



Prolonged exposure to social odours alters subsequent social interactions in crayfish (*Orconectes rusticus*)

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Chemical signals in aquatic systems are used to communicate social status and consequently alter aggressive behaviour. In addition, they may also function to influence the status roles of potential opponents or in part to predetermine the outcome of agonistic interactions between opponents. Here we examined whether exposure to social odours, in the absence of other sensory contact with a 'sender', alters subsequent agonistic behaviour in crayfish. Odour-receiver crayfish were exposed to one of four different odours from a 'sender' crayfish: odours from dominant (winner), subordinate (loser), or naïve crayfish, or water from a tank that did not contain a crayfish (no social odour). 'Receiver' crayfish were exposed to one of the aforementioned odours on five consecutive days. After this extended exposure, the 'receiver' crayfish was allowed to interact with an effect-evaluator crayfish that had been isolated during the 5-day exposure period. 'Receiver' crayfish that were exposed to dominant (winner) odours behaved analogous to a subordinate role in that these animals lost the majority of their fights, but they also fought more intensely compared to the other treatments. Conversely, when crayfish were exposed to subordinate (loser) odours, their agonistic interactions were less intense and they subsequently won more encounters compared to the other treatments. These results may suggest that the receivers of the dominant odours had exhausted their chemical signal reserves by responding to the signal and therefore were obliged to increase their levels of visual/tactile responses. Because their chemical reserves were no longer sufficient to reinforce the visual displays corresponding to increased fight intensity, these animals tended to lose fights. In contrast, the receivers of the subordinate odours probably had sufficient chemical reserves to communicate chemically during agonistic encounters, and consequently, won more fights.

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Intraspecific aggressive acts, such as attacking, threatening, submitting, or fleeing are usually termed agonistic interactions. It is through these types of interactions that dominance hierarchies are established (Hazlett 1966; Daws et al. 2002). A high social status, obtained through some advantage (i.e. size and/or prior experience), increases the likelihood that an animal will be able to defend food resources, shelters and mates, while suppressing success of subordinates (Maynard Smith & Price 1973;

Chase et al. 1994). Dominant status has been regarded as a reliable indicator for fitness and has been observed in a variety of organisms as increasing fitness in terms of augmented growth and mating opportunities (Bovbjerg 1953; Frey & Miller 1972; Tilson & Hamilton 1984). Dominant individuals experience an increase in fitness over subordinate individuals largely because of their increased access to and utilization of resources.

Agonistic interactions can be energetically costly and possibly injurious, but when used for the acquisition of resources, can be invaluable for increased growth or mating opportunities and decreased predation risk. Communication of social position when resources are the cause of conflict involves the exchange of information between a sender and a receiver, and the receiver's subsequent use of this information when deciding how to respond (Dawkins & Krebs 1978; Bradbury & Vehrencamp

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1998). By detecting or communicating with conspecifics of different social status, animals may avoid injury by reducing the number of competitive interactions or intensity of conflicts over resources (Salmon & Hyatt 1983; Enquist 1985; Beecher 1989).

Crustaceans are well suited for social chemical communication because they have a highly developed sense of olfaction used to detect chemicals for courtship (Atema & Engstrom 1971; Ameyaw-Akumfi & Hazlett 1975; Tierney & Dunham 1982; Bushmann & Atema 1997, 2000), maternal behaviour (Little 1975), identification and localization of food sources (Moore & Grills 1999), detecting moult status (Adams & Moore 2003), predator avoidance (Hazlett & Schoolmaster 1998), and agonistic interactions (Caldwell 1992; Karavanich & Atema 1998a, b; Breithaupt et al. 1999). Urine-borne chemical cues are likely to influence the progression and outcome of agonistic encounters in crayfish and lobsters. When crustaceans are deprived of the ability to detect chemical cues, either by the obstruction of chemoreceptors or prevention of urine release, agonistic bouts increase in duration and intensity, and the predictability of the victor is altered (Breithaupt et al. 1999; Zulandt Schneider et al. 1999, 2001; Bergman et al. 2003). These findings indicate that important information regarding status is transmitted through chemicals in the urine and that these chemicals play an important role in the determination and progression of agonistic bouts.

Chemical communication in crustaceans can be invaluable for expressing social status while competing for resources and in reducing the energetic costs of fighting, especially in a turbid habitat where an established hierarchy subsists and animals have repeated interactions with the same individuals (Karnofsky et al. 1989; Bergman & Moore 2003). However, crayfish do not appear to recognize individuals, but do recognize status (Zulandt Schneider et al. 2001); thus repeated chemical status exposure could affect crayfish in a stable habitat, such as a lake or within a laboratory. It is also possible that a cumulative effect could occur over time where repeated exposure to socially specific status odours alters the neurochemistry of the receiver and is observed through behavioural alterations. Thus the focus of this study was to examine how repeated chemical signal exposure from specific socially experienced animals altered the subsequent agonistic behaviour of receiving crayfish.

METHODS

Animals

All crayfish (*Orconectes rusticus*) used in this study were intermoult, form I (reproductively active) males collected using a seine net from the Portage River near Bowling Green, Ohio, U.S.A. Crayfish were physically and visually isolated for a minimum of 1 week prior to experiments to reduce the effects of prior social experience (Guiasu & Dunham 1997; Karavanich & Atema 1998b) and were isolated for a minimum of 1 week after trials to ensure intermoult status. Crayfish were housed in individually

ventilated pots (17.8-cm inner diameter) and maintained in a tank with recirculating water at a constant temperature (23°C) and a 14:10 h light:dark cycle. They were fed one commercial rabbit food pellet three times per week. Experiments were conducted in parallel from August 2002 to May 2003. Crayfish were marked individually on the dorsal carapace using correction fluid (Liquid Paper, Gillette Co., Boston, Massachusetts, U.S.A.) and were used once during the course of the study. If a crayfish moulted or died during the experiment or within 1 week before or after the trial, then the trial was removed from the study and an additional trial was conducted as a replacement. Three individuals died after the conclusion of the experiment and after being placed in communal tanks. This level of mortality is average for our laboratory and housing conditions. None of the crayfish were harmed during the course of this investigation, and at the conclusion of the study, the animals were returned to the branch of the Portage River from which they were collected.

Sender and Receiver Odour Exposure Set-up

Three crayfish were size-matched to within 95% of carapace and chelae length, and no less than a 90% difference for weight for each experimental trial (Table 1). Crayfish were then designated for a role as a 'sender', 'receiver', or 'effect evaluator' (Table 1). Senders either received experience winning ('dominant') or losing ('subordinate') a fight against a socially naïve opponent ('inducer') that was 30% smaller or larger, respectively, or received no fight experience ('naïve') on five consecutive days. Receivers were exposed to water-borne odours either from one of the three sender treatments or from a fourth control treatment (plain tank water), then paired with a naïve size-matched opponent ('effect evaluator').

We use the terms dominant/winner and subordinate/loser throughout interchangeably to refer to crayfish that had experienced wins and losses, respectively, on five consecutive days. We have tried to be consistent with the use of these social status terms (dominant, subordinate) as a predetermined history of either success or failure in previous encounters as defined by Francis (1988). However, we do not correlate any increased aggressiveness as a defining character of being dominant, even though it is quite evident that the establishment of dominance hierarchies is mediated by aggressive behaviour (Francis 1988). Dominant and subordinate social experience consists of either a series of wins or losses that have been shown to increase the likelihood that these animals will win or lose agonistic interactions with naïve opponents (Daws et al. 2002).

The three 'sender' social experiences took place in separate aquaria (40 × 20 × 14 cm), where after a 15-min acclimation period, the sender had access to the entire tank (naïve treatment) or a wall separating the sender and its naïve inducer was removed and the animals were allowed to interact for 5 min after first contact (dominant and subordinate treatments). A different inducer was introduced for each social experience trial (N = 200). All interactions occurred in a simplified dyad to control for complex interactions. All trials were

Table 1. Crayfish used in the experimental treatments

| Crayfish | Role | Number of individuals/treatment | | | | |
|-------------------|--|---------------------------------|-------------|-------|-------|-------|
| | | Dominant | Subordinate | Naïve | Water | Total |
| Inducers* | Animals used to produce social status of sender | 100 | 100 | | | 200 |
| Sender† | Individual in tank from which water was fed to receiver | 20 | 20 | 20 | | 60 |
| Receiver† | Individual that received water from sender tank | 20 | 20 | 20 | 20 | 80 |
| Effect evaluator† | Individual used in fights with receivers to evaluate behaviour | 20 | 20 | 20 | 20 | 80 |
| Total | | | | | | 420 |

*Inducers were 30% smaller and larger, respectively, in carapace and chelae length than 'dominant' and 'subordinate' senders.

†Size-matched to within 95% of carapace length ($\bar{X} \pm SE = 3.81 \pm 0.05$ cm) and chelae length (4.09 ± 0.08 cm), and no less than a 90% difference in weight (21.7 ± 0.7 g).

observed and a dominant–subordinate relationship ensued as predicted by size differential in all trials.

After each social experience, the sender crayfish was placed in a holding tank (20 × 20 × 20 cm) that was connected by a 1-mm diameter tube to a tank (20 × 20 × 20 cm) containing the receiver crayfish. Water was slowly and constantly gravity fed from the sender tank to the receiver tank (approximately 52 ml/min). All odours (urine or miscellaneous secretions) released into the water of the sender tank were also gravity fed along with the water to the receiver tank for 8 h/day for 5 days (40 h). The sender tank was refilled ad libitum throughout the day. During the remaining 16 h of the day, the flow was halted and the crayfish remained in their respective tanks until a new social experience trial was performed on the sender crayfish the subsequent day, after which the water feed was resumed. Sender and receiver tanks were never completely emptied during the 5 days. The receiver remained in its tank for all 5 days. Unfortunately, the final concentration of urine released by the sender and fed to the receiver tank was not quantified. It has been demonstrated that crayfish release urine spontaneously throughout a 24-h period (Breithaupt & Eger 2002). After the 40-h exposure, receiver crayfish fought a size-matched socially inexperienced fight opponent (effect evaluator) that had been isolated during the 5-day odour exposure portion of the experiment to evaluate the effect of the odour exposure (Table 1). Interactions between receivers from each treatment and their effect-evaluator opponents took place in separate aquaria (40 × 20 × 14 cm) equipped with a removable wall, where after a 15-min acclimation period, the wall separating the receiver and the opponent was removed and the crayfish were allowed to interact for 20 min.

Fight Analysis

A video camera, positioned 1 m above the test arena, recorded all trials on a VCR and displayed them on a monitor. Social experience trials were analysed to ensure that all social experiences were 100% accurate (i.e. winners always won and losers always lost), which was the case. All fight trials were analysed using a blind design where the observer who analysed the tapes did not have access to the experimental status of the crayfish. The identities of initiating and winning animals were

recorded for each encounter. The crayfish that first engaged an opponent in physical contact was deemed the initiator. The winner was deemed the crayfish that remained after its opponent (i.e. the loser) retreated or tail-flipped away. The intensity of fights was analysed using an ethogram modified in our laboratory from Bruski & Dunham (1987; Table 2; see Bergman & Moore 2003 for details). All interactions were analysed by examining the behaviour of both participants (receiver and effect-evaluator crayfish). Instances for initiating, winning, and fight intensity level reached were analysed 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 (see Zar 1999, pp. 185–200, 522–537). The multiple comparisons test was set up using a Microsoft Excel spreadsheet. Significant results are represented as $q_{0.05, \infty, 4} > 3.633$ for the multiple comparisons test and as $q > 3.633$ for the proportional comparisons. Initiation, winning, and intensity level reached were compared for all of the 'receiver' treatments. Fight intensity analyses were recorded for the interactions between crayfish, so when a participant changed its fight intensity from one level to the next, the fight level was recorded (Table 2). The time to reach these different fight intensities was analysed using a Kruskal–Wallis test (Statistica 6.0; StatSoft, Inc., Tulsa, Oklahoma, U.S.A.). Because time to reach intensity levels 2, 3 and 4 were correlated, we analysed these data using nonparametric Kruskal–Wallis tests (Zar 1999, pp. 176–179).

RESULTS

Initiation and Winning

The different receiver treatments (dominant-exposed, subordinate-exposed, naïve-exposed, tank water-exposed) had a significant effect on the tendency of a receiver to initiate and win fights. The multiple comparisons test for proportions revealed that crayfish exposed to dominant odour initiated fights significantly less often (0.40) than those individuals exposed to subordinate (0.70) and tank water (0.70) odours (dominant versus subordinate: $q = 5.28$; dominant versus water: $q = 5.28$; Table 3, Fig. 1). Crayfish exposed to dominant odours did not

Table 2. Agonistic bout intensity levels used to score fights

| Intensity level | Description |
|-----------------|--|
| 0 | No response |
| 1 | Approach |
| 2 | Approach with meral spread/threat display, usually accompanied by an antennal whip |
| 3 | Box, push and/or touch opponent with closed claws |
| 4 | Grab and/or hold opponent using claw |
| 5 | Unrestrained fighting: pulling at an opponent's claws or body parts |

initiate fights significantly more often than those exposed to naïve odours (0.60) against socially inexperienced (effect evaluator) opponents (dominant versus naïve: $q = 3.47$; Table 3, Fig. 1). In addition, crayfish exposed to dominant odours won significantly fewer fights (0.25) than crayfish exposed to either subordinate (0.80) or naïve odours (0.55), or control tank water (0.60) (dominant versus subordinate: $q = 10.02$; dominant versus naïve: $q = 5.37$; dominant versus water: $q = 6.24$; Table 3,

Table 3. Raw data and statistical results for the proportions of fights that were initiated and won by crayfish in each treatment

| Comparison | Number/ trials | Proportion | q | P |
|---|-------------------|------------|-------|-------|
| Fights initiated | | | | |
| Dominant-exposed vs subordinate-exposed | 8/20 | 0.40 | 5.28 | <0.05 |
| Dominant-exposed vs naïve | 8/20 | 0.40 | 3.47 | >0.05 |
| Dominant-exposed vs water (no odour) | 8/20 | 0.40 | 5.28 | <0.05 |
| Subordinate-exposed vs Naïve | 14/20 | 0.70 | 1.81 | >0.05 |
| Subordinate-exposed vs water (no odour) | 14/20 | 0.70 | 1.81 | >0.05 |
| Fights won | | | | |
| Dominant-exposed vs subordinate-exposed | 5/20 | 0.25 | 10.02 | <0.05 |
| Dominant-exposed vs naïve | 5/20 | 0.25 | 5.37 | <0.05 |
| Dominant-exposed vs water (no odour) | 5/20 | 0.25 | 6.24 | <0.05 |
| Subordinate-exposed vs naïve | 16/20 | 0.80 | 4.65 | <0.05 |
| Subordinate-exposed vs water (no odour) | 16/20 | 0.80 | 4.65 | <0.05 |
| Naïve vs water (no odour) | 11/20 | 0.55 | 0.87 | >0.05 |

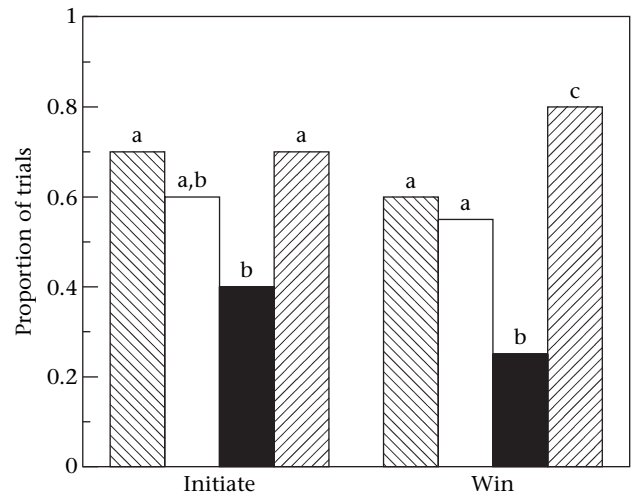
**Figure 1.** The proportion of trials during which receiver crayfish initiated and won fights after prolonged exposure (5 days) to tank water (control; ▨), or to water-borne odours from socially naïve (□), dominant (■), or subordinate (▩) conspecifics ($N = 20$ trials for each). Different letters above bars denote significant differences between treatments.

Fig. 1). Crayfish that were exposed to subordinate odours won a significantly greater proportion of subsequent interactions when compared to all other treatments (subordinate versus naïve: $q = 4.65$; subordinate versus water: $q = 3.78$). The proportion of interactions won by crayfish exposed to naïve odours did not significantly differ from that of crayfish exposed to control tank water (naïve versus water: $q = 0.87$).

Fight Dynamics

The proportion of agonistic interactions to reach intensity levels 0–5 (where 0 = no response; 5 = unrestrained fighting) was analysed using the multiple comparisons test and revealed a global significant difference. The proportion of fights that reached intensity level 2 differed significantly across odour exposure treatments. An intensity level 2 interaction is an aggressive display (i.e. meral spread of the chelae). Exposure to odours from socially experienced crayfish, dominant (0.85) and subordinate (0.90), increased the likelihood that an interaction with an effect evaluator would progress to intensity level 2 compared to those interactions where crayfish were exposed to naïve crayfish odour (0.65) or tank water (0.60) (dominant versus naïve: $q = 4.00$; dominant versus water: $q = 4.89$; subordinate versus naïve: $q = 5.25$; subordinate versus water: $q = 6.14$; Table 4, Fig. 2a). There were no significant differences in the proportions of agonistic interactions that reached intensity level 2 between naïve-odour and tank-water-exposed crayfish ($q = 0.89$) or between the dominant- and subordinate-odour-exposed crayfish ($q = 1.25$).

Intensity level 3 interactions occur when opponents use their chelae to push and 'box' each other. Interactions between crayfish exposed to subordinate odours and effect-evaluator crayfish (0.75) were less likely to escalate to

Table 4. Raw data and statistical results for fights that reached different intensities

| Comparison | Number/ trials | Proportion | <i>q</i> | <i>P</i> |
|---|-------------------|------------|----------|----------|
| Intensity 2 | | | | |
| Dominant-exposed vs subordinate-exposed | 17/20 | 0.85 | 1.25 | >0.05 |
| Dominant-exposed vs naïve | 17/20 | 0.85 | 4.00 | <0.05 |
| Dominant-exposed vs water (no odour) | 13/20 | 0.65 | | |
| Dominant-exposed vs Subordinate-exposed | 17/20 | 0.85 | 4.89 | <0.05 |
| Subordinate-exposed vs water (no odour) | 12/20 | 0.60 | | |
| Subordinate-exposed vs Naïve | 18/20 | 0.90 | 5.25 | <0.05 |
| Subordinate-exposed vs water (no odour) | 13/20 | 0.65 | | |
| Subordinate-exposed vs Naïve | 18/20 | 0.90 | 6.14 | <0.05 |
| Subordinate-exposed vs water (no odour) | 12/20 | 0.60 | | |
| Naïve vs water (no odour) | 13/20 | 0.65 | 0.89 | >0.05 |
| Intensity 3 | | | | |
| Dominant-exposed vs subordinate-exposed | 20/20 | 1.00 | 7.73 | <0.05 |
| Dominant-exposed vs naïve | 20/20 | 1.00 | 2.84 | >0.05 |
| Dominant-exposed vs water (no odour) | 19/20 | 0.95 | | |
| Dominant-exposed vs Subordinate-exposed | 20/20 | 1.00 | 4.36 | <0.05 |
| Subordinate-exposed vs water (no odour) | 18/20 | 0.90 | | |
| Subordinate-exposed vs naïve | 15/20 | 0.75 | 4.89 | <0.05 |
| Subordinate-exposed vs water (no odour) | 19/20 | 0.95 | | |
| Subordinate-exposed vs Naïve | 15/20 | 0.75 | 3.37 | >0.05 |
| Subordinate-exposed vs water (no odour) | 18/20 | 0.90 | | |
| Naïve vs water (no odour) | 19/20 | 0.95 | 1.52 | >0.05 |
| Intensity 4 | | | | |
| Dominant-exposed vs subordinate-exposed | 16/20 | 0.80 | 8.15 | <0.05 |
| Dominant-exposed vs naïve | 7/20 | 0.35 | | |
| Dominant-exposed vs water (no odour) | 16/20 | 0.80 | 4.65 | <0.05 |
| Subordinate-exposed vs water (no odour) | 11/20 | 0.55 | | |
| Subordinate-exposed vs Naïve | 16/20 | 0.80 | 7.26 | <0.05 |
| Subordinate-exposed vs water (no odour) | 8/20 | 0.40 | | |
| Subordinate-exposed vs Naïve | 7/20 | 0.35 | 3.29 | >0.05 |
| Subordinate-exposed vs water (no odour) | 11/20 | 0.55 | | |
| Subordinate-exposed vs Naïve | 7/20 | 0.35 | 0.89 | >0.05 |
| Subordinate-exposed vs water (no odour) | 8/20 | 0.40 | | |
| Naïve vs water (no odour) | 11/20 | 0.55 | 2.60 | >0.05 |
| Naïve vs water (no odour) | 8/20 | 0.40 | | |

the next intensity (level 3) when compared to interactions of crayfish exposed to dominant (1.00) or naïve (0.95) odours (subordinate versus dominant: $q = 7.73$; subordinate versus naïve: $q = 4.89$; Table 4, Fig. 2b). When crayfish were exposed to dominant odours, interactions with effect-evaluator crayfish were then more likely to reach this

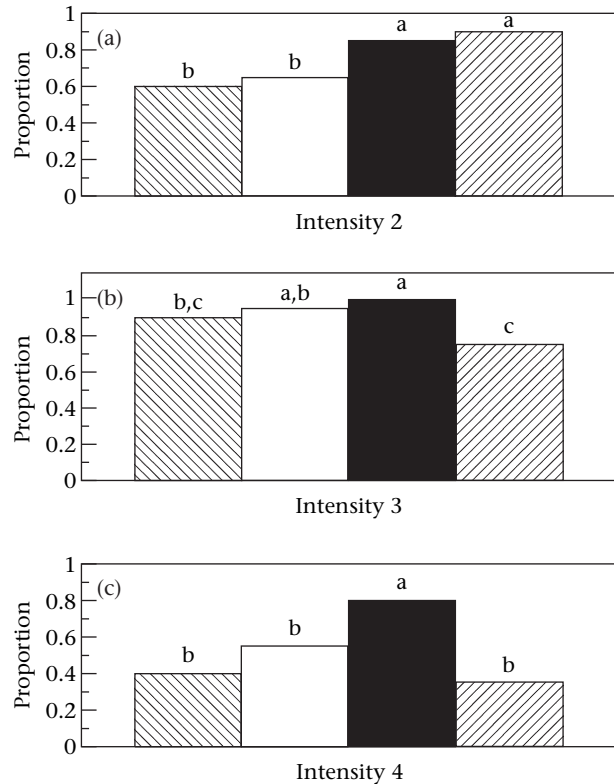


Figure 2. The proportion of interactions that escalated to intensities 2, 3 and 4 after prolonged exposure (5 days) to tank water (control; ▨), or to water-borne odours from socially naïve (□), dominant (■), or subordinate (▩) conspecifics ($N = 20$ trials for each). Different letters above bars denote significant differences between treatments.

intensity compared to interactions for those crayfish exposed to subordinate odours and tank water (0.90) (dominant versus water: $q = 4.36$). There were no significant differences for interactions of effect-evaluator crayfish with crayfish exposed to naïve odours and tank water ($q = 1.52$), dominant and naïve odours ($q = 2.84$), or subordinate odours and tank water ($q = 3.37$).

Intensity level 4 interactions are characterized by crayfish using their chelae to grab and hold an opponent. When crayfish that were exposed to dominant odours interacted with effect-evaluator opponents, fights were significantly more likely to reach intensity level 4 (0.80), compared to interactions between effect-evaluator crayfish and crayfish exposed to subordinate (0.35) or naïve (0.55) odours, or tank water (0.40) (dominant versus subordinate: $q = 8.15$; dominant versus naïve: $q = 4.65$; dominant versus water: $q = 7.26$; Table 4, Fig. 2c). There were no significant differences between subordinate, naïve, or tank water odour treatments (subordinate versus naïve: $q = 3.29$; subordinate versus water: $q = 0.89$; naïve versus water: 2.60).

Temporal Dynamics of Fights

A Kruskal–Wallis test revealed that there were no significant differences between treatments in the time it

took to reach intensity levels 2, 3 and 4. In addition, the time spent at each intensity level showed no significant differences across treatments (Fig. 3). Fights progressed in a fashion where lower fight intensities levels were skipped to reach higher fight intensities.

DISCUSSION

The results of this study show that extended (5 days) exposure to social odours alters subsequent agonistic behaviour. Social status odours have a substantial effect on the initiation and outcome of agonistic encounters, and affect the progression of fights in terms of fight escalation. Crayfish exposed to dominant (winner) odour showed a reduced initiative to engage in agonistic bouts and lost more interactions (Fig. 1). In contrast, crayfish exposed to subordinate (loser) odour showed an increased initiative to fight and subsequently won more interactions (Fig. 1). In both of these outcomes the receiver appeared to behave as the converse of the odour sender, suggesting that extended exposures to social odours alters the decision points of fights with socially inexperienced opponents (effect evaluators).

When crayfish were exposed to dominant odours, their interactions with socially inexperienced (effect evaluator) opponents consistently reached all fight intensity levels (Fig. 2). Conversely, when crayfish were exposed to subordinate odour and later fought the socially inexperienced opponent, interactions did not progress to the higher intensities (Fig. 2). At first glance these results would seem to conflict with the previous results of social odour exposure altering the decision points of fights. However, a possible elucidation for this conflict may be explained by the fact the latency to these fight intensities did not differ between treatments (Fig. 3). The mechanics underlying agonistic behaviour seem to remain intact, which support the premises of Bruski & Dunham (1987) and Huber & Kravitz (1995) that crustaceans follow strict rules of stereotypic conduct

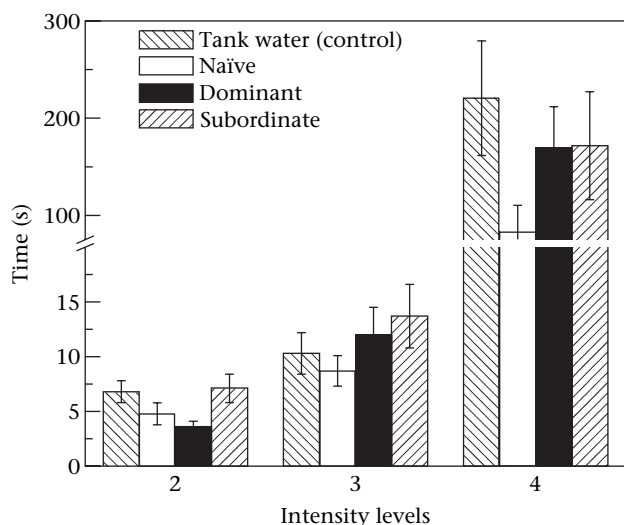


Figure 3. The mean \pm SE time to reach different fight intensity levels for crayfish in each treatment.

when fighting. Thus the temporal mechanics remained unchanged, suggesting again that the decision point of fights is the pertinent fight characteristic that was altered.

In crustaceans, it has been demonstrated that urine release (chemical signalling) is altered by social status (Bushmann & Atema 1997, 2000; Breithaupt et al. 1999; Zulantz Schneider et al. 2001). In fact these alterations in urine release can be visualized and show that crayfish release urine more often during social activities, and that eventual dominant crayfish will release urine more often (Breithaupt & Eger 2002). In addition, in crustaceans, when urine release is prevented (Breithaupt et al. 1999; Bushmann & Atema 2000; Zulantz Schneider et al. 2001) or olfactory reception is obstructed (Karavanich & Atema 1998a; Bergman et al. 2003), agonistic interactions become longer and more intense. Thus, if the signals sent by an odour receiver do not coincide with its behavioural output, this could lead to the differences we observed in fight intensities in the present study. A mismatch between the receiver's chemical and physical social experiences may have created a situation where the socially inexperienced (effect evaluator) opponent responded in a manner that created the observed differences in fight intensity. For example, upon receiving chemical signals from dominants over 5 days, receivers may have attempted to respond to (counteract) these signals by releasing chemicals, and when the receiver was ultimately allowed to interact with socially inexperienced (effect evaluator) animals, its chemical signal supply was already depleted.

Communication in crustaceans often involves multiple channels (e.g. visual and the chemical channels: Hughes 1996; visual and tactile channels: Bruski & Dunham 1987). The results of the present study suggest that the dominant-exposed receivers may have exhausted their chemical signals and therefore were obliged to increase their levels of visual/tactile responses during agonistic encounters. These receivers may have lost more fights because their chemical reserves were no longer sufficient to reinforce their visual displays. In contrast, the subordinate-exposed receivers probably had sufficient chemical reserves to reinforce their visual displays (i.e. meral spread of the chelae), possibly supported by some release of urine in order to win the final contests against the effect-evaluator crayfish.

At a mechanistic level, social interactions appear to cause changes in the serotonin levels of crustaceans (Kravitz 1988). Because subsequent agonistic acts are modified by serotonin, any resulting social status appears to be associated with serotonergic function (Kravitz 1988; Huber et al. 1997; Yeh et al. 1997) by possibly elevating or depressing levels of the biogenic amine. In this way, social status level can be potentially communicated during an agonistic encounter, through the release of urine that contains a signal possibly associated with internal serotonin levels. An intrinsic chemical change associated with changes in social status can consequently be extrinsically released with the urine to reduce fight intensity and decrease the chance of injury (Breithaupt et al. 1999; Zulantz Schneider et al. 1999, 2001).

In a broader context, instead of merely communicating social status, the results of this study show that social

signals (in this case chemical) can alter the subsequent social status of receivers. This phenomenon could be especially important in hierarchical conditions where social signalling is repeated throughout the habitat. The ability of social signals to alter a receiver's social status could lead to a reinforcement of social status and make hierarchies more stable in the absence of repeated agonistic encounters. Depending on the strength of this phenomenon, it is possible that social signals could replace the role of subsequent agonistic interactions in reinforcing social status. This in turn could lead to differences in the acquisition of defendable resources such as habitats or mates. The results here have demonstrated the importance of chemical signals for influencing future dyadic interactions. Under normal circumstances, chemical signals can function to reduce or increase both the intensity of fights and the possibility of injury. Urine-borne chemical cues appear to be expressed extrinsically as both a signal to communicate social status and an attempt to manipulate an opponent's neural state. The importance of urine for communicating status or causing neurochemical status alterations deserves more attention as a possible significant influence on social status within hierarchies.

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