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Corners and bubble wrap: the structure and texture of surfaces influence crayfish exploratory behaviour

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Summary

Touch is a principal sense in all animals. It is potentially important in species of freshwater crayfish that encounter murky waters or are nocturnal. Little is known about how tactile (touch) stimuli affect exploratory behaviour under these conditions. We placed animals in different tactile situations at the start of an exploration in a dark arena and tracked the position of the body and antennae to test whether subsequent search behaviour was affected. Individuals were exposed to differently textured walls, channelled out along a wall, or released in contact with no, one, or two walls. A corner arrangement of surfaces, where individuals started near two walls at right angles, produced behaviour that differed from that of other configurations; animals chose one wall and then maintained a close distance from the wall along which they were moving. The distance from a wall adopted by a crayfish walking parallel to it was affected by the texture of the wall. These results on the influence of tactile stimuli on crayfish exploratory behaviour may have implications for other taxa.

Key words: crustacea, haptic, antennae, exploration, tactile perception.

Introduction

Many animals use touch to interpret the surrounding environment. The way in which tactile (touch) stimuli are used have been studied in a range of species, e.g. crayfish (Zeil et al., 1985; Schmitz, 1992), rats (Carvel and Simmons, 1990), lobsters (Wilkens et al., 1996), cockroaches (Camhi and Johnson, 1999; Okada and Toy, 2000), moles (Kimchi and Terkel, 2002) and humans (reviewed by Goodwin and Wheat, 2004). Touch receptors positioned on moveable appendages increase the information available from surface contact. The location of an object can then be determined by combining the input from the receptors activated at the point of contact with that of position sensors associated with the joints of the appendage. This active touch, or 'tactile' perception, is also referred to as a haptic sense and the performance of some species resembles haptic perception in humans (e.g. Zeil et al., 1985).

The importance of tactile ability varies with the wild environment and behavioural patterns of the species. Its significance may increase if other sensory information is reduced or unavailable. For example, a number of crayfish species are most active during dark hours, e.g. *Procambarus clarkii* (Page and Larimer, 1972), *Orconectes virilis* (Hazlett et al., 1979), *Astacus astacus* (Abrahamsson, 1983), *Cherax destructor* (Merrick, 1993), *Austropotamobius pallipes* (Barbaresi and Gherardi, 2001). Even animals that are active during daylight may experience low light and turbid waters. In these circumstances, they may be dependent on non-visual information from chemical and tactile sensory systems to move about and orientate.

Antennae are an important source of tactile information for many invertebrates. Their function has been investigated in several species, including cockroaches *Periplaneta Americana* (Shaller, 1978), bees *Apis mellifera* (Kevin and Lane, 1985; Erber et al., 1997), crayfish *C. destructor* (Sandeman, 1985; Basil and Sandeman, 2000; McMahon et al., 2005), lobsters *Panulirus argus* (Wilkens et al., 1996), crickets *Teleogryllus oceanicus* (Balakrishnan and Pollack, 1997), stick insects *Carausius morosus* (Dürr et al., 2001) and aphids *Acythosiphon pisum* (Kunert and Weisser, 2005).

The second antennae of freshwater crayfish are morphologically and anatomically suited to tactile perception. They extend from either side of the rostrum at the animal's head (Bush and Laverack, 1982). Each antenna consists of a flagellum attached to a basal region articulated with the body. The flagellum is flexible and can be moved in three dimensions through nearly the entire space on one side of the animal by muscles at the base (Sandeman, 1985, 1989; Zeil et al., 1985). Its movement is monitored by proprioceptive neurons in the basal joints (Bush and Laverack, 1982; Mellon, 2000). When the flagellum makes contact with an object, touch receptors are activated at that position. Changes in location of sensilla stimulated over time also assist the animal to determine the direction of a stimulus (Masters et al., 1982). These features

provide an animal with sufficient information to determine the location of objects as it moves around (Zeil et al., 1985; Sandeman and Varju, 1988).

Crayfish antennae are active in many behavioural situations. *P. clarkii* and *Euastacus spinifer* sweep their antennae toward swimming prey animals (Breithaupt et al., 1995; Turvey and Merrick, 1997). Tactile cues assist *Orconectes rusticus* to find shelters (Alberstadt et al., 1995) and it is postulated that they help *Fallicambarus fodiens* to discriminate crayfish-made burrows from man-made ones (Punzalan et al., 2001). *C. destructor* can locate objects with its antennae and use that information to coordinate a physical attack (Zeil et al., 1985; Sandeman and Varju, 1988; Varju, 1989). Antennae are used during agonistic encounters between opponents of *Orconectes rusticus* and the way they wave them appears to have some behavioural significance (Bruski and Dunham, 1987, 1990).

A few of the possible situations in which crayfish could use antennal tactile stimuli for exploration and navigation have been investigated. One area of study involves thigmotactic behaviour – the use of touch to guide movements. An example is wall-following, which has been observed in walking crayfish (Basil and Sandeman, 2000). When walking close to a wall, animals trail the tip of a flagellum along it and navigate a path parallel to it. This activity has been described in a few species during studies on learning or exploration, e.g. *Astacus trowbridgii* (Gilhousen, 1929) and *C. destructor* (Basil and Sandeman, 2000; McMahon et al., 2005). The studies all took place in the laboratory and tests generally lasted in the order of tens of minutes – the start of an exploration being the time an animal is released into the test arena. Taking this into account, a synthesis of their results is that when crayfish are placed into a new environment, they start exploring close to walls. This suggests that a stereotyped search strategy is employed.

Search strategy may be influenced by tactile input when crayfish encounter new terrain because this is the time they are known to use tactile cues and remain close to walls. For example, animals will follow the walls of a test arena but this response diminishes as they learn the environment (Basil and Sandeman, 2000). Therefore, experiences during the start of an exploration could dramatically alter search outcome and the decisions crayfish make when they encounter familiar or unfamiliar terrain.

The importance of available tactile information in new terrain is suggested by studies that manipulated the antennae. When sensory stimulus is removed from one antenna of *C. destructor*, the individual turns in the direction of the intact flagellum from which it is still receiving tactile input (McMahon et al., 2005). When both flagella are denervated, crayfish meander around an arena and no longer follow walls (Basil and Sandeman, 2000). These studies have provided insight into how the animals function during early searching, but how different tactile input from objects affects behaviour when the antennae are intact, as in a wild situation, is unknown. For example, in the streams and creeks crayfish inhabit, surfaces range from rocks to wooden debris and soft mud. The texture of these will vary from coarse to smooth, and they will be arranged as the currents place them.

Here we investigated the behaviour of freshwater crayfish *C. destructor* as they set out to explore a new environment in darkness. We focused on changing the type of thigmotactic environment in which an animal started its exploration to test whether wall-following is a stereotyped response in this species. One experiment varied the number of vertical surfaces available, and a second altered texture.

Materials and methods

Animals

Freshwater crayfish, *Cherax destructor* Clark, of both sexes were obtained from commercial suppliers. Animals were maintained in fibreglass tanks (120 cm long×50 cm wide×20 cm high) for 4–8 weeks prior to experiments and fed *ad libitum* with aquaculture pellets. The husbandry room was on a reverse dark:light cycle (12 h:12 h) at 18°C ±1°C.

Apparatus

Experiments were conducted in a fibreglass tub 170 cm long×95 cm wide×45 cm high (the 'arena'). A peg-board-like system was used to create various configurations of walls (Fig. 1). An acrylic plate (160 cm×90 cm) formed the base, of which one side was etched to provide traction for crayfish when walking (Fig. 1A). Supports were inserted into grooves cut in the base plate and positioned at the back of walls so as not to interfere with the wall surface exposed to the crayfish. Acrylic sheets, 10 cm wide and of various lengths, were used to form walls. These were secured perpendicular to the base plate by fixing them to the supports with a clip at the top (Fig. 1B). Once each configuration was assembled, the peg-board was placed in the bottom of the experimental tub and weighted on each corner (Fig. 1C). The tub was filled with tapwater to the top edge of the walls (~10 cm deep).

Experiments were filmed using an infra-red CCD camera with a built in near-infra-red light source (Jaycar, Victoria, Australia), suspended from a tripod directly above each wall (Fig. 1C). Two cameras were spaced above the straight wall and laneway to provide coverage of the entire length of the wall. Camera footage was previewed on a monitor and recorded onto VHS tape using video cassette recorders.

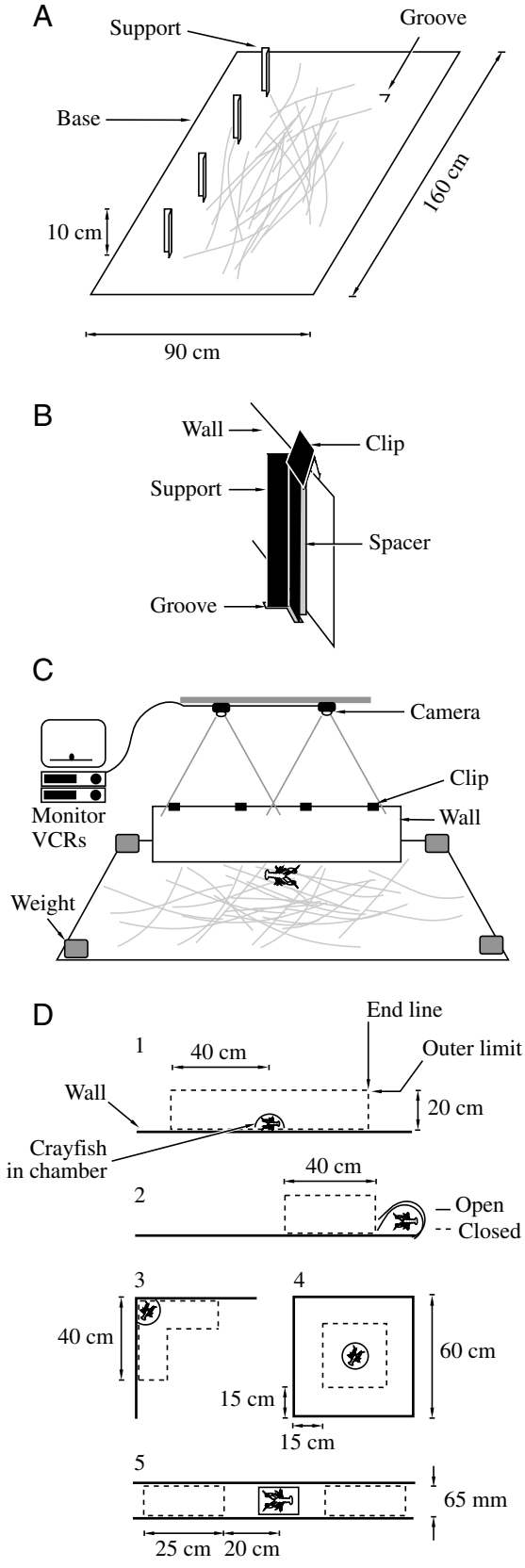
Wall configurations

Four different wall configurations were used to investigate the effect of the numbers of vertical surfaces available to touch at the start of an exploration. These varied the number of places, and directions, from which animals could receive tactile information (Fig. 1D).

Configuration 1: one straight wall 120 cm long. Animals were released from a semi-circular enclosure constructed from an ipsilaterally cut PVC pipe. The release point was the middle of the wall so animals could move in one of two directions (Fig. 1D).

Configuration 2: the same straight wall as the first

configuration but animals were released from one end. The release chamber was modified with a tapered exit to ensure crayfish were directed to exit beside the wall. At the outset, the chamber exit was closed. It was rotated by hand to produce an opening 5 cm wide through which crayfish could exit (see Fig. 1D). Individuals had only one surface to follow – the wall ahead.



Configuration 3: two straight walls, each 60 cm long, perpendicular to each other to form a right-angled corner. Crayfish started in the same enclosure as in the first treatment in the corner (see Fig. 1D). This ensured animals could touch two surfaces that projected in two different directions, giving them two paths to explore.

Configuration 4: a square arrangement of walls (60 cm×60 cm). Crayfish started in the centre, without touching any walls, in a PVC pipe placed on-end. This allowed each animal to find a surface at its own pace.

Wall textures

To determine if surface texture of the walls altered exploratory behaviour, a narrow laneway configuration was constructed (bottom Fig. 1D, similar to McMahon et al., 2005). Five different pairs of walls (120 cm long) with different textures were arranged in the arena. These included ones similar to those that might be encountered in the wild (e.g. rocks or wooden debris). We also arbitrarily selected a man-made surface (bubble wrap) with an exaggerated profile that might be detected by the animals.

Texture 1: PVC weatherboard (Formplex, Victoria, Australia). This was a rippled surface (Fig. 2).

Texture 2: sandpaper (40 grit; Fig. 2). One strip of sandpaper was attached to each acrylic wall surface with epoxy resin.

Texture 3: sandpaper control. To control for the possibility that the sandpaper contained manufacturing glue detectable to crayfish, smooth walls were also made from strips of the same sandpaper glued to the acrylic with the back, smooth paper side out.

Texture 4: bubble wrap packaging material (Clark Rubber, Victoria, Australia). Strips were cut and glued to the acrylic with the epoxy resin. This formed surfaces with 25 mm

Fig. 1. The peg-board system used to erect different configurations of walls. (A) Grooves were cut into the acrylic base plate so that supports could be positioned. Grey lines on base indicate scratched surface for traction. (B) Walls were attached by a clip at the top. Spacers were placed between the support and wall in the laneway configuration to standardise the width to 65 mm. (C) An example of one of the configurations of walls (straight) during a trial. Cameras were fixed to a tripod and suspended over a wall. Two cameras with overlapping fields were used over long walls, as shown. Footage was previewed on a monitor and recorded on video cassette recorder (VCR). Weights held the base plate to the bottom of the large tank (not shown). (D) Plan views of the five wall arrangements for the two experiments. Configurations are, from top to bottom: straight wall middle release (1), straight wall end release (2), corner (3), square (4) and laneway (5). Crayfish are shown in the release chamber, as they were positioned prior to a trial. The open and closed positions of the chamber in the end wall configuration are shown. End line and outer limit of observation zones that determined wall following are marked with dotted rectangles.

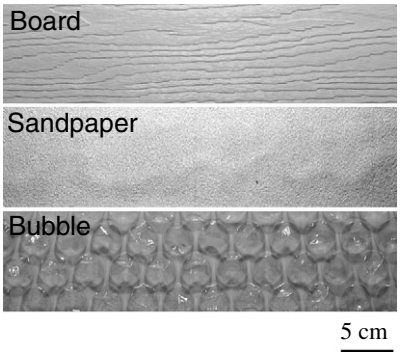


Fig. 2. Photographs of sections of three of the textured walls, board (PVC weather board, no.1), sandpaper (40 grit, no. 2), and bubble wrap (no. 4).

diameter, ~10 mm deep, semi-spherical protrusions at regular intervals (Fig. 2).

Texture 5: smooth acrylic walls made from naked cuts of the acrylic. The inner distance between the two walls was kept constant at 65 mm. This distance ensured crayfish touched a wall on both sides of their body at all times to control for evidence that *C. destructor* has a turning bias toward tactile input (McMahon et al., 2005). Adjustments for variation in the thickness of the walls, due to the different materials, were made by inserting acrylic spacer strips between the walls and supports (Fig. 1B).

Procedure

The arena was positioned in the husbandry room and treatments conducted at random periods between 1 and 6 h after the onset of dark. A red, 40 W light globe illuminated the room (1.5 lux 1 m from the source). Low-level light of this type creates minimal disturbance and behavioural response in *C. destructor*, while still allowing the experimenter to record data (McMahon et al., 2005). Callipers were used to measure the animals (±0.1 mm, antennae ±5 mm; values are mean ± s.d.): carapace length (37±2 mm), body width across the widest part of the carapace (18±1 mm) and length of both antennae (left 61±10 mm, right 59±10 mm). The flagella were at least 40 mm in length and of similar length on the left and right sides (mean difference between sides 8±6 mm). A crayfish was transferred to the release chamber and the red light was switched off and trials took place in darkness. After 1 min the enclosure was removed by hand with the assistance of a red-filtered (Lee, 106 gel, Lightmoves, Victoria, Australia) torch directed nearby.

The crayfish was observed after the entrance into, and until the exit out of, a rectangular zone adjacent to the walls (Fig. 1D). An arbitrarily defined boundary 20 cm from, and parallel to, the wall marked the outer limit. This line allowed the crayfish to wander a small distance from the wall if it was following. After 20 cm, it could not physically touch the surface and was deemed to be sufficiently far away to be no longer following. While animals could clearly not touch a wall once they were 10 cm away from it, some individuals wandered out of touching distance but still appeared to follow as they navigated parallel to the wall. This could represent following, so the more distant limit removed this ambiguity and allowed a quantifiable, clear distinction between 'followers' (those within the boundary) and 'wanderers' (those outside the boundary).

Other end boundaries were set perpendicular to the walls, 40 cm from the release point. This resulted in observations during the start of an exploration in a new environment and ensured that trials were concluded before the animal could touch the end of a wall, which would alter the sensory input received. In the straight wall configuration (no. 2), an end boundary was also set behind the enclosure opening because the target wall for following was in front of that point. In the square arrangement (no. 4) movement was monitored for 2 min in and out of a boundary, 15 cm from each wall because of the multiple number of surfaces (Fig. 1D). In the texture experiment no outer boundary was required; the zone was the width of the laneway. The start line was 20 cm from the release point and an end line another 25 cm thereafter (see Fig. 1D). This eliminated variation from animals that turned around when released, or that walked a short way and then turned around. If the crayfish walked more than 20 cm, then turned around before the end line, the trial was excluded (two animals in total).

After each trial, the crayfish was removed with a hand net and the arena was stirred with a plastic rod to disperse and dilute any odours that may have remained. Crayfish were measured and placed in the arena until each configuration or texture treatment was replicated 10 times with naïve animals. The tub was drained, refilled, and the peg-board reconfigured after each treatment. We did not consider it necessary to empty the arena more frequently because of the large volume of water it contained. Crayfish generally release odour through urine only intermittently (Breithaupt and Eger, 2002) and it is unlikely that a significant amount would have been released during the short duration of each trial. There is also evidence that over a similar time frame, the direction in which *C. destructor* walks is not influenced by previous paths taken by conspecifics (McMahon et al., 2005).

Analysis

Video footage was digitised to a PC computer. Trials were viewed frame by frame and a picture snapshot acquired every 1 s. In the square configuration (no. 4) images were taken every 2 s because of the larger area crayfish could cover. Picture files (768×576 pixel resolution) were loaded into ImageJ (National Institutes of Health, USA, download – http://rsb.info.nih.gov/ij). Landmarks were mapped to define locations on the crayfish and apparatus. The program's recording cursor was positioned over a given point so that the x and y coordinates could be logged. Coordinates were copied to a spreadsheet program and statistically analysed with Systat 11. Significance level was $P<0.05$.

Response to wall configurations

Movement was tracked to indicate the precision with which crayfish followed the walls (Fig. 3A; refer to Fig. 1D

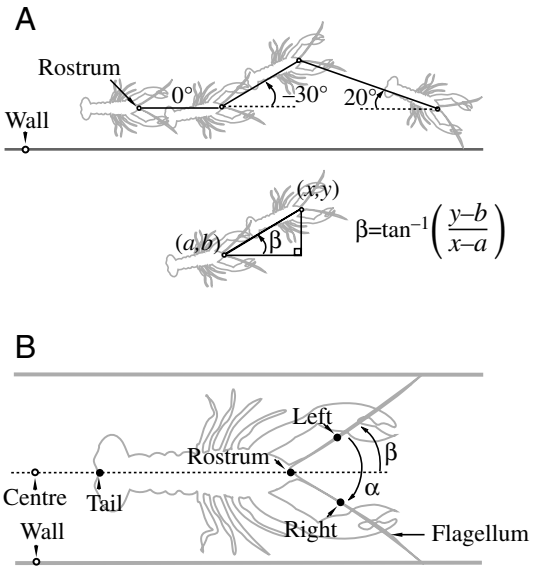


Fig. 3. Tracking of crayfish through the different environments. (A) Coordinates of the rostrum were tracked and angles measured between successive points. Three examples are shown for a crayfish that started parallel to the wall (0°), then moved away from the wall (negative angles up to –90°) and then moved back toward the wall (positive angles up to 90°). A reference coordinate was also logged (white circle on wall). An example of the calculation of angles is shown below. (B) A crayfish in the laneway of the texture experiment with its flagella spread to contact the wall on each side. Four points were tracked – rostrum, tailfan edge, left and right antennae – as indicated by solid circles. Apparatus reference coordinates of the centre line start point and one of the walls were also taken (white circles). Angles indicated for the antennae movement: α, angle at which each antenna was held with respect to the body axis, and β, angle between the antennae.

for zones). The rostrum was selected as a landmark because it was visible in all images. A reference point on the wall, where the crayfish started, was also recorded. Following a wall may be ambiguous to determine so we calculated heading angle to provide a quantifiable measure. The angle of walking with respect to the wall was derived from the coordinates. Angles were measured from 0 to ±90°; 0° was parallel to the wall, negative and positive values were movement away from and toward the wall, respectively (Fig. 3A). They were compared by two-factor ANOVA for configuration (nos 1–3) and zone exit (outer or end limit). Heading angles were not recorded in the square configuration because determining the nearest wall was difficult, particularly in the corners where some degree of human judgment would have been required. Instead, time inside the boundary was recorded (see Fig. 1D for boundary).

Response to wall textures

Body position and antenna angle were tracked from four landmarks. Coordinates of the rostrum, tailfan edge and one point on each of the left and right flagella (between the antenna base and wall) were recorded (Fig. 3B). Two coordinates on the apparatus were also logged so crayfish position could be referenced to the start position and walls (Fig. 3B).

With no *a priori* indication from the literature to suggest what aspect of behaviour might change in the laneway, we calculated several variables that we thought would be affected. For each trial we recorded: total time (s); total distance walked (mm); time walking straight (% total time heading 0±3°), distance walked backwards (mm) either faced forward and walked backwards, or turned 180° and walked forwards but in the direction of the start point; time walking backward (% total time); time stationary (% total time where movement change was less than 3 mm); heading angle (degrees); maximum heading (degrees) – the largest heading angle; mean change in distance (mm); angle made between antennae and rostrum (degrees); antenna angle with respect to body axis (degrees); position in laneway regions (% total time) – centre (±3 mm from laneway centre) and sides (>3 mm from centre line). Only the rostrum landmark was required to calculate non-angular variables. The derivation of angular variables is shown in Fig. 3B. Distance measurements were recorded in pixels but for convenience are reported in millimetres. Most variables were compared between the five textures by one factor ANOVA. Two factor ANOVA was used for region data (texture: nos. 1–5, and region: centre or sides) and antenna angle referenced to the body axis (texture: –1–5, and antenna: left or right).

Results

Response to wall configurations

The flagella stroked the vertical surfaces of the arena to some extent in most trials. Heading angles were different between crayfish that did and did not exit the observation zone at the end boundary (exit, P=0.001, Table 1). There was no difference in the angles across configurations (config, P=0.697, Table 1) nor an interaction between configuration and exit boundary (config × exit, P=0.224, Table 1). Crayfish that exited the observation zone at the end, rather than the outer boundary, had lower heading angles than those animals that did not (0°=parallel to wall, Fig. 4).

Crayfish were divided into groups. Those that did, and those that did not, follow the walls, based on whether or not they

Table 1. *Two-factor ANOVA comparing heading angle for different configurations of walls and whether or not crayfish exited at the end or outer boundary (i.e. followed the walls)*

	d.f.	Mean-square	*F*-ratio	*P*
Configuration	2	78.9	0.367	0.697
Exit	1	3223.9	14.981	0.001*
Config.×exit	2	343.3	1.595	0.224
Error	24	215.2		

*Significant difference in heading angle depending on whether or not crayfish followed the wall. Other comparisons were not significant.

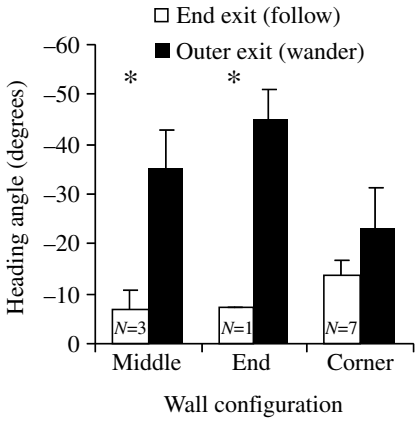


Fig. 4. Heading angles (means + s.e.m.) of crayfish that did and did not follow walls for the middle and end releases along the straight wall, and the corner treatment. Crayfish that followed walls had larger heading angles and these were away from the wall (negative). Angles of zero degrees indicate heading parallel to the wall. Number of animals out of 10 that were followers is given in each bar. Asterisks indicate groups of crayfish that did not follow walls (Fisher's exact $P<0.05$; see text).

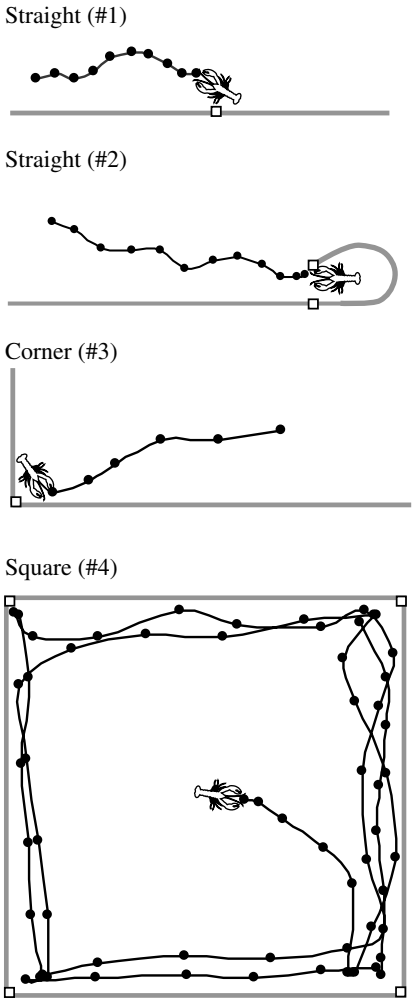


Fig. 5. Movement of crayfish through each of the four wall configurations. Wall configurations are shown by grey lines. The tracked path of one of the 10 animals that started from the crayfish icon is shown as a black line. Dots along the path represent subsequent 1 s measurements (2 s in the square configuration). White squares indicate apparatus reference points.

remained within the observation zone. Animals that exited via the outer limit boundary were wanderers and did not follow the wall, whereas those that exited the end line were followers. In the square treatment, followers were individuals that explored for >90% of the time in the zone near the walls. Using these definitions, the number of followers (out of 10) were 3, 1, 7 and 5 for the respective treatments 1–4. Examples of the paths that follower crayfish walked are shown in Fig. 5.

To investigate whether the pathway taken by crayfish is affected by different arrangements of walls at the starting point, we compared the number of animals that followed with the outcome that all animals would follow, as one might expect from previous experiments (e.g. Basil and Sandeman, 2000; McMahon et al., 2005). The corner configuration induced wall following; the number of followers was not significantly different (Fisher's exact test: $P=0.211$). Crayfish exploring the straight and square configurations (nos 1, 2, 4) did not follow the vertical surfaces (Fisher's exact tests: straight-middle: $P<0.001$, straight-end: $P=0.003$, square: $P=0.033$).

We noticed that in a small number of trials the crayfish was not touching a wall when the release chamber was removed. Animals were very close to the wall (within ~20 mm) and all touched the wall during the acclimatisation, but if no tactile information was received at this instant there might be no reason to follow. This did not affect the outcome as only four crayfish behaved in this way (from configurations 1 and 3) and half these were followers and half wanderers. In the other configurations all crayfish touched a wall at the start (straight no. 2) or could not touch any walls (square no. 4).

Response to wall textures

Crayfish waved their antennae when walking through the laneway, regardless of the texture, and their flagella stroked the walls frequently. In response to the board, rough sandpaper and bubble textures, the antennae were held behind the rostrum and trailed along the wall surface noticeably more frequently than in the presence of the smooth walls. This was most pronounced with the sandpaper texture where the flagellum vibrated as it was trailed.

To analyse wall texture effects on exploratory behaviour, we examined body position, antennal movement and time data (presented as means ± s.e.m.). Means from variables where no significant differences were detected are only reported for the 10 smooth texture trials to represent the outcomes across all treatments.

Body position

Crayfish moved differently in the five textured laneways: board (configuration no. 1), sandpaper rough (no. 2), paper

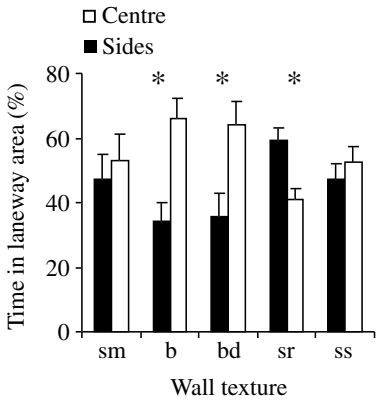


Fig. 6. Percentage of time spent in the two laneway regions (mean + s.e.m.), centre and sides. The time spent in a region was different, and this difference varied depending on the texture of the walls. Asterisks indicate significant differences between regions within each texture (see Table 2 and main text for *P* values). Crayfish spent about 50% of time in each region for the smooth (sm) and sandpaper smooth (ss) textures, more time in the sides in the bubble wrap (b) and board (bd) textures, and more time in the centre with the sandpaper walls (sr).

smooth (no. 3), bubble (no. 4) and smooth (no. 5). Time spent in the two regions (centre and sides) was different (region, P=0.008) and this difference varied depending on the wall texture (region × texture, P=0.001). These results are summarised in Fig. 6 and Table 2. *Post hoc* Bonferroni adjusted student *t*-tests compared data between regions within each texture (α adjusted to 0.010; Sokal and Rohlf, 1995). No difference was detected between the sides and centre for the two smooth surfaces (smooth, t_{18}=–0.509, P=0.617; paper smooth t_{18}=–0.806, P=0.431). Crayfish exposed to the board and bubble textures spent more time close to the walls than in the centre (board, t_{18}=–2.857, P=0.010; bubble, t_{18}=–3.649, P=0.002), and less time close to the sandpaper walls (t_{18}=3.442, P=0.003).

Heading angles did not differ between the textures. The mean heading angle was 14±4° ($F_{(4,9)}$=0.430, P=0.785) and the mean maximum heading was 5±1° ($F_{(4,9)}$=0.553, P=0.698) (Fig. 7). There was no difference in the time spent walking straight (heading angles of 0±3°, $F_{(4,9)}$=0.972, P=0.432). This occurred for 30±9% of the total time.

Table 2. *Analysis of time spent in the centre and sides of the laneway for the different wall textures*

	d.f.	Mean-square	*F*-ratio	*P*
Region	1	2840.9	7.463	0.008*
Texture	4	0.0	0.000	>0.999
Region×texture	4	2090.0	5.491	0.001**
Error	90	380.7		

*Significant differences in time spent in the two regions of the laneway.

**This difference varied depending on the texture (two-factor ANOVA).

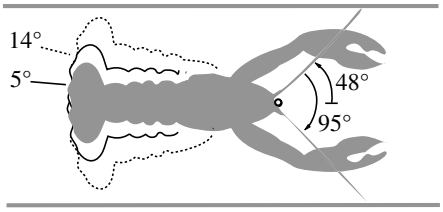


Fig. 7. Summary of angle measurements to indicate crayfish body and antenna position in the laneway. Heading and maximum heading angles are shown at the posterior end of the crayfish, 5±1° and 14±4° respectively (mean ± s.e.m.). Antennae were spread 48±3° either side of the body axis and with a mean angle of 95±7° between the two antennae.

Antennal movement

No difference was detected in antennal movement between textures (Fig. 7). Crayfish walked through the laneway and held their antennae 95±7° apart ($F_{(4,9)}$=2.047, P=0.104). The mean angle of the left and right antennae to the body axis (48±3°) did not change (two-factor ANOVA all P>0.05).

Spatial and temporal observations

The mean total distance walked by crayfish was not different between the textured laneways ($F_{(4,9)}$=0.841, P=0.507). The mean distance travelled was 272±4 mm; slightly greater than the 250 mm observation zone because measurements started just prior to, and finished just after, the boundaries.

The mean time to walk through the observation zone was 15±2 s and the mean distance travelled per second was 22±3 mm. The texture of the wall did not influence the time in the zone ($F_{(4,9)}$=0.996, P=0.420) or the change in distance ($F_{(4,9)}$=0.362, P=0.834). Crayfish were stationary for 5±2% of the time, which was not significantly different between textures ($F_{(4,9)}$=1.106, P=0.366).

Crayfish occasionally moved backwards in the laneway. This accounted for 0.7±0.7% of the total time in the observation zone and was not different between the treatments ($F_{(4,9)}$=1.944, P=0.119). The distance travelled by backward movement (0.2±0.2 mm) was also not different between the textures ($F_{(4,9)}$=1.081, P=0.377).

Discussion

We demonstrated that the exploratory behaviour exhibited by the crayfish *Cherax destructor* in a new environment was influenced by the number and types of vertical surfaces encountered at the start of an exploration. Different arrangement of wall position at the outset influenced the path travelled relative to walls, and different textured vertical surfaces affected body position in the laneway. The results indicate that *C. destructor* used different search strategies during exploration in the arena.

In another study that recorded the exploratory behaviour of *C. destructor*, flat partitions were placed so as to protrude perpendicular to the straight walls on each side of an arena

(Basil and Sandeman, 2000). Effectively, this produced corners and abrupt changes in topography and, as our result shows, may have induced following behaviour. This suggests that structural complexity of this kind is monitored by crayfish as they explore. In further support of this idea, there is evidence that the behaviour of *C. destructor* is altered by the complexity of, and changes in, topography (Basil and Sandeman, 2000; H. Baird, manuscript submitted).

The ways animals responded to aspects of the new environments reveal some previously unknown features of the exploration strategy of this species. The data suggest that the most probable explanation is that the antennae provided information about the structure of the terrain. In the corner environment, both flagella were able to touch two walls at the start. However, when *C. destructor* was released parallel to a single wall, only the flagellum nearest the vertical surface could make contact. One interpretation of this outcome is that when both antennae can contact surfaces the behaviour is more predictable than when touch information is received from only one side. That is, multiple sources of tactile information produce more stereotyped wall-following search strategies.

How crayfish used the antennae to detect the different surfaces and textures was not the object of this study, but our observations warrant comparison with other research. The base of the antennae and the setae along the flagella, are two areas from which thigmotactic information may have come. McMahon and colleagues (2005) splinted back one flagellum to the carapace so it could not touch any walls. This meant the antenna was receptive to vibrational information from the surrounding water but not to touch input from contact with a surface. From a stimulus viewpoint, this is the same as when a crayfish walked along a wall in our experiments (antenna adjacent to wall received tactile input from touch, the other was held by the animal in the water on the side away from the wall), but our animals' antennae were also free to move at the base. This suggests that the base of the antennae may provide critical information for interpreting tactile information from further along the appendage, and that it is used to generate a search strategy. The receptive setae along the flagellum may also be responsible for the observed differences. They have a range of specialised capabilities (Tautz et al., 1981; Bender et al., 1984) that could allow an animal to discriminate the fine detail of surfaces.

The antennae were not necessarily the only source of tactile information. Receptors on other body parts may have contributed. When crayfish were confined in the release chamber, for example, the abdomen and legs were commonly seen to contact the surfaces of the walls. Tactile receptors are found all over the body (Pabst and Kennedy, 1967; Wiese, 1976; Bush and Laverack, 1982) so their input could be expected to be incorporated in exploratory behaviour.

Besides tactile input from direct touching of the walls, at least three other factors may have influenced the observed behaviours. (1) Movement of appendages through the water could allow animals to detect the presence of nearby surfaces without touching them, for example using hydrodynamic information. Vibration receptors on the antennae and chelae can detect currents such as those reflected from surfaces (Tautz and Sandeman, 1980; Tautz et al., 1981; Tautz, 1987). (2) Other crayfish species, e.g. *Orconectes propinquus* (Stein and Magnuson, 1976), are known to alter their behaviour in response to predatory threats, so it is plausible that the physical handling in some studies (e.g. Basil and Sandeman, 2000; McMahon et al., 2005) may have provoked escape or avoidance behaviour and caused crayfish to walk near the walls. This could provide a direct path away from the danger, or at least one that offers some protection because of the physical presence of the structure. In our experiments, crayfish were released from chambers after a short acclimatisation period, rather than by hand, so stress from physical handling was likely to be minimal. (3) Crayfish may use walls as a reference point to come back to but not necessarily to follow (i.e. homing). Some crayfish 'bounced' off the walls as they moved away from the surface. While homing is not known in crayfish, it is a possibility because it occurs in sophisticated ways in other decapods (Zeil, 1998).

There is evidence in other crayfish, e.g. *Orconectes rusticus* (Moore and Grills, 1999) and decapods, e.g. *Homarus americanus* (Moore et al., 1991) that individuals have a propensity to walk close to surfaces when released into a new environment. There are also examples from land-dwelling species. Camhi and Johnson (1999) described how cockroaches use a precise system of antennal movement to navigate around protrusions along walls as they travel at high speeds. Therefore, different textures and structural designs may also be detected by other species during exploration.

Arthropods use tactile information in a manner similar to that revealed by our experiments with *C. destructor*. In some cases the behaviour is sophisticated. Bees can scan space differently with their left compared to their right antenna (Erber et al., 1997). They also use the tip of the flagellum to detect the fine microtexture of flowers (Kevin and Lane, 1985) and the more proximal region for learning about object position (Erber et al., 1997). Stick insects require antennae to touch the far side of gaps to make a successful crossing and the general search behaviour differs between species (Blaesing and Cruse, 2004). It remains to be seen if further complexity is also present in *C. destructor* and other species of decapods.

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