

# The use of the major chelae by reproductive male crayfish (*Orconectes rusticus*) for discrimination of female odours

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## Summary

The major chelae have been found to be important structures used for breeding and reproduction in crustaceans; however their role in the detection of conspecific sex odours is unknown. We implemented a behavioural bioassay to test whether male reproductive (form I) and nonreproductive (form II) crayfish (*Orconectes rusticus*) use their major chelae to detect reproductive female odours. We videotaped and analysed the behavioural reactions of form I and form II males to four different odour treatments: reproductive female-conditioned water, male-conditioned water, filtered fish homogenate (food odour; positive control), and water (negative control) delivered from an air stone ( $N = 20$  per treatment). In addition, all males were under two sensory conditions: intact or blocked chelae. Chelae were chemosensory-blocked by coating their dorsal surface with super glue. Normalized odour source handling time was measured, along with time spent handling the odour source between differing groups of sensory appendages: major chelae/first walking legs and maxillipeds/first walking legs. Our results indicate that both form I and II, intact and blocked, male crayfish significantly handled the odour source after a food stimulus was introduced. These results indicate the efficacy of the behavioural assay and sensory-blocks. Only intact form I males handled the odour source significantly when a reproductive female odour was delivered. Sensory-blocking of the chelae of form I males eliminated this difference. Intact and blocked form II males showed no differences when presented with reproductive female odours. Intact form I male crayfish spent more time handling the reproductive female-conditioned water source with their major chelae/first walking legs than those crayfish exposed to food odours. These results strongly suggest that the major chelae contain necessary chemosensory structures needed for female odour source recognition.

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## Introduction

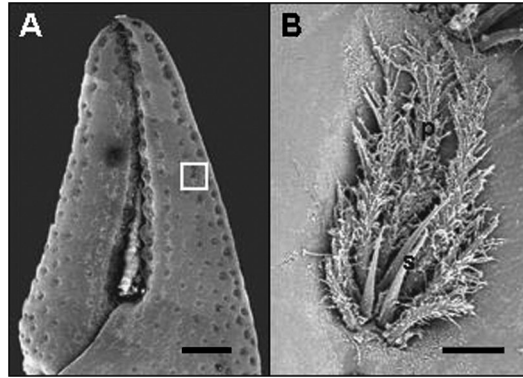
Crustaceans are known to utilize chemical communication for many aspects of their lives. Many behaviours of crustaceans have been shown to be mediated through chemical senses; these include: food acquisition and orientation (Moore et al., 1991; Kraus-Epley & Moore, 2002), conspecific recognition (Copp, 1986; Schneider & Moore, 2000), identification of conspecifics and/or social status (Schneider et al., 2001; Bergman et al., 2003), identification, localization, and sex recognition for mating purposes (Dunham & Oh, 1992; Stebbing et al., 2003), and detection of alarm cues (Hazlett, 1990, 1994; Schneider & Moore, 2000) and predators (Willman et al., 1994; Keller & Moore, 1999). Because crustaceans and other aquatic animals are considered 'leaky bags' (Atema, 1986), the chemicals they release into the environment may contain information about their internal states. This creates the potential for either active chemical communication with the sender actively releasing chemical information to convey information to the receiver or passive information released via normal metabolic processes. In either case, information about an animal's internal physiological state, including reproductive cues, could be transmitted to the receiving animal.

In crustaceans, reception of chemical cues occurs via peripheral chemoreceptors located within sensory hairs (or setae). Chemical stimuli reach the dendritic processes of sensory neurons by movement through pores in the cuticle covering the setae (Laverick & Ardill, 1965). Sensory setae are typically located on the cuticle of chephalothoracic appendages (Derby, 1982). These appendages, containing both mechano- and chemosensory setae, include antennae, antennules, maxillipeds (mouthparts), and pereopods (major chelae and walking legs). The most studied appendage, the antennules, are thought to be used for long distance orientation to prey items (Derby et al., 2001) and mate odours (Dunham, 1978; Gleeson, 1980; Kamio et al., 2005). In contrast, maxillipeds and walking legs have been found to respond to food odours (Derby & Atema, 1982; Corotto & O'Brien, 2002; Garm et al., 2005). Sensory receptors found on the walking legs can also function in detecting nearby food (Derby & Atema, 1982; Corotto & O'Brien, 2002), but have

also been implicated as potential sources of information for distance orientation (Moore et al., 1991; Keller et al., 2003). Major chelae of crayfish contain both simple and plumose setae. Simple setae have been demonstrated in crayfish and other crustaceans as having a bimodal chemo- and mechanosensory function (Weisbaum & Lavalli, 2004; Obermeier & Schmitz, 2004; Belanger et al., in prep.), while plumose setae are thought to be mechanoreceptors (Tautz et al., 1981). Currently, there is very little information about the role that the major chelae play in chemoreception, although observations from our laboratory suggest that the chelae may be involved in the detection of female odours.

Previous studies have attempted to isolate the sensory appendage responsible for peripheral perception of mate odours in crustaceans (Ameyaw-Akumfi & Hazlett, 1975; Dunham, 1978; Gleeson, 1980; Tierney et al., 1984; Kamio et al., 2005). In the crab, it is believed that the lateral antennule is responsible for pheromone detection (Dunham, 1978; Gleeson, 1980; Kamio et al., 2005). When this appendage was removed, response to a female odour source decreased significantly (Kamio et al., 2005). Similar results were demonstrated in two crayfish species, *Orconectes propinquus* and *Procambarus clarkii* (Ameyaw-Akumfi & Hazlett, 1975; Tierney et al., 1984). However, a study by Corotto et al. (1999) in crayfish (*P. clarkii*) did not find that the antennules are necessary for localization of females for the purpose of mating. When the crayfish had their antennules ablated, successful mating occurred in the same amount of time. These studies have ignored the potential role of the major chelae as chemoreceptive organs used for detection of conspecific odours.

Chelae are known to be important mechanical structures used for reproduction in crustaceans. In the freshwater prawn (*Macrobrachium rosenbergii*), males with larger chelae are more successful at courting and mating females (Ra'anan & Cohen, 1985; Ra'anan & Sagi, 1985). In crayfish, the major chelae are needed for the physical manipulation of females (stages 1-5, Stebbing et al., 2003). Because male reproductive success has been positively correlated with chelae length in crayfish, researchers have concluded that the most important purpose of the major chelae is their use in reproductive activities (Stein, 1976). Physiological studies on the major chelae of crayfish have indicated that the chelae have both mechano- and chemosensory neurons (Bauer & Hatt, 1980; Hatt & Bauer, 1980; Altner et al., 1983). Some species of crayfish moult from reproductive to nonreproductive forms



**Figure 1.** A. Scanning electron micrograph depicting the dorsal surface of the major chelae of a form I male crayfish containing sensory hair pockets (square). Scale bar in A is 5.6 mm. B. A sensory hair pocket (inset from A) found on the major chelae containing both simple (s) and plumose setae (p). Scale bar in B is 40  $\mu$ m.

and this may alter both the mechanical and sensory capabilities of the major chelae (Stein, 1976; Snedden, 1990).

Male *Orconectes rusticus* are dimorphic in that they moult continuously from season to season from displaying two different morphotypes (Crocker & Barr, 1968). The reproductive form differs from the nonreproductive form in that it has long white reproductive stylets and more robust major chelae in proportion to body size (Crocker & Barr, 1968; Stein, 1976). Belanger et al. (in prep.) have also shown that form I crayfish have a larger proportion of sensory hairs (plumose and simple setae) lining the dorsal surface of the major chelae (Figure 1). This sensory hair difference coincides with seasonal differences as sensory hairs are more abundant during the reproductive season. These findings create the possibility that male crayfish may use one or both of these sensory hairs types on the major chelae for mate localization, however this has not been tested behaviourally.

The goal of our study was to determine whether reproductive male crayfish, *O. rusticus* (Girard, 1852), use chemo- or mechanosensory structures, located on their major chelae, to detect and discriminate conspecific female cues. Stebbing et al. (2003) showed that male crayfish handled (made contact with, seized, or mounted) a female odour source more than the water; however their study did not investigate the sensory appendage responsible for the perception of female odours. To elucidate what role the major chelae have in the perception of female odours, we used a behavioural bioassay to test

this. Because it is known that the crustacean chemoreceptive system has the ability to discriminate many chemicals and chemical mixtures (Ache et al., 1976; Carr & Derby, 1986), it is important to decipher which peripheral appendage is important for distinguishing conspecific odours. Eventually, this will lead to an understanding of the peripheral processing of mate odours, as well as higher-order brain processing centres that are important for mate recognition.

## Materials and methods

### *Animals*

*Orconectes rusticus* (females, form I, and form II males) were collected by seining in the Portage River near Bowling Green State University in Bowling Green, Ohio, USA. Intermolt male crayfish used in experiments were visually and mechanically isolated in plastic flower pots that contained lids with holes (17.8 cm diameter and 9 cm depth) that were stored in a flow-through holding tank (48 × 154 × 31 cm) where water within the pots was continuously exchanged with water in the tank. The holding tank was in an environmental chamber (23°C, 14:10 hour light dark cycle). Crayfish males were held in population tanks with other males for 1 week to 5 months prior to being isolated for at least 48 h before behavioural experiments. Crayfish mass, carapace, and chelae lengths ( $\pm$  SE) were measured for form I and form II males used in this experiment (form I – 18.73  $\pm$  0.63 g; 3.63  $\pm$  0.04 cm carapace length; 3.70  $\pm$  0.06 cm chelae length, form II – 21.43  $\pm$  0.84 g; 3.87  $\pm$  0.05 cm carapace length; 3.83  $\pm$  0.08 cm chelae length) and only crayfish with intact appendages were used (i.e., antennae, lateral and medial antennule filaments, chelae, maxillipeds, and walking legs). As in Stein (1976) form I males had significantly larger major chelae in proportion to carapace length ( $p = 0.006$ ,  $t = 2.781$ ,  $df = 318$ ,  $N = 320$ ;  $t$ -test). Crayfish were fed a diet of rabbit pellets three times per week. Experiments using form I males were completed between August and October 2003-2005 and experiments using form II males were completed between February and July 2004. Form I and form II individuals were stored separately, as their availability was seasonal. All treatments were performed between 9.00 and 18.00 h.

Form I and form II males were identified by examining their reproductive stylets (Crocker & Barr, 1968). *O. rusticus* form I males have relatively long reproductive stylets that extend to the base of the second pereopods when the abdomen is flexed. The stylets of form II males differ from form I males in that they are shorter, yellow coloured, and are less structurally defined.

#### *Conditioned water stimulus and setup*

To examine male attraction to a female odour source and consequently chelae odour sampling ability, fresh female-conditioned water was obtained using a procedure similar to Stebbing et al. (2003). Conditioned water was obtained from six reproductive female *O. rusticus* ( $9.79 \pm 0.61$  g and  $3.22 \pm 0.10$  cm carapace length) by holding them individually in clear plastic pots ( $40 \times 20 \times 25$  cm) containing 500 ml of aerated dechlorinated water for a 24-hour period. Water from each pot was then collected, combined, and filtered (Whatman® 185 mm #1004185) to remove debris and stored at  $-20^{\circ}\text{C}$  until used. Conditioned water from 6 male (form I and form II) *O. rusticus* ( $11.07 \pm 1.08$  g and  $3.32 \pm 0.11$  cm carapace length) was also obtained, filtered, and stored using the same method. Different plastic pots were used for the collection of each odour type so that there was no cross contamination of the odours collected. There was no difference in mass or carapace length between the female and male crayfish odour donors used in this experiment ( $p = 0.322$ ,  $t = 1.042$ ,  $df = 10$ ,  $N = 12$ ;  $t$ -test). The negative control for this experiment was fresh dechlorinated water that had been aerated over a 24-hour period with no crayfish present. The water was then subsequently filtered in a similar manner to the previous conditioned water treatments. A positive control of filtered fish homogenate (food odour) was prepared fresh daily in a similar manner to Kraus-Epley & Moore (2002) by weighing 50 g of Pollock fish fillets pureed in a blender with 500 ml of dechlorinated water. This homogenate was then filtered as with all the other odours tested in this experiment.

#### *Blocking protocol*

The treatment groups consisted of the following: (i) Form I males with intact chelae; (ii) Form I males with chelae blocked; (iii) Form II males with intact chelae; (iv) Form II males with chelae blocked. Twenty individuals were used in each of the four treatment groups and were exposed to one

of the four randomized odours: female-conditioned water, male-conditioned water, dechlorinated water (negative control) and filtered fish homogenate (positive control). Each animal was used only once and a total of 320 crayfish (4 treatments  $\times$  4 odours  $\times$  20 crayfish) were used in this study. The blocking procedure, similar to Kraus-Epley & Moore (2002), was performed by restraining crayfish and covering the sensory hairs on their major chelae with superglue (Duro Quick Gel<sup>®</sup>). Using the applicator tip, glue was applied to the dorsal surface of chelae of experimental animals beginning at the base and extending to the tip, covering the sensory hairs (see Figure 1). Afterwards, a drying accelerator (Zip Kicker<sup>™</sup>) was applied to the superglue with a cotton swab in order to speed drying time. The dorsal surfaces of the major chelae were blocked because Solon & Cobb (1980) showed that lobsters (*Homarus americanus*) tend to position themselves so that the dorsal surfaces of the chelae would be more likely to receive stimulus events. Blocking animals by covering receptors with super glue inhibits both chemo- and mechanoreceptors (Kraus-Epley & Moore, 2002; Bergman et al., 2003). Intact animals had a similar amount of glue placed at the base of the carapace (on their back) to ensure that changes in behaviour were not due to the presence of superglue. Chelae of intact animals were washed with tank water using a syringe to simulate the physical stimulation of the chelae of experimental individuals during gluing. When the glue was completely dry (~2 min), each individual crayfish was placed in the test chamber for the acclimation period.

#### *Experimental trials*

Blocked and intact ( $N = 20$  trials per treatment) form I and form II male *O. rusticus* were placed in opaque, visually isolated tanks (25  $\times$  14  $\times$  14.5 cm). Each tank contained 2 litres of fresh dechlorinated water that was not aerated during recording periods. Males were allowed to acclimate for 1-hour to the test tank before the start of the experiment. As in Stebbing et al. (2003), the test tank contained a blue cylindrical aquarium air stone 2 cm in length attached to 30 cm of 5 mm-diameter silicone tubing. A syringe containing 20 ml of the test odour was attached to the air stone via 15 cm of 5 mm-diameter silicone tubing. Between trials, each of the test tanks and air stone odour sources were rinsed vigorously with distilled water. Different air stones and syringes were used for each odour type in order to prevent cross

contamination. The male's air stone handling time (odour source handling) was recorded for 15 minutes before the introduction of the treatment odour (pre-test period), and for another 15 minutes afterwards (test period) using a Canon XL-1 (digital video camera) mounted above the test tank. All odours were introduced by hand to the test arena at a rate of approximately 5 ml/s after removing the air from the tubing using negative pressure on the syringe. Preliminary dye trials demonstrated that the stimulus delivery procedure did not allow the odour to rapidly mix. Instead, a gradient was created from the diffusion of the odour away from the odour source. Experimental setup and recording periods for these bioassays were similar to those of Stebbing et al. (2003) and Belanger et al. (in press a).

#### *Data collection and analysis*

'Handling' of the odour source was defined as the animal making contact with, seizing, or mounting the air stone (Stebbing et al., 2003). As in Schmidt & Derby (2005), odour source handling time before the addition of odours in the 15 minute pre-stimulation period was subtracted from post-stimulation period in order to subtract the baseline activity from activity induced by stimulation. This data was analysed for normality and was assessed using a 3-way ANOVA with a Fisher LSD post-hoc test to look for differences across treatments. With this, overall handling responses comparing blocking status, odour type presented, and the reproductive form of the crayfish were compared. For those treatments that showed significant responses to the odour, appendage handling of the odour source was further analysed to differentiate these responses. For intact form I treatments with female-conditioned water stimulus, intact and blocked form I and form II's treatments with filtered fish homogenate stimulus, handling of the odour source with chelae/first walking legs and maxillipeds/first walking legs was recorded. We used data which examined chelae/first walking legs and maxillipeds/first walking legs because as soon as the major chelae contacted the odour source, walking legs were engaged within 3 seconds; this was the same for maxillipeds. For this reason, analysing individual chelae and maxillipeds handling was not a sufficient indicator of sensory appendage use. Being that we include walking leg data for examining both, the major chelae and maxillipeds, the use of walking legs for chemoreception of food and mate odours can be excluded. Appendage handling times were analysed separately using a 1-way ANOVA with a Fisher LSD post-hoc test to look for differences across treatments.

## Results

### *Odour source handling*

#### (1) Female-conditioned water responses

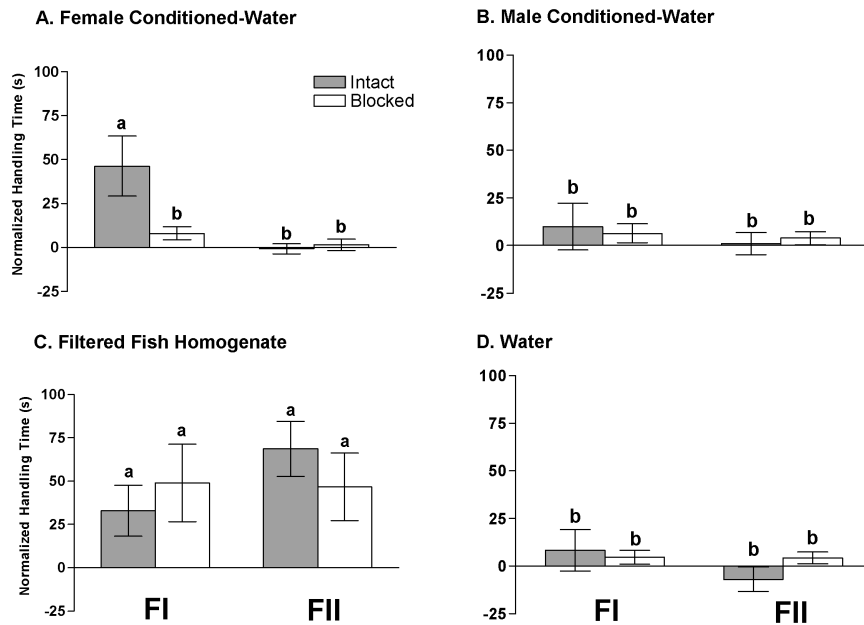
Intact reproductive (form I, FI) male crayfish responded to reproductive female-conditioned water with an odour source handling time of  $46.35 \pm 17.00$  seconds (s) (mean  $\pm$  standard error (SE)). Blocked form I males spent significantly ( $p = 0.018$ ) less time ( $8.00 \pm 3.80$  s) handling the female-conditioned water odour source when compared to intact form I males (Figure 2A). Intact and blocked nonreproductive (form II, FII) male crayfish did not respond to female-conditioned water ( $-0.75 \pm 2.91$  s and  $1.50 \pm 3.25$  s, respectively) and handled the female odour source significantly less ( $p = 0.004$  and  $p = 0.006$ , respectively) than intact form I males (Figure 2A). Female odour source handling times were similar when form I sensory-blocked individuals were compared to both form II treatments, intact, and blocked ( $p = 0.587$  and  $p = 0.687$ , respectively). There was also no difference ( $p = 0.703$ ) when blocked and intact form II treatments were compared. Therefore, the manipulation of the odour source by form I crayfish was eliminated when males had had their major chelae sensory-blocked, making blocked form I male responses comparable to those of form II males.

#### (2) Male-conditioned water responses

When both intact form I and form II males were exposed to conspecific male-conditioned water, there were no significant differences observed when compared to blocked individuals of the same form. Intact form I males spent  $9.95 \pm 12.31$  s handling the male-conditioned water odour source while blocked individuals spent  $6.45 \pm 5.05$  s ( $p = 0.828$ ; Figure 2B). The data for form II males was also not significantly different ( $p = 0.850$ ) where intact males spent  $0.95 \pm 5.81$  s handling the male-conditioned water odour source and blocked males handled the source for  $4.00 \pm 3.38$  s (Figure 2B).

#### (3) Food odour responses

When filtered fish homogenate, food odours (positive control), was tested, both intact and blocked form I male crayfish responded positively to food odours with increased handling time of the odour source. Intact form I males handled the odour source for  $32.95 \pm 14.71$  s, while blocked individuals



**Figure 2.** A comparison of normalized time in seconds (s) spent handling an odour source (mean  $\pm$  SE) for form I (FI) and form II (FII) males that were intact and sensory-blocked ( $N = 20$  per treatment) with respect to their major chelae. Significant differences ( $p < 0.0005$ ) between treatments were denoted as a and b. Shaded bars signify crayfish that were intact while white bars represent crayfish with sensory-blocked major chelae. A. Intact FI males spent significantly more time handling the female-conditioned water source than sensory-blocked FI individuals and both intact and blocked FII males. B. Conspecific male odours did not elicit a significant odour source handling response for both intact and blocked, FI and FII males. C. Fish odour source handling did not vary across all FI and FII treatments (intact and blocked) and were significantly greater than responses by form I blocked and form II intact and blocked males to female odours as well as, all responses to male odour and water. D. When FI and FII (intact and blocked) males were exposed to water, there were no significant increases in odour source handling time.

handled the odour source for  $49.00 \pm 22.39$  s (Figure 2C). These did not differ significantly from one another ( $p = 0.760$ ). Form II males handled the filtered fish homogenate similarly ( $p = 0.175$ ) with intact males spending  $68.60 \pm 15.96$  s handling the odour source and those males with their major chelae blocked handling the odour source for  $46.70 \pm 19.45$  s (Figure 2C). There was no difference across these treatments demonstrating that the chelae blocking did not affect responses to food odours.

#### (4) Water responses

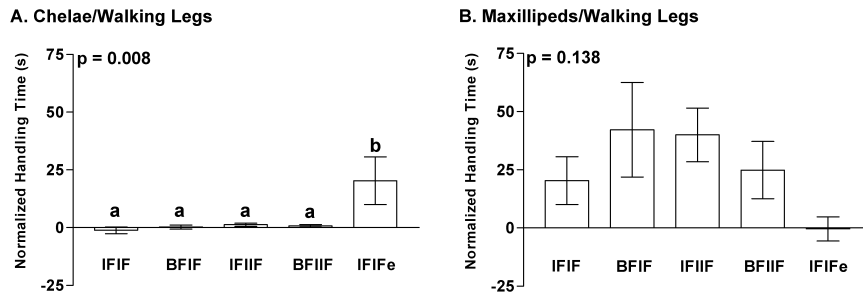
When exposed to water (negative control) intact form I male crayfish spent  $-6.90 \pm 6.43$  s handling the odour source while blocked crayfish handled the water source for  $4.30 \pm 3.15$  s (Figure 2D). Odour source handling times were not significantly different ( $p = 0.821$ ). For form II crayfish, there was no difference ( $p = 0.487$ ) between the time intact males spent handling the water source ( $8.35 \pm 10.94$  s) and blocked males ( $4.70 \pm 3.64$  s; Figure 2D). Overall, there was no significant difference between all of these treatments.

#### *Comparison across treatments*

Blocking of the major chelae significantly altered the response of form I males to female-conditioned water, but did not alter the response to fish homogenate ( $F_{(3,298)} = 15.54$ ,  $p < 0.0005$ ; 3-way ANOVA). Intact form I males responded significantly to both female-conditioned water and to fish homogenate (odour source handling time  $>25$  s), while blocked form I males only responded to fish homogenate and not female odours. Blocking the major chelae of form I males thus had an impact on the response by form I male crayfish to female odours however; this sensory-blocking technique did not affect their response to food odours. When comparing all treatments, odour source handling times were similar for form I blocked male crayfish to female odours, both intact and blocked form II males to female-conditioned water, all crayfish exposed to conspecific male odour, as well as all test animals exposed to water.

#### *Odour source handling by different appendages*

For those five treatments that showed significant increases in handling time ( $>25$  s) to the odour source (intact form I males to food odour (IFIF), blocked form I males to food odour (BFIF), intact form II males to food odour (IFIIF), blocked form II males to food odour (BFIIF), and intact form I males to female odour (IFIFe)), video footage was then further analysed to determine if there was a difference in the usage of sensory appendage for manipulation of the source. For those treatments, handling of the odour source with the chelae/first walking legs and maxillipeds/first walking legs was compared. This was done in order to determine if different appendages engaged the odour source when female and food odours were presented.



**Figure 3.** An examination of sensory appendage use for female and food odours for males that were intact or sensory-blocked with respect to their major chelae ( $N = 20$  per treatment). This figure includes normalized handling time (mean in seconds (s)  $\pm$  SE) from intact form I males to food odour (IFIF), blocked form I males to food odour (BFIF), intact form II males to food odour (IFIIF), blocked form II males to food odour (BFIIF), and intact form I males to female odour (IFIFe). A. Intact form I male crayfish handled a female-conditioned water odour source significantly more with their chelae/walking legs than male crayfish (FI and FII) exposed to food odours. Significant differences ( $p = 0.008$ ) between treatments were denoted as a and b. B. An increase in maxilliped/walking leg appendage use was observed for IFIF and BFIF treatments, as well as IFIIF and BFIIF treatments when compared to IFIFe.

When presented with female-conditioned water, intact form I males used their chelae/first walking legs significantly more ( $F_{(4,94)} = 3.671$ ;  $p = 0.008$ ) to handle the odour source (Figure 3A). Overall, intact form I male crayfish handled the female odour source with their chelae/first walking legs for  $20.30 \pm 10.32$  s (43.8% of total handling time). The response to female odour from intact form I males was statistically different ( $p = 0.002$ ) from form I intact males response to food odours. Those individuals did not respond to food odours with their chelae/first walking legs ( $-1.15 \pm 1.44$  s,  $-3.5\%$ ). Blocked form I males handled the food odour source for  $0.25 \pm 0.86$  s (0.5%) with their chelae/first walking legs. This differed from the time intact form I individuals spent handling the female odour source ( $p = 0.003$ ). Form II (intact and blocked) male crayfish also spent very little time handling the food odour source with their chelae/first walking legs also. Intact form II males handled the odour source for  $1.25 \pm 0.73$  s (1.8% of total time) while blocked form II males spent  $0.80 \pm 0.56$  s (1.7% of total time) handling the food odour. This differed significantly from the time spent by intact form I males responding to female odour ( $p = 0.005$  and  $p = 0.004$ , respectively).

Data from maxillipeds/first walking legs handling of the odour source was also examined ( $F_{(4,95)} = 1.785$ ;  $p = 0.138$ ) (Figure 3B). These data demonstrates that there is an increase handling of food odour sources with maxillipeds/first walking legs. Intact form I males handled a food odour source for  $20.37 \pm 10.29$  s (61.8% of total time) while blocked form I individuals spent  $42.20 \pm 20.34$  s (86.1%) handling the food odour source. Intact and blocked form II males handled the fish homogenate odour source for  $40.00 \pm 11.54$  s (58.3%) and  $24.85 \pm 12.33$  s (53.2%), respectively. When female odour was presented to intact form I males, they handled this odour source for  $-0.40 \pm 5.21$  s with the maxillipeds/first walking legs. Overall, there was an increase handling time of female odours with chelae/first walking legs (Figure 3A) while food odours were handled mainly with maxillipeds/first walking legs (Figure 3B).

## Discussion

The importance of the major chelae for the detection of female odours has been clearly demonstrated by the decrease in odour source handling by reproductive male *O. rusticus* following sensory-blocking of the setae on the major chelae. These results show that peripheral chemosensory input from the major chelae is required for the odour source grasping behaviour, rather than from receptors located on the lateral antennules (Tierney et al., 1984; Kamio et al., 2005). The deleterious effect of the blocking technique on the odour source handling behaviour is supported by previous observations on the loss of chemosensory mediated behavior following this type of blocking in *O. rusticus* (Kraus-Epley & Moore, 2002; Bergman et al., 2003) and subsequent blockage of sensory receptors. Using the blocking technique allowed us to clearly demonstrate that only reproductive male crayfish use their major chelae for detection of female odours. Also, we have found that crayfish can differentiate female odours from others, including food odours, utilizing different sensory appendages for handling of food odours. Differences in odour source handling, along with the fact that reproductive male crayfish have proportionally more sensory hairs on their major chelae (Belanger et al., in prep.), strongly suggests that the major chelae are chemosensory appendages that play a role in courtship and mating.

Both mechanical and chemical information presented to the major chelae may be important for mating and courtship activities. Courtship behaviour

in crayfish, as described by Stebbing et al. (2003), includes orientation, contact, seizure, turning, mounting, spermatophore deposition, and dismounting. During this orientation phase, male crayfish have been observed to wave their chelae horizontally through the water column in an odour-mediated sampling behaviour (T. Keller, pers. comm.). Chela waving behaviour has also been reported in *P. clarkii* by Itagaki & Thorp (1981) and it has been suggested by Dunham & Oh (1992) that chelae waves represent potential sex discrimination with this behaviour. Chela waving behaviour is believed to be a chemosensory event as chelae have been shown to have chemoreceptive properties (Bauer & Hatt, 1980; Hatt & Bauer, 1980; Altner et al., 1983) and crayfish exhibit movements of their chelae similar to other appendages (e.g., antennule flicking) that have been shown to be associated with chemosensory sampling (Kraus-Epley & Moore, 2002). Finally, in our study only reproductive forms of male crayfish exhibit this waving behaviour reactivity to female odours (Figure 2A). Taken together, these results indicate that the major chelae of reproductive male crayfish are chemosensory appendages used for the discrimination of female odours.

As well as being chemosensory appendages, the major chelae are also important physical structures that aid in reproduction in crustaceans (for review, see Mariappan et al., 2000). It has been shown that form I male crayfish (*O. propinquus*) with large chelae are more successful in copulating with females than those with small chelae (Stein, 1976). Also, Snedden (1990) showed that male crayfish (*O. rusticus*) with larger chelae are better able to secure and orient females for copulations (stages 1-5, Stebbing et al., 2003). It has been found that mating is unsuccessful in shore crabs (*Carcinus maenas*) if one or both of the major chelae are not functional (Sekkelton, 1988). In the freshwater prawn (*M. rosenbergii*) males with different chelae morphotypes have varying access to mates, with individuals displaying larger chelae effectively courting mates (Ra'anan & Cohen, 1985; Ra'anan & Sagi, 1985). Our data suggest that chemosensory function of the major chelae in mating may also be important for explaining some of the previous findings.

Several species of crayfish have been reported to be able to discriminate between male and female odours (e.g., *P. clarkii*, Dunham & Oh (1992); Ameyaw-Akumfi & Hazlett (1975); *O. virilis*, Hazlett (1985); *O. propinquus*, Tierney et al. (1984)) presumably through the use of sex pheromones. In studies concerned with the detection of mate odours in crayfish, researchers have focused on the antennules, but have found conflicting results (e.g.,

Corotto et al., 1999; Tierney et al., 1984). Tierney et al. (1984) demonstrated that *O. propinquus* perceive conspecific odours with their antennules, whereas Corotto et al. (1999) found that antennules were not necessary for localization and mating in *P. clarkii*. The contradictory results in regards to antennule use in these two studies may be explained by our results showing the potential role of the major chelae in the detection of female odours. Stebbing et al. (2003) showed that male crayfish (*Pacifastacus leniusculus*) are attracted to a conspecific female odour source and handle this source with their major chelae, however they did not investigate which sensory appendage was important for this response. Our data coincide with data presented by Stebbing et al. (2003) and Corotto et al. (1999) by showing that the major chelae are necessary for the discrimination of female odours in reproductive male crayfish. This was not the case however for food odours (positive control) where both sensory-blocked and intact reproductive and nonreproductive males still responded to food odours presented to them (Figure 2C), suggesting alternate sensory appendage(s) may be used for perception of food odours.

Overall, crayfish use chemical cues for many types of behaviours that are important to survival and reproductive success (Ameyaw-Akumfi & Hazlett, 1975; Hazlett, 1985; Tierney & Atema, 1988; Dunham & Oh, 1992, 1996; Corotto et al., 1999; Moore & Grills, 1999; Giri & Dunham, 2000; Stebbing et al., 2003). Odours from female crayfish are perceived and processed peripherally by the major chelae of reproductive male crayfish. These odours may be recognized by nonreproductive males; however, they, like sensory-blocked reproductive males, do not behaviourally respond to the odour source. Therefore the attraction to female odour by reproductive males may be a conspecific mating cue or pheromone used for reproductive purposes. Male crayfish, regardless of reproductive state, do not respond to conspecific male odours (mixed form I and form II). This is not surprising as previous work on naïve male crayfish shows that these males do not respond to a naïve male odour source (Bergman & Moore, 2005). This indicates that the response by reproductive male crayfish to the female odour source is not a general response to conspecifics, rather it is sex-related however, pure form I and form II odours must be analysed separately to elucidate if male crayfish can use chelae chemoreceptors to discriminate male reproductive status. Our data suggest that there is peripheral processing of conspecific female odours by the major chelae and that they use these large sensory organs

not only to aid mechanically in reproduction, but also for chemoreceptive purposes. Coupled with this research, future work on major chelae sensory responses to stimuli will lead to insight on how the brain processes and integrates chemosensory information from multiple sensory appendages. This will ultimately lead to a clearer understanding of how organisms make decisions about their environment.

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