



# Control of information flow through the influence of mechanical and chemical signals during agonistic encounters by the crayfish, *Orconectes rusticus*

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Animals use sensory information when making decisions regarding social status. In particular, crayfish use chemical and possibly mechanical signals to communicate past social history and status. These signals originate in the urine that is actively released during agonistic encounters. We examined the transfer of social information via chemical and mechanical signals by using (1) neutrally buoyant particles, along with a projected light sheet to visualize flow generation, (2) a fluorescent dye to observe urine release and (3) a unique combination of the two techniques to quantify the roles of urine and current generation during agonistic bouts. Both dominant and subordinate crayfish generated currents and appeared to use them to send and sample signals, but dominant crayfish generated more frequent currents than subordinates. Alterations in currents and behaviours suggest that currents are used to transfer social information. Dominant animals released urine during an encounter more frequently than subordinates, and usually did so while engaged with an opponent. In addition, when urine was released, it was transported within these self-generated currents. However, urine was not released every time a current was generated, making urine release relatively rare compared to current generation. A correlation of urine releases with current generation and agonistic behaviours appears to be an indicator for eventual dominant–subordinate relationships. Dominant crayfish showed agonistic behaviours more often when releasing urine. Consequently, chemical signalling influenced a fight opponent's behaviour.

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Communication occurs when information is exchanged between a sender and a receiver, resulting in an alteration of behavioural patterns in an adaptive way for one or both of the participants (Enquist 1985; Dusenbery 1992; Bradbury & Vehrencamp 1998). During communication, participants often control key aspects of the signal to facilitate the transfer of information. Signalling can

include behavioural acts by one animal that alter the subsequent behaviour of the receiver. This type of communication system evolved because the sender has some recognition that its behaviour affects the receiver and in part because the response of the receiver is simultaneously altered (Maynard Smith & Harper 2004). The key distinction between a signal and a cue is that animals can control when, where and how a signal is released into an environment, which subsequently affects the behaviour of both the sender and receiver.

Within a social framework, agonistic behaviour is regarded as a behavioural tool (i.e. a means of interacting with other conspecifics) to enhance the survival and/or reproductive effort of the individuals involved in the interaction (Wilson 1975). Communication of status or

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internal aggressive state can be used by sending signals to influence the outcome of an encounter without direct physical interaction (Wilson 1975). Displays using chemical, visual, mechanical, audible, or electromagnetic signals are used to confer a status relationship (Bossert & Wilson 1963; Cheney & Seyfarth 1988; Levenbach & Hazlett 1996; Yack et al. 2001; Dunlap & Larkins-Ford 2003). During agonistic encounters a contestant signals whether the encounter should escalate in intensity or whether a combatant should retreat. For example, a subordinate animal can signal submissive status and consequently be allowed to retreat without an agonistic bout (Wilson 1975).

A participant's social history or a resident's fighting ability can be revealed by using chemical or mechanical signals that contain information about social history or aggressive state (Enquist 1985). Male crayfish are influenced by such chemical information and respond with a typical aggressive response (i.e. meral spread when exposed to tank water from male conspecifics; Ameyaw-Akumfi & Hazlett 1975; Hazlett 1985; Dunham & Oh 1992). Moreover, crustaceans may release chemical information that conveys past social history to reduce agonistic intensity and the number of encounters (Breithaupt & Atema 1993; Karavanich & Atema 1998; Zelandt Schneider et al. 2001). Therefore, these chemical signals, alone or in combination with mechanical signals, could function as deterrents to potential opponents or as appeasement signals.

The movement of a crayfish's fan organs (maxillipeds), pleopods and gills, along with nephropore propulsion, can be used to generate flow fields, and these currents appear to control the transfer of chemical or mechanical cues that signal past social experiences (Breithaupt & Ayers 1996, 1998; Breithaupt 2001). Breithaupt & Eger (2002) found that crayfish engaged in agonistic encounters control urine release, particularly in terms of when, how long and how often to release urine. None of these previous studies, however, could robustly link changes in behaviour to current generation or precisely determine the sources of the currents.

Our study is the first to precisely determine the mechanism of current generation, and to explicitly show how current generation and urine release during agonistic interaction are correlated with social status in crayfish. We performed three experiments using a crayfish species, *Orconectes rusticus*, whose aggressive tendencies, social interactions and chemical communication system have been extensively examined (Thorp & Ammerman 1978; Bruski & Dunham 1987, 1990; Smith & Dunham 1996; Zelandt Schneider & Moore 2000; Zelandt Schneider et al. 2001; Bergman et al. 2003). In the first experiment, we used differential particle illumination velocimetry (DPIV) of neutrally buoyant particles to allow the visualization of currents generated by crayfish during paired interactions. In the second experiment, we injected crayfish with a fluorescent dye (fluorescein) to allow visualization of urine release during paired encounters. In the third experiment, we examined whether crayfish generate current and release urine in combination to facilitate communication with a conspecific.

## METHODS

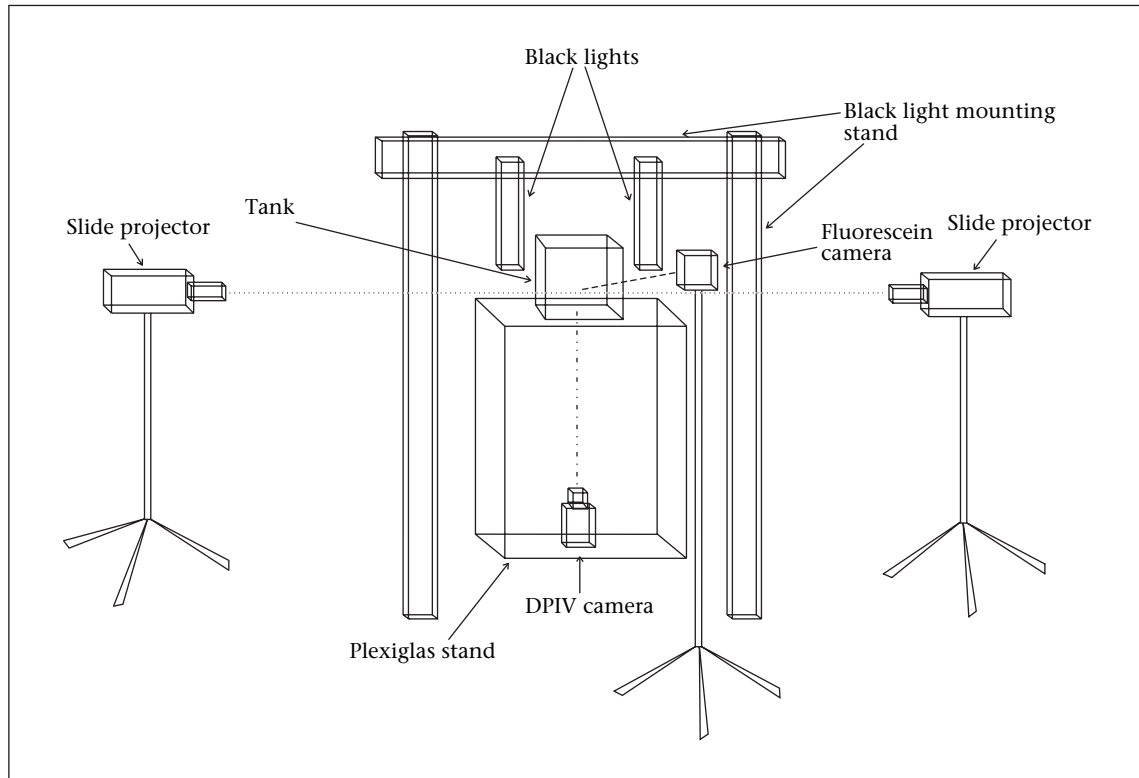
### Animals

We collected crayfish *O. rusticus* from the Portage River near Bowling Green, Ohio, U.S.A. by using a seine net. Intermoult male crayfish were physically and socially isolated in a flow-through holding tank. Crayfish had a mean  $\pm$  SE carapace size of  $36.3 \pm 0.4$  mm, chelae length of  $34.1 \pm 0.6$  mm, and a weight of  $15.0 \pm 0.5$  g. Crayfish were housed in an environmental chamber that regulated temperature ( $23^\circ\text{C}$ ) and light:dark cycle (14:10 h) for a minimum of 1 week prior to the experiments. Crayfish were fed one commercial rabbit pellet three times per week and were used once during the course of experiments. Crayfish were size-matched within 95% for carapace and chelae size, and no less than 90% for weight of participants. Interactions took place in an aquarium ( $22.9 \times 24.1 \times 22.8$  cm), henceforth 'fight tank', equipped with a removable wall, which visually and physically separated the opponents prior to each trial. In each trial, one crayfish was marked with reflective tape on the ventral side of one of the chelae to later distinguish between fight participants. All animals used in the DPIV trials were returned to the field. Fluorescein-injected crayfish were returned to the field only after all signs of fluorescein were absent, usually after a crayfish moulted (described below).

### Experiment 1

#### Flow-field visualization

We prepared particles from near-neutrally buoyant ABS stock material (acrylonitrile butadiene styrene; GE Polymerland no. FCCS0CP2002RBJ; 1.10 specific gravity;  $23^\circ\text{C}$ ). We froze the stock material with liquid nitrogen to make it brittle enough to be ground using a coffee grinder (Braun no. KSM2), then sorted the resulting particles using a series of mesh sieves. Prior to each trial, we added 1 g of ground particles (each approximately 0.006 cm in diameter) to the fight tank, which contained 2000 ml of dechlorinated water. After each trial, we rinsed and refilled the tank, and added new particles. To facilitate tracking of particles during each trial, we created a level plane of light 1.25 cm above the base of the tank using two slide projectors (Kodak Ekta Graphic IIIA) positioned on opposite sides and 70 cm from the tank (Fig. 1). We inserted into each projector a slide that had a thin horizontal slit ( $\sim 15 \mu\text{m}$ ) that passed a thin beam of light through to the opposite side of the tank. This light level was chosen because it illuminated particles that were excited by maxilliped movements, gill action and urine projection from the nephropores. Trials were performed in an enclosed environmental chamber where the slide projectors supplied the only source of light. A video camera was placed 30 cm underneath the tank to record particle and maxilliped movements. Black tarps were used to cover the top and sides of the tank to increase particle contrast. The tarps were raised 5 cm above the base of the tank on two sides to allow the light planes to pass through the tank. The result of the set-up was differential particle



**Figure 1.** Schematic of the experimental set-ups used for differential particle illumination velocimetry (DPIV) and fluorescein urine visualization.

illumination velocimetry (DPIV) similar to the design of Breithaupt & Ayers (1996, 1998). A comparison of fights with particles and numerous previous studies of fights without particles showed no observable change in the fight structure due to the presence of particles (Zulandt Schneider et al. 1999, 2001; Bergman et al. 2003).

#### *DPIV trials*

For each trial ( $N = 10$ ), we placed two size-matched naïve crayfish in the fight tank on opposite sides of the divider and allowed them to acclimatize for 15 min. We then removed the divider and allowed the crayfish to interact for 10 min. Size-matched crayfish were used to increase the level of intensity in aggressive interactions (Caldwell & Dingle 1979). All trials were videotaped and the currents generated by crayfish during the trial were later analysed by tracking individual particle movement during agonistic encounters.

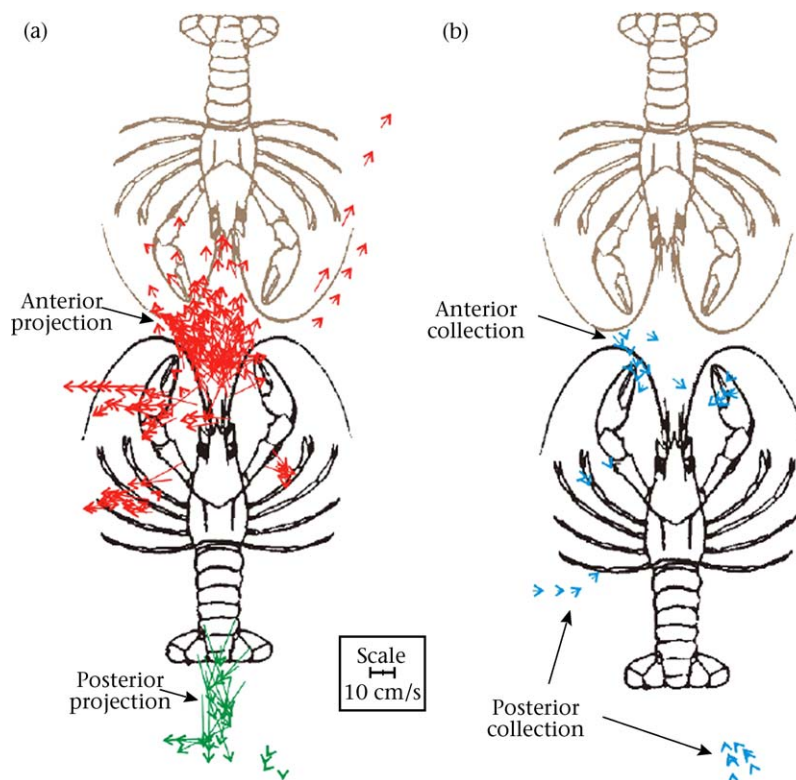
#### *Flow-field analysis*

Flow fields were analysed by tracking particles on a video monitor. The particles were tracked manually on grid transparencies attached to the surface of the monitor. Individual particle positions were recorded relative to two specific points on a crayfish: (1) the joint of the right chelae at the carapace and (2) the fifth walking leg on the left side. The two points were needed for a directional orientation of the crayfish in relation to particle movement. The time between successively recorded particle

positions allowed for the calculation of the speed of the particles. Particle location and angle of movement within the grid were transcribed relative to the crayfish to give a resulting velocity vector, where the length of the vector was proportional to the velocity. The actual distances that particles travelled were calculated using a calibration factor that was determined from an object of known size. Flow-field analysis was grouped in three general patterns of current production: (1) drawn towards the anterior and posterior of the crayfish or collection currents ( $N = 31$  tracked particles); (2) projected away ( $N = 203$  tracked particles) from the anterior or projection currents; and (3) flows away from the posterior, also considered projection currents ( $N = 26$  tracked particles). Sending and receiving currents (total = 260 particles) relative to crayfish during agonistic encounters were characterized on two separate flow-field diagrams (Fig. 2a, b).

#### *Behavioural analysis*

To correlate flow-field generation with behaviours, we determined behavioural events by analysing the DPIV trials. Flow fields were first characterized as either anterior or posterior projection currents. Anterior projection currents were generated by maxilliped movement, gill processes and/or nephropore release of urine. The currents were further subdivided into flows that occurred while in an agonistic bout (engaged) or while there was no physical contact. Posterior projection currents were defined as currents moving away from the tail region and generated by the modified swimmerets (pleopods). Aggressive behaviours were characterized for every second by using



**Figure 2.** Diagram of the flows generated by crayfish that resulted in particles being (a) projected away ( $N = 229$ ) and (b) drawn towards ( $N = 31$ ) crayfish during agonistic encounters in the DPIV trials. The length of the arrows indicates the relative velocity of the particles. Arrowheads indicate the direction of the particle flow towards or away from the crayfish. (Crayfish renditions were found at <http://www.state.ky.us/nrepc/water/crayfish.htm>.)

a modified ethogram (modified from Bruski & Dunham 1987; see Bergman et al. 2003).

### Statistical analysis

We performed a one-way MANOVA and a post hoc test on the velocities of the currents to determine differences between dominants and subordinates for the number and duration of current generations. The total time creating currents was analysed using a contingency table for proportions. The likelihood that a behaviour occurred during a current event was analysed using a correspondence analysis (van der Heijden et al. 1990; Moyaho et al. 1995) that gives the relational properties between the sequences of behaviours and currents. Correspondence analysis of a transitional matrix is a procedure that results in a graphical relationship between points (currents and behavioural acts), where all elements of behaviour are represented in one graph (van der Heijden et al. 1990). Two agonistic behaviours (retreat and tail-flip) were not displayed by dominant crayfish and consequently could not be included in the correspondence analysis. Any behavioural acts that had a low frequency ( $<5$ ) were included in the analysis, but were not elaborated upon in the data interpretation because of the uncertain nature of their correlations. The correspondence analysis decomposes the chi-square value of the matrix. The proportion of decomposition of the total chi-square for a two-way

frequency table divided by the total sum of observations in the table is called the inertia (van der Heijden et al. 1990). An eigenvector is a vector that produces a scalar multiple (eigenvalue) of the original vector for each event. This resulting eigenvalue is the scaling factor by which a linear transformation multiplies each of the eigenvectors, and thus results in a direction for each. An eigenvalue can then be interpreted as the proportion of variance accounted for by the correlation between respective weighted sums of the two sets of variables (currents and behaviours). The set of points in a correspondence analysis graph can be interpreted where the origin represents the mean profile for all acts (behaviours and currents) and therefore, any point near the origin is similar to the mean profile. In addition, when two behavioural points are near one another, it can be said that the two behaviours occupy a similar place in a behavioural sequence with regard to other acts. The proximity between all points indicates the degree to which one act tends to follow the other, thus how closely synched current events are to behavioural events in time. All statistical tests were performed using Statistica 6.0 (Statsoft Inc., Tulsa, Oklahoma, U.S.A.). As in any multidimensional scaling analysis, the axes are representations of those dimensions that combine the information in the rows and columns of the matrix used to compute the analysis. Theoretically, the analysis could use  $n - 1$  ( $n =$  number of rows or columns in the table) dimensions to generate spatial relationships between

points. The correspondence analysis is designed to reduce the number of actual dimensions without the loss of information in the 'higher' (second, third or fourth) dimensions.

## Experiment 2

### *Injection procedure for urine visualization*

We used the technique tested by Breithaupt & Eger (2002) to visualize urine release during agonistic encounters. A solution of sodium fluorescein (Sigma F-6377) was dissolved in dechlorinated water and injected at a dose of 0.01 ml/g of body mass into the pericardial region of the crayfish. To inject crayfish, we dried the carapace and placed a small strip of labelling tape over the injection site (dorsal carapace). We used a 1-ml syringe with a 26.5-gauge needle to inject the fluorescein solution through the pierced hole in the carapace. The needle was then removed and an additional piece of tape and superglue was placed over the needle hole. Each crayfish was allowed a minimum of 1 h to recover from the injection prior to use in a trial (Breithaupt & Eger 2002). Three of the 61 crayfish (4.9%) injected for use in preliminary and experimental trials died during or immediately after the injection procedure. Thus, the injection procedure probably resulted in the deaths of these three animals. However, as our injection technique improved, mortality rate from the injections decreased.

### *Urine visualization*

We used the DPIV tank set-up for the urine visualization experiments with the following additions (Fig. 1). We placed a second digital video camera in front of the tank to focus on the anterior nephropore region of the two crayfish. In addition, we positioned two black lights (15 W GE no. F15T8) vertically 2.5 cm from either side of the tank on the Plexiglas stand. The lights were used to maximize the illumination of fluorescein released with the urine.

### *Urine visualization trials*

We placed two size-matched crayfish in the fight tank on opposite sides of the divider and allowed them to acclimatize for 15 min. We then removed the divider and allowed the crayfish to interact for 15 min. Each trial ( $N = 13$ ) was recorded from front and bottom views to more accurately determine when fluorescein was released. Four trials were excluded from the analysis because there was no observable urine release by the subjects. Whether crayfish withheld their urine or whether the injection procedure failed was unknown.

### *Urine release and statistical analysis*

We recorded the social status and the duration and number of urine release events for each crayfish. We performed a behavioural analysis using the modified ethogram from Bergman & Moore (2003) to quantify the social status of crayfish. To test for significant differences between the duration, number and percentage of urine

releases for dominant and subordinate crayfish, we used a one-way MANOVA and a Tukey post hoc test.

## Experiment 3

### *DPIV and urine visualization*

To correlate urine release events with the generation of flow fields and agonistic behaviours, we modified the methods for DPIV and fluorescein as follows (Fig. 1). We placed 0.5 g of particles in the fight tank, which contained 9000 ml of water. The two black lights were positioned vertically about 6 cm above the base of the tank and the DPIV light plane was projected at 1.25 cm above the tank base and passed below the suspended black lights. The DPIV camera was positioned underneath and the fluorescein camera was positioned in front of the set-up. We temporally synched the two cameras using a laser (Spectra Rite II) and used correspondence analysis (van der Heijden et al. 1990; Moyaho et al. 1995) to correlate urine release by dominant and subordinate crayfish ( $N = 10$  for each) with current types and behaviours. Correspondence analysis of transitional matrices results in a diagram in which relationships between points (urine releases and behaviours) are based on chi-square distances.

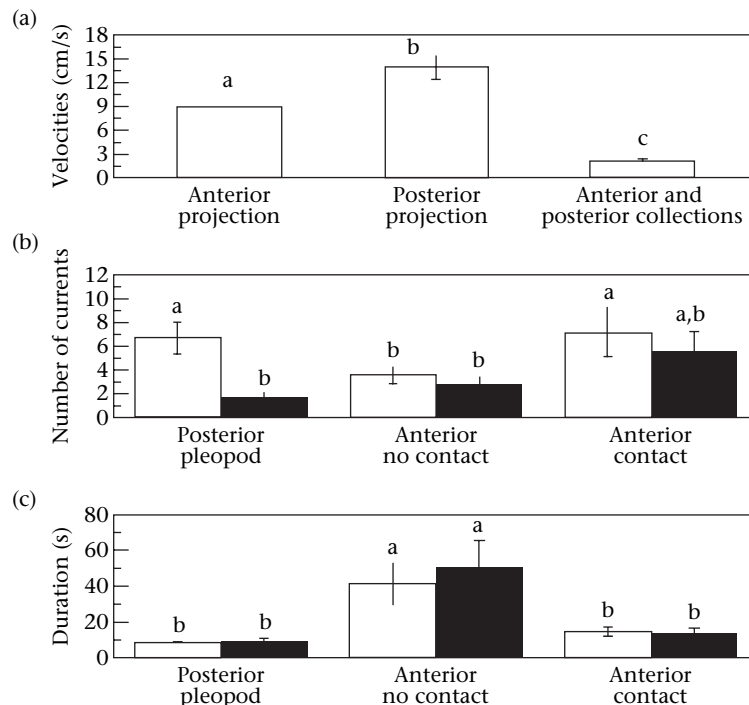
## RESULTS

### Flow-field Description

Currents were projected away and drawn towards crayfish during agonistic bouts at both the anterior and posterior portions (Fig. 2a, b). Projection and collection current velocities (mean  $\pm$  SE) differed significantly from one another ( $P < 0.05$ ; Fig. 3a). Anterior projection currents were significantly faster ( $13.9 \pm 1.5$  cm/s;  $N = 203$  particles) than posterior projection currents ( $8.8 \pm 0.4$  cm/s;  $N = 26$  particles) and anterior and posterior collection currents ( $2.2 \pm 0.3$  cm/s,  $N = 31$  particles).

### Agonistic Behaviours Associated with Flow-field Generation

In general, currents were generated more frequently and for shorter durations when crayfish were engaged with an opponent. Projection currents were only generated during contact with an opponent. Dominant crayfish generated significantly more posterior currents ( $\bar{X} \pm \text{SE} = 6.7 \pm 1.4$  per trial;  $N = 10$ ) than subordinates ( $1.7 \pm 0.5$ ,  $N = 10$ ;  $P < 0.05$ ; Fig. 3b) when opponents were in contact. No significant differences were found between dominant and subordinate crayfish for the average number of anterior currents generated when not in physical contact (dominant:  $3.6 \pm 0.8$ , subordinate:  $2.8 \pm 0.7$ ) or when in physical contact with an opponent (dominant:  $7.2 \pm 2.1$ , subordinate:  $5.5 \pm 1.8$ ). Anterior currents were generated less often when opponents were not in contact, but when they were generated, they were significantly longer in duration (dominant:  $41.2 \pm 12.0$  s, subordinate:  $50.4 \pm 15.3$  s) than those generated while



**Figure 3.** (a) The average  $\pm$  SE velocities of anterior and posterior projection and collection currents generated during agonistic encounters between crayfish. The average  $\pm$  SE (b) number and (c) duration of anterior and posterior currents generated by dominant ( $\square$ ,  $N = 10$ ) and subordinate ( $\blacksquare$ ,  $N = 10$ ) crayfish when they were in contact and not in contact during agonistic encounters. Different letters indicate a significant difference between treatments using a one-way MANOVA (a, c) and a contingency table (b).

opponents were in physical contact (dominant:  $14.8 \pm 2.6$  s, subordinate:  $13.4 \pm 3.4$  s) or than those generated at the posterior end (dominant:  $8.5 \pm 0.7$  s, subordinate:  $9.3 \pm 1.5$  s,  $P < 0.05$ ; Fig. 3c). Current durations of dominant and subordinate crayfish did not differ significantly for any current.

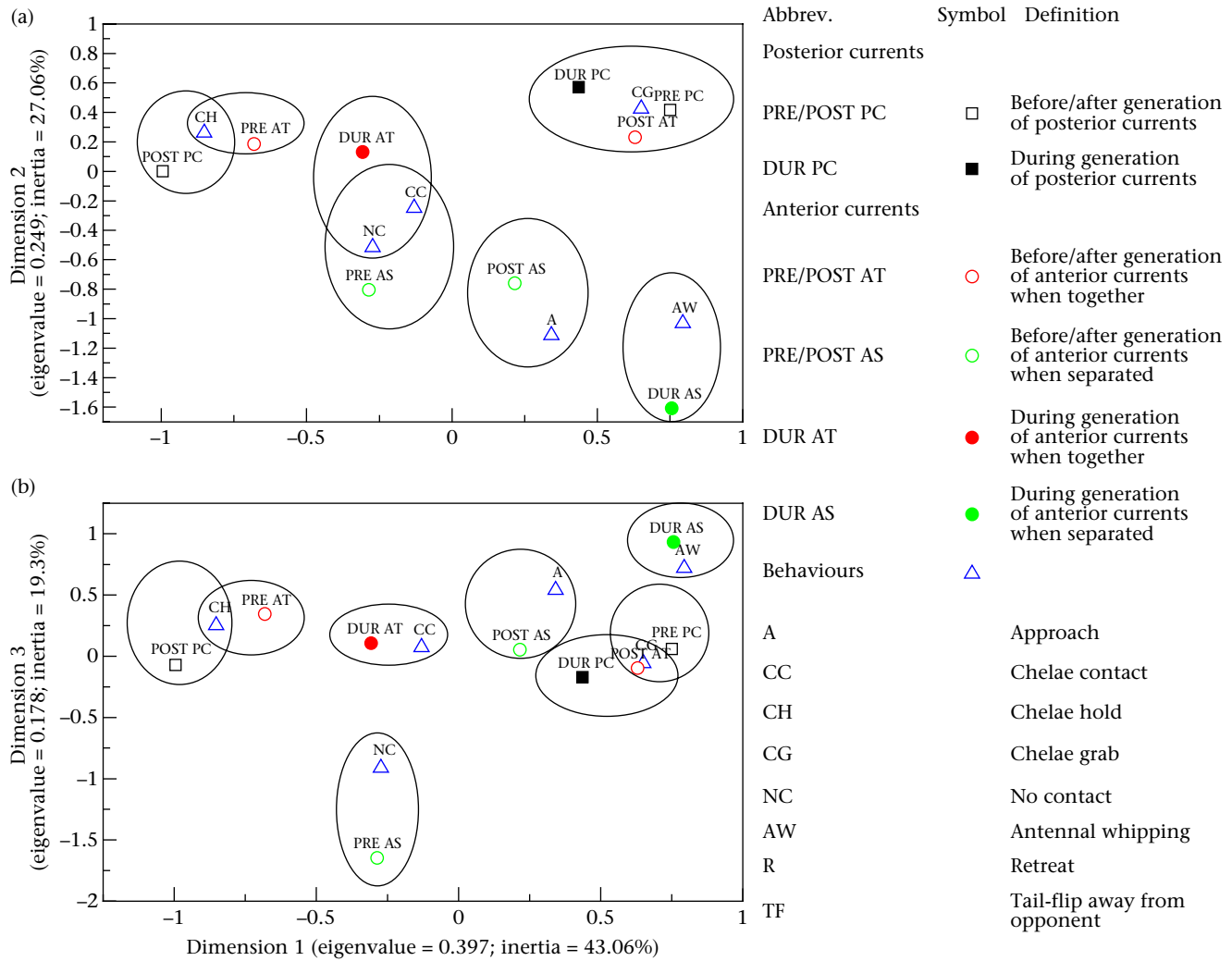
Results of the correspondence analysis of the behaviours and current types of dominant crayfish revealed a correlation between the two ( $P < 0.001$ ). Furthermore, these events were dependent upon social status. The two-dimensional plots revealed that chelae contact is the obvious transition from no contact to contact, and when in contact, dominant crayfish generated anterior currents (Fig. 4). During chelae contact there was a period when dominant crayfish locked chelae with subordinates and did not yet generate anterior projection currents. After chelae contact and holding periods, current generation subsided and fights increased in intensity, as indicated by grabbing with the chelae. Pleopod currents were generated more often by dominant crayfish before and during chelae grabbing periods. When pleopod currents subsided, the crayfish sustained chelae engagement. When crayfish were separated, dominant crayfish approached opponents before and after anterior current generation. When these currents stop, an antennal whip was used.

Results of the correspondence analysis of the behaviours and current types for subordinates differed from those of dominants ( $P < 0.001$ ; Fig. 5). When subordinate crayfish were engaged with an opponent, they held their opponent using their chelae before they generated anterior currents and after they generated posterior currents.

During anterior current generation, subordinate crayfish used an antennal whip. After this current subsided, subordinate crayfish tended to increase their fight intensity by grabbing with their chelae. Periods of no contact preceded anterior current generation. When currents were generated at the anterior end when opponents were not in contact, subordinates grabbed their opponent using their chelae and then usually retreated. After these currents subsided, subordinates not only continued to retreat, but also occasionally tail-flipped away. Subordinates used significantly fewer posterior currents than did dominants (Fig. 3b). However, when they did use posterior currents, they were associated with chelae contact. Before these posterior currents were generated, subordinate crayfish grabbed their opponent using their chelae and occasionally whipped their antennae. After posterior currents ceased, subordinates remained in chelae contact and held their opponent. Approaching opponents and tail-flipping away were infrequent. A weak association existed between the cessation of anterior currents and not being in physical contact.

### Dynamics of Urine Release

Dominants and subordinates released urine differently during agonistic encounters in terms of duration and the number of times urine was released. The average duration of urine release differed significantly between dominants ( $7.0 \pm 1.0$  s) and subordinates ( $4.1 \pm 0.9$  s) (Fig. 6a;  $P < 0.05$ ). Dominants released urine significantly more



**Figure 4.** Correspondence analysis of the behaviours and currents generated by dominant crayfish during agonistic encounters: (a) dimensions 1 and 2; (b) dimensions 1 and 3. The Euclidian distance between behaviours and currents indicates a higher correspondence value, which indicates a temporal connection. The analysis generates these spatial relationships between points by representing the data on axes of three-dimensional space. The circles were added to draw attention to the closest correlations.

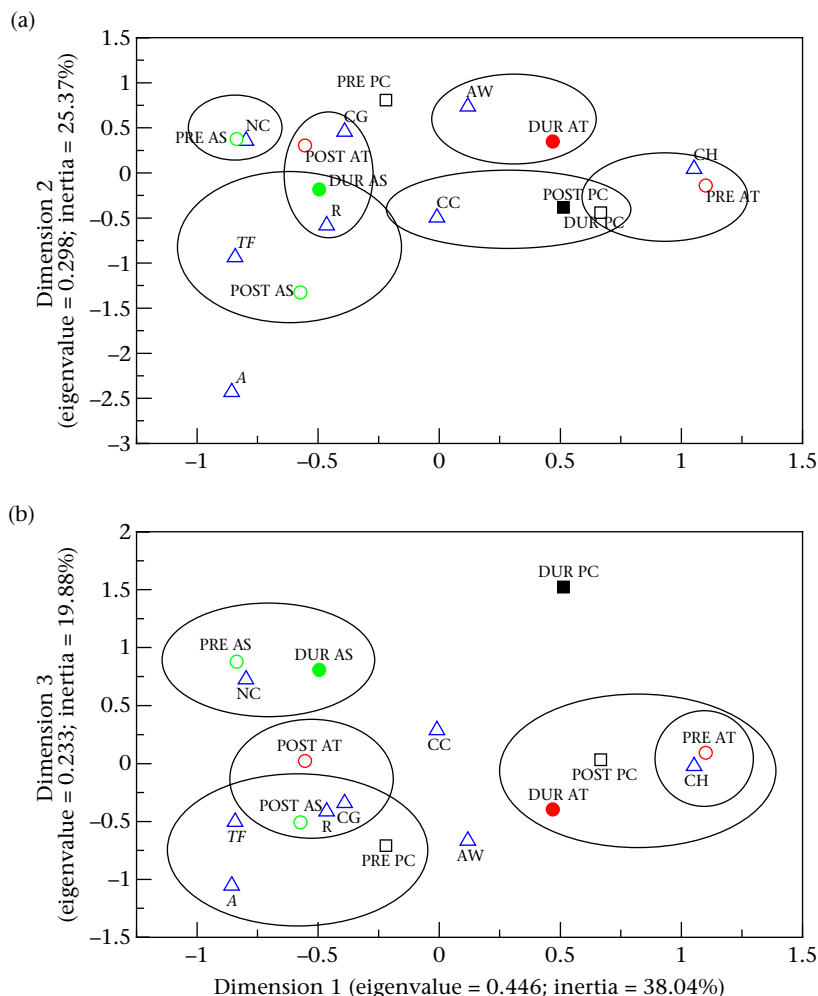
often ( $N = 75$  events) than did subordinates ( $N = 9$  events) during agonistic interactions (Fig. 6b;  $P < 0.05$ ). The timing of urine release did not differ between dominants and subordinates, but urine release occurred significantly more often while opponents were in contact ( $N = 49$  dominant,  $N = 7$  subordinate; Fig. 6c) than when they were not in contact ( $N = 26$  dominant,  $N = 2$  subordinate;  $P < 0.05$ ). In addition, dominants spent significantly more total time releasing urine (530.3 s,  $P < 0.05$ ) than did subordinates (36.7 s).

### Correlation of Current Generation with Urine Release

The combination of DPIV and urine visualization confirmed that urine is transported within anterior projection currents. This release was paired to specific agonistic behaviours and was dependent upon status roles. Results of the correspondence analysis showed that

dominant crayfish released urine while performing most agonistic behaviours (Fig. 7;  $P < 0.001$ ). Before releasing urine into anterior currents, dominants first approached an opponent and then made chelae contact. When dominants released urine with anterior currents, they did so either while holding the chelae of the opponent or when not engaged with their opponent. There were no agonistic behaviours closely correlated with the cessation of urine release during generation of anterior currents. Before urine releases with posterior currents, dominants first made initial chelae contact. Soon after, they released urine with posterior currents, then held and grabbed their opponent using their chelae. After posterior currents with urine release ceased, opponents separated. Antennal whips were not strongly correlated with urine releases.

Unlike dominants, subordinates did not release urine while generating posterior currents. In subordinate crayfish, agonistic behaviours and urine releases were not clustered and thus were more weakly correlated (Fig. 8;  $P < 0.05$ ). Chelae contact and chelae holding were performed more



**Figure 5.** Correspondence analysis of the behaviours and currents generated by subordinate crayfish during agonistic encounters: (a) dimensions 1 and 2; (b) dimensions 1 and 3. Behaviours in italics indicate that the behavioural events did not have a high enough frequency to be considered a confident measure of the events. All other symbol designations and abbreviations as given in Fig. 4.

often by subordinates before and during the generation of anterior currents with urine release. After anterior current generation with urine release, subordinates tended to continue to hold their opponent using their chelae. Approaching an opponent, chelae grabbing, antennal whipping, and no contact were performed at low frequencies during urine releases and consequently were considered to be invalid measures for agonistic behaviours.

## DISCUSSION

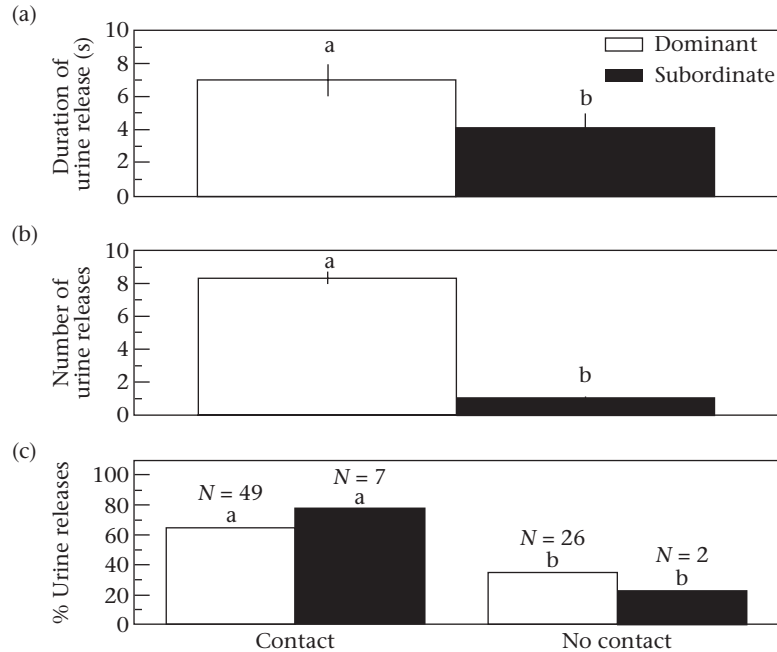
### Current Generation and Control

Our results show that crayfish actively control the delivery of potential information by controlling currents. Crayfish control these currents by generating different projection and collection flow fields. Anterior projection currents had the greatest velocity regardless of the social status of the individual, followed by posterior projections, and lastly collection currents. Anterior projections are generated using the maxillae and nephropores as a means

to send signals to the primary olfactory appendages (Rutherford et al. 1996; Breithaupt & Ayers 1998; Bergman et al. 2003). Posterior projections are generated by the pleopods and are associated more often with dominants than subordinates. Slower collection currents appeared to be generated by gill movements, as observed from the lack of appendage movement (maxillae and pleopods).

Crayfish used all of these self-generated currents to alter the delivery of either chemical or mechanical signals. These signals were also dependent upon social status. Dominant and subordinate crayfish generated different currents by projecting or drawing in signals. Dominant crayfish generated more posterior currents and more currents overall (posterior and anterior) than subordinates, suggesting that dominants project chemical information or mechanical information more often than subordinates. The results of our ethological analysis showed that the type and timing of generated currents and the delivery of signals were largely dependent upon the social status of the sender.

We have adopted the term 'signal' for both current generation and urine release because both were associated with behavioural changes in receivers (Maynard Smith



**Figure 6.** The average  $\pm$  SE (a) duration of urine release and (b) the number of urine releases per trial by dominant and subordinate crayfish during agonistic encounters. (c) The mean percentage of urine releases by dominant and subordinate crayfish when opponents were and were not in contact during agonistic encounters. Different letters indicate a significant difference between treatments using one-way MANOVA and Tukey post hoc tests.

& Harper 2004). In addition, the specific behaviours and agonistic levels associated with these signals differed between dominant and subordinate crayfish. Assuming that there are evolutionary consequences of being dominant or subordinate (i.e. mating, habitat selection, foraging constraints; see Bergman & Moore 2003), it appears as though these signals and their behavioural responses have evolved for their use within social situations.

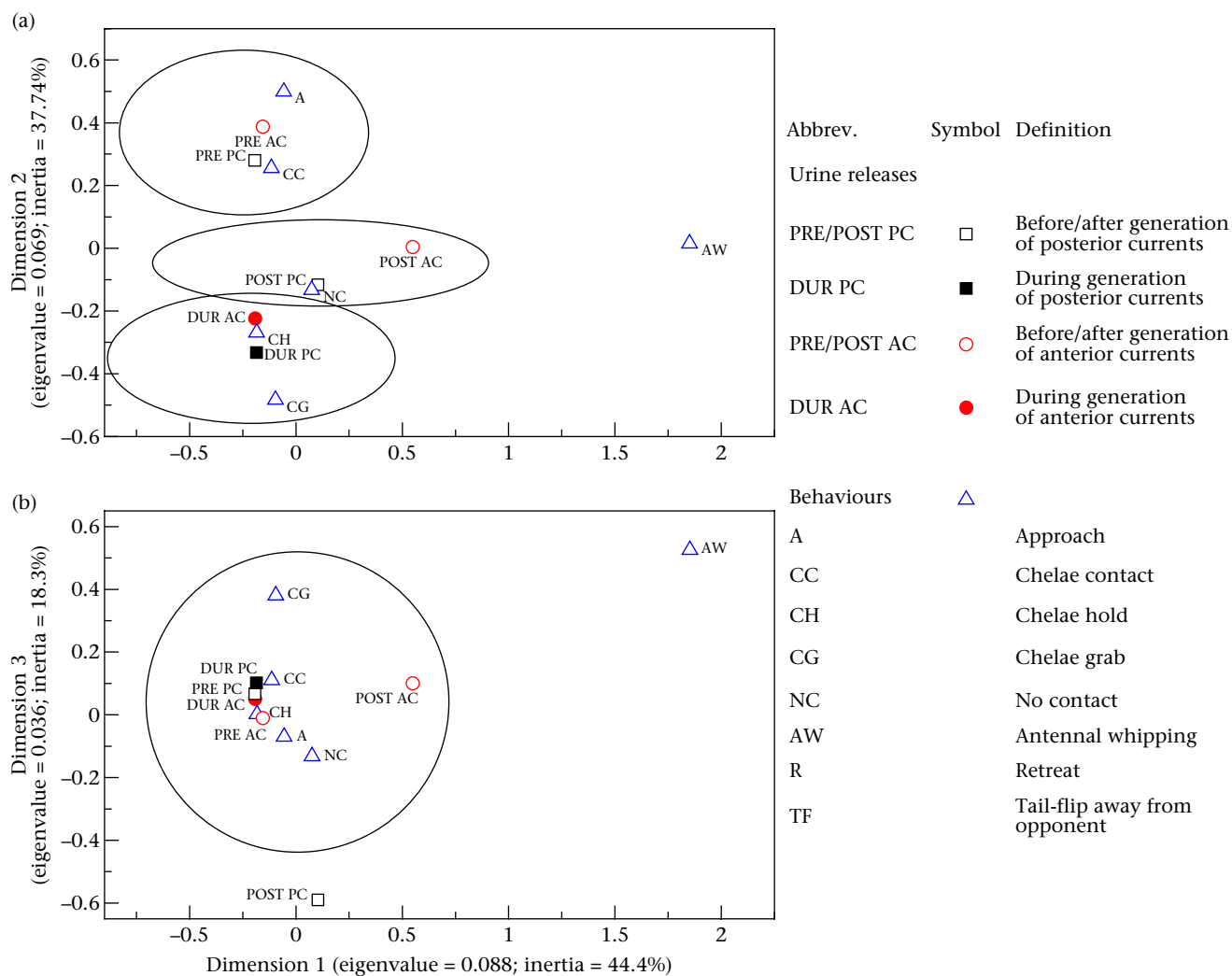
Agonistic behaviours associated with current generations were influenced by physical contact or absence of physical contact with an opponent. When two crayfish were engaged in a bout, they generated brief anterior currents relatively often. However, when crayfish were not engaged in a fight, they generated long, infrequent anterior currents. Since the types of anterior currents were correlated in time and space with specific agonistic behaviours, these currents contain information that is essential to controlling or influencing agonistic interactions.

Crayfish generated diverse patterns of flow during agonistic bouts that corresponded to certain social behaviours. Dominant and subordinate crayfish each displayed specific behavioural repertoires that were correlated with current generation. When two crayfish made initial agonistic contact, the eventual dominants locked chelae with the eventual subordinates and subsequently generated anterior projection currents. After this period, current generation subsided and the fight then increased in intensity, as indicated by chelae grabbing. The cessation of anterior currents while crayfish were physically separated led to antennal whipping, which was principally used by dominants. Antennal whips have been characterized as an appeasement display used by subordinates and are important for the facilitation of physical contact during

social interactions (Ameyaw-Akumfi 1979; Bruski & Dunham 1990). Our analysis shows that antennal whips can also indicate an increase in the intensity of agonistic encounters.

Current generation resumed when crayfish locked chelae during an agonistic encounter. When these anterior projections ceased, dominants continued to approach and pursue their opponent. Dominants did not retreat or tail-flip away from opponents, whereas subordinate crayfish consistently retreated and occasionally tail-flipped away from dominant crayfish after these currents subsided. Posterior currents were generated more often by dominants before and during chelae-grabbing periods. However, when subordinates generated posterior currents, they were used while in chelae contact. Status level appears to greatly influence current generation and the behaviours associated with currents. The coupling of flow and behaviour suggests that these currents are used for the transfer of information during social interactions. These differences in behaviour reinforce the 'principle of anti-thesis' suggested by Darwin (1872). Subordinate animals attempt to appease dominant animals by using postures and movements that are the opposite of aggressive displays used by dominants. In particular, this can be observed when a subordinate animal displays a lowered posture, by remaining nearer to the substrate, in response to the raised posture of a dominant animal, and is unwilling to engage in further agonistic interactions (Bruski & Dunham 1987; Edwards & Kravitz 1997).

Flow fields displayed a pattern of outgoing and incoming flow that appeared to carry mechanical or chemical information. In fact, Breithaupt & Ayers (1998) showed that isolated and tethered crayfish, whose major



**Figure 7.** Correspondence analysis of the behaviours and urine releases by dominant crayfish during agonistic encounters: (a) dimensions 1 and 2; (b) dimensions 1 and 3. The Euclidian distance between behaviours and currents indicates a higher correspondence value, which indicates a temporal connection. The circles were added to draw attention to the closest correlations.

chelae have been removed, create a variety of anterior currents. In our study, we visualized currents under more complex circumstances by using intact, untethered crayfish under agonistic conditions. Crayfish social status altered manipulation or generation of currents, which was evident from the fact that the agonistic behaviours and current types used by dominant crayfish differed from those used by subordinates. Our results suggest that current generation may serve as a tactile signal and function in dispersing chemical signals within an environment. Thus, crayfish may control currents for use as a potential vector for the exchange of information or possibly to conceal themselves from conspecifics at different times during the course of hierarchy establishment.

### Urine Release during Agonistic Interactions

Social status appeared to affect urine release during agonistic interactions. Dominants released urine longer

than did subordinates. In addition, dominants also released urine more frequently during fights than did subordinates, a finding which is similar to Breithaupt & Eger's (2002). Urine release during agonistic interactions appears to be an indicator for the eventual dominance of an animal. Urine releases appear to reveal past social history, such as previous social status (Zulandt Schneider et al. 1999, 2001).

The controlled release of urine during agonistic interactions suggests that there are chemical cues in the urine that reflect social status and mediate fight dynamics. Active urine release during agonistic interactions may indicate communication with and/or sampling of conspecifics (Breithaupt & Atema 1993). For example, Breithaupt & Eger (2002) found that urine release by crayfish occurs more frequently during agonistic encounters than during nonsocial activities. Urine appears to be of great importance in crayfish agonistic interactions, because when urine release is blocked, the duration and intensity of fights increase (Zulandt Schneider et al. 2001).



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