The functions of multiple visual signals in a fiddler crab

Running title: The functions of multiple visual signals

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Abstract

In many species, it is common for animals to have multiple signals within one channel of communication. Multiple signals may, however, be inefficient if they are redundant in nature. Identifying the functional significance of these multiple signals is therefore important if we are to understand the evolution of such elaborated behaviours. We proposed to identify the roles of movement-based multiple signals in a model animal system. Male fiddler crabs wave their sexually dimorphic enlarged claw during social interactions. Some species present multiple signals, where the level of complexity of the movement changes. Males of *Austruca mjoebergi* can perform a double wave consisting of a high- followed by a low-elevation lifting of the claw, or a single wave consisting of the high elevation movement alone. We first investigated structural differences between the double and single wave types, and found that single waves were lower in elevation than double waves. We then explored the adaptive meaning of the wave types by manipulating the social context in which males wave. We found that double waves were given in all contexts and in higher proportions at long distances, suggesting a function of broadcasting male location. Single waves, on the other hand, were mainly given at close range and in the presence of conspecifics, suggesting intraspecific communication. Female presence elicited the highest number and proportion of single waves, a likely result of a female preference for higher wave rates. Finally, we point out that there is an element of interaction between wave types that deserves future attention. This paper is an important contribution to expand our understanding of the adaptive meaning of multiple visual signals and help reach a unified theory of their evolution.

Keywords: signal multiplicity; courtship; sexual selection; broadcast signal; agonistic signal; signal evolution

Introduction

Effective communication between signaller and receiver puts signal structure under strong selection (Kirkpatrick, 1987; Endler, 1992; Guilford & Dawkins, 1991; Hebets & Papaj, 2005). Yet, some signals can evolve to extremely extravagant and costly forms (Andersson, Pryke, Ornborg, Lawes & Andersson, 2002). One surprisingly common, yet puzzling, strategy in animals is the production of signals that belong to the same sensory modality, defined here as multiple signals (Jennions & Petrie 1997; Candolin, 2003). Multiple signals are a supposedly ineffective behaviour, as they would entail seemingly unnecessary energetic costs (Mitoyen, Quigley & Fusani, 2019). Their adaptiveness is therefore a persistent question in behavioural ecology (Johnstone, 1996): why are multiple signals so prevalent in animals?

In some cases, multiple signals are targeted at distinct audiences (Murphy, 2006). In the leaf-folding frog, for example, males produce a two-part call; one part is used to attract females for mating and the other part is used in male-male communication (Backwell, 1988). Each two-part call is therefore simultaneously targeting two distinct audiences. In other cases, different signals target the same receiver, but do not carry the same information (Partan & Marler, 1999; Uetz, 2000). In the túngara frog, for example, the first part of the two-part call is sufficient for mate recognition; the second part of the call does not attract females when produced in isolation but, when produced immediately after the first part, the entire call is attractive to females (Wilczynski, Rand & Ryan, 1999). Finally, in other cases, multiple signals can be 'redundant': they are targeted at the same receiver and carry the same information. This 'signal redundancy' is surprisingly common in animal species (Jennions & Petrie, 1997) and may be beneficial because the duplication of information makes the signal more detectable (Moller & Pomiankowski, 1993), especially when the species lives in complex or fluctuating environmental conditions (Peters, Hemmi, & Zeil, 2007; Bro-Jørgensen, 2010). It has also been suggested that alternating between redundant signals may decrease the receiver's habituation (Partan & Marler, 2005).

Fiddler crabs (Crustacea: Ocypodidae) are classic species for testing ideas about signal structure and function. Males wave their single enlarged claw to attract females and to repel conspecific males (Pope, 2000). The wave structure differs vastly between species, but is highly stereotyped within a species (Doherty, 1982; Perez, Rosenberg, & Pie, 2012). Most fiddler crab species wave in two distinct contexts, mate attraction and territorial defence (Pope, 2000; Muramatsu, 2011), where signal multiplicity is clearly defined: the lateral wave employed solely in mate attraction and the vertical wave employed during mate attraction and territorial defence (How, Zeil, & Hemmi, 2007). The relative proportions of the two wave types is dependent on context, as males modify their waving behaviour differently depending on the receiver (How et al., 2007).

However, fiddler crabs present a more subtle form of wave multiplicity. Males can give two lateral wave types. In the perplexing fiddler crab (*Austruca perplexa*) the first is a single-part wave (single wave) consisting of a single low elevation lift of the claw and the second is a two-part wave (double wave) consisting of a low elevation followed by a high elevation claw lift. When females are far away, the high elevation part of the double wave enhances signaller's broadcast power (How, Hemmi, Zeil, & Peters, 2008). When females are close, choice is based on the height of the high elevation wave (Murai & Backwell, 2006). However, the function of the low elevation wave is unclear.

Other species of fiddler crab also produce two types of lateral waves (*A. mjoebergi*, Perez & Backwell, 2017; *Leptuca leptodactyla*, Rorato, Araujo, Perez, & Pie, 2017) and we advocate that the signal multiplicity in the group must be more carefully investigated. In particular, the Australian banana fiddler crab (*A. mjoebergi*) presents a combination of signals that are distinct from those of of *A. perplexa*: a single wave consisting of a high elevation motion; and a double wave consisting of a high elevation motion immediately followed by a low elevation motion (Perez & Backwell, 2017) (Supplementary video). We do not have a clear understanding of the adaptive meaning of this signal multiplicity. We know that the single wave is sufficient to attract females and that females are equally likely to approach a single and a double wave (Perez & Backwell, 2017). However, this was based on the displays shape only and we know that females also have preference for high and fast (leading) waves (Perez & Backwell, 2019). Wave displays are a predominant communicational channel in fiddler crabs (Pope, 2005), and we hypothesise that the social context has the potential to influence the type of signal that is given. Here we investigate the functions of the single and double waves by (i) determining the structural and temporal differences between all elements (the single wave, the first part and the second part of the double wave); and (ii) determining the effect of social context on the number and proportion of the two wave types given (and therefore determining the target audience).

Methods

Austruca mjoebergi is a small fiddler crab that lives on inter-tidal mudflats in large, mixed sex populations in Northern Australia. Approximately a quarter of the population in the study site (East Point Reserve, Darwin: 12°24’18”S; 130°49’45”E), occurs in an open area of sandy substrate where it overlaps with a larger fiddler crab species, *Tubuca elegans*. The two species are intermixed and often share territory borders (Sanches, Costa, Barreto, & Backwell, 2018). We collected data during the diurnal neap low tides (the periods of maximum mating activity) from October to December 2015.

Wave characterization

We filmed 36 naturally waving males on the mudflat. Prior to filming, a male was randomly chosen and a stick was placed next to his burrow to serve as a scale for measurements. The camera was supported on a tripod 50 cm above the ground, and at an approximate distance of 5 m from the filmed crab. The scale placed next to the male allowed us to measure the displays and correct for any distortions caused by differences in angle between the ground, the filmed male, and the camera (method used in Perez et al., 2016). We watched the videos frame-by-frame in a rate of 30 frames per second using the software *digilite* created in MATLAB (The MathWorks, Inc., Natlick, MA, USA) to measure wave amplitude and wave duration. Amplitude was measured as the difference in height between the lowest and highest point of the claw tip during a wave. Wave duration was measured from the wave start until the final resting position (parallel to the ground and motionless). We measured 1 to 20 waves per male. We also documented the shape of the wave by tracing the path of the claw tip in two representative waves, a single and a double wave.

Social context experiments

We experimentally manipulated the social context of 197 randomly selected *A. mjoebergi* focal males that were residents within the natural population. We selected a focal male and plugged the burrows of all of his neighbours within a 40 cm radius by placing small stones (naturally occurring on the mudflat) over their burrow entrances. This prevented interference from neighbouring crabs during the experiments. We randomly assigned a focal male to one of 10 treatment (with 18-23 trials for each treatment): either a conspecific female, a conspecific male or a heterospecific male (*T. elegans*) as a stimulus; placed at a distance of 10 cm, 20 cm, or 30 cm away from the focal male's burrow entrance. Heterospecific males are an important addition to this study, because although they are larger and do not fit in *A. mjoebergi*'s burrow, they still compete for territory. Heterospecific females were not used, as there is no evidence that males are able to tell heterospecific and conspecific females apart. In the final treatment, the focal male was filmed with no stimulus crab. The chosen distances of 10 cm, 20 cm and 30 cm fall within the range of visual acuity, where the focal male is able to detect the stimulus crab sex (How et al., 2007).

The stimulus crabs were collected from the mudflat prior to the trial. We measured their carapaces with callipers and put them in individual cups with a small amount of seawater, placed in the shade until used in a trial. We were cautious to ensure that all stimulus crabs were captured at least 2 m away from the focal male used in each experiment; this prevented the focal male from 'recognising' the stimulus crab and therefore treating it as a burrowless wandering individual. For the experiment, each stimulus crab was tethered to a nail by a short piece of cotton thread glued to its carapace. The nail was placed in the sediment at the specific distance for each trial, allowing the stimulus crab to move around no further than 2 cm radius from the nail. The stimuli crabs were mostly used only once and rarely a maximum of three times, but never more than once in any particular treatment. Stimulus individuality should have no impact of focal male response, because they were all previous burrow owners and behaved similarly when tethered to the nail (moving around the nail).

We video recorded (JVC GZ-EX355BAA) the focal male's waving activity directly from above for 2 minutes (starting from the re-emergence of the focal male from his burrow). We discarded trials when focal male retreated before the filming was complete, or gave <4 waves during the 2 minute test period. None of the focal males was filmed more than once. Following, we counted the number of single and double waves given by the focal male for the full two-minute recording.

Statistical Analyses

Wave characterization

To analyse males' natural wave characteristics, we compared the duration and amplitude of the waves of the 36 filmed males. We compared the average values for the single waves, and the two parts of the double waves (consisting of high and low amplitude elements) by running paired t-tests for each combination.

Social context experiments: number of single and double waves

We compared the number of single and double waves given under the 10 social contexts using ANOVAs and LSD posthoc multiple comparisons. Due to the number of posthoc tests, we applied the Benjamini-Hochberg procedure with $Q = 0.25$. This is a control of false discovery rates; it resulted in an α level of <0.01 for significance ($m = 45$ tests).

Social context experiments: proportion of wave types

We compared the proportions of the wave types given under different social contexts by running two Generalized Linear Models (GLMs) with quasibinomial distribution (probit link). We opted for this method as none of other more conventional options or data transformation had adequate residual fit (overdispersed). First, we ran a GLM where the proportion of double waves over the total number of waves was the response variable and the size difference between focal and stimulus, stimulus type (conspecific female and male; heterospecific male), distance (10cm, 20cm and 30cm) and an interaction between the last two were explanatory variables. We considered distance as a continuous variable and corrected the distance at the intercept from zero to the shortest distance to the stimulus (10cm). The treatment with no stimulus was excluded from this analysis because it does not have a value of distance. We ran back transformations to visualize the variation in wave type proportion produced at each stimulus presence in the three distinct distances from the focal male. Following this, we tested whether focal males gave the same proportion of wave types at the presence of heterospecifics and no stimulus treatments by running a second GLM with quasibinomial distribution (probit link) as described above. The proportion of double waves over the total number of waves was the response variable and stimulus type was the explanatory variable. We used the proportion of double waves, and not single waves, over the total number of waves as a response variable for the GLMs because of the large number of cases where males did not produce any single wave (value=0). We conducted all statistical analyses on R-3.2.4 (R Core Team, 2016).

Results

Wave characterization

Male *A. mjoebergi* produce two types of waves, a single wave and a double wave (Figure 1). In the single wave, the claw is raised high above the eyestalks and lowered in a circular path with constant speed, until it reaches the starting position (Figure 1). The double wave consists of a high element wave that is immediately followed by a low element wave (Figure 1). The high element of the double wave is similar to the single wave, but in the low element of the double wave the claw does not surpass the level of the eyestalks (Figure 1) (Supplementary video).

Comparison between the single wave and the high element of the double wave:

In both, the claw is unflexed so that the inner surface of the manus is visible to the approaching female (Supplementary video). They are also similar in duration (single wave ($\bar{x}$ = 1.22 s, s.d. = 0.23, n = 36; high element of the double wave $\bar{x}$ = 1.13 s, s.d. = 0.41, n = 36; t = 1.66, d.f. = 35, P = 0.106). There is, however, a difference in amplitude. Single waves are lower than the high element of double waves (single wave: $\bar{x}$ = 17.64 mm, s.d. = 5.49, n = 36; high element of the double wave: $\bar{x}$ = 19.45 mm, s.d. = 4.33, n = 36; t = -2.11, d.f. = 35, P = 0.042).

Comparison between the single wave and the low element of the double wave:

Unlike the single wave, the claw is not unflexed in the low element of the double wave so the inner surface of the manus is not visible to the female. The low element of the double wave is faster than the single wave (low element of double wave: $\bar{x}$ = 1.0 s, s.d. = 0.31, n = 36; single wave $\bar{x}$ = 1.22 s, s.d. = 0.23, n = 36; t = 3.63, d.f. = 35, P < 0.001). It is also significantly lower in amplitude (low element of double wave: $\bar{x}$ = 6.08 mm, s.d. = 2.98, n = 36; single wave: $\bar{x}$ = 17.64 mm, s.d. = 5.49, n = 36; t = 11.39, d.f. = 35, P < 0.0001).

Comparison between the high and low elements of the double wave:

The low element of the double wave has the same duration as the high element (low element of double wave: $\bar{x}$ = 1.0 s, s.d. = 0.31, n = 36; high element of double wave: $\bar{x}$ = 1.13 s, s.d. = 0.41, n = 36; t = -1.40, d.f. = 35, P = 0.169). The low element is, however, significantly lower in amplitude than the high element (low element of double wave: $\bar{x}$ = 6.08 mm, s.d. = 2.98, n = 36; high element of double wave: $\bar{x}$ = 19.45 mm, s.d. = 4.33, n = 36; t = 18.76, d.f. = 35, P < 0.0001).

Social context experiments: number of single and double waves

Males gave more double waves than single waves (3569:545; Binomial test, P<0.0001). Close females (10 cm) elicited the greatest number of single waves (mean = 9.86, s.d. = 5.87, n = 21) and this number decreases with distance (Figure 2). Close males (10cm) elicited the same number of single waves as females at 20 cm. All other stimuli (no stimulus crabs, females at 30 cm, males at 20 cm and at 30 cm, and heterospecifics at 10cm, 20cm and 30 cm) elicited the same number of single waves (Figure 2).

Females at 10 and 20 cm elicited the greatest number of double waves (mean 23.95 and 22.09, respectively) (Figure 2). All other stimuli elicited the same number of double waves as when no stimulus was present (Figure 2).

Social context experiments: proportion of wave types

Both, distance and stimulus affected the proportion of wave types given. Females elicited the highest proportion of single waves, and there was a decrease in the proportion of single waves as the distance between the focal and the female increased (proportion of single waves: 29% at 10 cm, 13% at 20 cm and 10% at 30 cm; GLM: Estimate=0.0415, S.E. = 0.0083, *P*< 0.001; Figure 3, Table 1). Conspecific male stimuli also elicited the highest proportion of single waves at closer distances, with a decrease as the distances got greater (proportion of single waves: 22% at 10 cm, 10% at 20 cm and 6% at 30 cm). Overall, there was no difference in the proportion of wave types given to male and female stimuli (GLM Estimate = 0.0023, S.E. = 0.014, $P = 0.87$; Figure 3, Table 1). When the focal male was presented with a heterospecific stimulus crab, they produced a lower proportion of single waves than when the stimulus was a conspecific female (proportion of single waves: 7% at 10 cm, 10% at 20 cm and 4% at 30 cm; GLM: Estimate = 0.6530, S.E. = 0.1904, $P < 0.001$; Figure 3, Table 1). Focal males gave more double waves at close distances when presented with a heterospecific stimulus and gave fewer double waves when the female distance increased (GLM: Estimate = -0.0298, S.E. = 0.0143, $P = 0.04$; Figure 3, Table 1). The size differences between focal male and type of stimulus (female, 0.17 ± 0.19 cm; conspecific male, -0.02 ± 0.19 cm; heterospecific male, -0.21 ± 0.17 cm) did not significantly affect the proportion of wave types given (GLM: Estimate = -0.3160, S.E. = 0.2546, $P = 0.22$; Table 1).

Lastly, the waving behaviour of focal males with heterospecific stimuli did not differ from the waving behaviour when no stimulus was present (proportion of single waves: 6%; GLM: Estimate = -0.1344, S.E. = 0.21, $P = 0.542$; Table 1).

Discussion

Males produce two types of waves: a single wave and a double wave. The single wave was similar to the high element of the double wave, the only difference being the higher amplitude (approximately 2 mm higher) of the double wave. The low element of the double wave was very different to both the single wave and the high element of the double wave: the claw was not unflexed during the low wave, and the amplitude of the low wave was a third of the amplitude of the other two wave types.

The number and the proportion of wave types showed similar patterns. Focal males gave more double waves (number and proportion) in all contexts (Figure 2 and 3). Conspecifics elicited a high number and proportion of single waves. The proportion of wave types given to conspecific males and females did not differ, although females elicited higher numbers of both wave types. All other stimuli elicited the same number of single and double waves as when there was no visible audience.

There was a strong effect of distance on the number and the proportion of wave types. Conspecific crabs elicited more single waves when they were close to the focal male and more double waves when they were further away from the focal male, which is supported by recent findings that males give more double waves in low densities (Chou et al., 2019). The proportion of double waves was lower when heterospecific males (as opposed to conspecific males) were presented at greater distances, suggesting that double wave may act as a 'broadcast' signal (given its higher amplitude).

Females have a strong preference for males that produce leading waves and high wave rates (Callander, Jennions, & Backwell, 2012), which indicate male condition (Mowles, Jennions, & Backwell, 2017; Takeshita, Murai, Matsumasa, & Henmi, 2018). Producing the shorter single wave would therefore allow males to wave at a higher rate when mate-searching females are nearby. Males do, in fact, wave at a much higher rate when females are nearby (double the rate when a female is at 10 cm than when no stimulus is visible). A similar effect may occur with a conspecific male audience: nearby males pose greater competition for female attraction as well as a threat on territory ownership than distant males. Faster single waves would presumably be more effective at signalling presence, quality and willingness to defend the territory against conspecific males.

While it is true that nearby conspecifics receive more single waves (number and proportion), they also receive a high number of double waves. This is especially true for females at 10 cm from the focal male (an average of 10 single waves and 24 double waves). This does not fit with the idea that focal males produce more single waves to nearby conspecifics in order to facilitate an increase in wave rate. Why do males direct *any* double waves at nearby individuals?

The amplitude of the high element of the double wave is approximately 2 mm higher than the single wave. We know that females also prefer higher displays, and producing double waves can be advantageous for female choice. This suggests that the double wave is not only a broadcast signal, but also an attractive signal. Likewise, high-amplitude displays can indicate male stamina to conspecific as well as heterospecific males, which are in fact larger than *A. mjoebergi* males. Another possible explanation is that by adjusting the ratio between signal types to context, a male continues to broadcast the display to all potential receivers. The operational sex ratio is highly male-biased: there are 45 waving males for each mate-searching female (Reading & Backwell 2007). When a male detects a female nearby, he concentrates his waving effort on courting her while also signalling to other mate-searching females in the vicinity. The same pattern/interpretation holds for nearby conspecific males. It is also possible that by displaying double and single waves at high rates, focal males decrease the chances that the receiver will habituate (Partan & Marler, 2005). We know that each signal type is individually attractive (Perez & Backwell, 2017), and they must interact and have a joint role in communicating to conspecifics (Mitoyen et al., 2019). Future preference experiments that manipulate the proportion of signal types displayed will be essential to test this hypothesis.

The low amplitude element of the *A. mjoebergi* double wave is unlikely to be a useful broadcast signal. Its low amplitude (does not exceed the eye height of the signaller) as well as its small lateral sweep (the claw is not unflexed) are not characteristics suitable for distance communication (How et al., 2008) (Figure 1). The addition of the low amplitude element does not make the wave more attractive to females (Perez & Backwell, 2017). Using unpublished data from Perez & Backwell 2017, the time taken to approach a single and a double wave do not differ (see Appendix 1), so the addition of the low amplitude element does not increase locatability or elicit a faster response from approaching females (see Rowe, 1999).

We could not find evidence that the second, low amplitude element of the double wave has a signalling function, although it is the most structurally distinct signal element of in *A. mjoebergi*. Signal structure is usually under strong selection for efficacy (Kirkpatrick, 1987; Endler, 1992, Hebets & Papaj, 2005), including the signals of fiddler crabs (Burford, McGregor, & Oliveira, 2000; Doherty, 1982; Pope, 2005). The low amplitude element of the double wave slows the rate at which males can signal, which suggests that it has a function in another context. One possibility is that it 'resets' the claw to the starting position so that the following wave can be precisely timed. A second suggestion is that the low amplitude wave re-establishes body balance after the higher amplitude element of the double wave. Like in fiddler crab claws, sexually dimorphic traits that are also used as weapons are selected for fight efficiency (Emlen, 2008). Thus, claw functionality and display is not only shaped through intersexual selection, but also constrained by fight efficiency and the coevolution of other body parts for balance (Perez, Heatwole, Morrell, & Backwell, 2015; Bywater, Wilson, Monro, & White, 2018). Future studies that address these points as well as investigations with other species that present the behaviour will be decisive to thoroughly unveil the adaptive significance of fiddler crab signal multiplicity.

Ethics

The work was conducted under the approval of the Animal Ethics Committee at the Australian National University (permit number A2015/54) and under a research permit from the Darwin City Council (permit number 2322876). We limited the handling and the amount of time each crab was used as much as possible. No crab was injured during the research, and they all continued their regular activities after release. The stimulus crabs were untethered and released immediately after their trials. We placed released crabs into temporary burrows that we created by thrusting a dowel rod into the sediment.

Conflict of interest statement

The author declares no conflict of interest.

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Table 1. Models results on the effect of (A) size difference between focal and stimulus, stimulus type (conspecific female and male-CM, heterospecific male-HM), distance (10cm, 20cm and 30cm) and an interaction between the last two; (B) stimulus type (heterospecific male-HM and no stimulus) on the proportion of double waves over the total. Estimates, standard errors (S.E.), t values and p-values are indicated.

	Estimate	S.E.	t value	p-value
(A)				
Intercept	0.6406	0.0896	7.146	<0.001
CM	0.1775	0.1465	1.212	0.22
HM	0.6530	0.1904	3.429	<0.001
Distance	0.0415	0.0083	4.997	<0.001
Size difference	-0.3160	0.2546	-1.241	0.22
CM : distance	0.0023	0.0145	0.164	0.87
HM : distance	-0.0298	0.0143	-2.078	0.04
(B)				
Intercept	1.6059	0.1979	8.113	<0.001
HM	-0.1344	0.2195	-0.612	0.542

Figures

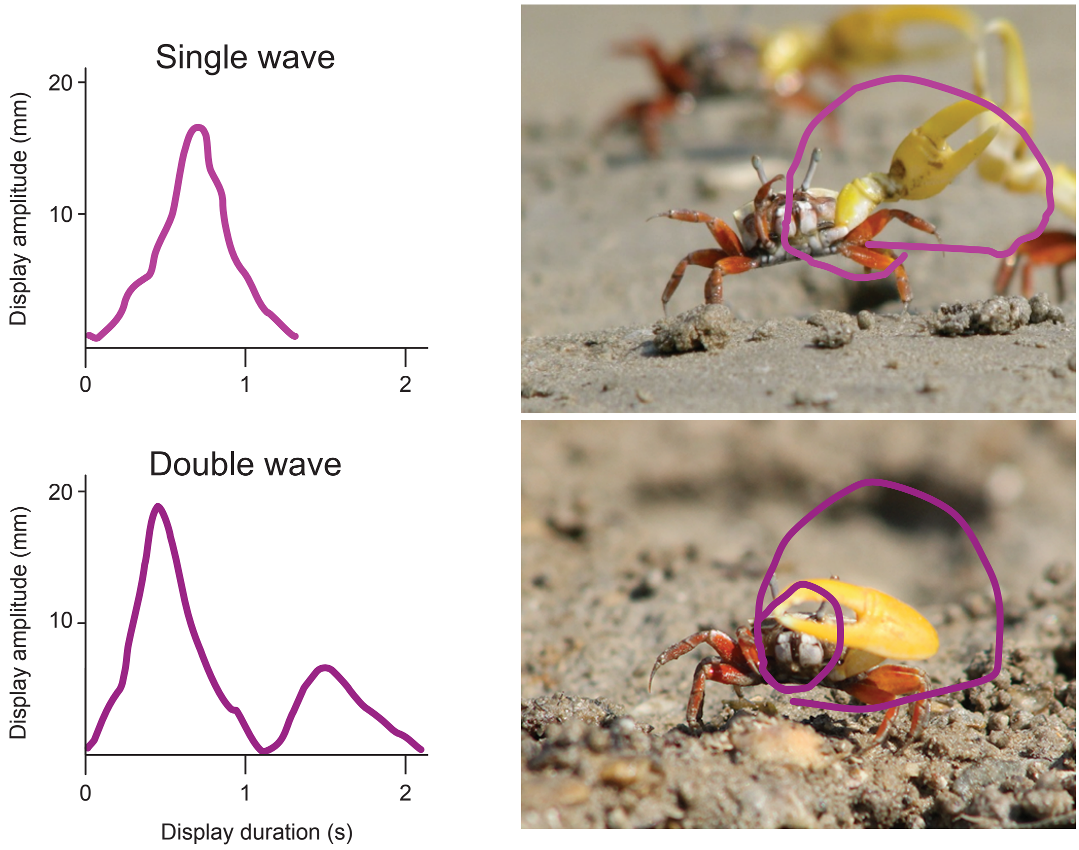

Figure 1. Representations of single and double wave displays: graphs of wave amplitude (mm) over time (s) (Left); frontal view of the movements (Right).

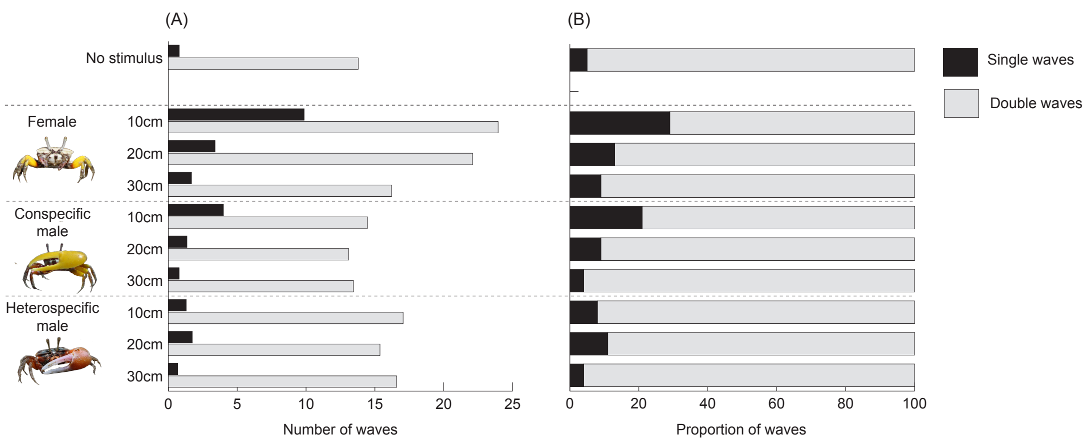

Figure 2. The absolute number with sample size and standard deviation of single waves (black) over double waves (grey) according to stimulus (female; conspecific male;

heterospecific male) and distances (10, 20 and 30cm). Letters between brackets group the treatments that are not significantly different from one another (results from Benjamini-Hochberg posthoc tests).

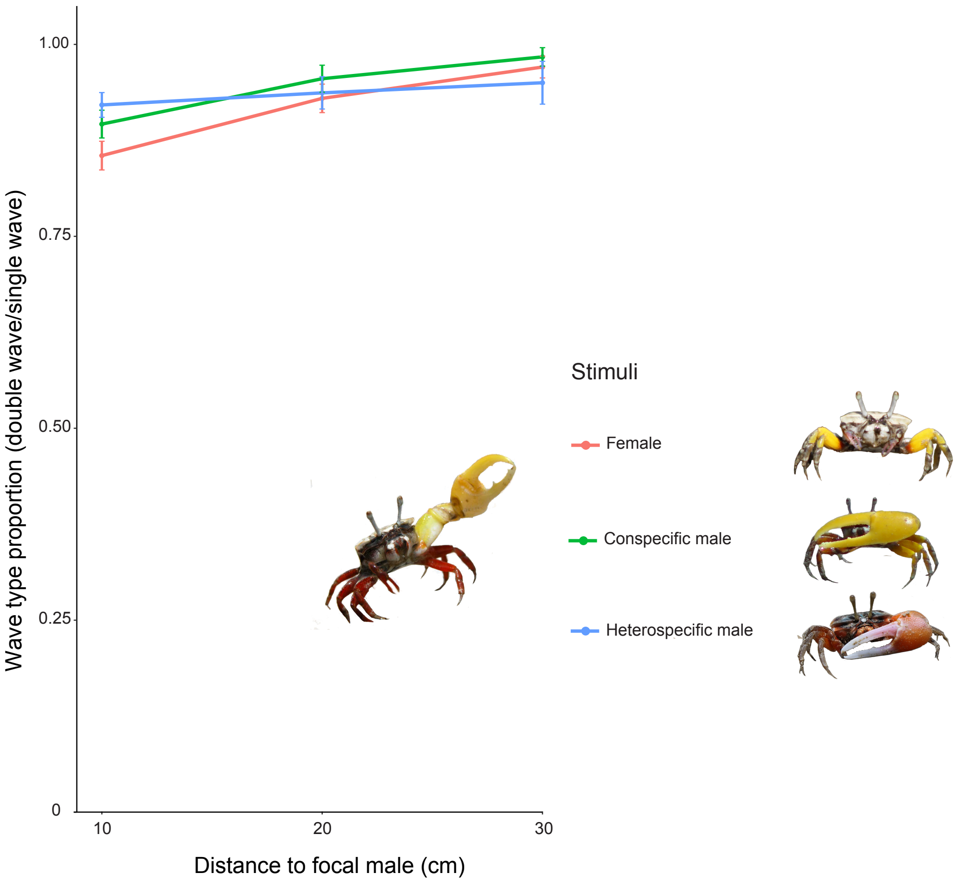

Figure 3. Plot of model predictions on the proportion of double waves over the total given by focal males in each trial (whiskers represent the SE).

Appendix 1. Unpublished data from female choice on single (1w) over double waves (2w) in robotic crab experiments by Perez & Backwell 2017. The time taken (in seconds) to approach a single and a double wave is indicated by Response time. The result of a t-test to test for differences in response time between the choices is shown with values for t, degrees of freedom and p-value.

Female number	Choice	Response time (s)
1	1w	81
2	2w	25
3	2w	10
4	1w	26
5	2w	74
6	1w	42
7	1w	38
8	2w	194
9	2w	70
10	1w	18
11	1w	34
12	1w	19
13	2w	111
14	1w	36
15	2w	25
16	2w	36
17	1w	35
18	1w	132
19	1w	34
20	2w	25
t-test (d.f,) P	-0.83 (12.11) 0.42	