

# Quantitative Behavioral Techniques for the Study of Crustacean Aggression

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## Introduction

Fighting in clawed decapod crustaceans has received considerable attention due to the presence of patent visual displays featuring conspicuous and potentially lethal weapons. General observation confirms that agonistic encounters in this group are characterized by a distinct shortage of diplomatic skills. With the exception of some mating behaviors, a meeting between two individuals of similar size invariably leads to agonistic interactions which escalate with a series of stereotyped behavior patterns until one of the combatants withdraws. To a large degree, it is physical superiority which determines the fight outcome, fostering a quick resolution in the presence of a mismatch in factors such as body and claw size, sex, or molt state. Moreover, social conditioning as a result of previous wins or losses influences both fighting behavior and the outcome of subsequent interactions on a time frame of up to several days. Even in group situations, fighting consists mainly of paired encounters; in crustaceans, interactions involving three or more individuals are extremely rare. The presence of this highly structured behavioral system offers unique opportunities to quantify the aggressive state of individuals, explore mechanisms underlying the formation and maintenance of dominance relations, investigate dynamic properties of hierarchy formation, and explore the significance of neural and neurochemical mechanisms in these behavioral phenomena. This chapter summarizes strategies for studying the neural mechanisms of aggression in crustaceans with the emphasis on a rigorous behavioral approach. These quantitative techniques focus on relatively stable, higher-order patterns in fighting behavior rather than relying simply on the frequency and duration of particular behavioral acts. It is the goal of this chapter to review and discuss previous work in the study of crustacean aggression with an explicit focus on integrating behavioral research with other subdisciplines of the neurosciences.

K. Wiese, University of Hamburg, Germany (Ed.)

## The Crustacean Nervous System

This unique selection of mini-reviews summarizes the current knowledge in all major fields of crustacean neurobiology and all levels of their CNS organization, using lobster and crayfish. It not only transfers theoretical knowledge but also describes all available contemporary and advanced techniques, such as patch clamp recordings, microelectrode techniques, immunocytochemistry and all methods of molecular genetics to identify cellular pathways of protein synthesis and peptidergic control. In summary, it is a comprehensive account of the research achievements in one of the major nervous systems alternative to the mammalian CNS.

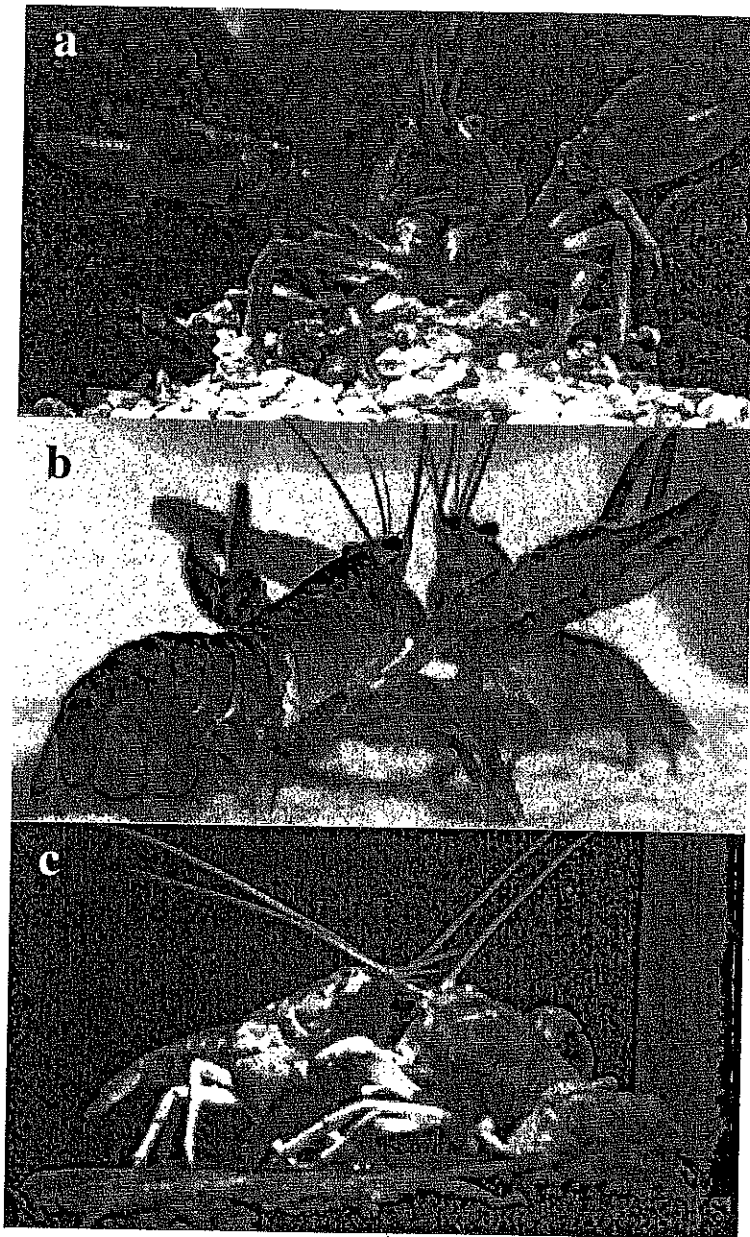
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## The Fighting Behavior of Clawed Decapod Crustaceans

Upon encountering a conspecific, clawed crustaceans will readily fight in a series of limited bouts. Individuals rely on a number of stereotyped and conspicuous agonistic behaviors with a striking resemblance across a wide range of decapod taxa, including crabs (Warner 1970; Lee and Seed 1992), lobsters (Scrivener 1971), and crayfish (Bruski and Dunham 1987; Zulantz-Schneider et al. 1999). Agonistic interactions are considered to begin when an individual approaches to within one body length of a conspecific. Forms of approach range from normal walking speeds without obvious threat displays, through rapid advances at elevated postures, to lunges with claws held straight forward and ready to grasp. In groups with established dominance relations, most challenges remain uncontested and are answered by a hasty retreat of the potential opponent. Asymmetries in variables such as body or claw size, sex, molt state, social experience, or physical condition, foster the quick resolution of a fight and have proved to be of predictive value for the eventual outcome (Barki et al. 1991, 1997; Ranta and Lindström 1992, 1993; Pavey and Fielder 1996). However, if neither attacker nor recipient retreats, a fight ensues that is characterized by a continued interchange of agonistic displays and fighting routines. Contested encounters commonly escalate through stages of increasing intensity and end when one of the combatants withdraws. Despite considerable variation in the precise form, order, and duration of the component behaviors, the typical scenario escalates with a temporal sequence of stereotyped agonistic patterns. Fight intensity increases progressively, beginning with threat displays upon first contact, continuing with phases of ritualized aggression and restrained use of the claws, and frequently ending in brief periods of unbridled combat (Bruski and Dunham 1987; Glass and Huntingford 1988; Huber and Kravitz 1995).

The beginning of a fight is marked by highly stereotyped threat displays. During meral spread (Fig. 1a) an animal directly faces its opponent with body lifted high above the substrate, claws pointing straight upwards, and antennae directed towards the back. As the opposing combatant mirrors this stance (Fig. 1b), both advance towards each other until they touch. Physical contact is initially limited to claws either held wide open or completely closed. Vigorous whips of the antennae precede escalation events when individuals signal their readiness to step up to a higher intensity. Restrained use of claws (Fig. 1c) gives way to grabbing and holding, with attempts to displace the opponent by pushing, pulling, or lifting. The highest levels of intensity, where claws are used in an unrestrained manner, tend to emerge only in later stages of fighting. At this point, individuals attempt to tear or break off the opponent's appendages using rapid movements of claws or short, upward-directed tailflips. The fight ends when one individual decides to retreat, an act which may also take a variety of forms from slowly turning or running away, to multiple tail flips followed by escape swimming.

Escalation during crustacean fighting is in close agreement with predictions from game theory models (Parker and Rubenstein 1981; Enquist and Leimar 1983; Leimar and Enquist 1984). While the escalation progresses in a stepwise manner, the animals presumably acquire increasingly detailed information concerning the



**Figure 1 a-c.** Images of fighting behavior in clawed decapod crustaceans. **a** Meral spread in the crayfish *Orconectes rusticus* - a common threat display during the early stages of an encounter. **b** Both opponents display threat towards each other in the American lobster, *Homarus americanus*. **c** Restrained use of the claws - a major component of fights in the crayfish *Orconectes rusticus*

opponents' strength and fighting abilities (i.e., assessment strategies). As individuals continue to match characteristics predictive of eventual success, increases in the intensity of encounters are accompanied by a greater incidence of injury. A highly structured behavioral system such as this effectively reduces the likelihood of damage in fighting between individuals bearing dangerous weapons. Therefore, timing of decisions by either animal to initiate, escalate, retaliate, or withdraw from encounters serves as key factor in determining the duration and progress of resulting encounters (Maynard-Smith 1974; Bishop and Cannings 1978). Encounters range from a brief meeting in cases where physical asymmetries are large, to prolonged fighting in the absence of a distinct mismatch (Scrivener 1971; Atema and Cobb 1980). Differences in body size (Ranta and Lindström 1992, 1993; Figler et al. 1995; Pavey and Fielder 1996) and claw size (Barki et al. 1991, 1997; Rutherford et al. 1995; Sneddon et al. 1997) serve as effective predictors of fight outcome, even when asymmetries are small and the opponents are otherwise matched closely (Vye et al. 1997). Prior residence by one of the opponents (Peeke et al. 1995), knowledge of resource value (Smith et al. 1994), molt stage (Tamm and Cobb 1978), behavioral strategies employed by the contestants (Guiasu and Dunham 1997), and previous social experience (Dunham 1972; Rubenstein and Hazlett 1974) also affect an individual's chances of winning.

Success in one or more prior encounters produces a lasting polarity in the outcome of future bouts between individuals - a dominance relationship has been established (Francis 1988; Drews 1993). Individual recognition, the mechanism underlying dominance in most vertebrates (Wilson 1975; Clutton-Brock and Harvey 1976), produces learned, pair-wise relationships. Although some decapods may communicate dominance in this manner (Vannini and Gherardi 1981; Karavanich and Atema 1991, 1998), a recognition of aggressive state is more common (Winston and Jacobson 1978; Copp 1986; Zulandt-Schneider et al. 1999). At a group level, dyadic relationships combine to form dominance hierarchies (Vannini and Sardini 1971; Atema and Cobb 1980), which are generally stable and (near) linear in many crustacean taxa (Wilson 1975; Schein 1975). The importance of relatively fixed individual characteristics, such as size (Bovbjerg 1953; Dingle 1983; Hyatt 1983), is often overshadowed by contextual factors (McBride 1958; King 1965; Francis 1988) and chance events (Landau 1951; Rushen 1982). Individuals frequently assume different ranks when identical groups are reconstituted repeatedly (Guhl 1953; Dugatkin et al. 1994), and social status is strongly contingent upon the order in which individuals are added to the group (Landau 1965; Bernstein and Gordon 1980). Social conditioning appears to play a central role in this, with recent winners more likely to win a subsequent fight (e.g. ~~Bark~~ Jackson 1991; Hollis et al. 1995; Hsu and Wolf 1999), and chances of future success significantly reduced in previous losers. Such winner/loser effects, along with evidence of the underlying physiological mechanisms, have been empirically demonstrated in several systems (e.g., Francis 1983; Jackson 1991; Chase et al. 1994). Support for the formation of linear hierarchies through self-assembly of initially similar entities has emerged from both theoretical models (Bonabeau et al. 1995; Hemelrijk 1999) and empirical data sets (Theraulaz et al. 1995; Goessmann et al. 2000). Animals losing to an opponent early in the formation of a hierarchy are thus likely to achieve only

relatively low rank, whereas winning early encounters predisposes individuals to obtain more dominant positions.

## **Behavioral Approaches for Studying Aggression and Social Status in Crustaceans**

A comprehensive, quantitative characterization of fighting behavior is a necessary first step in any attempt to explore the behavioral significance of neural mechanisms underlying aggression. Towards this goal we need to identify a set of key parameters that best characterize variation in fighting behavior, and devise criteria that allow us to assign numbers to the various behaviors. Furthermore, such an analysis needs to be based on clear and objective criteria that can be reliably judged by multiple observers, in different species, and in various behavioral contexts. The presence of a highly structured behavioral system in crustaceans offers unique opportunities for such a task. Over the years, ethograms have been proposed using a variety of experimental protocols in many species, including crayfish (Rubenstein and Hazlett 1973; Bruski and Dunham 1987), lobsters (Scrivener 1971), crabs (Huntingford et al. 1995), and stomatopods (Caldwell and Dingle 1975). In the majority of cases, lists of behavior patterns have been compiled and analyses have directly focused on their frequency, rate, and duration within individual bouts. Unfortunately, quantification of behavior that focuses on „what“ an animal does is intrinsically sensitive to the tremendous variability so common in behavioral systems.

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Methods detailed here attempt to extract higher order patterns that instead characterize “how” individuals conduct their fighting. Although the particulars of individual fights are variable, the underlying fight structure remains surprisingly constant. The techniques described here provide a general framework for the study of fighting which emphasizes the basic behavioral rules governing aggressive interactions in crustaceans. They aim to describe the underlying structure of fighting instead of relying on the particular course taken by individual encounters. Basic structural characteristics of fighting, such as estimates of the rate of escalation, are fairly stable even when fights feature different behavior patterns or vary in duration and intensity. Thus, a focus on structural characteristics assesses particular fighting strategies rather than providing a detailed account of activity on a minute-to-minute basis. Structural features are used to develop estimates for intrinsic aggressive tendencies, identify particular attack strategies, and determine the rules that govern decisions for escalation and retreat.

Experimental designs in aggression research commonly alter, assay, or make predictions about the aggressive state of individuals. However, by its very nature, fighting behavior is the concurrent interaction of two opponents - it cannot be observed in single individuals. Some measures do refer to the behavior of a particular individual, such as which animal initiates the fight, which first escalates to a particular level of intensity, or which animal finally wins or loses. These variables can be used in a variety of experimental designs and face few restrictions. The great majority of variables, however, characterize the complex interactions between internal states and particular decisions taken by both individuals, and thus cannot be assigned at the individual level. The inherent

ambiguity resulting from the dyadic nature of agonistic interactions can be avoided: when (1) both individuals of a fighting pair receive identical treatments; or when (2) the behavior of one individual is judged in interactions with a standardized opponent or model. In the former design, the characteristics of the interaction can be attributed jointly to both combatants. In the latter, the standardized opponent allows different characteristics of the interaction to be associated with particular attributes or characteristics of individual combatants. Experiments involving long-term manipulations present additional design problems. For instance, one cannot easily observe chronic effects of drug treatment on social status as dominant/subordinate relationships are generally unstable and require continued reinforcement (Goessmann et al. 2000). Without re-pairing, predictable asymmetries as a result of social status in lobsters and crayfish weaken over time and are all but undetectable after a few days of isolation (Karavanich and Atema 1998; Rutishauser, unpubl.). Measures of behavior that avoid the added complication of time-related changes in dominance relationships can be obtained relative to predictable, size-based relationships, by pairing treated animals with standardized randomly selected larger or smaller opponents. A.R.

For the purposes of quantification, individual dyadic interactions represent the basic elements of a behavioral analysis of aggression. The level of behavioral detail required for each interaction is determined by the particular question under study. For example, questions addressing aspects of communication during a fight usually require the collection of point-by-point information at fine-scale temporal resolutions for all individuals included, effectively reducing the total number of interactions that can be sampled for a given amount of effort. At the other extreme, a few select summary measures may be sufficient to characterize an extended number of interactions when studying the effects of behavioral or pharmacological manipulations in a large number of pairs or groups. Considering the extent of variability common to most behavioral systems, a faithful description will profit from maximized sample sizes and from sampling across a large number of individuals and pairs.

Laboratory settings allow us to optimize conditions fostering the occurrence of escalation events in dyadic encounters. Although field studies of crustacean aggression are limited, it appears that with proper housing conditions, fighting behavior in lab settings closely matches that observed in more natural surroundings. In both instances, individuals rely on the same stereotyped behavior patterns and escalate over time until dominance is established. While fighting in the field is usually resolved in brief encounters at relative low intensities, high-intensity encounters (Karnofsky et al. 1989) and even injurious fighting have been reported (Marden 1973). In order to characterize the full range of possible interactions, behavioral studies should include interactions ranging from the least intense, which are common in brief encounters, to the most intense, usually observed during extended bouts. Conditions for prolonged fighting are fostered when the sum of physical and experiential asymmetries is small (Scrivener 1971; Atema and Cobb 1980)- conditions rarely met during chance encounters in the field. Laboratory studies, however, provide greater scope for control of these asymmetries. In the lab, a careful selection of opponents for size, sex, and molt state greatly increases the chance that escalated fighting will be observed. As prior

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experience also influences the course of an interaction, social experience must be similarly controlled. Isolating experimental animals in individual containers can be an effective means of reducing the experiential factors resulting from previous dominance relationships. In our experience, visual and tactile isolation from conspecifics for a minimum of 3 days is sufficient to elicit interactions that reliably feature all levels of fighting intensity. When animals are not housed in isolation, care must be taken to ensure proper living conditions to minimize injury and incidence of cannibalism. Enclosures should be large and provide shelters that mimic the animal's natural habitat. Field observations suggest that individuals tend to encounter one another singly in brief interactions when substrates are rich in shelters. Animals housed in large aquaria with abundant opportunities to seek refuge seldom exhibit cannibalism, and claw banding is not required. Regardless of the housing method employed, simple selection procedures, such as using size-matched, intermolt individuals of the same sex, with intact and normal-sized appendages, ensure the maximum range of behavioral observations during controlled encounters, and are thus critical to experimental design.

## **A Quantitative Account of Aggression and Social Status in Crustaceans**

Interactions in clawed decapods begin when an individual approaches to within one body length of an opponent, which overtly reacts to the approach. A series of variables can be quantified for each interaction, including its duration and maximum intensity, identity of the initiating animal, time to first instance of different intensities, identity of the individual first escalating to a given intensity, count of instances at which the highest intensity events are reached; and the identity of the animal that eventually retreats (Table 1). Intensities of encounters are judged as follows: (0) no contest: neither animal attacks or one animal consistently retreats from the advances of the other; (1) threat postures: neither animal retreats and at least one individual uses threat displays or ritualized fighting without physical contact; (2) restrained physical contact: both animals contest the interaction and at least one individual touches the opponent with antennae or claws held open or closed; (3) claw lock: both animals contest the interaction and at least one individual uses its claws to grasp the opponent; (4) strike and rip: neither animal retreats and at least one individual makes unrestrained use of the claws in an attempt to rip or tear off the opponent's appendages. The rationale for this intensity scale is based on an observed increase in the incidence of injury from no physical contact to unrestrained use of claws. An interaction ends when an animal retreats from its opponent to a distance exceeding one body length and remains there for more than 5 s.

The behavioral effects of experimental manipulations may be examined with a multiplicity of statistical methods. Univariate analyses explore differences in behavioral variables as a function of treatments using either analysis of variance (ANOVA) for continuous data (i.e., duration) or negative LogLikelihood (-LLH) for categorical and frequency variables (i.e., intensity). Moreover, any statistical

**Table 1** Summary of behavioral measures used in the analysis of crustacean fighting

- Estimates of aggressive state and fighting strategies were based on an analysis of individual dyadic interactions. This approach aimed to attach probability estimates to a series of decisions, such as how likely an individual was to initiate an encounter, escalate from any given intensity to any other, retaliate when the opponent escalated, or to withdraw from further fighting. Dyadic interactions represent individual records in a relational database, subdivided into two sections. Interactions derived from the same experimental series share identical content in Fields 1-8 whereas Fields 9-19 are specific for each interaction
- 1. Experiment: denotes a particular experimental set of individual encounters. The identities of animals A and B were held constant for all interactions belonging to this series
- 2. Animal A: refers to a unique ID that matches an individual participating in the encounter with detailed records containing sex, size and molt records stored in another data base. Designation as A was used in all cases where a focal animal was subjected to a particular treatment (e.g., infusion of pharmacological substances, unique social experiences, or particular feeding regimes).
- 3. Animal B: refers to the ID that uniquely identifies the second individual in the encounter. When an individual represented a standardized opponents it was always designated as B.
- 4, 5. Weight A and Weight B: record body weights of animals A and B on the day of the experiment
- 6, 7. Sex A and Sex B: list the sex (male, female) of animals A and B
- 8. Experiment Start: registers the time of day (recorded on the tape with precision in seconds) at which the divider separating the two opponents is removed
- 9. Interaction Start: records the time (from the tape) at which the two opponents advance to within one body length and visibly react to each others' presence
- 10. Who Initiates: registers the individual playing the more active role in initiating the encounter. Designation of individuals as A or B matched with the content of fields 2 and 3. The field was left blank in situations where both individuals initiated at the same time
- 11. Initiation Speed: lists the speed with which the initiator commences the interaction: (1) slow approach (<1 body length -s), (2) rapid approach (>1 body length -s), (3) lunge
- 12. Intensity 2: marks the first instance of intensity 2 (restrained use of claws) during the encounter. Fields 12 and 13 are blank if restrained use of claws did not occur
- 13. Who Intensity 2: notes which individual first escalated to intensity 2
- 14. Intensity 3: marks the tape time of the first instance of intensity 3 (claw lock) during the encounter. Fields 14 and 15 are blank if claw lock did not occur
- 15. Who Intensity 3: notes which individual first escalated to intensity 3
- 16. Intensity 4: marks the first instance of intensity 4 (unrestrained use of claws) in the encounter. Fields 16 and 17 are blank if intensity 4 did not occur



- 17. Who Intensity 4: notes which individual first escalated to intensity 4
- 18. Maximum Intensity: lists the highest level of intensity that was reached in the encounter. Intensities (range 0 to 4) were judged according to criteria described above
- 19. N Intensity 4: records how often the fight included the highest intensity (instances of strike and rip)
- 20. Interaction End: records the time when an animal turned or walked away from its opponent, increasing the distance between them to more than one body length. Interactions were considered separate if they featured a break of at least 5 s
- 21. Who Retreats: marks the individual who walks or turns away increasing the distance between them to more than one body length. Designation of individuals as A or B matched the content of fields 2 and 3. The field was left blank in situations where both individuals retreated at the same time.
- 22. Retreat Speed: lists the speed with which the retreating individual withdrew from the interaction: (1) slow retreat (<1 body length/s), (2) rapid retreat (>1 body length/s), (3) tailflip
- The following measures are derived based on entries listed above
- 23. Relative Start Time: calculates the number of seconds elapsed between the start of the experiment (#8) and the start of the interaction (#9)
- 24, 25, 26. Start Intensity 2, 3, and 4: calculates the number of seconds elapsed between the start of the interaction (#9) and first instances of intensity 2 (#12), 3 (#14), and 4 (#16)
- 27. Duration: measures the length of the interaction in seconds between start (#9) and end (#20) of the interaction

analysis should include some form of nested design providing separate estimates for variation between treatments as well as between pairs within a treatment (Goessmann et al. 2000).

Aggression, by its very nature, is a multidimensional concept. A problem in assigning specific behavioral effects therefore arises when fighting characteristics are correlated to some degree. Escalating fights will automatically reach higher levels of intensity the longer they last (Huber and Kravitz 1995). Moreover, attacking crayfish have a higher chance of success (Rubenstein and Hazlett 1974), and the course a fight takes may depend on which animal initiates and which one retreats. Any specific primary behavioral effect thus leads to secondary changes in all variables correlated with the effect. When such dependencies exist, variables need to be considered simultaneously within a multivariate design in order to include information about these relationships. In addition, the degree to which variables occur together provides valuable information about the underlying behavioral structure of fighting. Principal components analysis (PCA) has been successfully employed to identify behavioral measures (dependent variables) that tend to vary as linked groups (Huber and Kravitz 1995). Multivariate analysis of variance (MANOVA) and discriminant function analysis (DFA) have also proved fruitful in explorations of crustacean fighting behavior (Huber et al. 1998; Huber and Delago 1998; ~~Delago et al., submitted~~). These techniques provide an objective and reproducible way in which to examine whether complex behavior differs among treatments and, if so, which behavioral characteristics best distinguish

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between them. Such analyses are of particular value in behavioral pharmacology where a substance of unknown specificity may alter behavior in complex ways, potentially affecting several different behaviors simultaneously.

### Summary of Recent Work Using Quantitative Measures of Aggression and Social Status in Crustaceans

Fighting behavior and underlying aggressive tendencies are undoubtedly influenced by a complex and diverse array of control mechanisms. The techniques described above were used initially to identify behavioral mechanisms in crustacean fighting and in the formation of dominance hierarchies, such as effects of social conditioning resulting due to previous wins/losses. Subsequently, changes in behavior resulting from acute and chronic manipulations of serotonergic systems were summarized.

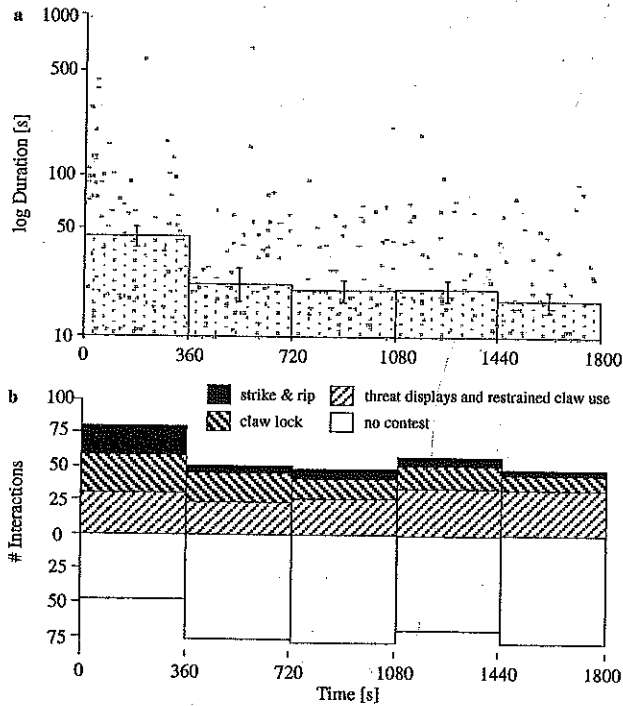


Figure 2 a, b. Characteristics of fighting behavior are summarized for 471 agonistic interactions in size-matched pairs of male crayfish *Orconectes rusticus*. a Durations are plotted for individual interactions observed during 30 min of fighting. Means ( $\pm$  SE) are displayed for consecutive 6-min intervals. b The frequency of interactions with different maximum intensities is graphed for 6-min intervals. Intensity 1 (threat displays) and 2 (restrained physical contact) are combined

Changes in fighting behavior associated with the development of dominance relationships over a period of days were analyzed in groups of crayfish. The number of agonistic challenges, their mean duration, and the maximum intensity reached were initially high, but decreased steadily as the hierarchy developed (Fig. 2). In all groups, linear hierarchies emerged and became increasingly stable over time. Winning influenced subsequent fighting behavior on two distinct time scales: in the short term, recent winners became progressively less likely to retreat; also, individuals that occupied dominant positions for several days became increasingly likely to escalate to higher intensities early in an encounter. Both effects biased the outcome of future interactions such that winning enhanced further success and losing decreased an individuals subsequent chances for the acquisition of dominant status. (Goessmann et al. 2000).

The ability to measure aggressive state (Huber and Kravitz 1995), combined with the identification of behavioral differences in need of a physiological explanation (Goessmann et al. 2000), provided the impetus to explore the neurochemical basis of decapod aggression. A quantitative analysis of behavior permitted (1) the identification of meaningful behavioral differences that can be matched with variability in brain mechanisms, and (2) exploration of the effects of physiological, molecular and biochemical manipulations at the level of intact, behaving organisms. In crayfish, as in other decapod crustaceans, increased serotonin function has been closely associated with heightened aggressive or dominant behavior (Edwards and Kravitz 1997). Direct injection elicited stereotypical agonistic behaviors (Antonsen and Paul 1997), and produced a posture resembling meral spread, a threat stance commonly seen in dominant animals (Livingstone et al. 1980). Serotonin may act concurrently as a gain setter in the nervous system and periphery, resulting in an enhanced performance of the motor programs that generate dominant threat postures (Kravitz et al. 1983; Harris-Warrick and Kravitz 1984; Kravitz 1988, 1990; Ma et al. 1992). Neurons within local circuits controlling tail flip, a common behavior of retreat, undergo changes in responsiveness in the presence of serotonin (Glanzman and Krasne 1983, 1986; Bustamante and Krasne 1991). These changes were characterized by a polarity in the direction of serotonin-induced postsynaptic activity between dominant and subordinate animals, and appeared to result from a time-dependent turnover in distinct serotonin receptor subtype populations (Yeh et al. 1996, 1997). Thus, in clawed decapod crustaceans, the effects of serotonin are broad, and findings suggested a likely role of the amine for the causation of aggression in this group of animals.

The infusion of small amounts of serotonin into the hemolymph of freely moving crayfish enhanced fighting behavior and reversed the natural dominance relationships predicted by size, such that larger animals no longer prevailed (Huber et al. 1997; Huber and Delago 1998). Serotonin-treated animals continued to engage their opponents in extended bouts of fighting, even in situations that carried a substantial risk of injury. This behavioral reversal resulted largely from an altered decision to retreat, without significantly changing the way in which fights were initiated, or how they escalated. Serotonin treatment thus changed an animal's aggressive motivation or willingness to fight by reducing its ability to behave like a subordinate. The behavioral characteristics of acute serotonin infusion (Huber et al. 1997; Huber and Delago 1998) closely match behavioral

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findings ascribed to short-term winner effects (e.g., dominants are less likely to retreat; Goessmann et al. 2000), raising the intriguing possibility that these are closely linked.

To further characterize the precise neurochemical mechanisms involved in such aggression-enhancing effects, serotonin reuptake mechanisms were explored using the selective serotonin reuptake inhibitor (SSRI) fluoxetine (~~Delago et al., submitted~~). Changes in fighting behavior were quantified in crayfish and lobsters that received chronic infusions of fluoxetine for up to 5 weeks. A combination of univariate and multivariate statistical techniques demonstrated that treatment with fluoxetine was generally accompanied by aggression-enhancing effects, similar to, but more subtle than those previously described for acute infusions of serotonin. Particularly during the initial days of treatment, individuals were less likely to retreat from larger opponents, resulting in longer, more intense fighting. These behavioral findings are in agreement with earlier studies which found that acute infusion of fluoxetine and serotonin blocks the aggression enhancing effects of serotonin (Huber and Delago 1998). Together, these studies confirm the central importance of serotonin reuptake in regulating short-term changes in decapod aggressive motivation.

A link between aggression and steroid hormones also appears likely. Studies reporting changes in the rate of escalation have commonly focused on the (steroid controlled) molt cycle (Tamm and Cobb 1978; Steger and Caldwell 1983), where measures of aggression matched the profile of ecdysteroids such as 20-hydroxyecdysone (Baldaia et al. 1984; Graf and Delbecque 1987; Snyder and Chang 1991), and direct injection of ecdysone increased aggression in stomatopods (R. Caldwell, pers. comm.). It is anticipated that this family of molecules is likely to make important contributions to changes in behavior over longer time scales during the formation of dominance hierarchies (e.g., the willingness of crayfish to escalate much more quickly after they have retained dominant status for several days).

The results of studies described here emphasize the benefits of the crustacean model. The ability to reliably characterize agonistic behavior, combined with the structural elegance of the crustacean nervous system, has provided key insights in our understanding of the complex neural basis of aggression. The ultimate challenge is now to understand aggression at the level of individual neurons. Continued exploration of the crustacean model systems described here promises to speed attainment of this eminently practical goal.

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