

TEMPORAL DYNAMICS AND COMMUNICATION OF WINNER-EFFECTS IN THE CRAYFISH, *ORCONECTES RUSTICUS*

by

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Summary

A variety of factors influences the formation of hierarchical structures, and can include an altered aggressive state, an ability to physically dominate, and previous agonistic experience. Using male *Orconectes rusticus*, we tested the duration of the winner effect by varying the time between a winning encounter and a subsequent encounter by a 20, 40 or 60-minute interval. Varying the time between the two fights significantly altered the probabilities of initiating fight behaviour and of winning a fight. A crayfish with a 20-minute delay between its winning experience and its subsequent fight was significantly less likely to initiate fight behaviour and significantly more likely to win its next fight than was an animal whose next fight was delayed for 40 or 60 minutes. We then investigated whether the dynamics of this winner effect were influenced by perception of odour signals during agonistic interactions by blocking the chemo- and mechanoreceptors on the antennae and antennules to prevent reception of relevant cues communicating social status. Individuals fighting an opponent with this loss of sensory information were significantly more likely to initiate a fight, but then escalated at a slower rate to a higher fight intensity level. In addition, individuals had a decreased chance of winning an agonistic bout against an opponent deprived of sensory input from the antennae and antennules.

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Introduction

Aggression plays an important role in the lives of many animals (Wilson, 1975). Activities relating to aggressive acts, specifically fight behaviour, are termed agonistic interactions (Scott, 1951). It is through these types of interactions that dominance hierarchies are established. When a high social status is obtained through these interactions, the status confers an individual with many advantages, including increased access to food, mates, and shelter, while decreasing access for subordinates. The use of dominant status to achieve a higher fitness is found in many invertebrate (Bell & Gorton, 1978; Trunzer *et al.*, 1999) and vertebrate taxa (Tilson & Hamilton, 1984). One invertebrate example, the American lobster *Homarus americanus*, acquires shelters by defeating opponents in agonistic bouts and thereby increases its access to available food and mates (Hyatt, 1983; Atema, 1986; Cromarty *et al.*, 1999). Because of such benefits, dominant individuals experience increased fitness over subordinate individuals (Wilson, 1975). Aggression can be costly and possibly injurious, but when used in an appropriate contextual manner can be quite invaluable to acquiring these benefits.

Crustaceans, particularly crayfish, have been used as a model system to study the formation and characteristics of dominance hierarchies because of the ritualized nature of their agonistic bouts (Bruski & Dunham, 1987), the formidable weaponry involved (Garvey & Stein, 1993), and the use of sensory information during such encounters (Zulandt-Schneider *et al.*, 1999, 2001). An agonistic encounter typically begins when an individual approaches a potential opponent. It may then progress to a series of threat displays and if neither individual retreats nor escapes, progress to a gradual increase in fight intensity (Huber & Delago, 1998). In the most intense interactions, crayfish rely on periods of unrestrained fighting where each individual attempts to injure an opponent by grasping at chelae, legs, or antennae. Fighting will continue until one individual retreats, usually signified by a tailflip away from the opponent (Bruski & Dunham, 1987). An individual is considered subordinate when it begins to retreat consistently, thus allowing unimpeded access to resources for the victor.

Asymmetries in fighting ability or resource holding power (RHP) may exist through some intrinsic feature or extrinsic circumstance that favors one contestant (Maynard Smith & Parker, 1976). Asymmetries serve as accurate predictors of dominance during interactions between pairs of crayfish and

may include physical body size (Bovbjerg, 1953, 1970; Rubenstein & Hazlett, 1974; Berrill & Arsenault, 1984; Pavey & Fielder, 1996) and chelae size (Garvey & Stein, 1993; Rutherford *et al.*, 1995). Other asymmetries such as prior residence (Peeke *et al.*, 1995), differing fight strategies (Guiasu & Dunham, 1997), and previous social experience (Rubenstein & Hazlett, 1974) or history in agonistic encounters (Daws *et al.*, 2002) determine the outcome of agonistic interactions.

An animal's previous winning or losing experiences may affect the outcome of subsequent interactions, namely the probability of winning the next agonistic interaction (Dugatkin, 1997; Hsu & Wolf, 1999). Individuals that experience a win during an agonistic interaction are more likely to win the next encounter against both familiar and naive opponents. This increased likelihood of winning is termed the winner effect. The converse is also true for the loser of an encounter, resulting in an increased probability of losing a subsequent fight. In general, both winner and loser effects have been observed in several species of fish, including the blue gourami, *Trichogaster trichopterus*, (Frey & Miller, 1972), paradise fish, *Macropodus opercularis*, (Francis, 1983), and the green swordtail fish, *Xiphorus helleri*, (Franck & Ribowski, 1987; Beaugrand *et al.*, 1991), birds (Jackson, 1991), and insects (Moore *et al.*, 1988, 1997; Whitehouse, 1997). However, winner effects do not always occur for all animals (Francis, 1983; Beacham & Newman, 1987; Schuett, 1997).

Moreover, winner and loser effects are time dependent. In some species, a prior losing experience causes an individual to lose subsequent encounters after as long as 15 to 24 hours (Francis, 1983, 1987; Beaugrand & Zayan, 1985; Beacham & Newman, 1987; Bakker *et al.*, 1989). In experiments with pumpkinseed fish, this winning effect was present immediately after a previous win, and after a time interval of 15 minutes. After 1 hour, however, previous winners no longer held an advantage against opponents (Chase *et al.*, 1994). However, Hsu & Wolf (1999) demonstrated that winner effects in a cyprinodont fish can last for at least 48 hours. Winner effect durations are variable and often species specific.

While the proximate causation of winner effects is not well understood, several factors may be contributing to a previous winner having an increased propensity to win future interactions. Winner effects may be a result of intrinsic changes or changes in the internal motivation of the winning individual. These changes could involve alterations in the way an individual perceives

the fighting ability of an opponent. For example, animals may associate the size of an opponent with its fighting ability (Otronen, 1990) or alternatively an animal may assess its own resource holding potential (RHP) compared to the population distribution of fighting abilities (Parker, 1974; Whitehouse, 1997). The causation of winner effects could also include changes in serotonin levels that have been shown to produce both heightened aggressive states and an increase in agonistic behaviours (Antonsen & Paul, 1997). If intrinsic causation is responsible for the results of agonistic interactions, an animal that has had a prior winning experience may function as a 'successful' fighter and thereby fight more readily in future agonistic interactions. These effects are likely caused by changes in internal motivation, but may also be mediated by a communication of status to which a potential opponent interprets and responds.

Alternatively, the winner effect may result from extrinsic changes or external characteristics. Such changes could include recognition of a conspecific with a heightened aggressive state (Copp, 1986). In Crustacea, recognition of aggressiveness could be accomplished visually by examining posture (Thorpe & Ammerman, 1978; Winston & Jacobson, 1978; Bruski & Dunham, 1987) or by detecting a change in the physiological state of the opponent that is expressed externally by chemical cues. For instance, serotonin plays different roles in the neurochemistry of dominant and subordinate individuals (Yeh *et al.*, 1996, 1997). This intrinsic alteration could possibly be expressed extrinsically through chemical signals, such as metabolites, that are released by an individual with a previous winning experience during an encounter in the environment (Zulandt-Schneider *et al.*, 1999, 2001).

Detection of chemical signals through the use of antennae and antennules has been well documented in crustaceans. In lobsters, chemical cues released with the urine are important in status and/or individual recognition (Karavanich & Atema, 1998) and mating behaviour (Snyder *et al.*, 1992, 1993). Karavanich & Atema (1998) also demonstrated that chemical cues influence the progression and outcome of agonistic encounters in lobsters. When individuals were deprived of the ability to detect odours by obstructing the chemoreceptors (anosmic), they were unable to recognize familiar opponents. This lack of individual recognition altered both the intensity and duration of the repeated agonistic encounters, producing second fights that were similar to the first interaction between the individuals. Zulandt-Schneider

et al. (2001) demonstrated that crayfish determine the social status of opponents by using chemical cues released with the urine of the crayfish, *Orconectes rusticus*. When these cues were removed, agonistic battles were longer and reached higher intensity levels (Zulandt-Schneider *et al.*, 2001). These studies indicate that information pertaining to the behavioural state of an individual can be transmitted through chemicals released with the urine and can influence the behaviour of its opponent. Zulandt-Schneider *et al.* (1999) have also shown that Louisiana red swamp crayfish, *Procambarus clarkii*, predominantly rely on urine chemical cues to communicate dominance status.

The goals of this study are two-fold. In male crayfish (*Orconectes rusticus*), we first explore the temporal dynamics of winner effects and then examine the roles of the antennae and antennules in receiving communication during agonistic encounters. First, by focusing on fight dynamics, we aim to determine whether an individual's probability of initiating and winning fights changes as a function of the time elapsed between a winning experience and a subsequent encounter. Secondly, by removing the ability of an opponent to detect chemical and mechanical cues, we tested whether the antennae and antennules influence the fight dynamics of agonistic encounters.

Materials and methods

Animals

All crayfish used in this study were male *Orconectes rusticus*, collected from the Portage River near Bowling Green, Ohio. All individuals consisted of intermolt, form I males with fully intact appendages (Avault & Huner, 1985). Animals were tactilely and socially, but not chemically isolated in individually ventilated plastic containers (177.8 mm ID), maintained in a tank with re-circulating water at a constant temperature (23°C) and a 14h : 10h (light : dark) cycle for a minimum of one week prior to the experiments. Crayfish were fed 1 rabbit food pellet three times per week. Descriptive statistics (mean \pm SEM) for a total of 234 crayfish used in this study are carapace length (31.1 \pm 5.4 mm), chelae length (29.7 \pm 7.7 mm) and weight (11.3 \pm 5.8 g). Crayfish were marked individually on the dorsal carapace using whiteout (Liquid Paper[®]) and were used only once in the course of the study. At the conclusion of this study, the animals were returned to the branch of the Portage River where they were collected.

Time dependency of the winner-effect

Fight trial

In order to determine the time dependence of a winner effect, three crayfish were used for each trial. Each crayfish was placed in a separate compartment of a fight arena (Fig. 1) that

was constructed of opaque Plexiglas ($40 \times 40 \times 14$ cm). The arena was divided into four quadrants of equal size ($20 \times 20 \times 14$ cm) using opaque retractable walls. The arena was filled with 15 liters of de-chlorinated water. After a fifteen-minute acclimation period, a wall (1 in Fig. 1) was removed and crayfish (A) and (B) were allowed to interact. Crayfish (B) was measured to be a minimum of 25% smaller in carapace length (24.1 ± 3.5 mm), chelae length (20.2 ± 4.6 mm) and body mass (4.83 ± 2.2 g) than crayfish (A) (carapace: 33.0 ± 4.1 mm; chelae: 32.5 ± 6.2 mm; weight: 13.19 ± 5.2 g). All three measurements were taken to ensure that crayfish (A) would have a winning experience and would thus emerge as the dominant individual at the end of the fight. These two individuals were allowed to interact for a single encounter; *i.e.* until one individual retreated or tailflipped away from its opponent creating dominant-subordinate relationship. Simultaneously, wall (2 in Fig. 1) was removed, and the future opponent, crayfish (C) (carapace: 33.0 ± 4.1 mm; chelae: 32.5 ± 6.0 mm; weight: 13.15 ± 5.3 g) was allowed to explore the same amount of area as crayfish (A) and (B). This was done to ensure that all animals had the experience of seeing the walls retracted. After the first encounter concluded, all three animals were returned to their respective starting positions. Forcing the animals into their original compartments was done so that all three crayfish received the same handling treatment.

After waiting periods of either 20, 40, or 60 minutes, a wall (3 in Fig. 1) in the test arena was removed, and crayfish (A) and (C) were allowed to interact. Crayfish (A) and (C) were size matched to within 10% of carapace length, chelae length, and body mass (see previous measurements). These two individuals were allowed to interact for a single encounter; *i.e.* one retreated or tailflipped from its opponent. The test arena was cleaned after each trial with deionized water and refilled for subsequent trials. Ten trials were conducted for each time interval, using a total of 90 crayfish.

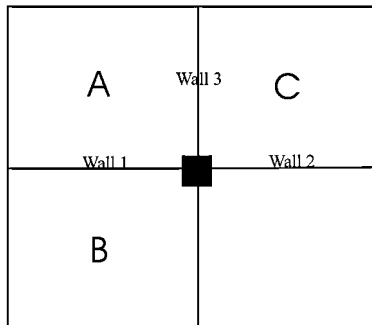


Fig. 1. The fight arena with retractable walls. Fight subject (A) was the test subject in which dominance was established by defeating the smaller crayfish (B). Fight subject (C) was the individual that (A) fought after a time interval of 20, 40 or 60 minutes in the temporal effect experiment and was also the individual with impaired chemo- and mechanoreceptors in the blocked antennae and antennules experiment.

The role of the antennae and antennules in winner effects

Chemo- and mechanoreceptor blocking technique for antennae and antennules

In the second part of this experiment, we examined the roles of chemical and mechanical communication, in relation to the antennae and antennules, in the maintenance of winner effects. First, crayfish (A) and (C) were placed in ice for ten minutes and then removed and restrained for fifteen minutes (see Fig. 1). During the restraint period, animals designated crayfish (C), received a superglue (Duro[®] containing cyanoacrylate) application to the antennae and antennules in the experimental trials. This would later serve to block this individual's chemo- and mechanoreceptors and subsequently inhibit communication involving the antennae and antennules between crayfish (A), the crayfish with a previous winning experience, and crayfish (C). The antennae and antennules of crayfish (A) were brushed with a Q-tip dipped in deionized water and also received a small dot of superglue on the carapace. The brushing was done to expose all individuals to a similar tactile treatment of their antennae/antennules and the glue was applied to control for the presence of the superglue odour. Both glue and water were allowed to dry before the animals were placed in their respective tanks. The control groups for (A) and (C) did not receive any of the experimental manipulations. A behavioural assay was performed by exposing the olfactory organs of crayfishes (A) and (C) to a directed pulse of food odour (homogenized fish) to examine if the glue had blocked the receptors. Crayfish (C), that was glued, exhibited no response to the odour whereas crayfish (A) responded by grasping at the syringe that was used to deliver the odour pulse. Both crayfish responded when the odour was directed at the walking legs. These results indicate that deionized water does not disrupt chemoreception in crayfish (A), whereas the superglue on the olfactory appendages of crayfish (C) was effective at blocking the chemo- and mechanosensory abilities of the antennae and antennules (Moore, unpublished data).

An additional experimental series was also performed that is not included in the results to address any concerns that the superglue adversely affects crayfish (C)'s behaviour. As a control, naive crayfish (A) fought naive crayfish (C) that received the superglue treatment, to examine if the glue was responsible for the experimental results shown to occur later. The trials indicate that the glue does not significantly affect the probability that crayfish (C) would initiate or win an agonistic encounter ($N = 12$, $\chi^2 = 0.0$, $p > 0.05$, $\beta = 0.07$). For both initiation and winning of fights there was an equal probability (50%) that either crayfish initiated or won an interaction. At the conclusion of the superglue experiment, all treated animals were housed in aquaria until moulting that removed the superglue from the olfactory appendages, and then these animals were returned to the wild.

Fight trial set-up

Fight trials were conducted in a similar manner described for the time dependent effects with the exception of the added superglue application and the following. The crayfish were placed in the fight arena and allowed to acclimate for fifteen minutes before any interactions took place. In fight one, crayfish (A) and (B) (same as temporal effect experiment) were now allowed to interact for 5 minutes following their first interaction to create a reinforced win. Another significant change from the previous experimental protocol included a uniform twenty-minute period between fights one and two. Following the twenty-minute period, wall (3) of the test arena was removed, and crayfish (A) was allowed to interact with crayfish (C), *i.e.* the individual whose chemo- and mechanoreceptors were blocked with superglue. Twenty

trials were conducted for both control and glue treatments, using a total of 120 animals. In the latter sections of the article, crayfish (A) will be referred to as the 'previous winner,' crayfish (B) will be the 'loser,' and crayfish (C) will be either the 'blocked' or 'intact' experimental animal.

Fight analysis and evaluation

A video camera (Panasonic wv-CL350), positioned one meter above the test arena, recorded the trials on a VCR (Panasonic AG-1980) and displayed them on a monitor (Sony PVM-1351 G). All taped fight trials were measured using a double blind design where neither experimenter nor the person analyzing the tapes had access to the particular experimental status of the individuals. The fights were analyzed using an ethogram modified in our lab (Table 1), which was based on that of Bruski & Dunham (1987). Temporal dynamics in behaviour were recorded, including the total time for the encounter and the time it took to reach different intensity levels. The identities of initiating and retreating animals were recorded for each encounter. A winner was determined when its opponent (*i.e.* the loser) retreated or tailflipped away. Instances for initiating and winning were analyzed using a multiple comparisons for proportions contingency table ($q_{0.05, \infty, 4} = 3.633$) that allows for testing analogous to the Tukey or Student-Newman-Keuls tests (Zar, 1999). Significant results are represented by giving a $q_{0.05, \infty, 4} > 3.633$ from the multiple comparisons test and a $p < 0.05$. In both experiments, initiation and winning were compared against an expectation of random behaviour (50% chance of winning or initiating) and against treatments. An analysis of time to different intensities was performed using a one-way MANOVA with a Tukey-HSD post-hoc analysis. An additional power analysis (Power = $1 - \beta$) was included for each statistical test.

TABLE 1. *Crayfish ethogram codes (used to score fight intensity levels)*

Intensity Level	Description
-2	Tailflip away from opponent or fast retreat
-1	Retreat by slowly backing away from opponent
0	Visually ignore opponent with no response or threat display
1	Approach without a threat display
2	Approach with meral spread threat display usually accompanied by an antennal whip
3	Initial claw use by boxing, pushing and/or touching with closed claws
4	Active claw use by grabbing and/or holding opponent
5	Unrestrained fighting by pulling at opponent's claws or body parts

Results

Winning effect and time dependency

A multiple comparisons for proportions contingency table was used on the proportion of fights initiated and won by the crayfish with a previous winning experience, crayfish (A), for the time intervals 20, 40, and 60 minutes ($N = 10$ for each). The proportion of fights won by the 'winner' after a twenty-minute time interval were significantly different from random, forty-minute, and sixty-minute interval crayfish ($q = 8.19, q = 4.43, q = 8.19, p < 0.05, 1 - \beta = 0.99$, respectfully); (Fig. 2). These crayfish won ten out of ten subsequent interactions. 'Winners' that experienced a forty-minute time interval won eight out of ten interactions and were significantly different from random and sixty-minute interval crayfish ($q = 3.76, q = 3.76, p < 0.05, 1 - \beta = 0.68$, respectfully); (Fig. 2). Crayfish with a sixty-minute interval between encounters won five out of ten subsequent interactions and were not significantly different from random.

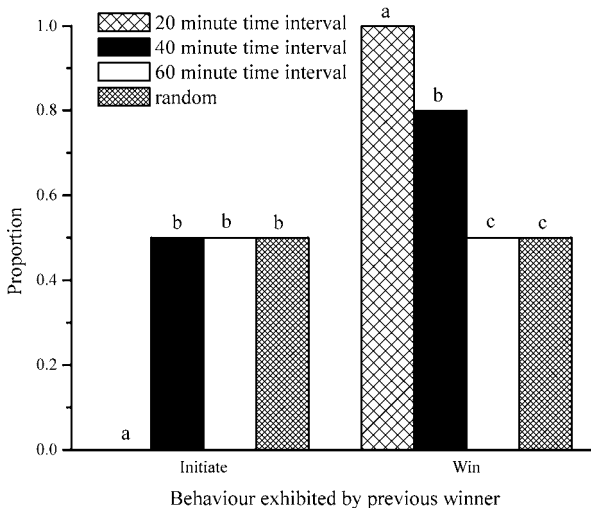


Fig. 2. Proportion of fights initiated and won by experimental crayfish 20, 40, and 60 minutes after a winning experience ($N = 10$ for each). The different letters indicate significant difference between groups. Crayfish with a 20-minute interval between fights were significantly different from all other treatments for fight initiation ($q_{0.05, \infty, 4} > 3.633, p < 0.05$). Crayfish with 20 and 40-minute intervals were significantly different from all other treatments in winning fights ($q_{0.05, \infty, 4} > 3.633, p < 0.05$).

The proportion of fights initiated by 'winner' crayfish increased along with the time interval between interactions. Zero out of ten crayfish, with a 20-minute interval between interactions, initiated in their subsequent encounters, which was significantly different from random, forty-minute, and sixty-minute interval crayfish ($q = 8.19$, $q = 8.19$, $q = 8.19$, $p < 0.05$, $1 - \beta = 0.99$, respectively); (Fig. 2). After both the forty and sixty-minute intervals, 'winners' initiated five out of ten fights and this result was not significantly different from random or against one another ($q = 0.0$, $p > 0.05$, $1 - \beta = 0.06$); (Fig. 2).

The differences in time (20, 40, 60 minute intervals) between a prior winning experience and a subsequent fight did not significantly affect the time it took the 'winner' crayfish to reach different intensity levels (refer to Table 1 for intensities measured). The average time taken to reach intensity two was 3.0 ± 0.23 s after a twenty-minute interval, 8.2 ± 2.11 s after a forty-minute interval, and 3.1 ± 1.07 s after sixty minutes. The average time taken to reach intensity three was 6.7 ± 0.45 s after a twenty-minute interval, 9.1 ± 1.69 s after a forty-minute interval, and 9.5 ± 2.25 s after sixty minutes. The average time taken to reach intensity four was 17.1 ± 1.12 s after a twenty-minute interval, 21.9 ± 4.69 s after a forty-minute interval, and 13.6 ± 1.47 s after sixty minutes. A MANOVA showed that the differences in the time taken to reach intensity levels 2, 3, and 4 between the crayfish after a twenty-, forty-, or sixty-minute time interval ($N = 10$ for each) were not significant ($p > 0.05$, $1 - \beta = 0.99$); (Fig. 3). Changes in the time interval between a previous winning experience and a second fight did not significantly affect the duration of the second encounter (MANOVA, $p > 0.05$, $1 - \beta = 0.99$).

Blocked chemo- and mechanoreceptor on antennae and antennules treatments

Blocking the chemo- and mechanoreceptors of the antennae and antennules of crayfish (C) significantly altered the outcome of the interactions (Fig. 4). Crayfish (A), that had had a previous winning experience, won 16 out of 20 (0.80) of their subsequent interactions against opponents with intact chemo- and mechanoreceptors, which was significantly more than a random distribution ($q = 5.52$, $p < 0.05$, $1 - \beta = 0.91$). When 'winning' crayfish fought against opponents with blocked chemo- and mechanoreceptors, they won 11

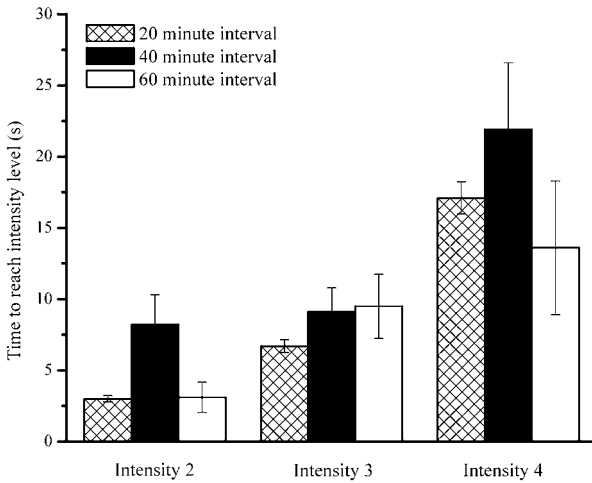


Fig. 3. Mean time (\pm SEM) to reach fight intensities 2, 3, and 4 for experimental crayfish with 20, 40, and 60-minute intervals after a previous winning experience. The differences in the time taken to reach intensity levels 2, 3, and 4 after a 20, 40, or 60-minute time interval ($N = 10$ for each) were not significant ($p > 0.05$, $1 - \beta = 0.99$) using a one-way MANOVA.

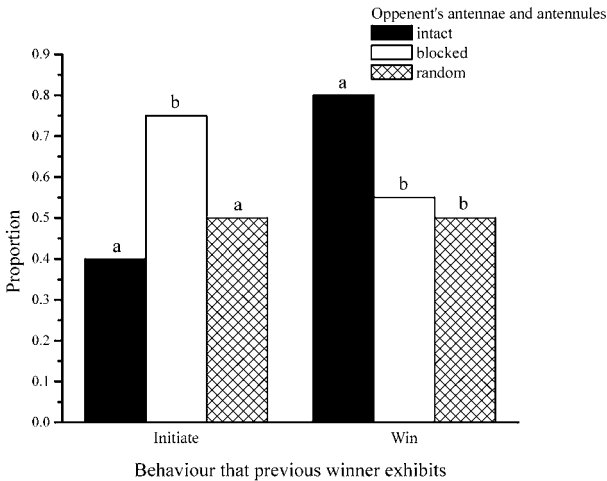


Fig. 4. The proportion of ‘winners’ that initiated and won fights after a subsequent winning experience. The different letters indicate a significant difference between blocked, unblocked, and random. ‘Winners’ that fought crayfish with blocked olfactory appendages initiated significantly more than against intact opponents or random ($N = 20$, $q_{0.05, \infty, 4} > 3.633$, $p < 0.05$). ‘Winners’ won significantly more against opponents with intact olfactory appendages than against blocked opponents or random ($N = 20$, $q_{0.05, \infty, 4} > 3.633$, $p < 0.05$).

out of 20 (0.55) of their subsequent encounters, which was significantly less than against the unblocked olfactory organ crayfish ($q = 4.65$, $p < 0.05$, $1 - \beta = 0.62$).

Blocking the olfactory organs of crayfish (C) also significantly altered the probability that the 'winner' crayfish would initiate an encounter. The crayfish with a previous winning experience, crayfish (A), initiated significantly more encounters with crayfish (C) when this opponent had received a glue application to its antennae and antennules than when it had not (Fig. 4). When 'winning' crayfish interacted with an opponent with chemo- and mechanoreceptors blocked with superglue, it initiated an interaction with crayfish (C) in 15 out of 20 (0.75) of the encounters. When crayfish (A)'s opponent had not received a glue treatment, 'winners' initiated interactions in 8 out of 20 (0.40) of the encounters. Fights against olfactory blocked opponents showed a significant difference that was greater than both random and unblocked opponents ($q = 4.50$, $q = 6.24$, $p < 0.05$, $1 - \beta = 0.79$, respectfully).

The mean time (\pm SEM) it took for 'winners' to reach levels two and three were significantly different when it fought against an opponent with its chemo- and mechanoreceptors blocked compared to an opponent with intact chemoreception. When 'winners' fought against an opponent with blocked

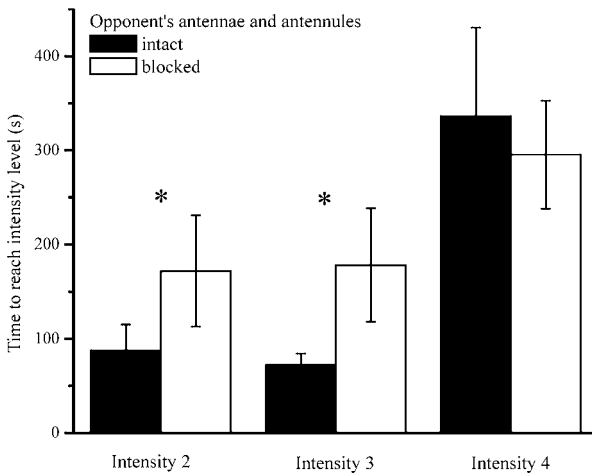


Fig. 5. Mean time (\pm SEM) to reach fight intensities 2, 3, and 4 for experimental crayfish ($N = 20$ for each). Asterisk indicates a significant difference using a one-way MANOVA with a Tukey-HSD post-hoc test. Time to reach intensity levels two and three were significantly different between the trials ($p < 0.05$, $1 - \beta = 0.99$).

antennae and antennules, it took 171.9 ± 58.9 s to reach fight intensity level two and 178.3 ± 60.1 s to reach intensity three (Fig. 5). Whereas, when 'winners' fought against the unblocked group it took 87.6 ± 27.5 s to reach intensity 2 and 72.4 ± 11.9 s to reach intensity 3, which was a significantly shorter time interval than against blocked opponents (MANOVA, $p < 0.05$, $1 - \beta = 0.99$). The time it took for either treatment to reach fight intensity 4 was 295.4 ± 57.4 s for encounters against blocked opponents and 336.4 ± 94.0 s against unblocked, which was not significantly different ($p > 0.05$, $1 - \beta = 0.99$); (Fig. 5). There was no significant difference found for the overall fight duration (MANOVA, $p > 0.05$, $1 - \beta = 0.99$).

Discussion

Our results illustrate that winner effects in agonistic interactions between male crayfish are observable after a single interaction, are time dependent, and are mediated by sensory information received through the antennae and antennules. As time increases between a crayfish's winning experience in an agonistic encounter and a subsequent one, its probability of winning diminishes, decreasing substantially over sixty minutes. Winner effects had the opposite effect on the probability that an animal initiated a subsequent encounter with an opponent. After twenty minutes, none of these individuals initiated interactions with their opponents. After forty minutes, however, this effect was no longer observed and the animal's probability of initiation was no longer different from random.

While the probability of winning and initiation by an individual were altered by previous winning experiences, the fight dynamics of the subsequent agonistic encounters were not. Both the time taken to reach different fight intensity levels, and the average duration of the second encounter were not significantly affected by the winner effect when the time between the two encounters increased.

Chase *et al.* (1994) found that in agonistic interactions between pumpkin-seed fish, the fish that won the previous interaction was more likely to defeat an opponent when the time between fights decreased. Winner effects were extinguished when the time between the first and second contest extended to one-hour. In addition, Hsu & Wolf (1999, 2001) showed that prior winning experiences affected both the probabilities of winning and the fighting

behaviours of contestants in subsequent interactions. However, these winner effects lasted for at least 48 hours. These results, along with ours, suggest plasticity in winner effect duration across different species.

An interesting finding of this study was the establishment of a winner effect after a single agonistic encounter. This was accomplished by allowing our experimental crayfish to interact with a significantly smaller opponent for a relatively short amount of time until a dominant relationship was established. After the establishment of dominance, the two crayfish were immediately separated. Since winner effects can be created after a brief agonistic encounter, this provides clues to the underlying cause of these effects.

The winner effects we examined may not be caused by long-term intrinsic physiological changes in the experimental animal. A mechanism of this type would likely require repeated exposure to winning experiences. Daws *et al.* (2002) demonstrated that crayfish, which experience two winning encounters a day for three consecutive days, have a higher likelihood of defeating significantly larger opponents in subsequent interactions than crayfish with a similar number of experiences. However, since we were able to establish winner effects with only one brief agonistic interaction, this implies that the effects seen in both studies are likely caused by short-term neurochemical changes that result from a single winning interaction and can be reinforced over repeated winning experiences. These changes may then bring about intrinsic changes in the subject that can change the probability of winning or be expressed extrinsically possibly as a chemical signal to manipulate an opponent's behaviour.

A likely intrinsic source of change underlying winner effects may be changes in biogenic amine levels. Biogenic amines, a family of chemicals found to be neurologically active, have been shown to influence the behaviour of decapod crustaceans. These compounds, which include serotonin (Brown & Linnoila, 1990), octopamine (Kravitz, 1986, 1988; Adamo *et al.*, 1995), norepinephrine (Barrett *et al.*, 1990), and dopamine (Nikulina & Kapralova, 1992; Shively *et al.*, 1997), have all been shown to be important in aggressive behaviour. Serotonin has been shown to affect the aggressive state of lobsters and crayfish (Edwards & Kravitz, 1997). Serotonin has also been demonstrated to decrease an animal's likelihood of retreat and tailflip behaviour (Huber *et al.*, 1997; Huber & Delago, 1998). If changes in neurochemistry occur as a consequence of winning, such as alteration in serotonin levels or regulatory mechanisms (production, reuptake, and receptor

up-regulation) then these changes may cause the observed short-term winner effects. It is likely that the neurochemistry is altered after a successful win in an agonistic encounter, and, that as the time between a winning encounter and a subsequent one increases, these functions return to normal. This could account for the short-lived nature of the winner effects that were observed.

Our results indicate that the dynamics of the second agonistic encounter remained the same and help to lend support to this hypothesis. If the observed winner effects were a result of a change in the information that a winner uses to assess the fighting ability of an opponent (Otronen, 1990) or a change in the manner that an animal assesses its own fighting ability (Parker, 1974; Whitehouse, 1997), one would expect that the winner would modify its fighting strategy in subsequent interactions. This should be reflected in temporal changes in fight dynamics such as the length of interactions and time taken to reach different intensity levels. Because no significant changes were observed in fight duration, it is more likely that the changes in probability of winning an encounter are a result of changes in intrinsic neurochemistry that are communicated extrinsically, rather than changing fight strategies.

The decreased tendency of crayfish to initiate interactions shortly after a winning encounter was unexpected, and contrary to the results of studies on other organisms. In studies with pumpkinseed fish, animals with a previous winning experience shortly before a second contest initiated the subsequent interaction with another fish (Chase *et al.*, 1994). This phenomenon was also observed in studies of winner effects of dark-eyed juncos (Jackson, 1991). In contrast, the second set of experiments help to clarify this anomaly. When chemical and mechanical communication between the two individuals, mediated primarily through the antennae and antennules, was prevented through the use of super glue, the crayfish with a previous winning experience initiated a significant percentage of interactions with its glue-blocked opponent. This result demonstrates that the decreased initiation seen earlier on the part of the previous winner is probably not a result of a decreased aggressive state. Rather, it is a result of the first agonistic encounter being communicated to this new opponent. When a crayfish with a previous winning experience interacts with a crayfish with its olfactory appendages intact, this individual may detect a dominant status signal from the previous winner and respond in defense.

Recognition of status has been demonstrated in many organisms. Male cockroaches use chemical cues in status recognition of potential opponents

(Moore *et al.*, 1997), and hermit crabs have been shown to recognize individual status (Winston & Jacobson, 1978). It has also been demonstrated that chemical cues contained in the urine of crayfish play a role in agonistic interactions and that through these cues an opponent's status is determined (Zulandt-Schneider *et al.*, 2001). If an opponent of the previous winner could not detect chemical cues used in status recognition, then the opponent may not display an appropriate response that would signal that the previous winner's status had been recognized. In this way, a lack of signal reception in the blocked opponent could lead to the increased tendency to initiate interactions on the part of the previous winner. When opponents were able to detect chemical cues from the previous winner, the opponent may act in a way that portrayed a subordinate status simply from the detection of the previous winner's chemical status, and thus the previous winner would no longer initiate fight behaviour more than by that expected from random.

An increase in initiation on the part of the previous winner against a crayfish with the olfactory impairment may also have been a result of a reluctance to initiate by its opponent. Due to a lack of mechanical or more likely chemical cues containing information about the previous winner's status, the opponent may have been uncertain regarding the status of the winner and therefore did not initiate contact with the winner. A decrease in initiation with the winner could also have been a result of the opponent's inability to perceive its own status or chemosensory signals. Without this information, the opponent would have been unable to make a comparison of its status to that of the winner and therefore may have been less likely to initiate contact. Regardless of a number of possible explanations behind this increase in initiation, it appears that status recognition is occurring.

The difference in fight dynamics observed between the crayfish with previous winning experiences and crayfish with and without the chemo- and mechanosensory impairment is most likely a result of status recognition as well. Crayfish that fought individuals without the ability to use their olfactory appendages spent a significantly longer time to escalate to intensity two and three when compared to those who fought non-impaired crayfish. This phenomenon has been observed in lobsters as well. Lesioning the chemoreceptors of lobsters, was shown to lengthen the duration and increase the intensity of agonistic encounters (Karavanich & Atema, 1998). Zulandt-Schneider *et al.* (2001) found that when crayfish were prevented from receiving chemical signals, they also fought for a longer period of time. These studies, along

with our results, demonstrate that information gathered from chemo- and mechanoreceptors on the antennae and antennules may help to mediate the decisions that an individual makes during an agonistic encounter. When an individual is deprived of these sensory cues, it has a reduced ability to determine the status of its opponent, and therefore may fight more intensely and for longer periods. Sensory deprivation has been shown to increase aggressiveness in other organisms as well. When visual cues used in individual recognition were removed, increased aggressiveness was demonstrated in both crayfish (Bruski & Dunham, 1987) and crabs, *Potamon fluviatile* (Vanini & Gherardi, 1981).

Sensory information received through the antennae and antennules appear to be crucial in determining the outcome of the agonistic interactions. Encounters in which crayfish were confronted with opponents with their olfactory appendages blocked lost significantly more than those who fought against crayfish with intact mechano- and chemoreceptors. This result demonstrates that winner effects observed in crayfish are likely due to chemical recognition by an opponent. Consequently, a lack of information reception decreased the probability that the previous winner would win the encounter against a blocked crayfish. The fact that the previous winner no longer consistently defeats a blocked opponent suggests that chemical or mechanical signals received by the antennae and/or antennules are a vital component to winner effects.

Because several biogenic amines have been found to play an important role in crayfish aggression in the past, it would be advantageous to examine the specific role these chemicals play in the development of winner effects. The expression of dominance could possibly be controlled by a single amine, a combination of amines or based on the relative proportions of the amines present in the urine. Whatever the controlling aspect of urine signaling is in the winner effect, we can conclude from this experiment that the winner effect is time and signal dependent, and that communication of past winning experiences appears to be a primary controlling factor of the winner effect.

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