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Making sense of electrical sense in crayfish

B. W. Patullo and D. L. Macmillan*

Department of Zoology, University of Melbourne, Parkville, Victoria, 3010, Australia

*Author for correspondence (d.macmillan@zoology.unimelb.edu.au)

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SUMMARY

The five sensory modalities of humans are also found in a wide range of invertebrates. Other vertebrates have evolved additional special senses, such as the magnetic sense, which are also found in some invertebrates. However, there remain a few sensory abilities that curiously appear to be found in either vertebrates or invertebrates, but not both. For example, electrosensitivity – the ability to detect electric fields in water – which should benefit vertebrates and invertebrates alike, is apparently only used by vertebrates. However, recent reports suggest that some invertebrates could have an electric sense. Here we examine that possibility further and demonstrate a behavioural threshold to low-level electrical fields in two freshwater invertebrates. The responses are not low enough for them to detect the Earth's magnetic field as some other electroreceptive species can do, but sufficiently low for them to use in navigation or prey and predator detection. This finding challenges the current view of the sensory world of aquatic invertebrates and has implications for the evolution of this ability.

Key words: Crustacea, electroreception, invertebrate, sensory biology.

INTRODUCTION

Animals communicate, navigate and search for food using sensory modalities such as vision, sound and odour. These main senses are used by vertebrates and invertebrates alike. A number of specialty senses are also evident in these two groups of animals. They enable a species to detect elements of the surrounding environment that would otherwise go unnoticed if only the core five senses humans share with invertebrates were available. For example the magnetic sense – the ability to detect the Earth's magnetic field – provides navigational information to species such as birds, sharks and lobsters (Lohmann et al., 1995; Walker et al., 1997; Freake et al., 2006).

A specialist sense exists underwater: electroreception, the ability to detect weak electric fields. Such fields are produced by biological and non-biological sources and conducted through the water. For example, muscular contractions that power swimming motion cause low-level electrical signals in the surrounding water (Fricke, 1984; Taylor et al., 1992; Herberholz et al., 2001; Finley and Macmillan, 2002). It is therefore hardly surprising that electrosensitivity evolved, so much so that it is widespread among aquatic vertebrates (e.g. Zakon, 1988; Bullock and Heiligenberg, 1986), is found in two monotremes (Gregory et al., 1987; Proske, 1990) and there is some evidence that it also exists in moles (Gould et al., 1993).

The role of electroreception in the biology of fishes is well documented (Bullock and Heiligenberg, 1986). A number of cartilaginous and bony fishes have evolved receptor systems to detect electrical signals and some have evolved specialised electric organs that generate voltages to extend the range of transmission (Bullock and Heiligenberg, 1986). Some aquatic species use the interaction between electrical emissions and the Earth's magnetic field for orientation (reviewed by Lohmann et al., 2008). Others employ them to communicate with conspecifics or to detect the presence of inanimate bodies or other animals, including predators and prey which distort the local field patterns (for reviews, see Bullock and Heiligenberg, 1986; Hopkins, 1999). Electroreception is therefore an important aspect of the ecology of all aquatic animals, whether they are the hunters or the hunted, and we still make new discoveries today in an attempt to fully understand this sense in vertebrates [e.g. neurobiology (Hofmann et al., 2008); evolution (Zakon et al., 2008); field description (Babineau et al., 2006)].

As far as invertebrates are concerned, the accepted position has been that they emit muscular signals detectable by electrosensitive animals (e.g. Fricke, 1984; Taylor et al., 1992; Gregory et al., 1997), but would be unable, for a number of reasons, to detect electrical signals themselves. For example, many of them have a hard exoskeleton that would be likely to make the transfer of signals to receptors inefficient (Bullock, 1999). There appears to be an evolutionary paradox here given an aquatic environment redolent with useful electrical information. The advantages are evident: being able to sense an animal before it is seen or smelt would assist with capturing prey or escaping a predator, and being able to navigate without needing physical landmarks would provide efficient travel through sparse open spaces. It is therefore puzzling that nearly 100 years of research into electroreception has uncovered no evidence of its use by invertebrates (Bullock, 1999).

Recent research, however, has demonstrated that aquatic invertebrates can respond to electrical signals (Patullo and Macmillan, 2007; Steullet et al., 2007). Although both reports demonstrated behavioural changes in freshwater crayfish to some extent, they have opened a debate on whether a true electrical sense exists: it could be argued that the detected signals are too large to be biologically important given the sensitivity documented in vertebrates. That is, crayfish responded to electrical signals but did so in response to fields that were higher in amplitude than those naturally produced by animals or features of the environment. Therefore, it is presently difficult to interpret how, why and in fact whether these animals use electrical fields in the wild. Any interpretation is further hampered because it is difficult to compare electroreception research because of different the methodologies used (Wilkens and Hofmann, 2005) and there is also evidence that the two species tested for electroreception, thus

far, may differ in other elements of their sensory biology (cf. Fields, 1966; Sokolove, 1973; McCarthy and Macmillan, 1999). Nonetheless, both reports suggest that electroreception is a plausible sensory ability in some invertebrates.

The lack of evidence of behavioural electroreception in invertebrates warrants further investigation, both for the insight it will provide to electrical biology and the wider interest in sensory abilities that exist in both invertebrates and vertebrates. Therefore we examined further the question of invertebrate electroreception. We tested behavioural responses to electric fields by *Cherax destructor* (the yabby), one of the species previously shown to have a degree of sensitivity (Patullo and Macmillan, 2007), and *Cherax quadricarinatus* (the redclaw cray), a tropical freshwater crustacean with no known response to electrical fields. We utilised a common form of stimulation used in vertebrate studies: a low-level field created from a dipole suspended in the water above the animal. We manipulated the two main characteristics that differ in biological electrical signals, size (amplitude) and frequency. We then monitored body movement when the signal was on and when it was off, anticipating no difference between the two if the animals were not sensitive to the electric fields.

MATERIALS AND METHODS

Animals

Australian yabbies, *Cherax destructor* Clark (Decapoda Parastacidae), were obtained from commercial suppliers in New South Wales, Australia and maintained in tubs at 18±1°C on a 12 h:12 h light:dark cycle for 2 weeks before experiments (experimental times were 0.5–6 h into the dark cycle). They were fed *ad libitum*. A sample of animals was measured and sexed (n=40; carapace length 28–35 mm; 55% female). Redclaw crayfish, *Cherax quadricarinatus* Von Martens (Decapoda Parastacidae), were obtained from Queensland and maintained in tubs at 24±1°C on a 12 h:12 h light:dark cycle for 2 weeks before experiments. A sample of animals was measured and sexed and the data were similar to *C. destructor*.

Apparatus

Variation in methodology, differences in the level of complexity of behavioural responses between species and the different conduction of fields in salt and fresh water make it difficult to compare research within the electroreception literature (Wilkens and Hoffmann, 2005). To minimise this problem and achieve results as comparable as possible to those in the literature, we followed the methodology used in investigations of the electric sense in fresh water.

A circular plastic tank (diameter 115 mm, filled with tap water 8 cm deep) was used as the test arena. This environment was similar to that used in vertebrate electroreception studies (e.g. Schlegel, 1988). Our preliminary observation of *C. destructor* in this tank revealed that the animals started and stopped walking periodically as they circled the arena. This meant that we could administer electrical stimuli at precise moments in walking behaviour. We opted to use this style arena and observe this walking behaviour because it is an uncomplicated element of crayfish movement that is common to both species studied.

Experiments were conducted in an enclosed earthed Faraday cage of aluminium sheets to ensure darkness inside (<0.5 lux). An infrared camera (Jaycar, Victoria, Australia) was suspended from the roof and connected to a computer that saved the footage as movies (mpeg). Tap water [*C. destructor*: 18–22°C, 100–120 μS cm k_{25} (conductivity at 25°C); *C. quadricarinatus*: 24–26°C, 191–214 μS cm k_{25}] filled the test arena and was replaced for each trial after the equipment was washed. The arena setup was located in one room and monitored on a playback monitor in an adjoining room. Electrical cables were also run into the adjoining room where a signal generator was located that was triggered to create the electrical fields in the test arena next door.

Creating electrical fields

A dipole (two carbon rods, 5 mm diameter, 15 mm exposed, 15 mm apart) was suspended 6 cm above the bottom of the tank. Stimuli were triggered after the crayfish started walking and observations from the footage assessed how long during the stimulus the crayfish's legs, chelae and body were moving. The absence of polarisation with this setup is addressed in previous work (Patullo and Macmillan, 2007).

The test signal was a waveform produced by a tadpole (*Rana* sp.), a swimming natural prey item of Australian crayfish species (Turvey and Merrick, 1997). This signal is known to evoke behavioural responses in *C. destructor* (Patullo and Macmillan, 2007). It was digitised in a computer editing program (Intuilink Waverform Editor, Agilent Technologies, Victoria, Australia) by tracing a graph of the biological signal published by Peters and Bretschneider (Peters and Bretschneider, 1972). The tadpole signal was a bioelectric field recording from an individual that was stationary and then started to swim. We traced a portion of the signal that was roughly constant in its maximum amplitude to ensure that the crayfish would be exposed to the test amplitude for the entire length of the stimulus, rather than a fraction of the time. This meant that we could accurately determine the threshold of any response while using a natural electrical signal. The resulting signal included four cycles over 1.5 s at 10,000 points per second.

The control signal was created by a similar process, except that a line was traced in the program, rather than a biological signal. The line corresponded to a signal of zero amplitude for the entire duration of the 1.5 s (resolution 10 K s^{-1}). This differed from the tadpole signal which had amplitudes that positively and negatively fluctuated. Effectively, the zero amplitude line exposed the crayfish to no electrical field with the same operating procedure as when the test signal was played. Both the test stimulus and control signals were saved on the computer and then uploaded to a waveform generator for playback (maximum output resolution 64 K s^{-1}; model 33220A, Agilent Technologies, Australia).

The field size was calculated by the dipole field equation commonly used in vertebrate studies (e.g. Kalmijn, 1982; Kaijura and Holland, 2002). An ammeter was wired in series with the positive electrode and the current, and together with the physical setup of the dipole, were used to calculate the field. This was also verified by computer analysis (PowerLab ML80, ADInstruments, New South Wales, Australia). For this, silver wire recording electrodes were chlorided at the tip and fixed 1 cm apart (diameter 1 mm, 2 mm exposed at the chlorided end). Measurements were taken spherically around the dipole with the recording electrodes parallel and perpendicular to the source dipole orientation. We report the largest field that was recorded from these orientations and locations at 4 cm from the electrodes, 2 cm above the bottom of the test arena. Most animals would have experienced fields smaller than the reported values because their maximum height from the bottom was less than 2 cm or they were in a location with lower amplitude when they received the stimulus due to the signal decaying the further it was from the source.

Experiments

Three control and three test stimuli were played in random order after a crayfish had been walking for a few seconds and at least 30 s

had passed since the previous stimulus. Natural variation in the walking patterns of the crayfish meant that stimuli were not played when a crayfish was in the same location, same orientation to the dipole or at the same time interval as the previous stimulus. This format of stimuli presentation resulted in random administration of the signals. Success of this randomisation was confirmed by recording the position of animals at the time the stimulus started in five trials (30 stimuli).

Three experiments (N=15 each) were chosen with the focus on being able to demonstrate a lower than previously observed response, to observe that response in multiple species and to compare the responses with those in existing literature on freshwater vertebrate electroreception.

(1) *C. destructor* threshold amplitude. Three stimuli were given in random order as described earlier: 450 μV cm^{-1}, 300 μV cm^{-1} and control (0 μV cm^{-1}). These stimuli represented our prediction of the lowest fields likely to produce a response, based on preliminary observations from a pilot study.

(2) *C. quadricarinatus* comparative sensory ability. To test whether another crustacean species is capable of electrical sensitivity similar to that found in *C. destructor*, random sets of two stimulus types were given to each redclaw crayfish: 400 μV cm^{-1} and control (0 μV cm^{-1}). We were unable to exactly match the test stimuli with those used for *C. destructor* because of the different temperature and conductivity of the water this species inhabits. Nonetheless, 400 μV cm^{-1} was a comparable amplitude to the threshold amplitude found in *C. destructor*.

(3) *C. destructor* threshold frequency. Field potentials generated in wild habitats vary in frequency, as well as amplitude. So we played sets of test stimuli that differed in frequency, at an amplitude that was above the threshold found in the previous experiments, to each crayfish. To do this, we adjusted the playback settings of the waveform generator to decrease or increase the duration of the digitised tadpole signal so that the cycles in the new signal reflected three different frequencies. These were: 3 Hz, 20 Hz, 40 Hz and the control signal (0 μV cm^{-1}).

Observation and analysis

Digitised movies of each trial were watched and movement was scored without observer knowledge of the type of stimulus; a blind observation. This was achieved by watching an LED, concealed from the test arena but viewable on the playback video, that was triggered to be illuminated by the generator when a signal started to play. Behaviour was recorded for the period when this LED was on. If there was no change in the movement of the crayfish's appendages and body between two frames, one stationary moment was scored. This included the legs, the antennae, the abdomen and the tailfan. Effectively, the animal was motionless. Otherwise one moving moment was recorded. Monitoring this range of movement, in a binary manner, was advantageous because we had no *a priori* expectation of how the crayfish might alter their behaviour to the low-level fields. Furthermore, there is already the suggestion that more complex behaviours, e.g. feeding, do not change when low-level fields are present (Steullet et al., 2007).

The stationary moments were summed and converted to seconds, based on the frame rate (12 frames s^{-1}), for analysis. This method has been verified by independent observers who were asked to score responses with no knowledge of the hypothesis or nature of the stimuli [three observers, 91–93% accuracy, 46 stimuli (Patullo and Macmillan, 2007)]. The statistical tests that were applied were repeated measures ANOVA or paired *t*-tests as outlined in the Results, all being two-tailed and computed in Systat 11 with alpha set at 0.05.

RESULTS

General response to test stimuli

Both of the species of crayfish mainly moved around the perimeter of the arena when first placed in the tank, prior to the observation period. This walking usually slowed within the first 5 min, after which the crayfish moved in bursts for 5–60 s, periodically stopping in between walks. Crayfish would cut through the centre of the arena or change direction of their circular walking track, but did so infrequently. When the electrical field was on, a few animals spun around to face the centre of the arena from where the signal originated, or rapidly raised their claws, or performed both of these acts. This was only observed in a small number of animals, i.e. during two or three stimulus presentations for each species. Although these behaviours may be reliable indicators of responses to electrical signals, a different test paradigm would be required to test this because they were observed too infrequently in this setup to warrant a statistical comparison. As a result, we focussed the observation on body movement, as described in the Materials and methods, and discuss the data in the following section.

Analysis of the electrical field

Fig. 1 summarises the electrical field characteristics of the test signal. The signal that was traced and digitised on the computer is shown below to the original tadpole field potential (top trace) recorded by Peters and Bretschneider (Peters and Bretschneider, 1972) (Fig. 1A). We verified the output signal by recording from electrodes in the arena, shown below the other two signals (Fig. 1A). The generated signal and the recorded signal both closely approximated the original electrical signature of the tadpole (Fig. 1A).

To summarise the field recordings taken around the dipole, we plotted a selection of the data radiating outwards from the dipole down to the bottom of the arena. This produced a decay in amplitude typical of aquatic environments, starting at 18 mV cm^{-1} between the dipole and diminishing to about 300 μV cm^{-1} 6 cm away on the bottom of the arena (Fig. 1B). When the position of animals was tracked in the arena, from still frames off the video, crayfish were about 4 cm from the dipole that emitted the test signal. The body was between 4.0 and 5.7 cm from the electrodes (4.8±0.1 cm; mean ± s.e.m.; Fig. 1B).

Analysis of crayfish movement

Three experiments were carried out. First, we varied the amplitude of the electric field. We compared two different sized fields with a control (null signal played in the same manner as the electrical signals). When the control stimulus was played, crayfish were rarely motionless during the observation time (0.04±0.01 s, 4% of the observation period). When the 450 μV cm^{-1} field was active, crayfish were not moving for 0.28±0.09 s (20% of the observation period). During the 300 μV cm^{-1} electrical signals, crayfish were motionless for 0.10±0.04 s (8% of the observation period). This meant that *C. destructor* significantly decreased their movement when the field was 450 μV cm^{-1} (repeated measures ANOVA between subjects: $F_{3,45}$=6.007, P=0.005; Mann U-test comparison: U=1286, P<0.001; Fig. 2A). There was also a trend for crayfish to respond at 300 μV cm^{-1} but this was not significantly different from the control (Fig. 2A).

Second, to verify the low threshold response to the electric field found in *C. desctructor*, we examined another invertebrate species – *C. quadricarinatus*. The test stimulus field was 400 μV cm^{-1} which was a similar amplitude to that which produced a significant change in the behaviour of *C. destructor*. When this field was played, *C. quadricarinatus* stopped moving for 0.34±0.07 s (24% of the

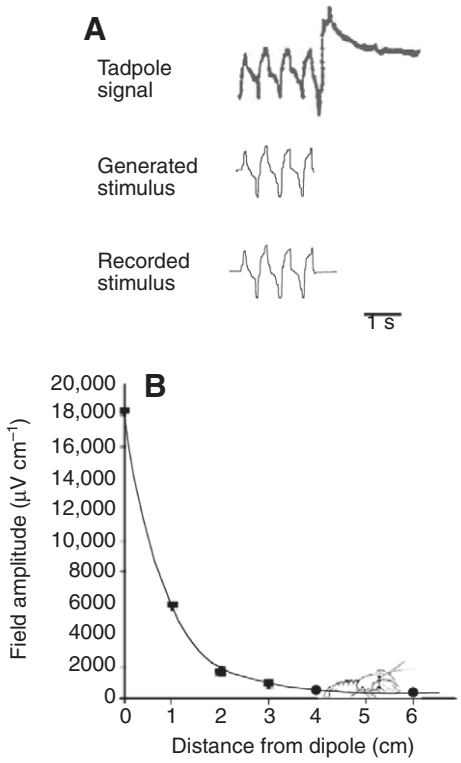

Fig. 1. Detail of the test electrical field. (A) A tadpole signal (top) was used because it had a section of constant amplitude and was a signal of the kind the crayfish could encounter in the wild [signal from Peters and Bretschneider (Peters and Bretschneider, 1972)]. The test signal (middle) was created by tracing the tadpole signal (top) in a computer program. We verified the test signal in the arena by recording with two electrodes and capturing the electrical field on a computer (bottom signal). (B) Graph of the test signal amplitude and its distance from the dipole. The electrical field decayed as a function of 1/distance, typical of aquatic environments.

observation time). In the control field, crayfish were moving for most of the test time (motionless 0.07±0.06 s, 6% of the observation time). Therefore, *C. quadricarinatus* movement significantly decreased in the presence of signals of the same order of magnitude as those that were effective with *C. destructor* (400 μV cm^{-1}, paired *t*-test t_{14}=–2.790, *P*=0.014; Fig. 2A).

The third experiment varied the frequency of the test signal. We compared responses to three frequencies with a control signal. Responses at 3 and 20 Hz frequencies were similar. At 3 Hz, *C. destructor* were motionless for 0.34±0.09 s (24% of the observation period) and at 20 Hz they were stationary for 0.34±0.11 s (24% of the observation period). At 40 Hz, the crayfish were not moving for 0.04±0.05 s (5% of the observation period). During the control null signal, *C. destructor* were motionless for 0.04±0.05 s (5% of the observation period). *C. destructor* therefore significantly decreased their activity when fields of 3 and 20 Hz were in the water, but not when the signal was 40 Hz (repeated measures ANOVA between subjects: $F_{3,40}$=4.274, *P*=0.011; Mann *U*-test comparison control-3 Hz: *U*=303, *P*=0.006; control-20 Hz: *U*=262, *P*=0.001; Fig. 2B).

DISCUSSION

We demonstrated that two species of freshwater crayfish change their behaviour when electrical fields are present in the surrounding water. One species was from tropical waters in northern Australia, the other, was a temperate animal found in the rivers and dams of south-eastern Australia. This is the strongest suggestion to date of a widespread response to low-level electrical fields in invertebrate species.

Electrical sensitivity in invertebrates has been suggested by two types of research: stimulation of animals with higher voltages than we know animals or environments to produce [shrimp (Kessler, 1965; Poleta et al., 2005); *C. elegans* (Sukul and Croll, 1978; Gabel et al., 2007); cockroach (Newland et al., 2008)] and the use of low-level voltages to simulate biological events or relationships (Patullo and Macmillan, 2007; Steullet et al., 2007). The responses to high voltages offer little for the interpretation of a specialist sense that detects naturally occurring electrical signals because they cause involuntary or abnormal behaviour, e.g. forced tailflip swimming by shrimp (Kessler, 1965). We therefore focus our discussion here on the few studies that targeted behavioural responses to low-level fields.

Our study, as with two others, aimed to investigate a biological and evolutionary purpose to responses to electrical fields that may occur in the environment in which crayfish live. There is a demonstration of a similar response to those obtained in our study, but the fields were an order of magnitude higher (Patullo and Macmilan, 2007). A second report showed that the crayfish *P. clarkii* could also respond to electrical signals, but once again the voltages were a little higher and the stimulus paradigm was also different (Steullet et al., 2007). This suggests that when considering electrical sensitivity in crustaceans, and other invertebrates, there may be a number of important experimental design elements that make

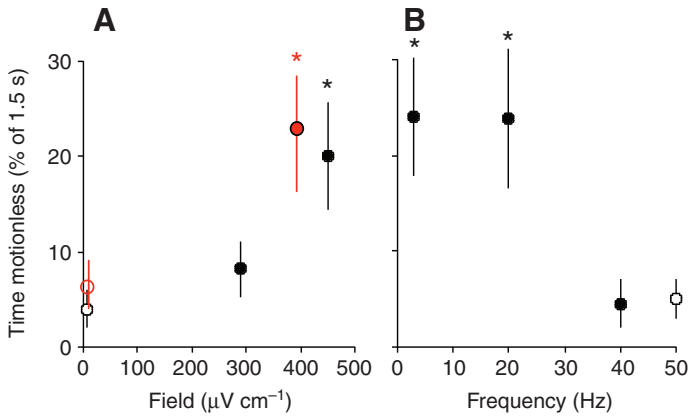

Fig. 2. Responses to electric fields by the two crayfish. *C. destructor*'s movement was recorded when electrical signals were played and we summarise here the percentage of time crayfish were motionless, for (A) different field amplitudes [control, 300, 450 μV cm^{-1} peak to peak for *C. destructor* (black circles) and *C. quadricarinatus* (red circles)] and (B) different frequencies (3, 20, 40 Hz, control). Values are mean ± s.e.m. *P<0.05, see text.

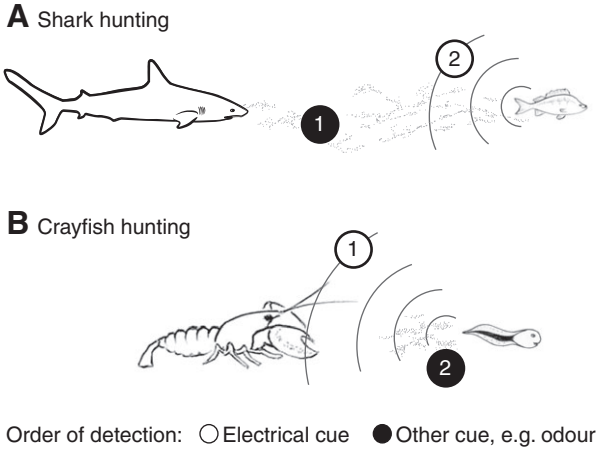

Fig. 3. Electrosensing behaviour of crayfish compared with vertebrates. For example, multimodal hunting – multiple senses are used in a specific order to capture prey. (A) A fish emits a smell in the surrounding water, if it is swimming, vibrations will also transfer through the water. The fast-swimming shark is capable of sensing the odour or vibrations from afar. Nearer the prey animal, the electrical field is detected to pin-point a precise attack. (B) Hypothesis of crayfish hunting. A crayfish slowly ambling along the riverbed senses the electrical field of a potential meal nearby. It then could use the smell or vibrations emitted by the prey, or its sensitive tactile ability, to pin-point the exact location of the food. Thus, the specialist electrical sense is used after the other hunting senses for many aquatic vertebrates, but before the other senses for *C. destructor* and *C. quadricarinatus*.

responses to low-level signals more readily observed, as we outline below.

First, the size of the arena may alter a behavioural response. One of the reports that monitored the behaviour of crayfish used a large arena, 40 cm×40 cm×20 cm (Steullet et al., 2007). For our study reported here, the arena was deliberately made small (diameter 11 cm), but sufficiently large that it did not disturb an animal walking. This meant that irrespective of where or how an animal was walking, or how it was positioned when a field was switched on, it would be surrounded by electrical activity. This was advantageous compared with a larger arena because we observed an animal in an electrical field for the entire observation time, rather than waiting for it to walk into the field.

The likelihood of a response in our arena was further maximised because the environment had minimal sensory stimuli other than the test signal. There was no light, no substrate to dig, no shelter to hide in and no objects to navigate or touch. Many crustaceans are known for their acute sensory abilities: chemical (e.g. Breithaupt and Eger, 2002; Díaz and Thiel, 2004), visual (e.g. Cronin et al., 2001; Van der Velden et al., 2008), tactile (e.g. Basil and Sandeman, 2000; Patullo and Macmillan, 2006) and sound (e.g. Popper et al., 2001; Montgomery et al., 2006). Any of these could alter behaviour during an experiment. The crayfish in our study, therefore, were not able to be distracted by multiple stimuli. This may have heightened the response to the electrical field by the crayfish, or been the reason that we could observe a behavioural change at thresholds that previous researchers could not. We can use this information when interpreting our data to suggest how the crayfish use electrical cues in the wild.

The electrical biology of crayfish and vertebrates

We can infer from our results how the two species use their electrosensory ability and how they could be advantaged by it. Some vertebrates use electroreception in very directed and obvious ways. For example, sharks are lured to a potential prey item by smell or vibration and then they use the electrosense to pin-point the food (Wilkens and Hofmann, 2005) (Fig. 3A). Such attack behaviour was less evident during our research; individuals were occasionally observed turning toward signals, grasping the electrodes and sweeping the chelae back and forth in some trials, albeit not statistically significantly so (D. Semmens and B.W.P., unpublished observations). The only other published report on crayfish electrosensitivity was likewise unable to reveal a reproducible movement directed toward a signal source of small amplitudes similar to those used in our study (Steullet et al., 2007).

Here, our demonstration that two species of crayfish only change body motion in response to an electrical field and do not move toward or attack the signal source, suggests that the crayfish are not using their electrosensitivity in the directed way of many vertebrates. Instead, we propose that an electrical signal alerts the crayfish to the proximity of a biologically significant presence, such as food, that can then be more precisely monitored with one of the other senses (Fig. 3B) – it pauses to 'listen' to the signal so that it can heighten the attention paid by other sensory modalities to find the stimulus. This is an unexpected conclusion given the numerous reports that infer the opposite in vertebrate species. However, this hypothesis would provide sufficient advantage for the behaviour to be selected, particularly in species that live in habitats as variable for the other sensory modalities as those in which these crayfish species are found, as is also the case for so many other aquatic vertebrates.

Comparing the sensitivity to the vertebrates

So how low is our threshold in *Cherax* compared with the vertebrates? To address this, we examined the literature on vertebrate electroreception in freshwater habitats to establish where the sensitivities of our species fit within a wider biological context. Vertebrate species range in their electrical sensitivity from fields of low nanovolts to high microvolts per centimetre (Wilkens and Hofmann, 2005) (Fig. 4A). Both crayfish species in this study are sensitive within this range. We also found that they are capable of responding to fields of an amplitude produced by potential prey items (i.e. other small species in their environment; Fig. 4A). Furthermore, their behavioural sensitivity to these signals is tuned to the main frequencies those prey animals produce (Fig. 4B,C). Even decomposing fish and leaf material can emit electrical signatures that are within the range and could guide these omnivorous animals to a meal.

The sensitivity of marine vertebrates is more difficult to compare with our results because of the different water conductivity between salt and fresh water (see Wilkens and Hofmann, 2005). A brief comparison suggests that there are cases of animals that are more and less sensitive to the crayfish in this study. Species such as sharks and rays that use the Earth's electrical field to navigate, detect signals down to a few nanovolts (Kalmijn and Kalmjn, 1981; Kalmijn, 1982). Fish with electric organs that emit fields to communicate with conspecifics also have a low threshold of sensitivity, down to tens of microvolts (Hopkins, 1999). However, some species detect comparatively large signals, e.g. the electrical emission from the plaice, *Pleuronectes platessa*, is 1000 $\mu V\,cm^{-1}$ (Kalmijn, 1966). So although our threshold was higher than that required for detecting the Earth's magnetic field and electric organ discharges in marine ecosystems, it is considerably lower than some of the values in predator–prey interactions in that environment.

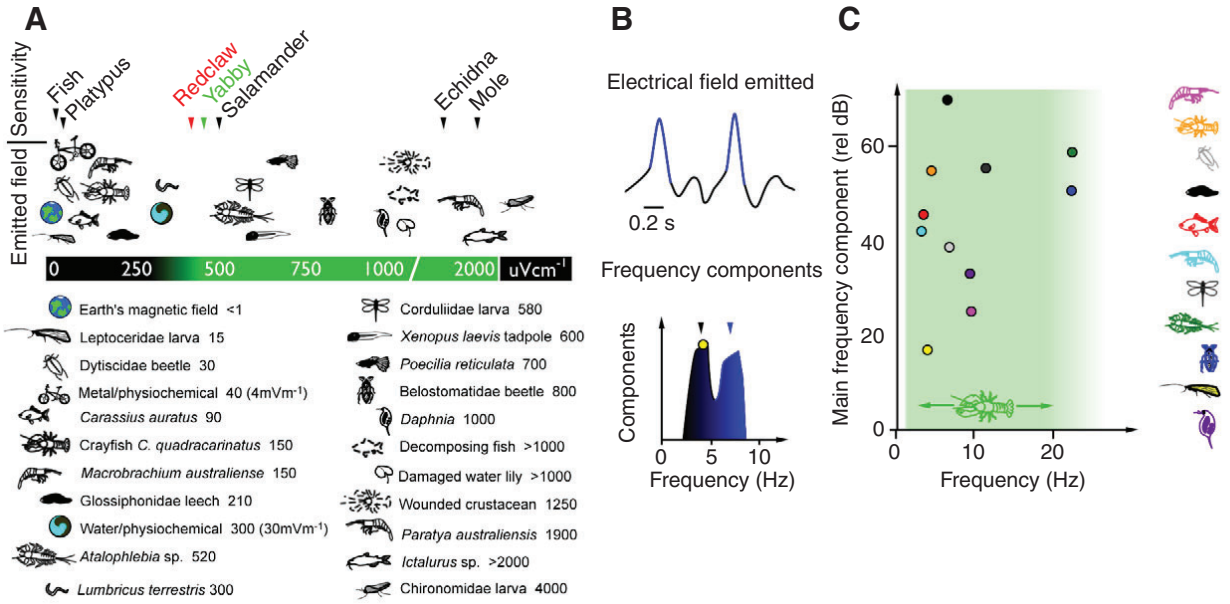

Fig. 4. Summary of electroreception in freshwater ecosystems. (A) Electrical fields are created by three main sources: induction of the Earth's magnetic field, physiochemical differences in the water, and animal movement. The size of these electrical fields is plotted along the green scale bar. For small animals it is between about 5 and 2000 $\mu V\,cm^{-1}$ (Taylor et al., 1992; Wilkens and Hofmann, 2005) and fields created by induction of the Earth's magnetic field and physiochemical sources, e.g. different temperature or ionic sections of water and rusting metals such as bicycles, are at the lower end of the scale (Peters and Bretschneider, 1972). The threshold of detection of freshwater electroreceptive animals also varies, as indicated by the arrows and species across the top of the diagram. Our behavioural tests showed that *C. destructor* (yabby) threshold was between 300 and 450 $\mu V\,cm^{-1}$ and *C. quadricarinatus* (redclaw) was 400 $\mu V\,cm^{-1}$. These crayfish could therefore detect any of the animals emitting fields above these values (the range highlighted in green on the scale). (B) Electrical fields are made up of different frequency components. For example, two main components make up the emitted electrical field from a caddis fly larva displayed in the top graph; one of ~4 Hz (black), the other of ~7 Hz (blue). In this case, the first peak on the frequency plot below (yellow dot) represents the largest frequency component [fast Fourier transform (Taylor et al., 1992)]. (C) The main frequencies for some animals have been documented and are summarised on the graph [from Taylor et al. (Taylor et al., 1992); *Daphnia* from the range in Wilkens and Hofmann (Wilkens and Hofmann, 2005)]. *C. destructor*'s sensitivity to different frequencies, 3–20 Hz in our behavioural tests, is highlighted in green and includes the main frequency components of various small animals. Coloured points on the graph correspond to colours of the animals shown on the right.

This comparison of the behavioural threshold of the two species studied here with those of other electrosensitive animals that live in similar fresh water habitats, shows that two Australian crayfish possess an ability that permits them to be players in the rich electric world they inhabit. Whether this represents an electric detecting ability that is a true sense, such as touch, is debateable. However, the animals clearly responded to the changing electrical fields of an amplitude and frequency that would be found in their wild habitat. Nevertheless, we are lacking evidence of a receptor or sensory pathway that uses known receptors and a demonstration of how the sensitivity is integrated with wild behaviour. We did investigate these issues in experiments of the type reported throughout the vertebrate literature on electro-sensitivity, for example buried electrodes and choice experiments, however, the results were not statistically conclusive to warrant publication in this report. Either way, the data in this report indicate that these crayfish species react to electrical fields of a level that would assist them to sense a number of features of their environment and now also offer the prospect of discovering how this is achieved.

The evidence here of electroreception in two crustacean species challenges long-existing data that are drawn from interactions involving crayfish or other aquatic invertebrates. Our data support the hypothesis that an additional underwater channel for communication is available to some crustaceans. There are many instances where discovery of a sense at one threshold level is followed by examples of greater sensitivity when researchers look for and find it in species where it has been selected for particular behavioural advantage [e.g. Kalmijn (Kalmijn, 1966) compared with Kalmijn and Kalmijn (Kalmijn and Kalmijn, 1981)]. Our report suggests that there is another case here. The present finding has implications for our understanding of the evolution of electroreception and the search for, and the investigation into, other senses that co-exist in vertebrate and invertebrate species.

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