dominance in social interactions has been documented in many taxa, and in many cases plays a key role in social mating of many species. As a result, this trait may be subject to sexual selection in these taxa. While inter- and intrasexual selection may act independently on an assortment of traits, some male traits have been shown to confer both advantages in competition with other males and in mate selection by females (Berglund et al., 1996). This has been seen in coloration in birds, pheromone composition in cockroaches and mice, and the major chelae of male fiddler crabs (reviewed in Berglund et al., 1996). Dominance is known to confer advantages in competition in males (Wong & Candolin, 2005; Trivers, 1972), and females of many taxa have been shown to prefer males that are successful in agonistic contests (reviewed in Berglund et al., 1996; but see Qvarnström & Forsgren, 1998 for possible reasons why females should not prefer dominant males). Given this, intra- and/or inter-sexual selection are likely to act on dominance if dominance a) is at least partially heritable, and b) confers advantages for reproductive success to one or both sexes.

Male dominance has been shown to be heritable in cockroaches (*Nauphoeta cinerea*; Moore et al., 2002; Moore, 1990), pigs (*Sus scrofa*, Jonsson, 1985), mice (*Peromyscus maniculatus*, Dewsbury, 1990), and Japanese quail (*Coturnix japonica*, Nol et al., 1996), and may be heritable in other taxa. If the formation of dominance hierarchies through agonism involves considerable risk and/or effort on the part of those participating, it is reasonable to assume that possessing high dominance status confers some advantage on those willing to take the risk to achieve it. For example, success in agonistic interactions has been shown to increase reproductive success in males of several taxa, including cockroaches (*Nauphoeta cinerea*; Breed et al., 1980), elephant seals (*Mirounga angustirostris*, Cox & LeBoeuf, 1977), and vervet monkeys (*Cercopithecus aethiops sabaeus*; Raleigh & McGuire, 1989), and increased access to other resources such as food in rainbow trout (*Oncorhynchus mykiss*; Johnsson, 1997) and spotted hyenas (*Crocuta crocuta*; Tilson & Hamilton, 1984; reviewed in Zulandt Schneider et al., 2001), and preferred shelters in crayfish (Levenbach & Hazlett, 1996; Statzner et al., 2000). Male reproductive success is thought to be limited by the number of females a male is able to successfully fertilize, and thus may be increased either through enhanced attractiveness to mates, or through a greater ability to monopolize matings (Trivers, 1972). Given this, intra-sexual selection will likely act to promote the expression of those traits that are helpful in competition with other males, or traits correlated with such an advantage (i.e. size, aggressive state, etc.) (Trivers, 1972).

Unlike males, female reproductive success increases little with additional matings, but is instead limited by the number of offspring she is physiologically capable of producing, and the overall quality of those offspring (Trivers, 1972). Social structures that are mediated by dominance hierarchies may confer advantages to females if selective mating based on male dominance is beneficial; for example, mating with a dominant male may increase a female's access to resources (such as food, shelter, or preferred breeding territory), or may provide her with indirect benefits (good genes, sexy sons, higher offspring survivability) (Kokko et al., 2003; Jennions & Petrie, 2000; Wong & Candolin, 2005). Mating with dominant males has been shown to benefit females through access to preferred resources or increased contributions to offspring in taxa where direct benefits play a role in the mating process (reviewed in Trivers, 1972). Dominance is also commonly accepted as an honest indicator of high quality in males (Wong & Candolin, 2005; Kokko et al., 2003; Berglund et al., 1996) as it is likely difficult or costly to fake due to repeated testing in agonistic contests (Berglund et al., 1996; Wong & Candolin, 2005). The ability to produce and maintain features that contribute to high dominance status (i.e. large weaponry in crayfish) has also been suggested to indicate male quality, in that such features may be costly to produce, and males possessing such features may have qualities such as higher energy reserves or lower parasite loads in order to produce them (reviewed in Berglund et al., 1996; but see Qvarnström & Forsgren, 1998 for features of dominant males that may not be linked to intrinsic quality). If this quality is heritable, females may accrue indirect benefits through genes conferring higher survivability, fecundity, or attractiveness for her offspring (Kokko et al., 200), even when no direct benefits are provided by the male. Females should therefore selectively mate with males possessing traits correlated with such high quality as choosy females would receive these benefits over females who mate indiscriminately (Berglund et al., 1996).

It should be noted that traits such as dominance may also be heavily influenced by environmental effects. Factors such as size, motivational state, and resource availability have been shown to influence the outcomes of agonistic interactions in dominance establishment (Moore, 2007), and are likely influenced by environmental conditions surrounding an individual. For example, size may be affected by age and/or resource availability. It is therefore possible that dominance rank may be influenced by these non-genetic factors, and may not necessarily indicate high genetic quality. In such cases females may benefit little from such a preference, depending on the relative contributions of genetic and environmental effects. Some have also argued that such a preference should not exist, as mating with dominant males may incur additional risks for the female (Qvarnström & Forsgren, 1998; Wong & Candolin, 2005). For

example, if dominant males are more likely to obtain multiple mates, this may increase their exposure to sexually transmitted diseases which would then be transferred to a female choosing such a male for a mate. Multiple matings may also lead to sperm depletion in these males (Qvarnström & Forsgren, 1998). They also make the argument that mating with dominant males may represent a trade-off for females in species where males provide direct benefits to females. If dominant males are better able to gain access to preferred resources, and thus are potentially attractive to several females, a given female may have to share those resources and therefore gain proportionally less by mating with the dominant male (Qvarnström & Forsgren, 1998). Similarly, dominant males may invest heavily in competition to ensure success, and may therefore invest less in other activities such as parental care (Qvarnström & Forsgren, 1998; Qvarnström, 1997). Preference for dominant males may also vary with environmental conditions, as dominant males may require greater resources to compensate for energy stress imposed by increased aggressiveness or size (Qvarnström & Forsgren, 1998), and availability of such resources may influence the health and survival, and thus attractiveness, of dominant individuals. Given this, female choice likely represents a balance between potential costs and benefits of mating with a given mate that may vary with environmental conditions.

The strength of selection on females to be choosy depends on the magnitude of the disparity between male and female parental investment and tends to be stronger when this difference is great, as females stand proportionally more to lose from making a poor mate choice (Trivers, 1972). If dominance accurately signals some aspect(s) of male quality, females should prefer dominant males (but see Qvarnström & Forsgren, 1998). This has been demonstrated in several taxa, including rock shrimp (Diaz & Thiel, 2003), American lobsters (*Homerus americanus*; Bushmann & Atema, 2000), and house crickets (*Acheta domesticus*, Savage et al., 2004). In the case of *Orconectes quinebaugensis*, where males seem to provide no direct (i.e., material) benefits to females or their offspring, choosy females may still receive indirect benefits. In order to benefit from such choice, females must first be able to accurately assess male genetic quality through the expression of male traits (Kokko et al., 2003).

Odor cues have been used by females of many taxa to evaluate potential suitors (reviewed in Wong & Candolin, 2005), and in crayfish in particular, chemical cues have been known to play a large role in the sending of signals involved in behavioral contexts such as foraging (Zulandt Schneider et al., 2001), predator avoidance (Zulandt Schneider et al 2001; Pecor, 2006), agonism (Zulandt Schneider et al., 2001; Bergman & Moore, 2005), sex determination (Hazlett, 1985a), determination of reproductive status (Durgin et al., 2008), and mating (Zulandt Schneider et al., 2001). During agonistic encounters, males release urine that

can be readily detected by other conspecifics, which may convey information regarding an individual's aggressive state or status. Zulandt Schneider et al. (2001) suggested that urine cues may be used to convey status and thus to avoid escalated physical fighting as agonistic encounters between males were both longer and of higher intensity when urine cues were blocked (see also Breithaupt & Eger, 2002). Similarly, prolonged exposure to odor cues from dominant and subordinate males has also been shown to alter agonistic behavior, with urine from dominants or subordinates eliciting opposing changes in the subsequent agonistic behavior of receivers (Bergman & Moore, 2005). This evidence suggests that there may be a difference between urine signals generated by dominant and subordinate males that is detectable to conspecifics.

Winning or losing an encounter may cause neurochemical changes in male crayfish, such as increased serotonin levels associated with winning (reviewed in Moore, 2007; Moore & Bergman, 2005), and that these changes are likely to be reflected in the chemical composition of the urine released (Moore & Bergman, 2005), whether through the presence of specific compounds or signal combinations or the relative composition of components within the urine (Zulandt Schneider et al., 1999). Such signals may also facilitate female choice based on male dominance, or on characteristics correlated with male dominance. Differences in dominance status have been shown to be detectable through odor cues in both male and female crayfish (Zulandt Schneider et al., 1999, 2001; Bergman et al., 2005; Bergman &Moore, 2005; Breithaupt & Eger, 2002), with females showing differential association with odors of dominant males over subordinates (Zulandt Schneider et al., 1999). Similar results have also been shown for other taxa, including the closely related American lobster (*Homerus americanus*), in which females use male odor cues to both detect and distinguish between potential mates based on dominance rank (Bushmann & Atema, 2000), and the cockroach (*Nauphoeta cinerea*), in which the ratio of components within the male pheromone determines dominance status and has been shown to affect attractiveness to females (Moore et al., 2002; Moore et al., 2001). Females may therefore use these cues contained in male urine signals in assessing potential mates.

In some cases, despite female preferences, females may not always be able to choose their desired mate. In species where the level of male competition is high, female choice may be overridden, with dominant males either isolating females from other males or interrupting copulation with subordinate males (Trivers, 1972). Such cases may result in females being prevented from mating with preferred males, thus decreasing potential benefits. Alternatively, mating with superior males resulting from the exclusion of inferior subordinates may serve to increase potential benefits to females (Wong & Candolin, 2005). In either case, females may employ a form of secondary female choice to ensure that they maximize their own benefits in

relations to copulations they have received. For example, females may adjust investment in egg size or number with regard to the identity of her mate in order to benefit higher quality offspring and maximize long term fitness (Sheldon, 2000). Such differential investment is expected to occur frequently in species where females do not receive direct benefits (such as O. *quinebaugensis*) and may be coerced into mating (Mφller & Thornhill, 1998).

Egg size may have a critical influence on offspring fitness, with larger eggs increasing offspring survival because they provide greater energy reserves, allowing for larger progeny that may be adept in competition and foraging, or generating progeny better able to survive harsh environmental conditions (Bernardo, 1996). This may represent a greater investment than producing many, smaller eggs, and females may undertake such investment when mated to higher quality males in order to maximize survival of fitter offspring (Galeotti et al., 2006). Alternatively, investment in greater numbers of eggs may also require considerable energy input, favoring the production of many high quality offspring, and greater resources may alternatively be allocated to egg size for offspring of less fit males in order to promote a higher survival rate when no other option is available (Galeotti et al., 2006). Female crayfish of *Austopotamobius italicus*, a species where male coercion is frequent, have been shown to adjust investment in egg size and number in relation to male size traits (Galeotti et al., 2006), and differential primary reproductive effort has also been observed in relation to attractiveness of mates in other taxa (reviewed in Galeotti et al., 2006). It is likely that females have a limited resource pool from which they can allocate maternal resources, and thus may face a tradeoff between egg size and number produced (Bernardo, 1996). The costs and benefits of fitness for females and offspring through increasing either egg size or number will therefore depend on the relative energetic costs of each and the relative contribution of each to overall offspring fitness (Galeotti et al., 2006).

Other constraints may be placed on female investment in offspring that influence her ability to allocate resources preferentially based on the identity of her mate. Female condition, such as size and age (which may be under selection for reasons other than reproduction; Bernardo, 1996), or environmental effects, such as resource availability, may also affect resource allocation and offspring fitness.   Higher fecundity has been shown to be associated with larger body size in many taxa, such as lizards (*Anolis garmani*; Trivers, 1972) and other species of crayfish (reviewed in Aquiloni & Gherardi, 2007). Smaller females may additionally be subject to anatomical constraints in the number or size of eggs they are able to produce and store (Bernardo, 1996). Female diet, though partially dependent on resource availability, has been known to contribute to variation in nutrient content of eggs (Bernardo, 1996), and may also play a role in differential offspring fitness. Environmental conditions have also been shown to influence egg

size and quality, with those females with greater access to resources having more options for investment. This may be advantageous, as the relative fitness of offspring generated under a certain resource allocation regime may vary given different environmental conditions (e.g.. smaller eggs may produce fitter offspring if food is scarce since smaller offspring require less to survive) (Bernardo, 1996).   In addition, there is a conflict between the fitness of the progeny and of the mother, as additional investment in any one offspring decreases the relative investment a female may be able to make in additional progeny (Bernardo, 1996). Given these constraints, both maternal and environmental effects may influence female investment patterns. For the purposes of this study, environmental conditions and resource availability were kept as consistent as possible between females in order to focus primarily on differential choice in investment.

Other forms of secondary, or cryptic, female choice (Eberhard, 1996) may also be possible, such as the ability to bias paternity in favor of one male over another (Eberhard, 1996; Kokko et al., 2003), or to delay spawning until after a suitable mate has been obtained (Thiel & Correa, 2004) in instances where polyandry occurs. However, this study looks only at data obtained from single matings, and as such focuses on primary reproductive investment only.

As previously mentioned, increased male reproductive success associated with dominance rank has been shown in several taxa, and in some cases this is apparently attributable to increased attractiveness to females. However, dominant males may be able to gain matings for themselves regardless of female preference. When male competition is high, dominant males may be able to interrupt matings of subordinate males or sequester females for themselves (Trivers, 1972), as has been shown in crayfish (*A. pallipes*, Villanelli & Gherardi, 1998; *A. italicus*, Rubolini et al., 2006; *O. rusticus*, Berrill & Arsenault, 1984), rock shrimp (*Rhynchocinetes typus*, Thiel & Correa, 2004), tiger salamanders (*Ambystoma tigrinum tigrinum*, Howard et al., 1997), sword-tailed newts (*Cynops ensicuada popei*, Sparreboom, 1997), and guppies (*Poecilia reticulata*, Houde, 1997). Dominant males have also been shown to be less likely to be displaced during mating than subordinates (Cox & LeBoeuf, 1977; Trivers, 1972; Thiel & Correa, 2004). Dominant males may also, through competition, exclude subordinate males from being easily evaluated by females (Kodric-Brown, 1992; Houde, 1997; Wong & Candolin, 2005), thus removing them from the selection process and potentially overriding female choice if subordinate males are preferred.

In cockroaches, dominance status has shown to be associated with differences in behaviors marking different components of courtship, and some of these behavioral components were found to be heritable in association with dominance rank (Moore, 1990). It is therefore possible that dominant males may have certain advantages in mating that subordinates may lack,

and these result in fitness advantages for such males. For example, dominant male cockroaches were found to attract females and complete courtship more quickly than subordinates (Moore, 1990). In the black field cricket, *Teleogryllus commodus*, males with shorter latency to mating were able to obtain significantly more copulations than other males (Shackleton et al., 2005). This suggests that dominant males may be able to mate more quickly and efficiently than subordinates potentially allowing them to obtain a greater number of copulations. By being able to find and mate quickly with females, a male may also decrease the chances that a female has been previously mated, thus potentially increasing his own fertilization success if the first male to mate gains an increased paternity share of the offspring or if females are less likely to re-mate after their first mating (Trivers, 1972). It has been shown in *O. quinebaugensis* that females who have previously mated are less attracted to male odors than unmated females (Durgin et al., 2008), and this may indicate a change in receptivity that accompanies this change in reproductive status.

Females may also resist mating with males in order to mate with only those males that can overcome this initial resistance (Holland & Rice, 1998; Gavrilets et al., 2001). In crayfish, females have been known to show resistance to mating (personal observation; Villanelli & Gherardi, 1998), and play an active role in the mating process by choosing whether or not to become immobile (receptive) upon contact with the male (Villanelli & Gherardi, 1998). Dominant males may be more vigorous or persistent in courtship (Trivers, 1972), and this may play a role in overcoming this resistance more quickly (Shackleton et al., 2005).

Fertilization success may not only depend on success in mating, however, but may also depend on success in post-copulatory sperm competition in species where mating is promiscuous. Such success may be dependent on time of mating relative to other males (e.g., first or last), or on the amount or intrinsic quality of sperm transferred. As previously stated, being able to mate quickly and efficiently may benefit dominant males if first male sperm precedence is common. In rock shrimp, dominant males guard females and delay transfer of the spermatophore until spawning, thus increasing their chances of fertilization success for most of the eggs (Thiel & Correa, 2004). Subordinate males do not have this advantage, as they are more likely to be displaced by a more dominant individual before female spawning occurs. In many crustaceans, last male sperm precedence is common (Koga et al., 1993; Urbani et al., 1998; Murai et al., 2002), and this would favor post-copulatory mate guarding. In the case of the rock shrimp, dominant males also guard the female for an extended period of time after mating has occurred, presumably to prevent future matings by other males (Thiel & Correa, 2004). This has also been

seen in *N. cinerea*, where dominant males had longer post-copulatory associations with females and secured longer matings overall than subordinates (Moore, 1990).

Particularly in species where females store sperm internally and sperm competition may be fierce, males have been suggested to adjust overall copulation length to help ensure paternity and/or to decrease the likelihood of future contributions to sperm competition from other males (Andres & Cordero-Rivera, 2000; Trivers, 1972). Being able to mate for longer overall may confer advantages to males in that they may ensure that a female remains monopolized for an extended period of time, which may preclude subsequent matings by other males if female receptivity is limited in time (Snedden, 1990). Longer matings may also allow males to increase their investment in the female through additional sperm expenditure, thus again increasing their likelihood of fertilization success. Sperm expenditure has been shown to correlate with overall mating duration in the crayfish *A. italicus*, where duration was also correlated with the value of the female being mated (Rubolini et al., 2006). Lastly, in other taxa, post-copulatory insurance mechanisms such as sperm plugs (e.g. crayfish [*O. rusticus;* Snedden, 1990], garter snakes [*Thamnophis sirtalis parietalis*; O'Donnel et al., 2004]) or potentially manipulative chemical compounds (Johansson & Jones, 2007) may be transferred by the male to decrease the chances a female will re-mate, thus decreasing sperm competition and potentially increasing the fertilization success of the focal male. Longer copulations may facilitate the transfer or increase the efficiency of such mechanisms. These measures may be of particular importance in species where last male sperm precedence is common, as has been mentioned previously for crustaceans (Koga et al., 1993; Urbani et al., 1998; Murai et al., 2002).

The model organism selected for this study is *Orconectes quinebaugensis*, a crayfish native to eastern North America that is typically found in rocky lakes and streams (Mathews & Warren, 2008). Crayfish constitute an ideal model system for studying such dominance effects, as they readily engage in the formation of dominance hierarchies, both in and outside of the lab, through a system of stereotyped, well-documented behaviors (Bruski & Dunham, 1987). In addition, the disparity between male and female parental investment is great in this species (males provide no parental care), which may results in selection for females to be choosy and for males to compete (Trivers, 1972). Mating is assumed to be promiscuous, as this has been observed commonly in other crayfish species, and because multiple paternity has been reported in a congener, *O. placidus* (Walker et al.,2002). Mating occurs in the fall, from approximately October - January (personal observation). Females store sperm over the winter months until eggs are fertilized and extruded in early spring. Embryos are cared for by the mother for up to several months, and after hatching, mothers continue caring for juveniles until they reach independence

during the summer months. Both males and females undergo cyclic dimorphism of form, alternating between a reproductively active (form I) state in the fall breeding season, and the reproductively inactive (form II) state in the late spring. Both form alteration and growth are achieved through molting (Hamr, 2002).

This chapter examines the possible benefits of dominance in sexual selection for both males and females throughout the reproductive process. I tested the predictions that, prior to copulation, females would prefer odors produced by dominant over subordinate males, and that, during mating itself, dominant males would either be more efficient at mating or able to mate for longer than subordinates. I also conducted a pilot study examining post-copulatory reproductive advantages of dominance, which tested the predictions that females mated to dominant males would invest more heavily in egg production, and have a greater number of offspring surviving to independence, than females mated to subordinates. Each of these investigations may help to gain insight into reproductive advantages conferred on either males or females through the dominance trait, and would therefore yield further support that either or both intra- and inter-sexual selection act on the evolution of male dominance.

MATERIALS & METHODS

Collection and Maintenance of Organisms

Crayfish for all experiments were collected from the Quinebaug River in Sturbridge, MA in Aug - Oct 2007. All animals were inspected to ensure sexual maturity. Male reproductive status was assessed by the morphology of the gonopods, known to vary in form between reproductive and non-reproductive states (Hamr, 2002), and female reproductive status was assessed by the development of glair glands, known to show similar variation (Wetzel, 2002). In addition, all females were collected by early September and isolated from males in the laboratory and were thus likely to be unmated since their last molt and unlikely to be carrying viable sperm.

Crayfish were housed at the laboratory at Worcester Polytechnic Institute (WPI) in closed, re-circulating freshwater systems with biological, mechanical and UV filtration. All crayfish were held for at least 2 weeks before use in a trial. Each crayfish was housed individually in a 4 L plastic tank with a clay pot for shelter, such that, during the holding period, individuals were in chemical contact with other crayfish but never in physical contact. Crayfish were fed 3 times per week on an alternating diet of commercial shrimp pellets and frozen broccoli from Aug. – mid Dec. 2007 and from mid May – July 2008. This was reduced to 2 times a week

from Dec. – May due to decreased organism activity over the winter months. Light conditions mimicked those occurring naturally. Crayfish used in dominance and female preference trials were also isolated both physically and chemically for one week prior to use to avoid any effects of chemical signaling on behavior. During this time, each animal received a 50% water change on each day that it received food.

Establishment of Dominance in Males

Trials to establish male dominance levels were carried out from 9/25/07 – 10/16/08. Carapace length and the length of the right chela were measured for all animals collected. All animals that were missing >1walking leg, or had clearly asymmetrical chelae, were excluded. Crayfish were divided into small and large size categories based on the approximate median carapace length of animals collected to minimize any effects of size during agonistic encounters. For each size category, I calculated the mean carapace and right chela lengths. Any individuals not within +/- 10% of the mean carapace length and within +/- 15% of the mean right chela length for each group were excluded from the group. Final group sizes were 22 large males and 14 small males for a total of 36 males.

Males from each final size group were subjected to four rounds of agonistic encounters in order to obtain a dominance score for use in subsequent experiments. A random number generator (www.random.org) was used in each round to generate a random sequence of unique numbers between 1 and the total number of crayfish in each group. Crayfish assigned the first two numbers were paired as opponents, followed by the next two, etc., until all were matched. Males faced a novel opponent in each round. If randomization was such that a male would face a previous opponent, the entire sequence was re-generated until there were no such instances occurring. Rounds were spaced one week apart to eliminate any effects of previous social experience on agonistic behavior (Dawes et al., 2002; Moore, 2007). In each round, pairs of opponents were placed in 30.5-cm wide x 40.6-cm long x 14.6-cm deep plastic tanks filled with fresh, filtered tap water. Within the tanks, opponents were separated with transparent dividers and were allowed to acclimate for 2 min. Dividers were then lifted and agonistic interactions were video-recorded for 10 min. At the conclusion of each trial, both animals were returned to their holding tanks. All tanks were thoroughly rinsed and refilled with fresh, filtered water between each trial.

Videos were analyzed by three separate scorers to identify the winner of each trial. Males were awarded a dominance score ranging from 0 – 4 based on points awarded as follows, summed for all four rounds: win = 1 point, draw = 0.5 points, loss = 0 points. A "win" was

defined as an individual consistently not retreating after an encounter, or if the approach of the individual consistently resulted in retreat of its opponent. A "loss" was defined as an individual consistently retreating from an encounter, or if the individual consistently retreated from the approach of its opponent. Trials were considered a "draw" if encounters were prolonged so that there was no clear winner, or if the identity of the retreating male alternated between individual encounters within a trial. Males receiving a score of 0 or 1 were designated as low dominant, while males receiving a score of 3 or 4 were designated as high dominant.

Any male dying during the course of the four rounds was replaced by a substitute male of suitable size to preserve the scores of the remaining males. Substitutes did not receive scores. Trials that did not have at least a 2/3 consensus between scorers as to the identity of the winner were excluded from the analysis. In addition, a trial was also excluded if a 2/3 consensus was reached but the answer in disagreement was in direct conflict with the majority decision (e.g. the third reviewer concluded that crayfish A was the winner, where the majority had identified the winner to be crayfish B). Any trial where a crayfish molted within 2 weeks before or after use was also excluded from the dataset, as were any trials in which a crayfish died within 6 days after use. These parameters resulted in a total of 28 males receiving final dominance scores (Figure 1). These males were used in the following subsequent experiments involving males of varying dominance level.

In addition, dominance rank of males obtaining a score was analyzed with respect to both carapace and right chela length. Measurements for each parameter were normalized by calculating the percent of the mean within each size group that each measurement represented. Data from both groups was then combined into a single set for analysis. Data were analyzed using a linear regression model, with percent mean carapace and chela length as independent variables and dominance as the response variable.   The regression was carried out via the backward input method, with variables dropped from the model at $p > 0.100$.   Those comparisons producing significant models were tested for the assumptions of normality and equality of variances through plotting of the residuals. All assumptions were found to be met. All analyses were run using SPSS v.14.0.

Experiment 1: Test of Female Preference for Dominant Male Odors

Preference of sexually mature females for dominant male odors was tested 10/31/07 – 11/15/07 using a flow-through Y-maze apparatus consisting of a base arm and two equidistant test arms (Figure 2). A continuous flow of fresh, filtered water was provided from the test arms to the base, and was maintained through continuous re-filling of the source water throughout

trials. A detailed description of the apparatus can be found in Durgin et al. (2008). Test arms were modified from the original design to include plastic barriers creating containment chambers for males for use as odor sources, approximating the size of the acclimation area provided for females. These barriers were perforated with several holes to allow for flow-through of water and chemical contact of subjects, but prevented physical contact between males and females during the trials. Y-mazes were tested after modification with food coloring to ensure that flow from each test arm mixed within the juncture of the two arms and did not flow back into either test arm. White lab tape was also added in a strip along the center of all arms to aid in visualization of subjects within the maze.

Fifty-six sexually mature females were randomly assigned to one of two separate rounds for evaluation with two mazes being run simultaneously in each round. This was done to maximize the sample size for female response. No female was used more than once throughout the experiment. Rounds took place 1 week apart to avoid any residual effects on the behavior of males used as sources in the previous round. Within each round, females were given a choice between control water (no male present) and treatment water (containing a male) from one of the 28 randomly assigned males previously receiving a dominance score. Randomization was achieved through random sequence generation as before, with each number corresponding to one of the scoring males. Males and females were added to the apparatus simultaneously (females in the gated chamber at the end of the base arm and males in the containment chamber of the designated test arm) and allowed to acclimate for 10 minutes to prevent any effects of disturbance pheromones caused by stress (Hazlett, 1985b, 1989, 1990). Males were randomly selected to inhabit the right or left test arm in each trial to avoid any effects of side bias. After acclimation, the gate containing the female was lifted and the female was allowed to explore the maze freely for 10 minutes, during which time videotaping occurred. The trial animals were then returned to their home tanks and the system was allowed to run clean for 5 minutes with filtered water to ensure no residual chemical signal remained for the subsequent trial.

Videos were analyzed by reviewers blinded to the dominance score of the source male. Timestamps were noted for when the female left or entered an arm, and the identity of the arm was recorded. A female was considered to have entered or left an arm when the tip of the rostrum crossed the base of that arm (as indicated by the juncture line of the apparatus). Total time spent in each of the three arms was calculated for each trial. The difference between time spent in the treatment arm (containing the male) and the control arm (containing only filtered water) was used as a measure of preference for male odor, with a larger difference indicating a greater preference over the control and a negative difference indicating repulsion towards the male. Any trial in

which the female failed to leave the base arm within the trial time was excluded from the dataset. Any trial in which either animal died within 10 days, or molted within 7 days, of use was also excluded. This resulted in a total of 50 trials available for analysis.

Data were analyzed using a multiple linear regression model, with dominance, male carapace length, and female carapace length as independent variables and difference in time spent between treatment and control arms as the response variable. The regression was carried out via the backward input method, with variables dropped from the model at p> 0.100 in order of descending p-value. Those comparisons producing significant models were tested for the assumptions of normality and equality of variances through plotting of the residuals. All assumptions were found to be met. Comparisons were also made for average time spent in treatment vs. control arms and right vs. left arms across all trials and within each maze using the Student's T-test. Significance was evaluated at α=0.008 to account for the number of comparisons made through T-tests via Bonferroni correction. All analyses were run using SPSS v.14.0.

Experiment 2: Test of Dominant Male Advantage in Mating

Trials examining advantages conferred on dominant males during mating were carried out 12/3/07 – 12/20/07. Due to the limited number of females I was able to collect before the breeding season, and the use of some of these females in subsequent experiments, my ability to size match males and females for this experiment was limited. Therefore, a group of females was selected from those available such that differences in size between females were minimized. Females were then selected to within +/- 10% of the average carapace length of that group for inclusion in trials. This resulted in a total of 20 qualifying females. Of these 20, 17 had been previously used in the female preference experiment, again due to the limited number of females collected prior to the breeding season. Males were selected from those surviving to this point that had received dominance scores to match this number. All high dominant (n=7) and low dominant (n=5) males remaining were included to ensure maximal variance in male dominance, with 8 additional, randomly chosen males added to complete the requisite 20 males total. Females were randomly assigned to males using the random sequence generation as before, with each number corresponding to a previously assigned male.

During trials, both members of a pair were simultaneously placed in 17.0 cm-wide x 27.5cm-long x 12 cm-deep plastic containers lined with opaque dividers to prevent visual contact between pairs. Pairs were allowed to interact freely for 3 hours, during which time videotaping took place. At the end of the three hour period, any pair not engaged in physical mating behavior

(see below) was removed and individuals were returned to their home tanks. Any pair still engaged in physical mating behavior at the end of the three hours was filmed until mating had been completed. Pairs completing mating during the three hour period were removed to home tanks immediately after mating had been completed to prevent multiple matings (the subsequent experiment required that females only be mated once). All containers were filled with filtered fresh water and were thoroughly rinsed and the water replaced between each trial.

Videos were scored for whether or not a male was successful in mating, and for time spent in three different stages of physical mating behavior. Stage transitions were identified by the occurrence of a series of behaviors as detailed in Table 1.   Stage one, "Time to Successful Contact", was defined as the time from the start of the trial to the time of the male making a contact with the female that resulted in successful mating. Stage two, "Time to Position", was defined as time from the point of successful "contact" to the point where the "position" behavior occurred. Stage three, "Time to Release", was defined as the time from the point where "position" occurred to the occurrence of "release". Matings were only considered successful if all three stages above were completed.

Data on dominance score, male carapace length, female carapace length, and difference in carapace length within pairs relative to mating success was analyzed using the Student's t-test. Both the assumptions of normality and equality of variances were tested using the Shapiro-Wilks W and the Levene's Test for Equality of Variances, respectively, and were found to be met. Significance was evaluated at $\alpha=.05$. Data on time spent in each stage of mating behavior were analyzed using multiple linear regression, with dominance, female carapace length, and difference in carapace length as fixed factors and time spent in each of the three stages as a response variable. Both male and female carapace length could not be included along with difference in carapace length, as both contribute to this variable. As such, female carapace length was selected as the included factor, as males may adjust their reproductive strategy according to female size (Rubolini et al., 2006) and male carapace length is partially accounted for by dominance score (see below). All regressions were carried out via the backward input method, with variables dropped from the model at p> 0.100 in order of descending p-value. Those comparisons producing significant models were tested for the assumptions of normality and equality of variances through plotting of the residuals. Again, all assumptions were found to be met. All analyses were run using SPSS v.14.0.

Experiment 3: Test of Post-copulatory Advantages to Dominance

Females that were mated successfully in any of the three rounds of mating trials (n=27) were held in their home tanks over winter from 12/21/07 – 5/30/08, during which time they were monitored every other day for the development of eggs and juveniles. Non-mated females from prior experiments were also housed over winter in home tanks to serve as controls. All crayfish were treated identically and cared for according to the protocol outlined in the "Collection and Maintenance of Organisms" part of this section. Any female failing to extrude eggs during the course of the experiment was excluded from further data collection as it could not be confirmed that successful insemination had occurred, and therefore no inferences could be made relative to the dominance level of her mate.

 Females were taken for measuring on the first day after egg extrusion where secreted glair residue (present in a sac-like structure around the clutch when first extruded) was absent and eggs could be easily accessed. Clutch mass, average egg mass, average egg size, and clutch size were measured as indicators of female reproductive investment. Clutch mass was calculated by comparing the wet mass of the female before and after extrusion. Females were massed in fresh water to ensure that no change in overall clutch mass occurred due to loss of water from eggs. Excess water was removed from the clutch by blotting the eggs gently with a paper towel before weighing. A sample of 10 haphazardly chosen eggs was taken from each clutch to assess egg mass and size. The diameter of each of the 10 eggs was measured around the widest point using calipers accurate to 0.01 mm, and an average was taken over the entire sample. Eggs were then placed in 1.5mL Eppendorf tubes containing 1mL of water for weighing. Tubes had been previously weighed to obtain initial mass. Egg mass was measured accurately to 0.0001g, and an average mass was taken over the sample. Clutch size was extrapolated by dividing the calculated clutch mass for each female by the average egg mass of the sample.

Females were then returned to their home tanks to allow eggs to develop, during which time they were checked every other day for juvenile development. The approximate percentage of rotten and developing eggs was noted for each female (actual numbers could not be obtained as counting of eggs and juveniles would require removal of some of these from the female, therefore interfering with natural development). Occurrence of juvenile independence, defined as the point at which no juveniles remained attached to the female's pleopods (and accompanied by the female molting in all but one case) was recorded for each female. Females were then temporarily removed from their home tank to allow juveniles to be counted, after which time juveniles were removed to empty tanks for later release and females were returned to the home tank. The

number of juveniles surviving to independence was recorded and used as a measure of early offspring fitness.

Data on dominance score relative to developmental status of eggs were tested for the assumptions of normality and equality of variances, and the data were not normally distributed. Therefore they were analyzed using the Mann Whitney U Test. Data were included from all females that extruded eggs. Eggs were considered to be developed if 50% or greater of the total clutch had developing eggs. There was only one female with developing eggs that did not meet this criterion, in which case approximately 95% of the clutch was rotten. This individual was therefore grouped with those females that possessed rotten clutches. Significance was evaluated at $\alpha=0.05$.

Data on female investment and juvenile survivorship were analyzed by multiple linear regression. Only data from females that produced developing eggs were included, as I could not confirm that clutches producing only rotten eggs were successfully fertilized. Dominance score and female carapace length were included as independent variables, with average egg size, average egg mass, clutch size, and number of juveniles surviving to independence each as separate response variables. All regressions were carried out via the backward input method, with variables dropped from the model at $p > 0.100$ in order of descending p-value. Those comparisons producing significant models were tested for the assumptions of normality and equality of variances through plotting of the residuals. These analyses were run using SPSS v.14.0.

RESULTS

Establishment of Dominance in Males

A significant relationship was found between overall male size and dominance score. Males with greater carapace lengths achieved significantly higher dominance scores than males with smaller carapace lengths ($p=0.005$) (Figure 3). Similarly, males with greater right chela length achieved significantly higher dominance scores than males with smaller right chela lengths ($p<0.001$) (Figure 4).

Experiment 1: Test of Female Preference for Dominant Male Odors

Overall, females spent more time in the arm containing the male than the arm containing control water regardless of dominance level (Figure 5), but at a significance level of $\alpha= 0.008$, this difference was not significant ($p= 0.042$). When given a choice between control water and

water treated with odors from males of varying dominance level, no significant relationship was found between the difference in time spent in each arm and the dominance level of the male providing the odor cue (p= 0.685; Figure 6). In addition, no significant relationship was found between either female (p= 0.717) or male (p= 0.281) carapace length and the difference in time females spent in the treatment vs. the control arm of the y-maze. When testing for side bias, a significant difference was found in time spent in the right vs. left arm overall (p< 0.001), with females spending more time associated with the right arm (Figure 7). To investigate this difference further, time spent in the right and left arms overall was compared for each of the two mazes used (Figure 8). It was found that in maze 2, but not maze 1, females spent significantly more time in the right arm than the left, regardless of male location (p< 0.001; p= 0.091, respectively). Individual mazes were also tested for overall treatment vs. control water preference (Figure 9),but there was no significant difference in time spent in either arm based on male presence in either maze (maze 1, p= 0.637; maze 2, p=0.028) at α=0.008.

Experiment 2: Test of Dominant Male Advantage in Mating

No significant difference was found in average dominance level of males who were successful and males who were unsuccessful in mating (p= 0.859) (Figure 10). Similarly, there was no significant difference in average male carapace length (p= 0.692), female carapace length (p= 0.686) (Figure 11), or difference in carapace length within pairs (p= 0.589) (Figure 12) between successful and unsuccessful matings. In examination of duration of mating stages, only Time to Release produced a significant relationship with male dominance level (p= 0.050), with more dominant males having longer associations with females after copulation had begun (Figure 13). Both Time to Contact and Time to Position showed decreased duration with increasing dominance rank (Figure 13), but these trends were not significant (p=0.637 and p=0.251, respectively). In all cases, female carapace length and difference in carapace length within pairs were found to have no significant relationship to time spent in a given stage of mating (Time to Contact: p=0.399, 0.248; Time to Position: p=0.650, 0.332; Time to Release: p= 0.410, 0.128, for female carapace length and difference in carapace length, respectively).

Experiment 3: Test of Post-copulatory Advantages to Dominance

For females that produced rotten versus developing clutches, there was no significant difference in the dominance scores of the males they mated with (p=0.092) (Figure 14). In examination of female investment parameters relative to the dominance score of the contributing male, no significant relationship was found between male dominance score and either average

egg mass (p=0.569) (Figure 15), average egg size (p=0.416) (Figure 16), or clutch size (p=0.891) (Figure 17). Similarly, no significant relationship was found between the number of juveniles surviving to independence and male dominance score (p=0.395) (Figure 18). In all cases, female carapace length was found to be non-significant in its relationship to investment or survivability parameters (avg. egg mass: p=0.601; avg. egg size: p=0.944; clutch size: p=0.603; number of juveniles surviving to independence: p=0.915).

DISCUSSION

Establishment of Dominance in Males

A significant effect of size was found for both male carapace length and male chela length relative to the mean of each size group used to establish a dominance hierarchy, despite efforts to minimize the size range within groups. This highlights the possible importance of size differences in dominance establishment, even when differences are quite small. Size has been shown to be an important predictor of dominance in many crayfish species (Pavey & Fielder, 1996; Moore, 2007; Villanelli & Gherardi, 1998; Figler et al., 1995), with larger males gaining an advantage in competition for resources such as shelter or mates, and thus in dominance establishment (Figler et al., 1995; Villanelli & Gherardi, 1998; Moore, 2007; Klocker & Strayer, 2004). However, in some cases other factors, such as prior social experience in agonistic encounters, have been shown to supersede this size predisposition in influencing fight outcomes (Daws et al., 2002), and thus this relationship may not be absolute under all circumstances. Other factors, such as sex, reproductive state, previous social experience, motivational state, and extrinsic signals (reviewed in Moore, 2007) have also been shown to influence dominance and may do so either in concert with or independently of size. It is therefore difficult to isolate dominance as a single component in this, or any, study. While many of these factors were controlled for by experimental design and the seasonal nature of the experiments conducted, size remains a significant factor in my trials that may interact with any hereditary predisposition for or against dominance. Therefore, it is possible that female choice or advantages conferred on males based on dominance may be associated either with direct selection on dominance itself, or indirect selection for traits, such as size, that may be correlated with dominance status if such traits are heritable. A third possibility is that factors like size are likely influenced by environmental factors such as age or resource availability. In this case, preferences or advantages based on size may result in only weak selection for male traits, depending on the relative influences of the genetic and environmental components.

Experiment 1: Test of Female Preference for Dominant Male Odors

Our data presented no evidence that females associate preferentially with the odors of dominant males over subordinates. Among many possible explanations that may exist for this finding, three seem particularly likely given our data: (1) that females do not prefer dominant males through odor cues, (2) that odor cues alone are not sufficient for females of *O. quinebaugensis* to make an adequate mate assessment to facilitate choice, or (3) that any necessary discriminatory signal was not present in sufficient amounts for such choice in my trials. Despite evidence for female preference for dominant males in other species (Diaz & Thiel, 2003; Bushmann & Atema, 2000; Savage et al., 2004), arguments have also been presented that females may not prefer dominant males due to additional risks that can be incurred from such a pairing (Qvarnström & Forsgren, 1998; Wong & Candolin, 2005). Qvarnström & Forsgren (1998) argue that some of these risks may include reduced fertilization success, reduced paternal care, increased chance of disease transmission, sharing of potential resources, and decreased lifespan or fecundity. I have no evidence to suggest that any of these potential risks are present in *O. quinebaugensis*, but as no direct benefits are apparent in this species, risk of reduced parental care or potential resource sharing seem unlikely factors to affect mate choice. Similar to my findings, female *P. clarkii* showed no preference for males differing in dominance when given a choice between male a pair of males visually and chemically available but physically isolated (Aquiloni & Gherardi, 2007).

However, a preference for dominant males has been shown to be communicated through odor cues in related taxa (*P. clarkii*, Zulandt Schneider et al., 1999; *H. americanus*; Bushmann & Atema, 2000), and therefore an alternate explanation may be that additional cues may be required for mate assessment. In the lobster *H. americanus*, females show an initial mate preference for dominant males when both the physical male and urine cues are present (Bushmann & Atema, 2000). However, this preference was reduced or absent when either the male or the chemical signal was removed from the scenario. By re-introducing a catheterized male (unable to release urine) into scenarios where chemical signals alone were released, the initial preference for dominant males was able to be partially restored, suggesting that both the physical availability of the male and chemical cues play a role in mate choice. Similarly, females of the crayfish *P. clarkii* make mate choices based on information gained through eavesdropping on male fights, and were found not to exhibit a preference for dominant males when visual or odor cues alone were provided (Aquiloni et al., 2008). In cockroaches, males possess both distance and contact pheromones, the latter of which require physical contact to sample. In measuring heritability of

the duration of several stages of mating, it was found that duration of behaviors occurring after females had contacted males were highly heritable, where duration of behaviors involving the general attraction to the male broadcast pheromone showed no significant heritability (Moore, 1990). In addition, males were either consistently accepted or rejected based on female behavior after contact had been made, but not before. This suggests some detectable (and heritable) variation in the contact pheromone that females responded to behaviorally. It may therefore be that mate preference is chemically influenced, but that physical contact may be required for full signal interpretation. Since females were only allowed access to broadcast chemical information in my experiment, a preference for dominant males may not have been seen if such additional cues are required for complete mate choice.

It is also possible that females prefer some trait correlated with dominance that is not indicated by odor cues. In *P. clarkii*, females were shown to prefer larger males but not dominant males when exposed to both chemical and visual cues (Aquiloni & Gherardi (2007), and male size has been shown to correlate with dominance in other crayfish taxa (Pavey & Fielder, 1996; Moore, 2007; Villanelli & Gherardi, 1998; Figler et al., 1995). Body size has also been shown to be correlated with attractiveness and fighting ability in house crickets (Savage et al., 2004), and females of the crayfish *A. pallipes* have been shown to prefer larger males and males with two intact chelae (Villanelli & Gherardi, 1998). It is therefore possible that females may use cues such as size (or others not examined in my experiments) that may be correlated with dominance in making their mate choice, and thus dominance information in chemical cues alone may not elicit a strong preferential response.

A third possibility is that the male odor signal required for female choice based on dominance was not adequate to allow females to make an assessment in the duration of the trials. While females in my experiment did spend more time associated with male water over control water overall, this difference was not significant at $\alpha=0.008$, and it is possible that it was not great enough to elicit a preferential response based on dominance. A similar study found that odors from a single male were sufficient to elicit a preference for dominant males over subordinates (Zulandt Schneider et al., 1999), and this approach was initially taken to better represent natural signaling levels that females would encounter. However, a similar study using *O. quinebaugensis* (and from which I modified my design for this experiment) found a significant preference for male treated water over control by using water treated for 24 hours with multiple crayfish of interest as the chemical source, and this was dispersed fully throughout the source water for the duration of the trials (Durgin et al., 2008). It is therefore possible that male signal was not present for long enough or in sufficient amount to elicit a measurable preference under

the modifications made in my experimental design.   For example, crayfish of *Astacus leptodactylus* were shown to emit urine one to two times an hour and spontaneously, with this output being increased in agonistic situations (Breithaupt & Eger, 2002) and the presence of conspecifics (Breithaupt & Eger, 2002; Bergman et al., 2005). While conspecifics were present in my experiment, females were located downstream of the flow through the maze, and thus males may not have been able to sense the female until she was at the end of the arm the male occupied. In addition, a similar study using crayfish of *Austropotamobius pallipes* found that odors from a single receptive crayfish did not elicit a mating response in males, while water treated with 20 such females elicited a significant response (Villanelli & Gherardi, 1998). As such urine release may have been too infrequent or in too low a volume to elicit a significant response from the females. It is also possible that urine may have been released during the time the female was in the opposite arm to that in which the male was located, in which case the odor would be unavailable to the female.

Despite randomizing the arm that source males occupied during trials, when testing for possible side bias, a significant right side preference was found overall for trials conducted in either maze. Upon closer examination, this preference seems to be generated in maze 2 alone, as there was no significant side bias in maze 1. While the cause of this bias is unknown, it is possible that it stemmed from slight alterations in flow resulting from the modified gate added to each maze to contain the males. Both mazes were tested to ensure that flow from both arms mixed where each met the base of the maze, and that no water from either experimental arm flowed into the one. They were not tested explicitly for equality of flow in each arm, though each arm was visually inspected to ensure approximately equal water levels in each male reservoir and arm. Still, it is possible that slight variations in water flow may have existed between arms and may have contributed to this side bias. By chance, it also happened that in maze 2, males were assigned to the right arm more often than the left, though the lack of preference for male water over control in maze 1 suggests that this is not the cause of the bias and may in fact be the contributing factor for the near significance of the male preference in maze 2.

As urine output could not be quantified or visualized, I cannot be sure of whether or not the biases seen (or not seen) in these data are due to functionally relevant detection of odors present or to artifacts of the experimental design. As such, I do not favor one explanation over the other in my interpretations. In the future, this experiment could be conducted using a direct choice between dominant and subordinate males to test female preference, as this has produced significant results in other experiments (Zulandt Schneider et al., 1999; Durgin et al., 2008) and may be more effective in showing any biologically relevant differences in female response. It

may also be prudent to run this experiment using pre-treated water, as was done in (Durgin et al., 2008), to determine the signal strength and frequency necessary to elicit a measurable female response.

Experiment 2: Test of Dominant Male Advantage in Mating

No significant difference in mating success was found based on dominance score, male size, female size, or size difference within pairs, suggesting that mating success is not likely to be determined by any one of these factors independently. Similarly, there was no significant relationship between dominance score and either the time to contact the female or the time to get the female into the proper position for mating, suggesting that dominant males were neither better at eliciting female receptivity or at efficiently maneuvering the female to ensure faster mating. However, males that obtained higher dominance scores spent significantly more time associated with the female after copulation than did subordinates. This may be adaptive if by doing so the male increases his own chance of fertilization of the majority of the female's eggs over that of other males. Such an advantage has been shown in the damselfly, where males who mated for longer gained increased fertilization success within a clutch, and copulation duration was increased relative to male density and mount of sperm previously deposited (Andres & Cordero-Rivera, 2000).

In species where females store sperm and the incidence of sperm competition is likely high, males may increase fertilization success by increasing the volume of sperm deposited, mating selectively with virgin females, or adjusting the duration of copulation (reviewed in Aquiloni & Gherardi, 2008). In the crayfish *Austropotamobius italicus*, males who associated with females longer both delivered a greater amount of sperm and monopolized the receptive female longer from mating with other males (Rubolini et al., 2006). Sperm competition is an important component of male competition in species where females store sperm (Trivers, 1972), and males may increase their chances of fertilization success in this form of competition by depositing more sperm (Rubolini et al., 2006; Snedden, 1990). In *A. italicus*, males have been known to increase their sperm expenditure relative to the value of the female they were mating with, with larger, more fecund females receiving a larger deposit (Rubolini et al., 2006), supposedly for this purpose. Due to the quality of my video recordings, and my need to keep mated females alive for the subsequent experiment, I was unable to assess the amount of sperm deposited by males in this experiment.

An alternate strategy in species where sperm storage occurs is to prevent other males from mating with a female altogether, thus decreasing the amount of sperm present to compete

with that of the focal male (Trivers, 1972). As in *A. italicus*, the longer mating duration in *O. quinebaugensis* likely results in the female being monopolized by the current male throughout the duration of mating and may preclude other males from mating during this time. Mate guarding has been seen in other taxa, such as the rock shrimp *Rhynchocinetes typus*, where dominant males prolong associations with the female both before and after mating, delaying spermatophore transfer until the female is ready to spawn and guarding afterwards to ensure their own fertilization success (Thiel & Correa, 2004); or in the cockroach *N. cinerea*, where dominant males were shown to have prolonged associations with females after mating (Moore, 1990). However, this is likely to benefit males only if female receptivity is limited after mating, and the likelihood of females mating subsequently within the reproductive season is low. I have observed matings in *O. quinebaugensis* to occur throughout the course of the mating season, but I have no data suggesting the length of time an individual female may be receptive. In *O. rusticus*, the number of receptive females available for mating decreases as the breeding season progresses and females sequester themselves in preparation for egg extrusion (Berrill & Arsenault, 1984), which may indicate that individual females are not receptive throughout the entire breeding period. In addition, females of *O. quinebaugensis* who had mated were found not to show a preference for male odors, while virgin females showed a significant preference for male odors over control water (Durgin et al., 2008). This may represent a change in female receptivity or motivational state following mating.   Despite this possible advantage for prolonged association with females, it should be noted that a trade-off likely exists between time spent with each female and the number of mates a male can obtain within a given breeding season (Trivers, 1972).   Duration of copulation therefore likely represents a balance between time spent mate guarding and time spent searching for new mates in species such as *O. quinebaugensis* where males do not provide parental care.

Success in fertilization may also depend on the timing with which sperm is deposited. Last male sperm precedence has been shown to be the most common form of this competitive advantage for crustaceans (Koga et al., 1993; Urbani et al., 1998; Murai et al., 2002), and extended associations with a female during and after copulation may increase a male's chance of being the last male to mate with that female. This may occur through monopolization of females as presented above, provided a male is copulating with a female that has previously mated. However, prolonged copulation duration may also allow for the transfer of post-copulatory insurance measures that may decrease the likelihood of subsequent mating for a given female. Male *O. rusticus* have been known to deposit sperm plugs after sperm deposition has occurred (Snedden, 1990), which may deter subsequent males from depositing sperm if removal of such a

structure is either impossible or energetically costly (Durgin et al., 2008). It has been suggested that male gonopod morphology in reproductively active crayfish may have evolved to facilitate removal of such structures. However, in *O. rusticus*, the male sperm plug crystallizes over time and thus may become increasingly hard to remove as time passes after mating (Snedden, 1990). Prolonged associations with females after copulation has occurred may then also serve to extend the interval between female matings, thus increasing the effectiveness of such a barrier in species where sperm plugs are used.

Other post-copulatory insurance mechanisms may include the transfer of chemical compounds by a male to the female during mating that may affect either female receptivity or how a female is perceived by subsequent males (Johansson & Jones, 2007). For example, males of many butterfly species transfer compounds to females along with their spermatophores that decrease both female receptivity and attractiveness to other males (Andersson, et al., 2000). In addition, male damsel flies with longer mating durations were able to increase overall fertilization success in relation to males who had shorter mating durations, and this was suggested to be due to some stimulatory effect of the male on the female (Andres & Cordero-Rivera, 2000). In crayfish, Durgin et al. (2008) showed that male *O. quinebaugensis* reacted preferentially to water treated with odors from virgin females, but showed no preference when given a choice between control water and females that had been previously mated. It was suggested that one reason for this may be that females may no longer produce a signal advertising her receptivity or location to males. Chemical signals have been suggested to change with changes in female body chemistry following mating in other taxa (reviewed in Johansson & Jones, 2007), and this may be facilitated by chemical or physiological factors associated with mating. As stated previously, the same study also showed that virgin, but not mated females, preferred water treated with male odors over control water, which may indicate decreased female receptivity after mating that may or may not be due to such mechanisms.

As in *O. rusticus*, it is likely that female availability and receptivity may change as the breeding season progresses. As such, the importance of each of these advantages may vary depending on the length of individual female receptivity, and at what point during the breeding season interactions occur. As previously mentioned, a trade-off for males may exist between time spent in associations with females and time spent acquiring additional mates (Trivers, 1972), and males may adjust their behavior accordingly. For example, males may extend copulatory lengths and mate guarding later in the season where females are likely to have already mated and the chance of finding additional mates is low, but may allocate more time to acquiring additional mates and guard less at the beginning of the season (Snedden, 1990). My experiments were

conducted near the end of the breeding season. Further experimentation is needed to make direct comparisons on whether progression through the breeding season affects the timing of mating behaviors in this species.

Though no conclusion can be drawn as to the genetic basis of the difference in the mating behavior observed from my data, genetic differences in mating behavior related to dominance have been observed in other taxa. In the cockroach *N. cinerea*, genetic variation found in the dominance rank of males was found to correlate with a shorter time to female attraction, quicker initiation of courtship, quicker courtship duration, and longer post-copulatory associations and mating overall (Moore, 1990). The same study also found that overall attractiveness/acceptability of a male to a female was at least partially genetically based and heritable. As dominance has been shown to be heritable in other taxa (pigs (*Sus scrofa*), Jonsson, 1985; mice (*Peromyscus maniculatus*), Dewsbury, 1990; Japanese quail (*Coturnix japonica*), Nol et al., 1996), it is possible that there may be some heritable component of this mating behavior relative to dominance in *O. quinebaugensis*. Additional studies aimed specifically at this premise would have to be conducted for this to be determined.

Despite the possibilities already outlined for dominant male advantage in extended post-copulatory associations with females, the design of my experiment may not have allowed for detection of all possible advantages conferred on males by social status. In nature, mating occurs in a social context, with the potential for both multiple males and females to be present and influence mating outcomes. Some advantages conferred by dominance, if present, may only manifest in this social context. In many taxa, dominant males have been shown to increase mating success by excluding subordinates from mating opportunities, or by making subordinates unavailable for choice by females (reviewed in Wong & Candolin, 2005). For example, in the three-spined stickleback, dominant males had a mating advantage over subordinates when allowed to move freely about the enclosure, but this advantage was non-existent when males were tethered and females were allowed unhindered choice (Ostlund-Nilsson & Nilsson, 2000). Similarly, dominant males have been shown to have a mating advantage through courtship interference in tiger salamanders, sword tailed newts, and guppies (reviewed in Wong & Candolin, 2005). Alternately, dominant males may also have an advantage in sperm competition through increased competitive ability of sperm regardless of timing or volume of expenditure (Jennions & Petrie, 2000), but this was not examined in this experiment.

Although this experiment was designed to examine possible advantages conferred on males by high dominance status, I cannot rule out the influence of female inter-sexual selection. Both latency to mating and length of post-copulatory associations have been used as a measure of

attractiveness in other studies (Moore, 1990), with a shorter courtship interval and longer post-copulatory association indicating a more attractive male to females present. Though I found no significant relationship of time spent in pre-copulatory stages (Time to Contact, Time to Position), it is possible that the significantly longer post-copulatory associations made by dominant males may be in part due to some previously undetected female preference. Females may benefit from allowing dominant males to mate longer if by mating longer, dominant males are able to maximize their fertilization success.   This is under the assumption that dominant males are of superior quality and thus provide indirect benefits to females, which has been generally accepted for many taxa (Wong & Candolin, 2005; Kokko et al., 2003; Berglund et al., 1996). Additionally, mating success in crayfish has been said to be determined by the female assuming the receptive position, in which she becomes immobile, allowing the male to mate (Villanelli & Gherardi, 1998). In my personal observations of the mating process, both males and females were apparently able to terminate the mating process, with females either successfully resisting male advances, or females becoming mobile and either escaping from or fending off the male successfully once the mating process had begun. This occurred during any of the stages examined, and often resulted in mating failure for the male. Though I saw no significant difference in mating success based on dominance score or any other factor, females may still play a potentially important role in the timing and mechanics of the mating process.

It should be noted that interpretations presented here are not meant to be strongly conclusive, as the small sample size used in this experiment precludes definitive conclusions from being drawn. However, with a larger sample size, further investigation may be able to provide a clearer insight into the possible advantages that dominance may confer to males during the mating process and what selection factors may act on them.

Experiment 3: Test of Post-copulatory Advantages to Dominance

Due to constraints on the experimental design, this experiment was treated as a pilot experiment. Firstly, the sample size of females that were successfully mated, and subsequent laid developing eggs, was very small (n=7), representing only 17.5% of original females used. This made meaningful analysis difficult in regard to trends in investment and juvenile survivability. In addition, some of the females that produced developing eggs were mated to the same male, and therefore the seven clutches were non-independent (n=5 sires).   If this experiment were to be carried out as a full study in the future, a much larger sample size of initial males would have to be used, such that a sufficient number of males achieving dominance scores would be available in order to avoid such complications.

In the pilot analyses run, no significant difference was found between the average dominance score of males contributing to clutches that had 50% or greater developing eggs (n=7) and clutches where eggs were entirely rotten (n=9).   However, 4 out of a total of 5 sires of fertile clutches were highly dominant males (obtaining a score of 3 or 4), while the dominance scores in the rotten group represented a range of values (mean=1.5). Also, the single low dominant male (dominance score of 1) contributing to the developing group sired a clutch with just over 50% developing eggs, where all clutches sired by high dominant males in this group were almost entirely composed of developing eggs. In polyandrous species, it is suggested that mating multiply may increase a female's chance of fertilization by males who are of "higher intrinsic quality," and that may produce offspring with greater viability, survivability, or attractiveness (Tregenza & Wedell, 2000). In the Swedish adder, *Vipera berus,* this was supported, with different males having varying success rates in siring viable offspring (Madsen et al., 1992). If dominant males are of "higher intrinsic quality" (Wong & Candolin, 2005; Kokko et al., 2003; Berglund et al., 1996), this suggests that there may be some advantage in viability of eggs sired by more dominant males, which may be worth investigating under conditions where sample size and independence constraints do not limit the interpretation of the data.

Similarly, no significant relationship was found between the dominance score of sires and egg size, egg mass, clutch size, or juvenile survivability. This is unsurprising, as the majority of the developing clutches analyzed were sired by high dominant males (rotten clutches were not analyzed for female investment), and thus any real trends would be unlikely to be detected. In light of this, there still seems to be a general trend for increasing egg size and decreasing clutch size with dominance score, as well as increasing numbers of surviving juveniles. This may represent a trade-off between egg size and number (Bernardo, 1996), with females mated to dominant males favoring the production of larger eggs, which may lead to increased juvenile survivability and overall fitness (Bernardo, 1996; Galeotti et al., 2006). This may similarly contribute to possible increased offspring survivorship seen, though this may also be caused by the greater percentage of developing eggs produced by females mated to dominant males (this study), or may be due to some component contributed by the male that increases juvenile survivability (reviewed in Jennions & Petrie, 2000). Again, in polyandrous lizards, (*Lacerta agilis,* Olsson et al., 1994) females mated multiply to different males produced juveniles with greater survivability, presumably due to superior genetic quality transferred by some males, which supports this possibility. These conclusions are only speculative at best, however, as at this small sample size one cannot make a conclusive statement as to whether or not these trends would be robust enough to persist under further investigation.

Other points of interest that were not examined in this study, but that should be considered for further investigation, include timing of juvenile development and long term survivability of juveniles relative to the dominance score of the male sire. In the cockroach *N. cinerea*, male dominance pheromone has been suggested to affect female fecundity and the development rate of eggs and juveniles, and female mate choice may respond to these changes (Moore et al., 2001). In addition, females of some taxa may alter time of extrusion based on mate choice in order to maximize fertilization by a preferred male (Thiel & Correa, 2004; Galeotti et al., 2006). Given this, it may be of interest to examine parameters such as the length of time from mating to extrusion of eggs, and also from extrusion to hatching, through larval development, and to independence relative to dominance level of the sire. Independence was the stage selected to count juveniles for my survivorship assay, and was chosen due to juveniles achieving resemblance to the adult stage and for ease of identification, as well as due to time constraints posed by other experiments that required space in the laboratory. However, it is possible that differences in survivorship based on male dominance may not manifest until later stages, and perhaps not until adulthood. It would therefore be interesting to explore long term survivability of offspring relative to dominance and other possible parameters of interest. Ultimately, it would be ideal to be able to raise offspring sired from such experiments as detailed above, and enter male offspring into contests similar to those that males participated in this study to establish dominance. In this way, dominance level of sons could be compared to that of the father, and would be a direct test of the heritability of dominance as a sexually selected trait.

Conclusions

Overall, the majority of data do not support my hypothesis that dominance interactions are shaped by sexual selection pressures.. However, I did find evidence for significant differences in the duration of the mating process relative to dominance score, which may signal an advantage in fertilization conferred on dominant males, and which may be subject to either or both intra- and/or inter-sexual selection. If dominance is at least partially heritable in this species, such selection may act on this trait to shape both dominance and mating interactions for future generations. Further investigation is needed to elucidate which of these selection methods may be acting specifically on these interactions, and to explore if other factors related to dominance, both examined here and as yet unexplored, may still have some contribution to the development of this trait as a whole through sexual selection.

ACKNOWLEDGEMENTS

REFERENCES

Andersson, J., Borg-Karlson, A. K., Wiklund, C. 2000. Sexual cooperation and conflict in butterflies: a male-transferred anti-aphrodisiac reduces harassment of recently mated females. Proceedings of the Royal Society London B. 267: 1271-1275.

Andres, J. & Cordero-Rivera, A. 2000. Copulation duration and fertilization success in a damselfly: an example of cryptic female choice? Animal Behavior. 59: 695-703.

Aquiloni, L. & Gherardi, F. 2007. Mutual mate choice in crayfish: large body size is selected by both sexes, virginity by males only. Journal of Zoology. p.1-9.

Aquiloni, L., Buric, M., Gherardi, F. 2008. Crayfish females eavesdrop on fighting males before choosing the dominant mate. Current Biology. 18: R462-R463.

Berglund, A., A. Bisazza, and A. Pilastro. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. Biological Journal of the Linnean Society 58: 385–399.

Bergman, D., and P. Moore. 2005. Prolonged exposure to social odours alters subsequent social interactions in crayfish *Orconectes rusticus*. Animal Behaviour 70: 311-318.

Bergman, D. A., and P. A. Moore. 2003. Field observations of intraspecific agonistic behavior of two crayfish species, *Orconectes rusticus* and *Orconectes virilis*, in different habitats. Biological Bulletin 205: 26–35.

Bergman, D., Martin, A., Moore, P. 2005. Control of information flow through the influence of mechanical and chemical signals during agonistic encounters by the crayfish, Orconectes rusticus. Animal Behaviour. 70: 485-496.

Bernardo, J. 1996. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. American Zoologist. 36: 216-236.

Berrill, M. & Arsenault, M. 1984. The breeding behavior of the northern temperate orconectid crayfish, *Orconectes rusticus*. Animal Behaviour. 32: 333-339.

Breed, M. D., S. K. Smith, and B. G. Gall. 1980. Behavioral strategies during inter-male agonistic interactions in a cockroach. Animal Behaviour 28: 1063-1069.

Breithaupt, T. & Eger, P. 2002. Urine makes the difference: chemical communication in fighting crayfish made visible. Journal of Experimental Biology. 205: 1221-1231.

Bruski, C. & Dunham, D. 1987. The importance of vision in agonistic communication of the crayfish *Orconectes rusticus*, I: an analysis of bout dynamics. Behaviour. 63: 83-107.

Bushmann, P. & Atema, J. 2000. Chemically mediated mate location and evaluation in the lobster, *Homerus americanus.* Journal of Chemical Ecology. 26: 883-899.

Cox, C. R., and B. J. LeBoeuf. 1977. Female incitation of male competition: a mechanism in sexual selection. American Naturalist 111: 317-335.

Daws, A. G., J. Grills, K. Konzen, and P. A. Moore. 2002. Previous experiences alter the outcome of aggressive interactions between males in the crayfish, *Procambarus clarkii*. Marine and Freshwater Behaviour and Physiology 35: 139-148.

Dewsbury, D. 1990. Fathers and sons: genetic factors and social dominance in deer mice. Animal Behaviour. 39: 284-289.

Diaz, E.R. & Thiel, M. 2003. Female rock shrimp prefer dominant males. Journal of the Marine Biological Association of the United Kingdom. 83: 941-942.

Durgin, W., Martin, K., Watkins, H., Mathews, L. 2008. Distance communication of sexual status in the crayfish Orconectes virilis: female sexual history mediates male and female behavior. Journal of Chemical Ecology. 34: 702-707.

Eberhard, W. 1996. Female control: sexual selection by cryptic female choice. Princeton University Press. Princeton, NJ.

Figler, M. H., J. E. Finkelstein, M. Twum, and H. V. S. Peeke. 1995. Intruding male red swamp crayfish, *Procambarus clarkii*, immediately dominate members of established communities of smaller, mixed-sex conspecifics. Aggressive Behavior 21: 225-236.

Galeotti, P., D. Rubolini, G. Fea, D. Ghia, P. Nardi, F. Gherardi, M. Fasola. 2006. Female freshwater crayfish adjust egg and clutch size in relation to multiple male traits. Proceedings of the Royal Society London B. 273: 1105-1110.

Gavrilets, S., Arnqvist, G., Friberg, U. 2001. The evolution of female mate choice by sexual conflict. Proceedings of the Royal Society London B.   268: 531-539.

Hamr, P. 2002. *Orconectes*, pp. 585-608. In D. M. Holdich, (ed.), Biology of freshwater crayfish. Blackwell Science, Oxford.

Hazlett, B. 1985a. Chemical detection of sex and condition in the crayfish *Orconectes virilis*. Journal of Chemical Ecology. 11: 181-189.

Hazlett, B. 1985b. Disturbance pheromones in the crayfish *Orconectes virilis*. Journal of Chemical Ecology. 11: 1695-1711.

Hazlett, B. 1989. Additional sources of disturbance pheromone affecting the crayfish *Orconectes virilis*. Journal of Chemical Ecology. 15: 381-385.

Hazlett, B. 1990. Source and nature of disturbance-chemical system in crayfish. Journal of Chemical Ecology. 16: 2263-2275.

Holland, B. & Rice, W. 1998. Chase-away sexual selection: antagonistic seduction versus resistance. Evolution. 52: 1-7.

Houde, A. 1997. Sex, color, and mate choice in guppies. Princeton University Press. Princeton, NJ.

Howard, R.D., Moorman, R.S., Whiteman, H.H. 1997. Differential effects of mate competition and mate choice on eastern tiger salamanders. Animal Behaviour. 53: 1345-1356.

Jennions, M. and M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. Biological Reviews. 75: 21-64.

Johansson, B. and Jones, T. 2007. The role of chemical communication in mate choice. Biological Reviews. 82: 265-289.

Johnsson, J. I. 1997. Individual recognition affects aggression and dominance relations in rainbow trout, *Oncorhynchus mykiss*. Ethology 103: 267-282.

Jonsson, P. 1985. Gene action and maternal effects on social rankings and its relationship with production traits in pigs. Journal of Animal Breeding and Genetics. 102: 208-220.

Klocker, C. A., and D. L. Strayer. 2004. Interactions among invasive crayfish (*Orconectes rusticus*), a native crayfish (*Orconectes limosus*), and native bivalves (Sphaeriidae and Unionidae). Northeastern Naturalist 11: 167-178.

Kodric-Brown, A. 1992. Male dominance can enhance mating success in guppies. Animal Behaviour 44: 165-167.

Koga, T., Henmi, Y., Murai, M. 1993. Sperm competition and the assurance of underground copulation in the sand-bubbler crab *Scopimera globosa* (Brachyura, Ocypodidae). Journal of Crustacean Biology. 13: 134-137.

Kokko, H., R. Brooks, M. Jennions, J. Morley. 2003. The evolution of mate choice and mating biases. Proceedings of the Royal Society London B. 270: 653-664.

Levenbach, S. & Hazlett, B.A. 1996. Habitat displacement and the mechanical and display functions of chelae in crayfish. Journal of Freshwater Ecology. 11: 485-492.

Mathews, L.M., Warren, A.H. 2008. A new crayfish of the genus Orconectes Cope, 1872 from southern New England (Crustacea: Decapoda: Cambariidae). Proceedings of the Biological Society of Washington. 121: 374-381.

Maynard Smith, J. 1978. Optimization theory in evolution. Annual Review of Ecology and Systematics 9: 31-56.

Møller, A. & Thornhill, R. 1998. Male parental care, differential parental investments by females and sexual selection. Animal Behaviour. 55: 1507-1515.

Moore, P. A. 2007. Agonistic behavior in freshwater crayfish – the influence of intrinsic and extrinsic factors on aggressive encounters and dominance, pp. 90-114. In E. J. Duffy and M. Thiel (eds.), Evolutionary ecology of social and sexual systems – crustaceans as model organisms. Harvard University Press, New York.

Moore, A., Haynes, K., Preziosi, R., Moore, P. 2002. The evolution of interacting phenotypes: genetics and evolution of social dominance. The American Naturalist. 160: S186-S197.

Moore, A., Gowaty, P., Wallin, W., Moore, P. 2001. Sexual conflict and the evolution of female mate choice and male social dominance. Proceedings of the Royal Society London B. 268: 517-523.

Moore, A. 1990. The inheritance of social dominance, mating behavior and attractiveness to mates in male *Nauphoeta cinerea*. Animal Behaviour. 39: 388-397.

Nol, E., Cheng, K., Nichols, C. 1996. Heritability and phenotypic correlations of behavior and dominance rank of Japanese quail. Animal Behaviour. 52: 813-820.

Murai, M., Koga, T., Young, H. 2002. The assessment of female reproductive state during courtship and scramble competition in the fiddler crab, *Uca paradussumieri*. Behavioral Ecology and Sociobiology. 52: 137-142.

O'Donnel, R.P., Ford, N.B., Shine, R., Mason, R.T. 2004. Male red-sided garter snakes *Thamnophis sirtalis parietalis* determine female mating status from pheromone trails. Animal Behaviour. 68: 677-683.

Olsson, M., Madsen, T., Shine, R. 1994. Rewards of promiscuity. Nature. 372: 229-230.

Ostlund-Nilsson, S. & Nilsson, G.E. 2000. Free choice by female sticklebacks: lack of preference for male dominance traits. Canadian Journal of Zoology. 78: 1251-1258.

Pavey, C. R., and D. R. Fielder. 1996. The influence of size differential on agonistic behavior in the freshwater crayfish, *Cherax cuspidatas* (Decapoda: Parastacidae). Journal of Zoology 238: 445-457.

Pecor, K. W. 2006. The mate pursuit-predation avoidance tradeoff in the virile crayfish, *Orconectes virilis*. Marine and Freshwater Behavior and Physiology 39: 229-233.

Qvarnström, A. 1997. Experimentally increased badge size increase male competition and reduces male parental care in the collared flycatcher. Proceedings of the Royal Society London B. 264: 1225-1231.

Qvarnström, A., and E. Forsgren. 1998. Should females prefer dominant males? Trends in Ecology and Evolution 13: 498-501.

Raleigh, M. J. and M. T. McGuire. 1989. Female influences on male dominance acquisition in captive vervet monkeys, *Cercopithecus aethiops sabaeus*. Animal Behaviour 38: 59-67.

Rubolini, D., P. Galeotti, G. Ferrari, M. Spairani, F.Bernini, and M. Fasola. 2006. Sperm allocation in relation to male traits, female size, and copulation behavior in a freshwater crayfish species. Behavioral Ecology and Sociobiology 60: 212-219.

Savage, K., Hunt, J., Jennions, M., Brooke, R. 2004. Male attractiveness covaries with fighting ability but not with prior fight outcome in house crickets. Behavioral Ecology. 16: 196-200.

Shackleton, M., Jennions, M., Hunt, J. 2005. Fighting success and attractiveness as predictors of male mating success in the black field cricket, Teleogryllus commodus: the effectiveness of no choice tests. Behavioral Ecology and Sociobiology. 58: 1-8.

Sheldon, B. 2000. Differential allocation: tests, mechanisms and implications. Trends in Ecology and Evolution. 15: 397-402.

Snedden, W. A. 1990. Determinants of male mating success in the temperate crayfish *Orconectes rusticus*: chela size and sperm competition. Behaviour. 115: 100-113.

Sparreboom, M. 1997. Sexual interference in the sword-tail newt, *Cynops ensicuada popei* (Amphibia: Salamandridae). Ethology. 102: 672-685.

Statzner, B.., Fievet, E., Champagne, J.Y., Morel, R., Herouin, E. 2000. Crayfish as geomorphic agents and ecosystem engineers: biological behavior affects sand and gravel erosion in experimental streams. Limnology and Oceanography. 45: 1030-1040.

Thiel, M. & Correa, C. 2004. Female rock shrimp *Rhynchocinetes typus* mate in rapid succession up a male dominance hierarchy. Behavioral Ecology and Sociobiology. 57: 62-68.

Tilson, R. H. and W. J. Hamilton III. 1984. Social dominance and feeding patterns of spotted hyenas. Animal Behaviour 32: 715-724.

Tregenza, T., Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage: Invited Review. Molecular Ecology. 9: 1013-1027.

Trivers, R. L. 1972. Parental investment and sexual selection, pp. 139-179. In B. Campbell (ed.), Sexual selection and the descent of man. Aldine, Chicago.

Urbani, N., Sainte-Marie, B., Sevigny, J.M., Zadworny, D., Kuhnlein, U. 1998. Sperm competition and paternity assurance during the first breeding period of female snow crab (*Chionoecetes opilio*)(Brachyura: Majidae) Canadian Journal of Fisheries and Aquatic Sciences. 55: 1104-1113.

Villanelli, F. & Gherardi, F. 1998. Breeding in the crayfish, *Austropotamobius pallipes*: mating patterns, mate choice, and intermale competition. Freshwater Biology. 40: 305-315.

Walker D., Porter B.A., & Avise J.C. (2002) Genetic parentage assessment in the crayfish *Orconectes placidus*, a high-fecundity invertebrate with extended maternal brood care. Molecular Ecology 11: 2115-2122.

Wetzel, J. 2002. Form alteration of adult female crayfishes of the genus *Orconectes* (Decapoda: Cambaridae). American Midland Naturalist. 147: 326-337.

Wong, B. & Candolin, U. 2005. How is female mate choice affected by male competition? Biological Reviews. 80: 559-571.

Zulandt Schneider, R.A., Schneider, R., Moore, P. 1999. Recognition of dominance status by chemoreception in the red swamp crayfish, *Procambarus clarkii*. Journal of Chemical Ecology. 25: 781-794.

Zulandt Schneider, R. A., R. Huber, and P. A. Moore. 2001. Individual and status recognition in the crayfish, *Orconectes rusticus*: the effects of urine release on fight dynamics. Behaviour 138: 137–153.

FIGURES & TABLES

Table 1. Ethogram of behaviors used to mark stage transitions for time spent in each stage of mating.

Behavior	Description
Contact	Approach of the male to the female with chelae spread wide and ending in a distinct grabbing motion towards the female upon physical contact
Position	The point at which both the male and female abdominal segments were properly aligned to allow gonopod interaction *
Release	The point at which the male and female were no longer in contact or when the interaction clearly became agonistic

*This was usually marked by the bending of the male's tail 90° to be flush with the female
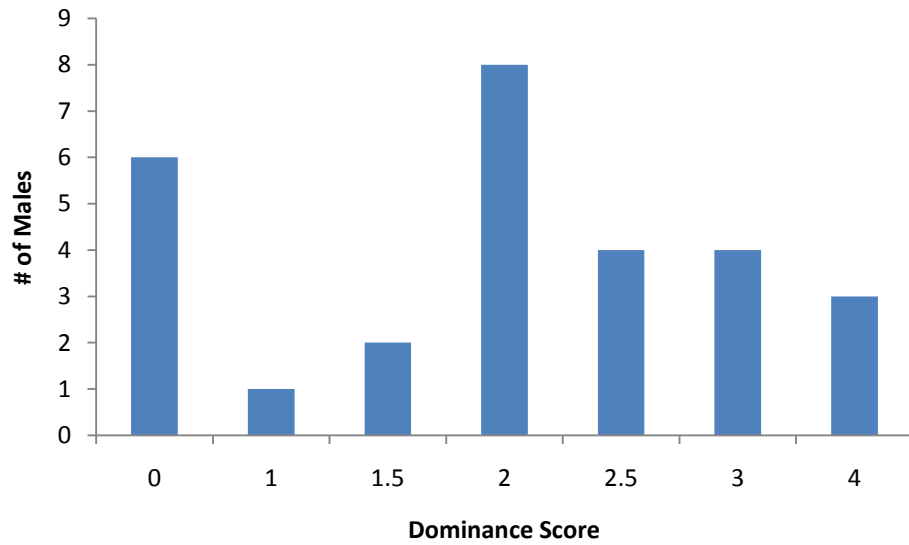
Figure 1. Distribution of dominance scores assigned to males completing all four rounds of dominance trials. n=28 males received scores. Males achieving a score of 0 to 1 were considered low dominant and males achieving a score of 3 to 4 were considered high dominant.
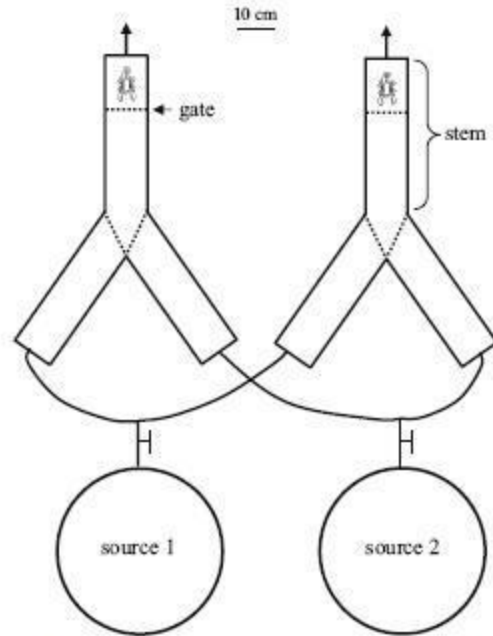
Figure 2. Diagram of Y-maze apparatus used to test female preference for dominant male odors. Figure taken from Durgin et al. (2008). The apparatus used is the same as in Durgin et al. (2008) with the following modifications: opaque plastic barriers perforated with several small holes were added to the ends of the test arms to serve as containment chambers for males providing the odor source, such that only chemical contact was permitted between males and females. Fresh, filtered water flowed from source water reservoirs through each maze, mixing evenly at the juncture of the two test arms and then flowing into a catch basin at the base.
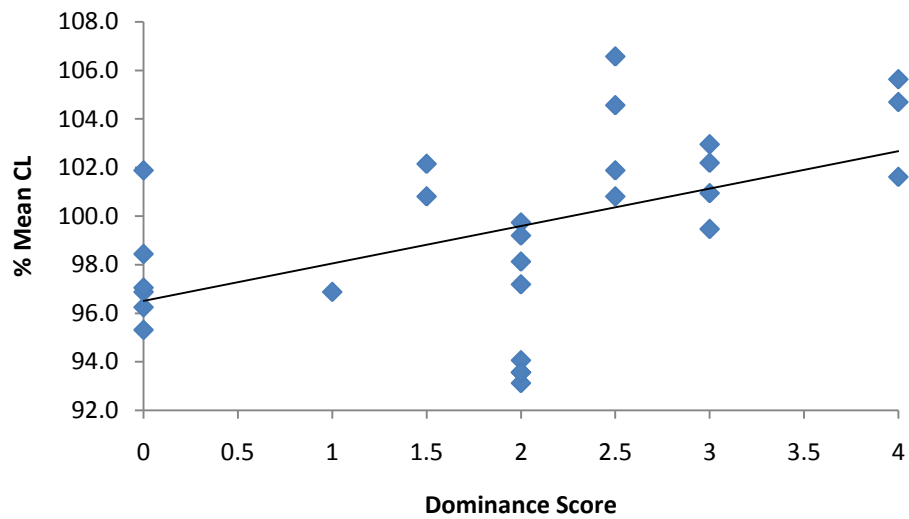
Figure 3. Percent mean carapace length relative to dominance score of both large and small males. Carapace length of individuals was normalized by mean carapace length calculated within each size group (n= 28 males total). Data were analyzed via linear regression. The relationship was found to be significant (p=0.005, R^2=0.254) .
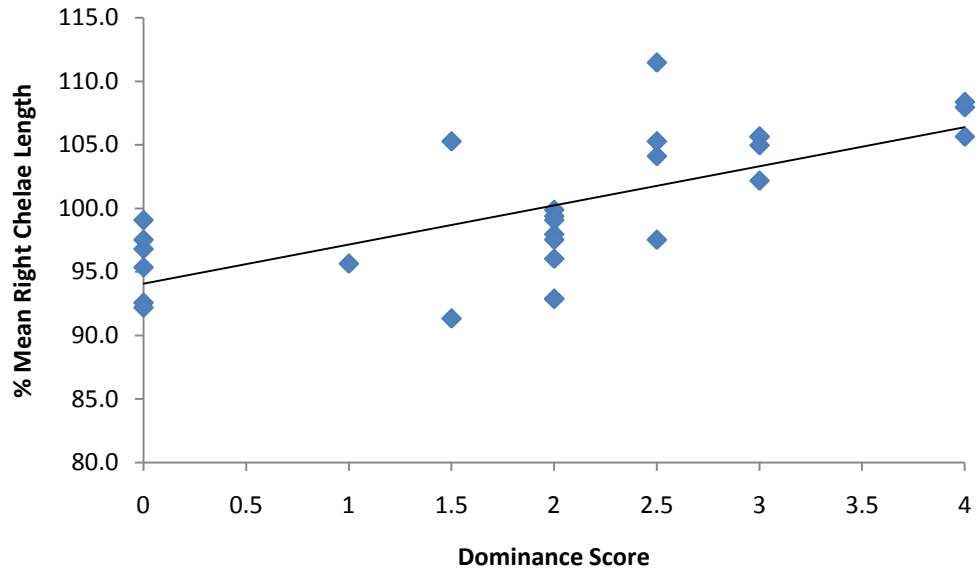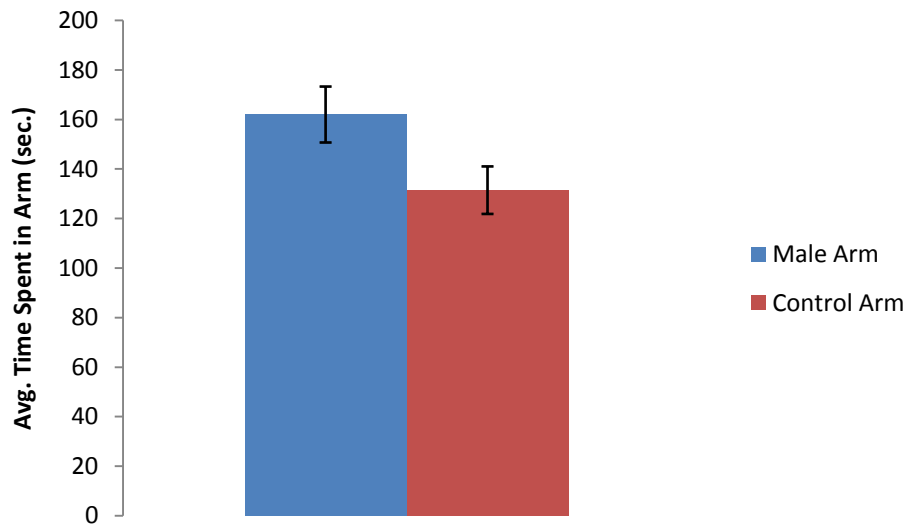
Figure 4. Percent mean right chela length relative to dominance score of both large and small males. Carapace length of individuals was normalized by mean carapace length calculated within each size group (n= 28 males total). Data were analyzed via linear regression. The relationship was found to be significant (p<0.001, R^2=0.474).

Figure 5. Average time spent in treatment vs. control arms for all trials. Total time spent in each arm was taken from each useable trial for comparison (n=50 for each arm). Data were analyzed via the Student's T-test. Error bars represent standard error. The difference was found to be significant at α=0.05 (p=0.042), but not at α=0.008.
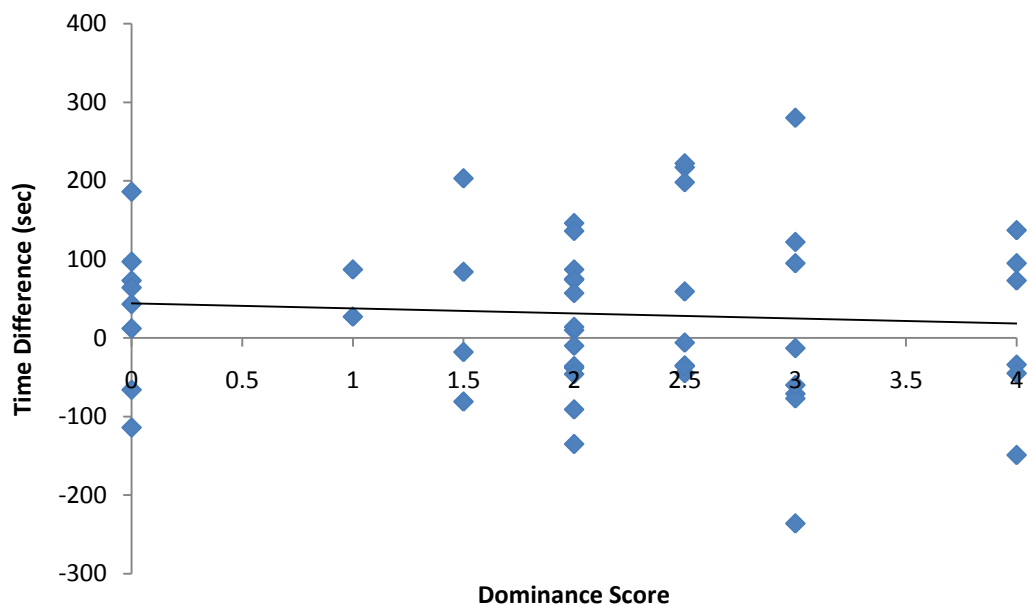
Figure 6. Difference in time spent in treatment vs. control arms by females exposed to odors of males with varying dominance score. Positive values denote a greater amount of time spent in the arm containing the male (treatment), while negative values denote a greater amount of time spent in the arm with no odor (control). n= 50 trials. Data were analyzed via multiple linear regression. No significant relationship was found between dominance score and difference in time spent in maze arms (p=0.685, R^2=0.030).
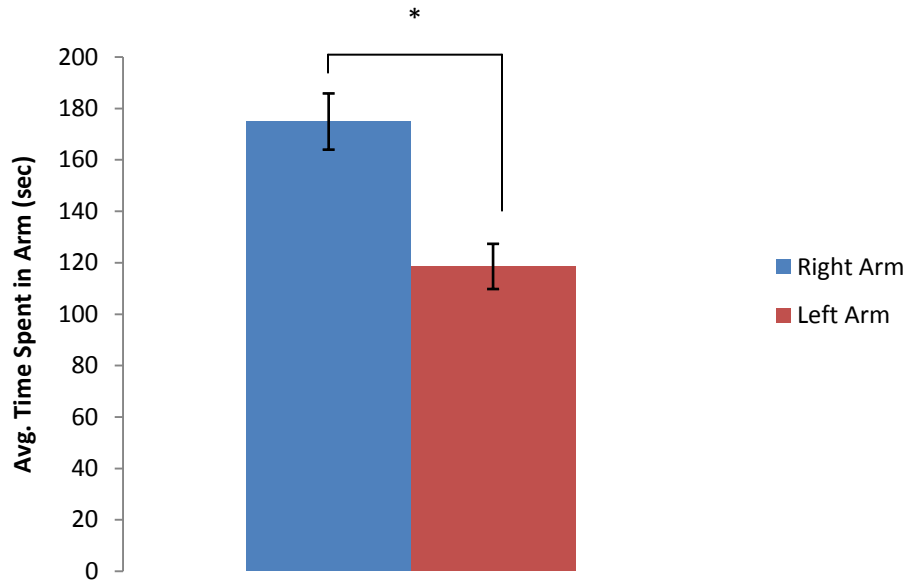
Figure 7. Average time spent in right vs. left arms for all trials irrespective of male location. Total time spent in each arm was taken from each useable trial for comparison (n=50 for each arm). Data were analyzed via the Student's' t-test. Error bars represent standard error. Asterisks indicate significant differences at α=0.008 (p<0.001).
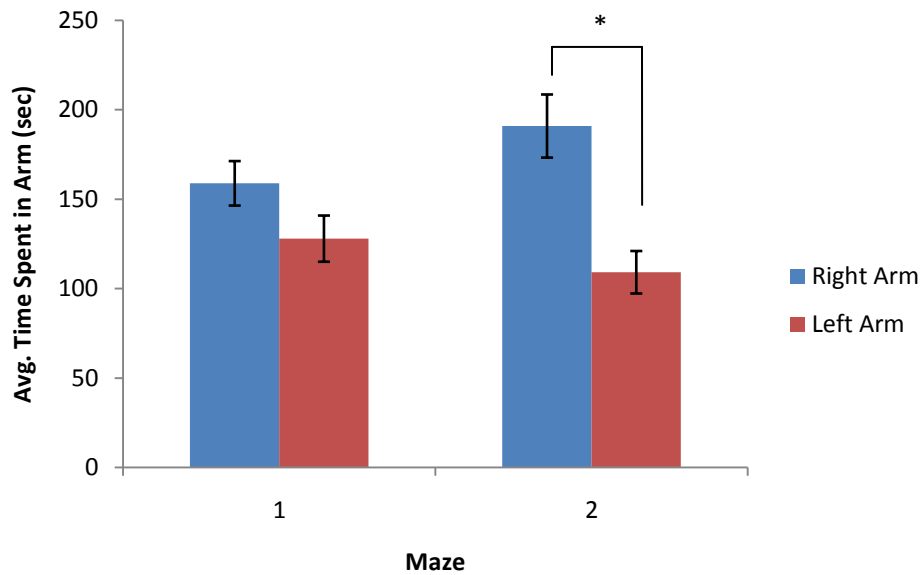
Figure 8. Average time spent in right vs. left arms for all trials subdivided by maze. Total time spent in each arm for each trial was compared within the individual maze in which the trial was conducted (n=25for each arm in each maze). Data were analyzed via the Student's t-test. Error bars represent standard error. Asterisks indicate significant differences at α=0.008 (p=0.091 Maze 1; p<0.001 Maze 2).
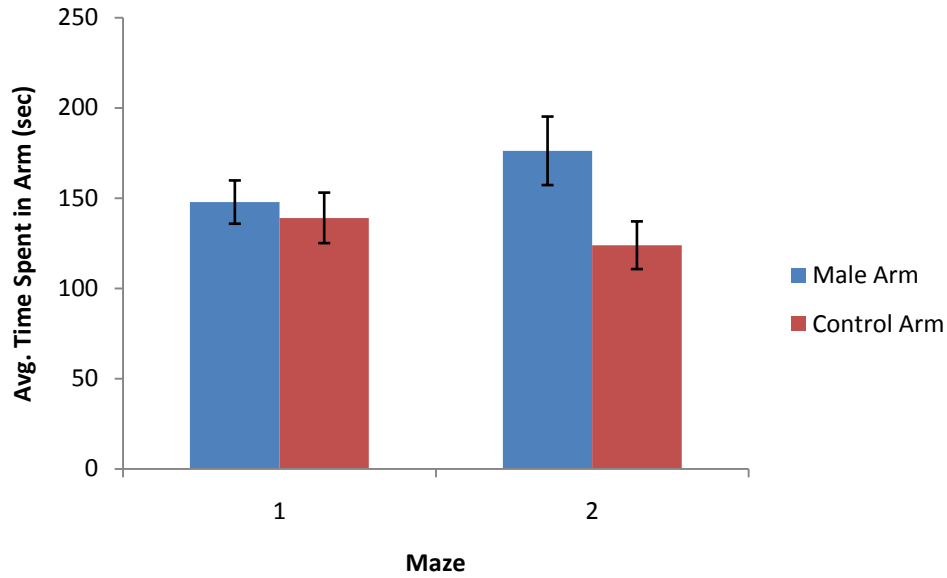
Figure 9. Average time spent in treatment vs. control arms for all trials subdivided by maze. Total time spent in each arm for each trial was compared within the individual maze in which the trial was conducted (n=25 for each arm in each maze). Data were analyzed via the Student's t-test. Error bars represent standard error. Differences in Maze 2 were found to be significant at α=0.05 (p=0.028), but not at α=0.008. Differences in Maze 1 were not found to be significant (p=0.637).
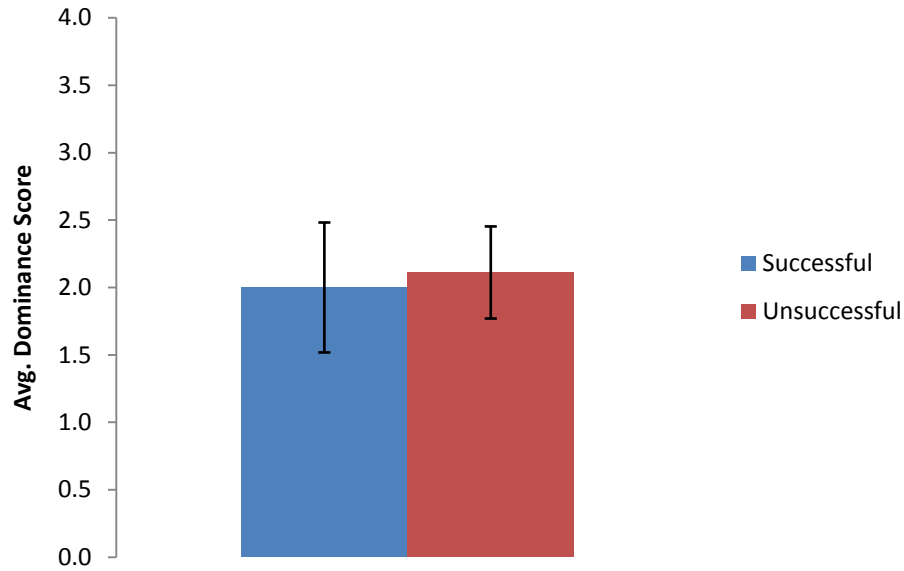
Figure 10. Average dominance score of males relative to mating success. Mating was considered to be successful if all three stages of mating were observed. n=9 and 7 for unsuccessful and successful matings, respectively. Data were analyzed via the Student's t-test. Error bars represent standard error. No significant difference was found in mating success based on dominance score (p=0.859).
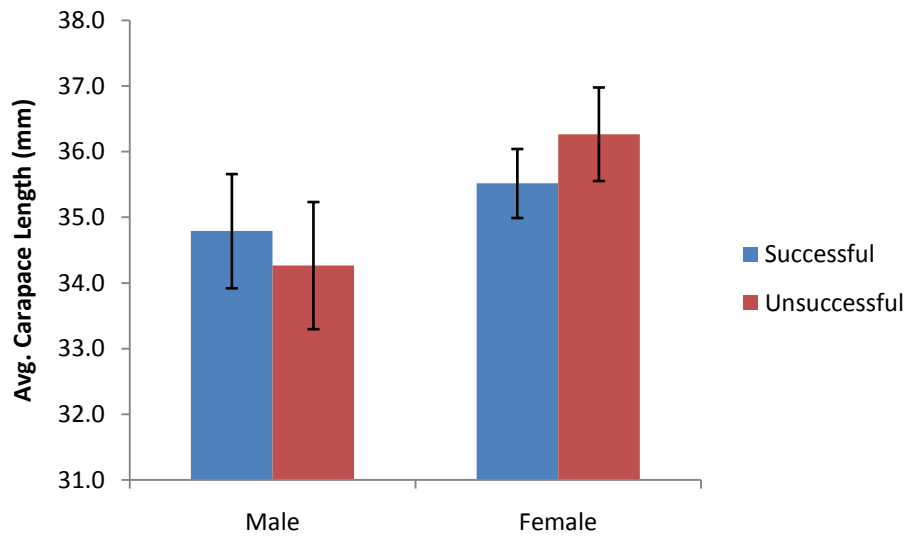
Figure 11. Average carapace length of males and females relative to mating success. Mating was considered to be successful if all three stages of mating were observed. n=9 and 7 for unsuccessful and successful matings, respectively. Data were analyzed via the Student's t-test. Error bars represent standard error. No significant difference was found in mating success based on either male or female size (p=0.692 and p=0.686, respectively).
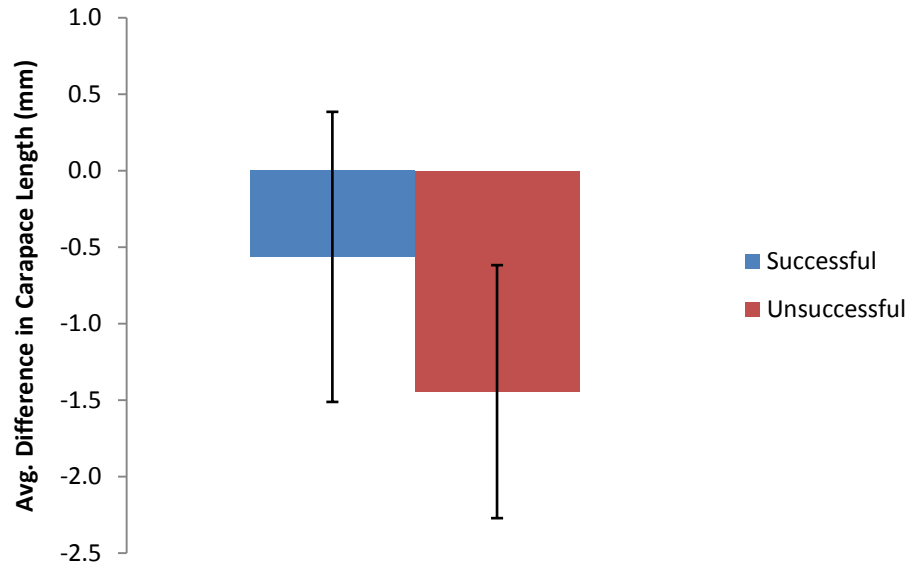
Figure 12. Average difference in carapace length within pairs relative to mating success. Mating was considered to be successful if all three stages of mating were observed. Negative values indicate instances in which the female was the larger member of the pair. n=9 and 7 for unsuccessful and successful matings, respectively. Data were analyzed via the Student's t-test. Error bars represent standard error. No significant difference was found in mating success based on difference in carapace length (p=0.589).
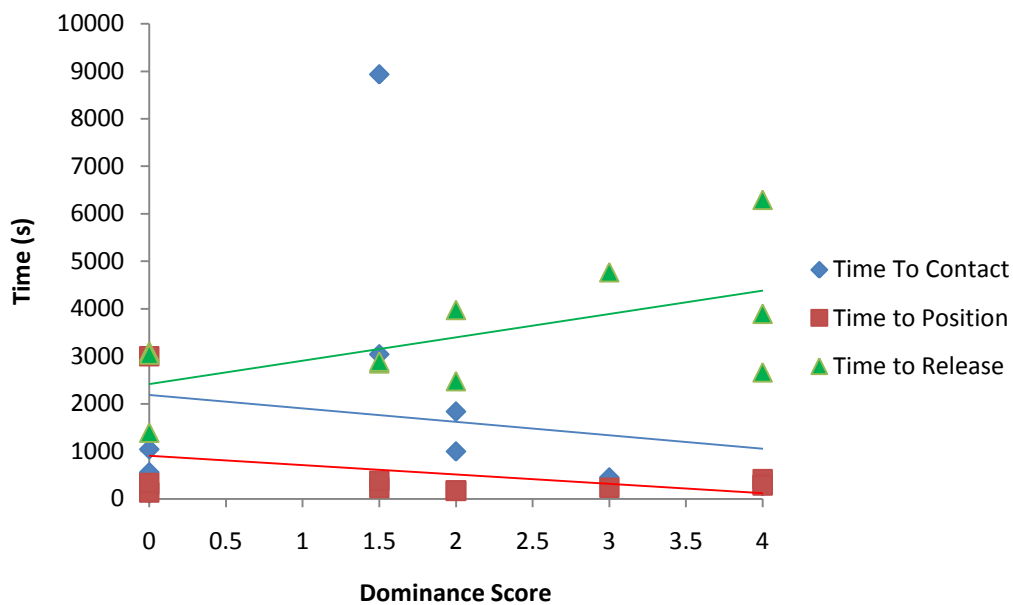
Figure 13. Time spent in various stages of mating relative to dominance score of males. n=11 for all parameters. Data were analyzed via multiple linear regression. The relationship between Time to Release and male dominance was found to be significant ($p=0.05$, $R^2=0.577$). No significant relationship was found between Time to Contact or Time to Position and dominance score ($p=0.637$, $R^2=0.255$ and $p=0.251$, $R^2=0.268$ respectively).
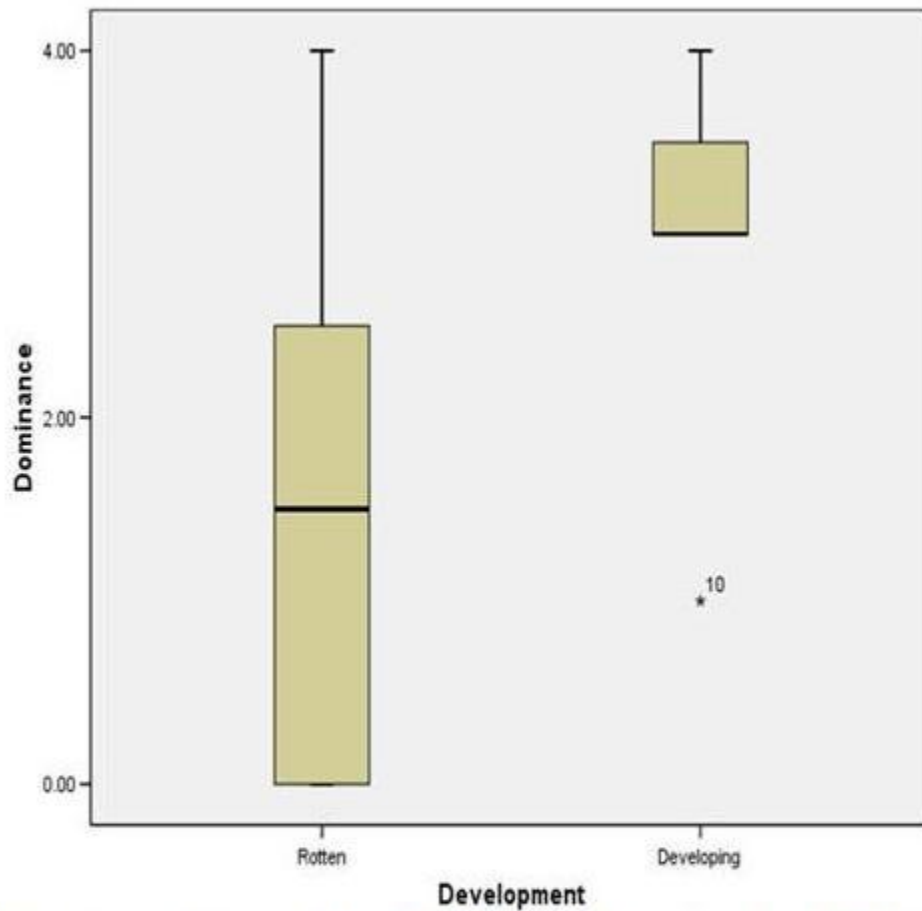
Figure 14. Dominance score of males relative to developmental status of clutches sired. Clutches were considered to be developing if approximately 50% or greater of the eggs present were observed to be developing. n=9 rotten and n=7 developing clutches. Data were analyzed using the Mann-Whitney U test. (*) indicates an outlier in the developing data set (male dominance score = 1). All other males siring developing clutches were considered to be high dominant. No significant diference was found in dominance score of males siring rotten or developing eggs (p=0.092).
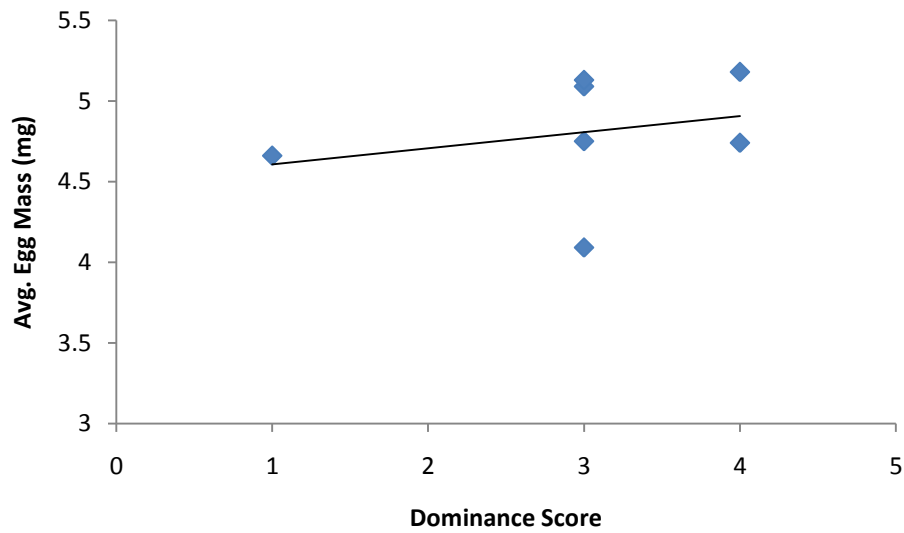
Figure 15. Average mass of eggs within a clutch relative to dominance score of contributing male. Averages were taken from a total of 10 eggs randomly sampled within a clutch. n=7 females with developing clutches. Data were analyzed via multiple linear regression. No significant relationship was found between dominance score and average egg mass (p=0.569, R^2=0.138).
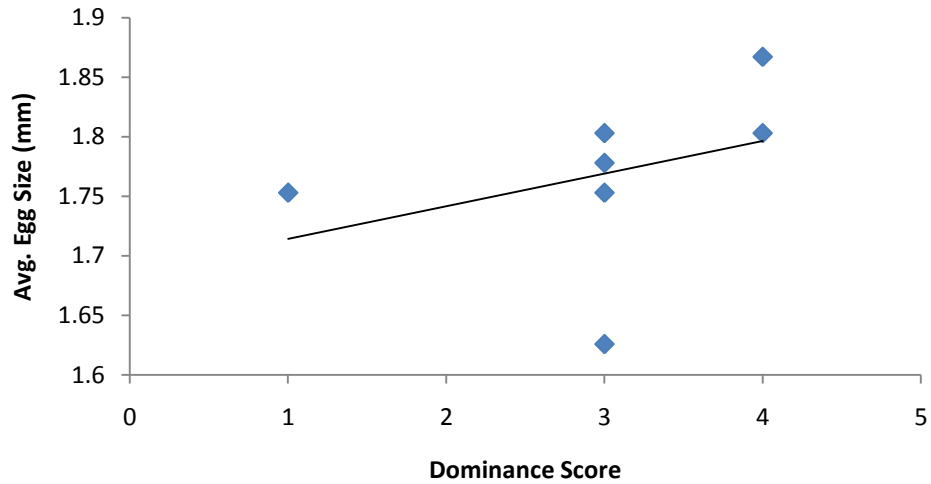
Figure 16. Average size of eggs within a clutch relative to dominance score of contributing male. Eggs were measured around their widest point. Averages were taken from a total of 10 eggs randomly sampled within a clutch. n=7 females with developing clutches. Data were analyzed via multiple linear regression. No significant relationship was found between dominance score and average egg size (p=0.416, R^2=0.137).
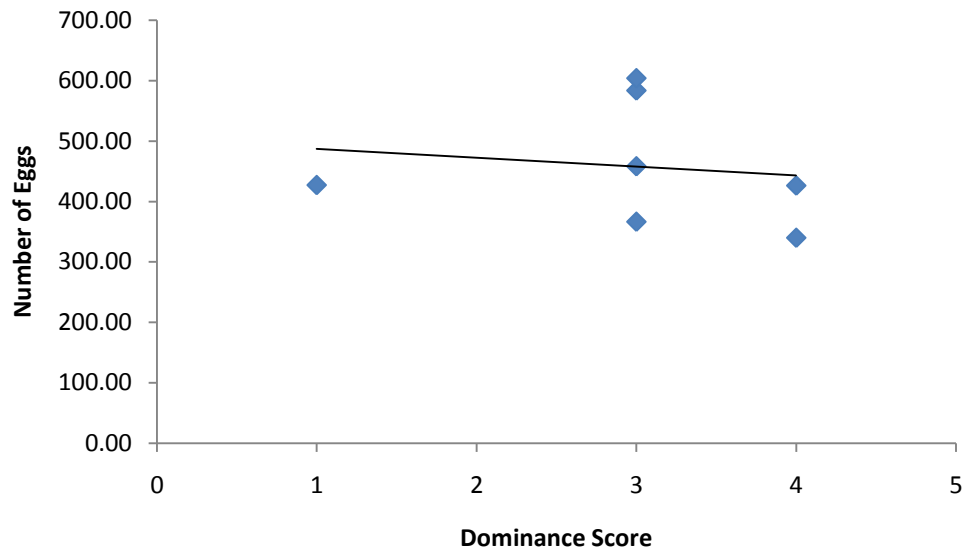
Figure 17. Average clutch size produced by females relative to dominance score of contributing male. Total clutch size was extrapolated from measurements of total clutch mass and average mass of eggs from that clutch. n=7 females with developing clutches. Data were analyzed via multiple linear regression. No significant relationship was found between dominance score and average clutch size (p=0.891, R^2=0.063).
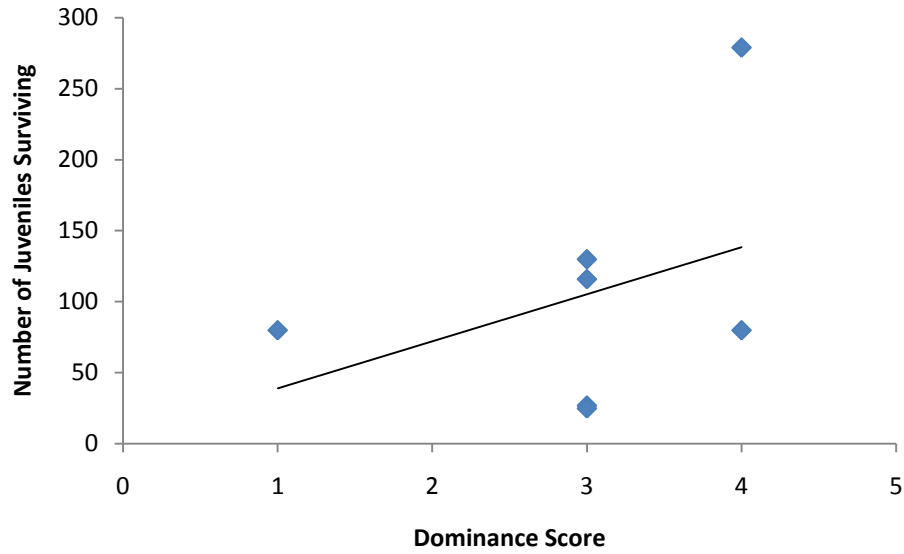
Figure 18. Total number of juveniles surviving to independence relative to dominance score of sires. n=7 females with developing clutches. Data were analyzed via multiple linear regression. No significant relationship was found between dominance score and the number of juveniles surviving to independence from the mother (p=0.395, R^2=0.150).

CONCLUSIONS

The research presented in this thesis was intended to gain insight into the role of dominance and sexual selection in the crayfish *Orconectes quinebaugensis* by testing the hypothesis that high male investment in dominance interactions evolved as a result of intra- and/or inter-sexual selection pressures.   Chapter 1 of this work sought to examine whether or not investment in agonistic interactions differ between sex and season in the crayfish *O. quinebaugensis* in light of possible sexual selection acting on the evolution of this trait, specifically testing the prediction that reproductively active males invest more in agonistic interactions than both reproductively active females and non-reproductive forms of both sexes. I reported evidence that agonistic interactions used in the development of dominance hierarchies in this species may be under sexual selection, though perhaps not as originally expected. As predicted, reproductively active males were found to spend more time in agonistic interactions and had a higher proportion of fights reaching maximum intensity than reproductively active females, and as such, dominance interactions may be under sexual selection, with males investing more during the reproductive season than females in order to maximize their own reproductive success through increased mate number. However, I also report other unexpected inferences from this experiment. Specifically, by including data from both the reproductive and non-reproductive seasons, I found no evidence for increased aggression in Form I males relative to Form II males, and no difference between sexes in the non-reproductive season. An alternate explanation may then be that females invest less in agonistic interactions during the reproductive season, when they are presumably investing in energetically expensive eggs. It is also possible that the increased investment seen in the non-reproductive season, particularly in large animals, represents investment by both sexes in some mutually valuable resource such as food or shelter.

Overall, these data indicate that investment in agonism differs by sex and by reproductive status, and may represent a dynamic system in which the identity and value of resources vary according to both sex and season, and in which dominance interactions may be under both sexual and natural selection.

Chapter 2 of this thesis examined possible influences of intra- and inter-sexual selection on dominance through advantages conferred, specifically testing the predictions that females prefer odors produced by dominant males over those of subordinates and that dominant males gain an advantage in mating through either mating more efficiently or for longer durations than subordinates. A pilot study was also conducted to test the prediction that females mated to dominant males invest more heavily in eggs through increased egg size, mass, or number than

females mated to subordinates, and that juveniles sired by dominant males will show higher survivability to independence. Though the majority of the data were unsupportive of hypotheses, it was found that dominant males spent significantly more time in post-copulatory associations with females during the mating process than subordinates. This may represent an advantage to dominant males over subordinates, as dominant males are less likely to be displaced than subordinates (Trivers, 1972; Cox & LeBoeuf, 1979; Thiel & Correa, 2004) and may allow dominant males to monopolize the female longer after mating. This may in turn increase a male's fertilization success through decreased sperm competition, achieved through either increased sperm expenditure prevention of subsequent males from mating, or from securing last-male sperm precedence. However, I cannot rule out the influence of female behavior and possible female choice in these trials. It is therefore possible that the mating process, such as time spent in post-copulatory associations, may be influenced by both intra- and inter-sexual selection if such behaviors have a heritable component.

Future work is needed to more fully explore the influences and effects of both intra- and inter-sexual selection on dominance in this and other species. Most notably, sample sizes, particularly in experiments concerning the post-copulatory female investment and juvenile survival, would need to be improved in order to be able to obtain more reliable and informative results. Other related work of interest may be to explore what sensory cues are required for mate choice in this or related species, as odor cues alone were found not to elicit a significant preference based on dominance score of males in my experiment; or to examine whether or not dominant males possess mating advantages in a more natural, social situation, as certain advantages may only manifest themselves when multiple males or females are present. It would also be ideal to examine juvenile survivorship beyond independence from the mother, as differences in survivability may manifest at later stages in development. Rearing of juveniles beyond this point and into adulthood would also allow the testing of male offspring in agonistic contests similar to those used to establish dominance in these experiments, and could be used as a direct test of heritability of the dominance trait from father to son in this species.

REFERENCES

Bergman**,** D. A., and P. A. Moore**.** 2003. Field observations of intraspecific agonistic behavior of two crayfish species, *Orconectes rusticus* and *Orconectes virilis*, in different habitats. Biological Bulletin 205: 26–35.

Bernardo, J. 1996. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. American Zoologist. 36: 216-236.

Breed, M. D., S. K. Smith, and B. G. Gall. 1980. Behavioral strategies during inter-male agonistic interactions in a cockroach. Animal Behaviour 28: 1063-1069.

Cox, C. R., and B. J. LeBoeuf. 1977. Female incitation of male comp5etition: a mechanism in sexual selection. American Naturalist 111: 317-335.

Galeotti, P., D. Rubolini, G. Fea, D. Ghia, P. Nardi, F. Gherardi, M. Fasola. 2006. Female freshwater crayfish adjust egg and clutch size in relation to multiple male traits. Proceedings of the Royal Society London B. 273: 1105-1110.

Hamr, P. 2002. *Orconectes*, pp. 585-608. In D. M. Holdich, (ed.), Biology of freshwater crayfish. Blackwell Science, Oxford.

Koga, T., Henmi, Y., Murai, M. 1993. Sperm competition and the assurance of underground copulation in the sand-bubbler crab *Scopimera globosa* (Brachyura, Ocypodidae). Journal of Crustacean Biology. 13: 134-137.

Moore, A. 1990. The inheritance of social dominance, mating behavior and attractiveness to mates in male *Nauphoeta cinerea*. Animal Behaviour. 39: 388-397.

Nol, E., Cheng, K., Nichols, C. 1996. Heritability and phenotypic correlations of behavior and dominance rank of Japanese quail. Animal Behaviour. 52: 813-820.

Murai, M., Koga, T., Young, H. 2002. The assessment of female reproductive state during courtship and scramble competition in the fiddler crab, *Uca paradussumieri*. Behavioral Ecology and Sociobiology. 52: 137-142.

Raleigh, M. J. and M. T. McGuire. 1989. Female influences on male dominance acquisition in captive vervet monkeys, *Cercopithecus aethiops sabaeus*. Animal Behaviour 38: 59-67.

Rubolini, D., P. Galeotti, G. Ferrari, M. Spairani, F.Bernini, and M. Fasola. 2006. Sperm allocation in relation to male traits, female size, and copulation behavior in a freshwater crayfish species. Behavioral Ecology and Sociobiology 60: 212-219.

Stocker, A. M. and R. Huber. 2001. Fighting strategies in crayfish *Orconectes rusticus* (Decapoda, Cambaridae) differ with hunger state and the presence of food cues. Ethology 107: 727-736.

Trivers, R. L. 1972. Parental investment and sexual selection, pp. 139-179. In B. Campbell (ed.), Sexual selection and the descent of man. Aldine, Chicago.

Urbani, N., Sainte-Marie, B., Sevigny, J.M., Zadworny, D., Kuhnlein, U. 1998. Sperm competition and paternity assurance during the first breeding period of female snow crab (*Chionoecetes opilio*)(Brachyura: Majidae) Canadian Journal of Fisheries and Aquatic Sciences. 55: 1104-1113.