

THE ROLE OF THE MAJOR CHELAE IN THE LOCALIZATION AND
SAMPLING OF FEMALE ODOURS BY MALE CRAYFISH, *ORCONECTES*
RUSTICUS (GIRARD, 1852)

BY

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ABSTRACT

Research has shown that the major chelae of male crayfish, *Orconectes rusticus*, are important chemosensory appendages and may aid in the perception and discrimination of female odours. However, it is unclear whether the major chelae are important for localization of female odour sources. In this study, we examined the role of sensory information from the major chelae in the localization and discrimination of conspecific female odours. We analysed the behavioural reactions of form I male crayfish to two different odour treatments: reproductive female-conditioned water or water (controls) delivered from one end of a test arena. Also, all male crayfish either had their chelae intact or sensory blocked. We measured locomotory behaviours in response to odours and chelae treatments. In addition, behaviours performed at the source of the odour were quantified. Male crayfish with intact chelae spent more time closer to a reproductive female odour source, whereas crayfish with blocked chelae showed no preference. There was no response to water. Male crayfish demonstrated differences in chelae waving and other local behaviours in response to female odours. These results suggest that the major chelae are important sensory structures, which aid in the localization and discrimination of female odours.

RÉSUMÉ

Des recherches ont montré que la pince principale de l'écrevisse mâle *Orconectes rusticus* est un important appendice chémorécepteur et pourrait aider à la perception et la discrimination des odeurs des femelles. Cependant, il n'est pas clair si la pince principale est importante pour la localisation de la source des odeurs femelles. Dans cette étude, nous avons examiné le rôle de la pince principale dans l'information sensorielle comme la localisation et la discrimination des odeurs femelles con-spécifiques. Nous avons analysé le comportement de la forme I des mâles d'écrevisse à deux différents traitements olfactifs: de l'eau de femelles conditionnées à la reproduction ou de l'eau (contrôle) provenant de la fin de la zone de test. De plus toutes les écrevisses mâles avaient soit

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leur pince intacte ou sensoriellement bloquée. Nous avons mesuré le comportement de locomotion en réponse aux traitements odorants et de la pince. De plus les comportements effectués à la source de l'odeur ont été quantifiés. Les mâles avec une pince intacte passent plus de temps près de la source d'odeur de femelles en reproduction, tandis que les écrevisses avec des pinces bloquées ne montrent aucune préférence. Il n'y a eu aucune réponse à l'eau témoin. Les mâles d'écrevisse démontrent des différences dans le mouvement des pinces et d'autres comportements locaux en réponse à l'odeur des femelles. Ces résultats suggèrent que les pinces principales sont des structures sensorielles importantes, qui pourraient aider à la localisation et la discrimination des odeurs des femelles.

INTRODUCTION

Observations of animal behaviour, both terrestrial and aquatic, have implicated chemical communication as a significant mode of communication in most animal phyla (Dusenbery, 1992). Chemical cues play an important role in guiding the behaviour of many animals, such as attraction to conspecifics (Hansson, 1994), and many animals rely on chemical cues for mate choice and species recognition (Wyatt, 2003). Behavioural studies indicate that many crustaceans rely on chemical cues for localization of food odours (Devine & Atema, 1982; Moore et al., 1991; Kraus-Epley & Moore, 2002), discrimination of different odorants and sources (Derby et al., 1989; Adams et al., 2003; Wolf et al., 2004), communication with conspecifics (Atema, 1986; Schneider et al., 2001; Bergman et al., 2003), and identification, localization, and sex recognition for mating purposes (Atema & Engstrom, 1971; Dunham, 1979). Detection of chemical cues in crustaceans occurs via peripheral chemoreceptors located within the sensory setae of cephalothoracic appendages (Laverack, 1988; Derby, 1989).

Several studies have investigated the use of chemical reproductive cues by female crayfish and subsequent behavioural responses by conspecific males (e.g., Ameyaw-Akumfi & Hazlett, 1975; Gaudioso Lacasa & Cabello, 1979; Tierney & Dunham, 1982, 1984; Bechler et al., 1988; Dunham & Oh, 1992; Villanelli & Gherardi, 1998; Acquistapace et al., 2002; Stebbing et al., 2003a, b; Belanger & Moore, 2006). Responses to reproductive chemical cues include attraction (Ameyaw-Akumfi & Hazlett, 1975; Gaudioso Lacasa & Cabello, 1979; Tierney & Dunham, 1982, 1984; Bechler et al., 1988; Stebbing et al., 2003a, b), attempted copulation (Gaudioso Lacasa & Cabello, 1979; Villanelli & Gherardi, 1998), decreased aggression (Ameyaw-Akumfi & Hazlett, 1975; Hazlett, 1985; Dunham & Oh, 1992), increased motility (Acquistapace et al., 2002; Stebbing et al., 2003a), increased urine output and current generation (Simon & Moore, 2007), and increased handling of a female odour source (Stebbing et al., 2003a; Belanger & Moore, 2006). Mating cues are believed to be released in the urine of female crayfish (Stebbing et al., 2003a) and output may be increased during reproductive

pairings (Simon & Moore, 2007). It is thought that these mating cues are detected by aesthetasc hairs found on the lateral flagellum of the antennule (Ameyaw-Akumfi & Hazlett, 1975; Tierney et al., 1984; Dunham & Oh, 1992); however, there is evidence that suggests that the major chelae may also aid in female odour perception by providing feedback to the antennules (Belanger & Moore, 2006).

One of the behavioural indicators that suggests the importance of chelae in sex recognition is the chelae sampling behaviour in the presence of conspecific odour. Chelae waving behaviour has been described by Thorp & Ammerman (1978) and Itagaki & Thorp (1981) in the crayfish, *Procambarus clarkii* (Girard, 1852). These authors demonstrated that chelae waving increased in response to a conspecific odour. Later, Dunham & Oh (1992) suggested that chelae waves may represent a potential for sex discrimination in this crayfish species, and that these crayfish may use structures on their chelae for chemoreception. Chelae waving behaviours may be similar to antennular flicking (Snow, 1973; Moore et al., 1991; Goldman & Koehl, 2001; Kraus-Epley & Moore, 2002) where the waving may facilitate the movement of chemicals to the microenvironment of the sensory cells, contained within the sensory setae of the major chelae. We have shown that reproductive male crayfish (Form I) have more chemosensory setae on their chelae than non-reproductive (Form II) males, and that they use sensory information, obtained from their major chelae, to aid in the discrimination of female odours (Belanger & Moore, 2006; Belanger et al., 2008). However, it is unclear whether chelae sampling is important for localization of sex cues, or whether male crayfish can detect the location of such cues without sensory information from the chelae.

The goal of this study was to determine the role of sensory information obtained from the major chelae of reproductive (form I) male crayfish, *Orconectes rusticus* (Girard, 1852), in the localization of a female odour source. To accomplish this, we examined how male crayfish localize and respond to female odours and whether these behaviours are altered by lack of sensory information from the chelae. We hypothesized that male crayfish with intact major chelae will be able to localize the source of a conspecific female and will exhibit chelae waving in response to this odour, and that these behaviours will be eliminated without chemosensory information from the major chelae.

MATERIAL AND METHODS

Animals

Orconectes rusticus (reproductive females and form I males) were collected from the Portage River near Bowling Green State University in Bowling Green, Ohio. Intermoult reproductive (form I) male crayfish used in these experiments were identified by examining their reproductive stylets (Crocker & Barr, 1968).

Crayfish were visually and mechanically isolated from each other in a flow-through holding system (each container was 11 cm depth \times 17 cm width \times 27 cm length). The flow-through tanks were housed in an environmental chamber (23°C, 14 : 10 hr light dark cycle). Crayfish males were isolated for at least 48 hr before the start of an experiment. Crayfish mass, carapace, and chelae lengths (\pm S.D.) were measured. Only form I males (16.65 \pm 5.0 g; 3.54 \pm 0.32 cm carapace length; 3.50 \pm 0.52 cm chelae length) with intact appendages (e.g., antennae, lateral and medial antennule filaments, chelae, maxillipeds, and walking legs) were used in this experiment. Reproductive female crayfish (13.42 \pm 2.01 g and 3.43 \pm 0.15 cm carapace length) were housed in a similar manner prior to collection of odour. Crayfish were fed a diet of rabbit pellets three times per week. Experiments were completed between January and February 2007. All treatments were performed between 0900 hr and 1800 hr.

Conditioned water stimulus and setup

To examine male attraction/localization and chelae sampling behaviours in response to a conspecific odour source, female-conditioned water was obtained using a procedure similar to that described in Belanger & Moore (2006). Female-conditioned water was obtained from eight reproductive female *O. rusticus* held individually in clear plastic pots (40 cm \times 20 cm \times 25 cm) containing 500 ml of aerated dechlorinated water for a 24-hr period. Water from each pot was then collected, combined, and filtered (Whatman[®] 185 mm #1004185) to remove large debris and subsequently used for behavioural trials. The control for this experiment was dechlorinated water that had been aerated over a 24-hr period in the same style of clear pots, with no crayfish present. The water was then subsequently filtered in a similar manner to the previous conditioned water treatments.

Experimental design

Experiments consisted of a 2 \times 2 design with two treatments (intact chelae and blocked chelae) and two odour treatments (female-conditioned water and dechlorinated water (control)). Ten crayfish were tested within each treatment/odour combination. Each crayfish was used only once and a total of 40 crayfish (2 treatments \times 2 odours \times 10 crayfish) were used in this study.

Sensory blocking protocol

The term "intact" refers to a crayfish with the ability to derive sensory information from its major chelae as opposed to "blocked", which refers to crayfish with chemo- and mechanosensory information from their major chelae eliminated. The blocking procedure, similar to the one described in Belanger & Moore (2006), was carried out by applying a thin layer of superglue (Duro Quick Gel[®]) to the dorsal

surface from the base to the tip of the major chelae, covering the pockets of sensory setae (Belanger et al., 2008). A drying accelerator (Zip Kicker™) was applied to the superglue with a cotton swab to speed drying time. Intact animals were handled and manipulated in a similar fashion with a similar amount of glue placed at the base of the carapace as a control for 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.), a spot of liquid correction fluid was applied to the carapace of each individual crayfish to facilitate visualization and tracking during trials. Following this, each crayfish was placed in the test arena to acclimate.

Test arena

The flow-through test arena (120 cm long \times 21 cm wide \times 19 cm high) contained an inflow and outflow valve and was filled initially with 10.5 l of aerated dechlorinated water (fig. 1). The inflow valve was located in the centre of one end

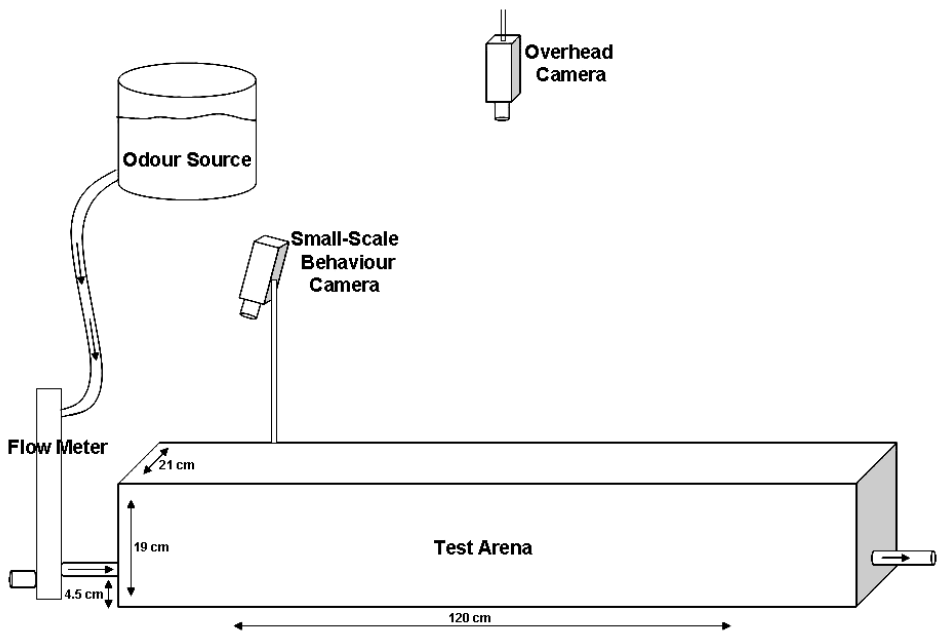


Fig. 1. Schematic of the experimental test arena setup used for examining odour source recognition and sampling behaviours in male crayfish, *Orconectes rusticus* (Girard, 1852). Chemical stimuli, female-conditioned water or water control, were gravity-fed into the experimental test arena. Flow rates were controlled by a flow meter. Two cameras were positioned above the test arena in order to observe large-scale and small-scale behaviours of the crayfish. The overhead camera was used to examine locomotory responses exhibited by each crayfish. The small-scale behaviour camera was focused in the proximal region of the test arena and was used to determine chelae sampling and other localized behaviours.

of the test arena, henceforth referred to as the proximal end. The inflow valve was 4.5 cm above the bottom and was connected via plastic tubing to a flow meter (Manostat Riteflow #2, Manostat, Peaquannock, New Jersey). Attached to the flow meter was a 3 l vat containing the test odorants: female-conditioned water or water (control). After each trial, the test arena and tubing were thoroughly rinsed. Each trial was recorded using a Canon XL-1 digital video camera (overhead camera) mounted above the test arena, which was used to monitor large-scale locomotory behaviours. A Sony 3CCD digital video camera (small-scale behaviour camera) was used for monitoring behaviours that occurred near the odour source (fig. 1).

Experimental trials

Blocked and intact male crayfish were placed individually at the distal end (furthest away from the odour source) of the test arena in a cage made of egg crating (8.5 cm wide \times 8.5 cm long \times 7 cm high) for 30 min. Following this, the cage was removed from the test arena and crayfish were allowed to acclimate to the test arena for at least 15 min. (range 15-17 min.) before the introduction of the treatment odour. Once the crayfish returned to the distal area of the arena (the area the crayfish was in when the acclimation period was initiated) the test odour was introduced (flow rate 50 ml/min.) and movements and behaviours were recorded for 15 min. during odour delivery (procedure modified from Thorp & Ammerman, 1978). Following experimental trials, crayfish were returned to the holding system, where they were housed for several months. Their general health, feeding behaviours, and survival were unaffected by the superglue treatment.

Data collection and analysis

Videos obtained from the overhead and small-scale behaviour cameras were analysed using a JVC MiniDV/S-VHS Dual Deck VCR (model #HR-DVS3U) and a Sony Trinitron Color Video Monitor (model #PVM-1315Q). Large scale locomotory behaviours were quantified in relation to the position of the odour source. The periods of time spent in the proximal area (0-60 cm from source) versus the distal area (60-120 cm from source) were calculated from the overhead camera view during the 15 minutes when the odour was delivered. Further analysis was performed using Peak Motus Motion Analysis System (Peak Performance Technologies Inc., Englewood, Colorado) in order to compare the behaviour of males in treatments where they displayed a preference for the proximal region of the test tank. A spot on the carapace (correction fluid mark) was digitized at 1 point/3 s (Moore & Grills, 1999; Kraus-Epley & Moore, 2002). The test arena was divided into four sections: 0-30 cm, 30-60 cm, 60-90 cm, and 90-120 cm from odour source and the time spent in each of these sections was collected and analysed further.

TABLE I

Behavioural responses recorded from male crayfish, *Orconectes rusticus* (Girard, 1852) in response to a conspecific conditioned water odour source (modified from Thorp & Ammerman, 1978; Itagaki & Thorp, 1981)

No behaviours present	Crayfish positioned in front of the odour source with no chelae movement or contact
Chela(e) waving	Moving the right and/or left chela back and forth in front of the body horizontally in the water column, perpendicular to the substratum
Chelae opening and closing	Pincers of both chelae opening and closing
Chelae open	One or both chelae held apart but not raised from the substratum
Chelae odour source contact	Making contact with, seizing, or mounting the odour source with the chelae
Meral spread	Chelae raised from the substratum and held apart

Small-scale behaviours performed near the odour source (within 10 cm) were quantified from the small-scale camera. These behaviours included: chelae waving, chelae opening and closing, chelae open, chelae odour source contact, and meral spread (see table I for definitions of each behaviour).

Statistical analysis

A three-way ANOVA (factors: odour type, treatment group, and test arena location) with a Fisher-LSD post-hoc test was used to investigate differences in the amount of time spent in each area of the test arena. A two-way ANOVA (factors: treatment group and 30 cm sections of the test arena) with a Fisher-LSD post-hoc test was used to investigate differences in the amount of time spent in each 30 cm area of the test arena when female odour was presented.

The proportion of time spent performing behaviours (time doing a specific behaviour/total time doing all behaviours for a particular treatment) near the odour source was analysed using a multiple comparisons for proportions contingency table ($q_{0.05, \infty, 4} = 5.301$). This is a common statistical analysis for proportional data that allows for testing analogous to the Tukey or Student-Newman-Keuls tests (Zar, 1999). Significant results are represented by giving $q_{0.05, \infty, 4} > 5.301$ from the multiple comparisons test, which is equivalent to $p < 0.05$.

RESULTS

Time in the proximal and distal sections of the test arena

Results of the three-way ANOVA test showed that male crayfish spent different amounts of time in the proximal and distal sections of the test arena depending on

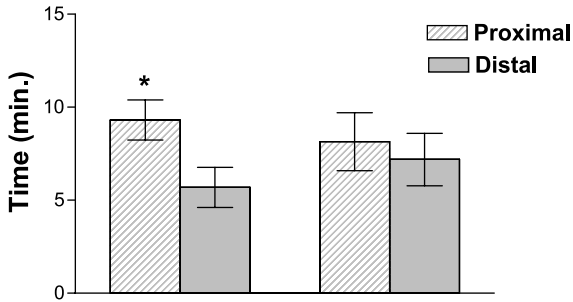
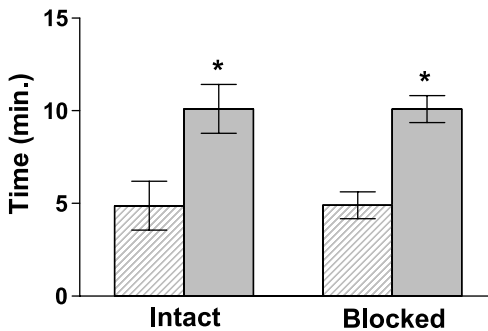
A. Female-Conditioned Water**B. Water (Control)**

Fig. 2. Amount of time (min. \pm S.E.) spent in proximal (hatched bars) and distal section (solid bars) of the test arena by male crayfish, *Orconectes rusticus* (Girard, 1852). A, male crayfish with intact chelae spent significantly more time in the proximal end of the test arena versus the distal end when a female-conditioned water odour source was present; there was no difference in the amount of time spent in the proximal versus distal region of the test arena when sensory blocked males were presented with female-conditioned water; B, male crayfish, with both intact and sensory blocked chelae, spent significantly more time in the distal section of the test arena when water (control) was introduced; *represents that there was a significant difference ($p < 0.05$; three-way ANOVA with Fisher-LSD post-hoc test) in the time spent in the proximal versus distal section of the test arena.

the odour delivered. There was a significant interaction between odour type and sections of the test arena ($F_{(1,72)} = 18.01$, $p < 0.0001$).

(1) Female-conditioned water responses

Intact male crayfish spent significantly more time (mean \pm S.E.) in the proximal region (9.31 ± 1.08 min.) of the test arena than in the distal section (5.69 ± 1.08 min.) when female odour was present ($p = 0.04$). There was no significant difference in the amount of time blocked male crayfish spent in the proximal (8.14 ± 1.57 min.) versus the distal section (7.19 ± 1.41 min.) of the test arena ($p = 0.72$, fig. 2A).

Because intact male crayfish demonstrated a preference for the proximal regions of the test arena, a digitized video tracking system was used to further analyse their

distribution within the test arena. A two-way ANOVA test showed that there was a significant difference among the 30 cm sections of the test arena that male crayfish were found in when males had either intact or blocked chelae ($F_{(3,72)} = 21.91$, $p < 0.0001$). Intact male crayfish spent 8.32 ± 1.08 min. within 30 cm of the odour source. They spent significantly less time in the 30-60 cm (0.99 ± 0.23 min., $p = 0.01$), 60-90 cm (1.00 ± 0.30 min., $p = 0.01$), and 90-120 cm (4.69 ± 0.92 min., $p = 0.01$) regions of the test arena when compared to the time spent in the section of the arena 0-30 cm from the odour source (fig. 3A). Blocked male crayfish exposed to female-conditioned water spent 6.60 ± 1.49 min. within 0-30 cm of the test arena. They spent 1.21 ± 0.29 min. within 30-60 cm, 1.03 ± 0.22 min. within 60-90 cm, and 6.16 ± 1.66 min. within 90-120 cm sections of the test arena when exposed to a female odour source (fig. 3B). Unlike with intact males, there was no difference in the amount of time blocked males spent between 0-30 cm and 90-120 cm of the test arena ($p = 0.75$). Blocked males spent significantly more time within 0-30 cm of the test arena when compared to the time spent in the 30-60 cm ($p = 0.0003$) and 60-90 cm ($p = 0.0003$) sections.

(2) Water (control) responses

Male crayfish spent significantly more time in the distal portion of the test arena when water (control) was delivered at the proximal end of the tank. Intact males spent 4.87 ± 1.32 min. in the proximal region of the tank, while they spent 10.11 ± 1.32 min. in the distal region ($p = 0.003$). Blocked males spent 4.91 ± 0.73 min. in the proximal region of the test arena and spent 10.09 ± 0.73 min. in the distal portion of the arena ($p = 0.004$; fig. 2B).

Chelae sampling behaviours

Overall, intact male crayfish performed behaviours for 34.80 ± 13.33 s within 10 cm of the female odour source, while blocked males spent 13.70 ± 6.97 s performing behaviours. In order to determine if crayfish had a different behavioural repertoire while at the odour source, we compared the proportion of time spent performing behaviours using a multiple comparisons for proportions contingency table (table II). Results of this test indicate that male crayfish with intact major chelae spent a significantly higher proportion of their time chelae waving at a female odour source than did crayfish from all other treatments (61%, $p < 0.05$). Conversely, blocked male crayfish, presented with female odour, spent more time (74%) opening and closing their chelae, instead of waving. There was no difference in the amount of time male crayfish spent performing meral spreads in response to all odours presented ($p > 0.05$). Overall, there were no recorded behaviours by male crayfish (intact or blocked) in response to water (control).

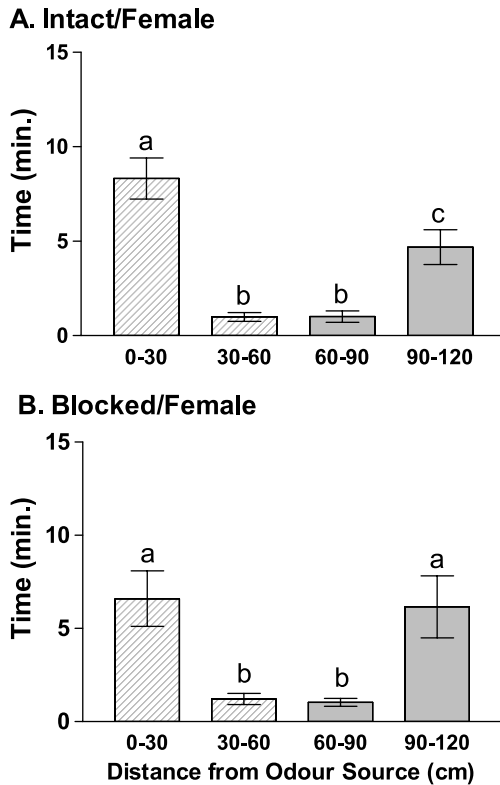


Fig. 3. Time (min. \pm S.E.) spent by male crayfish, *Orconectes rusticus* (Girard, 1852), in each of the four 30 cm sections (0-30 cm, 30-60 cm, 60-90 cm, and 90-120 cm from the odour source) of the test arena when presented with female-conditioned water. Hatched bars denote the proximal section of the test arena while solid bars signify the distal section (as shown in fig. 2). Intact male crayfish spent significantly more time within 30 cm of the odour source when presented with female-conditioned water. Significant differences ($p < 0.05$) are denoted as a, b, and c.

DISCUSSION

This study demonstrates that reproductive (form I) male *Orconectes rusticus* with intact major chelae can locate and are attracted to a reproductive female odour source. Form I male crayfish spent more time in the area of the test arena closest to the odour source and demonstrated more chemosensory sampling behaviours when presented with female odours (fig. 2). After the major chelae were blocked from receiving sensory information, there was no significant increase in the time males spent in the proximal region of the test arena. Male crayfish with blocked major chelae spent a similar amount of time in the proximal and distal portions of the test arena and did not perform chemosensory sampling behaviours. Digital video analysis revealed that intact male crayfish spent significantly more time within 30 cm of the female-conditioned odour source when compared to all other areas

TABLE II

Proportion of total time spent by *Orconectes rusticus* (Girard, 1852) performing behavioural responses within 10 cm of the female-conditioned water odour source

Chelae treatment/ odour type (N = 10)	Time (s) performing behaviours	Chela(e) waving	Chelae open/close	Chelae open	Chelae odour source contact	Meral spread
Intact/female	34.8 ± 13.33	0.61 ^{1a}	0.12 ^{2a}	0.16 ^{2a}	0.10 ^{2a}	0.01 ^{2a}
Blocked/female	13.7 ± 6.97	0.23 ^{1b}	0.74 ^{2b}	–	0.02 ^{12a}	–
Intact/water	–	–	–	–	–	–
Blocked/water	–	–	–	–	–	–

– Behaviour not observed.

Different numbers (1, 2) denote significant differences ($p < 0.05$) across rows.

Different letters (a, b) denote significant differences ($p < 0.05$) across columns.

of the test arena (fig. 3). Also, intact male crayfish demonstrated increased chelae waving behaviours when they were positioned within 10 cm of a female odour source when compared to blocked male crayfish. Taken together, results from this study suggest that the major chelae of form I males are important chemosensory appendages that aid in the identification, attraction, and localization of conspecific female odours.

Previous studies have demonstrated that male crayfish are attracted to and localize conspecific female odours, e.g., *Austropotamobius pallipes* (Lereboullet, 1858) (cf. Gaudioso Lacasa & Cabello, 1979), *Orconectes propinquus* (Girard, 1852) (cf. Tierney & Dunham, 1982, 1984), *O. rusticus* (cf. Belanger & Moore, 2006), *Pacifastacus leniusculus* (Dana, 1852) (cf. Stebbing et al., 2003a, b), *Procambarus clarkii*, and *P. acutus* (Girard, 1852) (cf. Bechler et al., 1988). In this study we found that form I male *O. rusticus* with intact major chelae spent more time in the area proximal to the odour source than in the distal section of the test arena (fig. 2A). Furthermore, when presented with a female odour source, only intact male crayfish spent significantly more time within the first 30 cm region of the test arena than in the 90-120 cm section. This result is supported by previous work done by Bechler et al. (1988) where they showed that male *Procambarus clarkii* and *P. acutus* displayed a preference for female conspecific odour in a Y-maze. Also, Stebbing et al. (2003a) found that reproductive male crayfish (*Pacifastacus leniusculus*) were attracted to and exhibited increased levels of activity and handling behaviour in response to a reproductive female odour source. In the current study, once the major chelae of form I males were sensory blocked, there was no difference in the amount of time spent in the proximal and distal sections of the test arena. This finding was previously demonstrated by Belanger & Moore (2006), who showed that once the chelae of form I male crayfish

were sensory blocked they behaved similarly to non-reproductive (form II) males where there was no response to female odours. Also, form I male *O. rusticus*, with both intact or blocked chelae, showed a preference for the distal portion of the test arena when they were presented with water (fig. 2B). These results indicate that the major chelae of form I male crayfish contain chemosensory structures that may aid in the discrimination and localization of conspecific female odours.

Along with spending more time in the proximal area of the test arena, male crayfish with intact sensory abilities displayed chelae waving behaviours when they were positioned in front of a female odour source (table II). Previously, *P. clarkii* have been shown to perform chelae waving behaviours in the presence of conspecific odour (Itagaki & Thorp, 1981) and it has been suggested that chelae waving may be a sampling behaviour that is used for sex discrimination (Dunham & Oh, 1992). We believe that chelae waving behaviour is a chemosensory sampling act, for three reasons: chelae have been previously shown to have chemoreceptive properties (Bauer & Hatt, 1980; Hatt & Bauer, 1980; Altner et al., 1983), the major chelae are important for discrimination of female odours (Belanger & Moore, 2006), 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 (Snow, 1973; Moore et al., 1991; Goldman & Koehl, 2001; Kraus-Epley & Moore, 2002). Therefore, chelae waving may aid in the transport of conspecific odours to the chelae sensory cells and thus male crayfish may employ chelae waving behaviours in order to enhance sensory perception of female odours.

Generally, male crayfish are attracted to conspecific female odours (e.g., *A. pallipes* (cf. Gaudioso Lacasa & Cabello, 1979); *O. propinquus* (cf. Tierney & Dunham, 1982, 1984); *P. leniusculus* (cf. Stebbing et al., 2003a, b); *P. clarkii* (cf. Bechler et al., 1988)). However, males are only attracted to females that are sexually receptive (Gaudioso Lacasa & Cabello, 1979; Villanelli & Gherardi, 1998) and exhibit increased motility and handling behaviour in response to a reproductive female odour source (Stebbing et al., 2003a). Also, there is evidence that demonstrates that form II male *Orconectes rusticus* are not attracted to conditioned water from reproductive females (Belanger & Moore, 2006) and form II males do not display mating behaviours with either reproductive or non-reproductive female conspecifics (Simon & Moore, 2007). This suggests that form I male crayfish recognize and use chemical cues, released from sexually receptive female conspecifics, for the discrimination and localization of mates. Sensory blocking the chelae of reproductive male *O. rusticus* eliminates this ability, making their suite of behaviours similar to those of non-reproductive males (Belanger & Moore, 2006). Given the importance of the major chelae for discrimination and localization, chelae waving behaviour may also be an important sampling behaviour used for mating and mate recognition. If so, removing the ability to

sense female odours with their chelae, by utilizing sensory blocking, should inhibit the ability of male crayfish to perform behaviours essential for reproduction.

The results from this and other studies indicate that the major chelae of the crayfish are important chemosensory appendages mediating crayfish behaviours in regard to recognition of sex and/or mating odours. This is evident as a suite of behaviours occurring when a form I male crayfish comes into contact with a reproductive female odour source. First, male crayfish use sensory information, obtained from their major chelae, to discriminate a female odour source from other odours (Belanger & Moore, 2006). Secondly, they localize the female odour source and begin sampling the odour using chelae waving (this study), and they lastly grab and handle the female odour source with their major chelae (Stebbing et al., 2003a; Belanger & Moore, 2006). Congruent with this theory is the finding that form I male crayfish have more robust chelae and a higher number of chemosensory setae on their chelae compared to the chelae of form II males (Belanger et al., 2008). In addition, it has been shown that loss of the chelae or chelae function can affect mating success (Sekkelsten, 1988; Abello et al., 1994; Juanes & Smith, 1995; Keller & Hazlett, 1996). It is possible that chemoreceptors, found on the major chelae, may work with other appendages (e.g., antennular aesthetascs, Ameyaw-Akumfi & Hazlett, 1975; Tierney et al., 1984; Dunham & Oh, 1992) by providing important feedback information on the sequence of behavioural decisions necessary for mate choice and localization. Finally, several studies have demonstrated the use of reproductive odours by female crayfish to attract conspecific males (reviews by Dunham, 1988; Bechler, 1995). Overall, it can be concluded that the major chelae are not only necessary for the mechanical manipulation of females during mating interactions, but may also aid in discrimination, localization, and even selection of reproductively receptive females.

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