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## RESEARCH ARTICLE

# Differences in the escape response of a grapsid crab in the field and in the laboratory

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## ABSTRACT

Escape behaviours of prey animals are frequently used to study the neural control of behaviour. Escape responses are robust and fast, and can be reliably evoked under both field and laboratory conditions. Many escape responses are not as simple as previously suggested, however, and are often modulated by a range of contextual factors. To date it has been unclear to what extent behaviours studied in controlled laboratory experiments are actually representative of the behaviours that occur under more natural conditions. Here, we have used the model species *Neohelice granulata*, a grapsid crab, to show that there are significant differences between the crabs' escape responses in the field compared with those previously documented in laboratory experiments. These differences are consistent with contextual adjustments such as the availability of a refuge and have clear consequences for understanding the crabs' neural control of behaviour. Furthermore, the methodology used in this study mirrors the methodology previously used in fiddler crab research, allowing us to show that the previously documented differences in escape responses between these grapsid species are real and substantial. *Neohelice granulata*'s responses are delayed and more controlled. Overall, the results highlight the adaptability and flexibility of escape behaviours and provide further evidence that the neural control of behaviour needs to be addressed in both the laboratory and field context.

KEY WORDS: Multi-stage response strategy, Escape behaviour, Predator avoidance, Crabs, Vision, Limited information

## INTRODUCTION

An important step towards understanding the neural mechanisms that underlie behavioural responses is identifying the inherent differences between field and laboratory research and the results they generate. The practical and logistical difficulties of studying neural response patterns in the field means that most neurobiological studies to date have been confined to the laboratory. By comparing behaviours that can be observed both in the laboratory and in the field, the interactions between neural processing and the ecological pressures that drive animal behaviour can be better understood. Escape behaviours are considered a good target for such investigations because they are easily and reliably evoked. Moreover, to be successful, escape behaviours must be fast, meaning that the underlying circuits tend to be relatively simple (but see Card, 2012) and often contain giant neurons amenable to

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electrophysiological recording (Herberholz and Marquart, 2012). This assumed simplicity begs the question whether there is a significant difference between behaviours evoked in the laboratory and those evoked in the field.

The escape behaviour of grapsid crabs in response to visual threats has been extensively studied in two species belonging to different families: the crab *Neohelice granulata* (Dana 1851) (previously *Chasmagnathus granulatus*) (Grapsidae) from Argentina and the fiddler crab *Uca vomeris* McNeill 1920 (Ocipodidae) from Australia. Both species are central place foragers that emerge from their burrows at low tide, live at high densities, and are predated upon by seabirds such as gulls and terns. The species have comparable exposure to predation pressure and display similar social interactions (e.g. Fathala and Maldonado, 2011; Zeil and Hemmi, 2006).

For both species, field research has underscored the critical role of the burrow in predator avoidance (Fathala et al., 2010a,b; Hemmi, 2005a; Hemmi and Zeil, 2003a) and has shown that the escape response habituates (Hemmi and Merkle, 2009; Raderschall et al., 2011; Tomsic et al., 1993, 1998). There are, however, differences between the observed behaviours that are significant. These differences are difficult to interpret because studies on the escape behaviour of *N. granulata* were predominantly done in the laboratory, in combination with neurophysiological analyses (Berón de Astrada et al., 2013; Oliva et al., 2007; Sztarker and Tomsic, 2008, 2011; Tomsic et al., 2003), whereas the majority of studies on *U. vomeris* have been carried out in the field (Hemmi, 2005a,b; Hemmi and Merkle, 2009; Hemmi and Pfeil, 2010; Smolka et al., 2012, 2011).

Field research in fiddler crabs has highlighted that they respond very early to approaching predators. They initiate their escape when the predator is only seen by one or a few ommatidia (Hemmi and Pfeil, 2010) and they run towards their burrow rather than away from the predator (Hemmi, 2005a). The decision criterion that triggers escape is multifaceted, taking into account the predator's speed, size and elevation in the visual field, as well as the individual crab's situation (Hemmi, 2005b; Hemmi and Pfeil, 2010; Smolka et al., 2011). They respond earlier, for instance, when they are further away from their burrow. The crabs also use different response criteria at different stages in their multi-stage response sequence (Hemmi and Pfeil, 2010).

*Neohelice granulata* have shown a different response repertoire when tested for predator escape in the laboratory. Crabs on a treadmill run directly away from the stimulus, and the speed (Oliva and Tomsic, 2012) and direction (Medan et al., 2015) of their escape are continuously adjusted according to visual information. In comparison to the field results for *U. vomeris*, *N. granulata* escape responses are triggered much later, when the stimulus has reached a much larger angular size (Hemmi and Tomsic, 2012; Oliva and Tomsic, 2012). The response criterion that initiates escape is based on a fixed increase in angular size of the stimulus (Oliva and Tomsic, 2012).

It is unclear whether observed differences in the escape behaviours of the two species reflect fundamental differences between the two species or whether they are a consequence of the contextual differences in which the animals were tested. To examine this question, we analysed the escape response of *N. granulata* in the field using the same experimental techniques previously used in fiddler crabs. These experiments allowed us to compare (a) the escape performance of the two species using the same visual stimulus and method of analysis and (b) the escape performance of a single species, *N. granulata*, in both field and laboratory conditions.

## MATERIALS AND METHODS
### Study species
*Neohelice granulata* is a robust mid-sized grapsoid crab, reaching a size of up to 3.6 cm across the carapace. The species inhabits both the intertidal zone (mud and sand flats) and salt marshes (areas densely vegetated with cord grasses) along the Atlantic coast of South America. The crabs live in dense colonies of up to 100 individuals per $m^2$ (Luppi et al., 2013; Spivak, 2010). Individual animals dig burrows for protection and are preyed upon by several species of seabirds (Bachmann and Martinez, 1999; Spivak and Sanchez, 1992). In certain regions, including the area used for this study, the distribution of *N. granulata* overlaps with that of the fiddler crab *Uca uruguayensis*.

### Experimental procedure
During experiments, crab behaviour was filmed with two video cameras (Sony Handycam HDR-CX550VE) fixed to a central metal pole approximately 1.6 m above the mud flat, covering an area of approximately 1.2 $m^2$ each (Fig. 1). Black, hollow plastic balls were used as dummy predators (e.g. Hemmi, 2005a; Hemmi and Pfeil, 2010). A pulling line, wrapped around a motorized driving wheel, allowed the dummies to be moved at constant speed along a dummy track made from tightly strung monofilament fishing line (Fig. 1A). The dummy track was mounted at a height of approximately 20 cm above ground. Two dummy sizes and two dummy speeds were used in a 2×2 balanced design. The dummies were either 3 or 4 cm in diameter (Fig. 1C) and moved at either a

slow or fast speed (Fig. 1B, 32.8±0.9 and 64.5±1.9 cm s$^{-1}$, respectively, means±s.d.). The same dummy predators, modelled on the hunting behaviour of the gull-billed tern (Land, 1999), were previously used effectively to elicit anti-predator responses in fiddler crabs (e.g. Hemmi, 2005a; Hemmi and Pfeil, 2010). The scaled-down design takes advantage of the crabs' low spatial resolution (Smolka and Hemmi, 2009) and their inability to accurately measure the relevant distances to objects above the horizon (Hemmi, 2005b).

Once the experimental setup was completed, crabs were given at least 10 min to resume normal foraging behaviour before the experiment started.

During what we call a 'run', a dummy approached the recording area from a distance of 6–7 m, moved past the two cameras and then returned to its starting position (Fig. 1A). These runs were repeated every 2–3 min. We replicated this setup in three different locations. At each location, 24 runs were performed in six randomized blocks. Each four-run block consisted of a 2×2 combination of two speeds and two dummy predator sizes in a randomized sequence. A total of 54 crabs contributed to the data.

### Video analysis and response measures
Accurate 3D position information of crabs, burrows and dummies was calculated at 160 ms time intervals from calibrated video information. Video footage was converted to AVI format using ffmpeg and then analysed with custom-made Matlab software (J.M.H.). All cameras were calibrated for lens distortion effects by the use of a checkerboard test pattern with the Matlab camera calibration toolbox (Bouguet, 2005). The toolbox was also used to determine the position of the cameras relative to each other and to the ground. The ground was assumed to be flat, with the dummy at a height of 20 cm above the ground.

For frames where the dummy was not visible in the video, dummy positions were reconstructed from dummy movement information. The pulling line that moved the dummy also moved two patterned wheels, each visible to one of the video cameras. Analysing the wheel rotations allowed us to calculate the exact position of the dummy relative to the crabs for the entire duration of the experiment.

Consistent with previous studies, a home-run was considered to have occurred whenever a crab moved at least 3 cm towards its burrow during a 3 frame period (480 ms). The start of the response was assigned to the first of
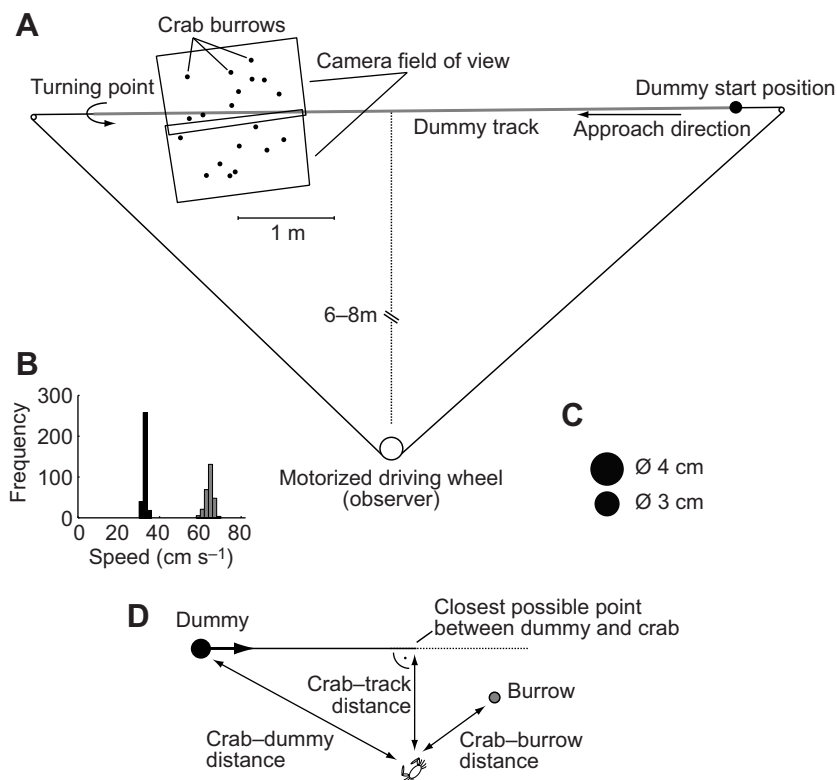


Fig. 1. The experimental setup. (A) A bird's eye view of a typical experimental setup, showing the fields of view of the two video cameras, the dummy track along which the dummy was moved towards the crabs and the position of the observer. Crab burrows are marked as small black dots in the fields of view of the cameras. (B) The distribution of dummy speed, separated into the slow (black) and fast (grey) dummies. (C) The two different dummy sizes used in the experiments. (D) An explanation of the various measurements used to describe the approach direction of the dummy relative to the burrow and the crab.

these frames in which the crab had moved at least 1 cm during one 160 ms interval. A burrow descent was recorded when a crab entered its burrow to the point where it became invisible. Responses were only counted if they occurred during the incoming dummy movement, i.e. while the dummy moved from its start position towards the turning point (Fig. 1A) and before the dummy had passed the closest point to the crab (i.e. it was still approaching). The decision to run home was assumed to have occurred 1 frame (160 ms) prior to when the response was measured. The burrow-descent decision was considered to have occurred 3 frames (480 ms) before a crab had fully disappeared. This was necessary as our criterion for a crab to have descended underground is very conservative and required the crab to become completely invisible. In most situations, the crabs were already clearly heading underground 1 or 2 frames before the response was registered. Changing the criterion by a few frames did not change the interpretation of the results. The biggest effect of varying this criterion was on the crab–dummy distance for the fastest dummy speeds.

As in previous experiments, only crabs that were at least 5 cm away from their burrow at the start of the run were considered in the analysis (Hemmi and Pfeil, 2010). This ensured that the criterion for initiating a run home could in principle be achieved. For each valid run, we determined the crab–dummy, crab–burrow and crab–track distance. All crabs that left the cameras' field of view at any stage before the dummy had reached the closest point to the crab or that were involved in an interaction with another crab were excluded from the analyses. From the geometry of the situation, we could then calculate the apparent size (the angular size of the dummy predator as seen from the crab's point of view) and apparent speed of the stimulus (the angular speed of the dummy as seen from the crab's point of view).

### Statistics

Data from the decision both to run home and to disappear below ground were analysed in the context of a survival analysis using a Cox model (Collett, 2003). The Cox proportional-hazards model allows analysis of the effects of several risk factors on the probability of responding to an ever more threatening stimulus. It allows an integrated analysis of response probability and response timing. Each crab–dummy interaction only contributes to the data over the range of stimulus values that can potentially be experienced by the crab. In other words, a data point for a crab that is 50 cm away from the dummy line only contributes to the shape of the survival curve until the dummy has reached the closest point to the crab (i.e. 50 cm). The last point of contribution is indicated by a cross in the figures, representing the censor value (see below). Each cross therefore indicates a non-response.

For statistical analysis using the Cox models, dummy speed was fitted as a factor with two values, and all other terms as continuous variables. For the burrow-descent decision, crab–track distance was the same as burrow–track distance.

As in a typical regression analysis, the contribution of each model term was judged by comparing equivalent models that did or did not contain the term in question, but contained all other relevant terms. All terms of the final model made a statistically significant contribution to that model at the 5% level. Statistical significance was judged using a simple permutation approach applied to the full Cox model (Collett, 2003). This non-parametric approach allowed us to avoid making assumptions about the underlying distribution of our data. The only assumptions were that observations were independent and identically distributed under the null hypothesis. While there were often several crabs on the surface during a dummy's approach, there was no clear neighbour effect. For the 83% of runs where there were ≤4 crabs contributing to the analysis for any given dummy approach, response probability stayed at or above 90%, regardless of the number of crabs on the surface.

In order to take into account the experimental and repeated measures design, we restricted permutations within crabs. To test statistically for effects of approach speed, dummy speed was randomly permuted 10,000 times across all the responses for each individual crab – not across crabs. The exception to this rule was the distance between the crab and the dummy track (crab–track distance). Because crabs are associated with a particular burrow, there is very little variation of this measure within a crab and therefore

resampling was done across crabs. For each crab, a mean crab–track distance was calculated and this single value was permuted across crabs. The score of our statistical measure, the log-likelihood estimate of the Cox model, a measure of residual variance left in the data, was used as the score for our resampling. The log-likelihood estimate was computed on our original, un-permuted data set and was then compared with the scores of the permuted data sets. Significance was judged by calculating the percentage of permutations that resulted in a score that was more extreme than or equal to the score calculated from the unpermuted data set. As usual, *P*-values thus indicate the probability that the measured effect was due to chance alone. By permuting strictly within individual crabs we eliminated crab-to-crab variability from the analysis. Computations were performed in Matlab R2014a (The MathWorks, Natick, MA, USA).

### RESULTS

*Neohelice granulata* crabs reliably responded to the approaching dummy predator by running home to their individual burrows (Fig. 2A). In 533 responses, 83% of crabs ran within 5 deg of the direction to the burrow and only one escape run deviated by more than 30 deg from the direction to the burrow. Overall, in 89.9% of all crab–dummy interactions (*N*=593, 54 crabs) where the crabs were away from their burrows, crabs responded before the dummy passed by them. Most of the crabs that did not respond to the dummy were relatively far away from the dummy track, i.e. they had a relatively large crab–track distance. This is indicated by the censor points
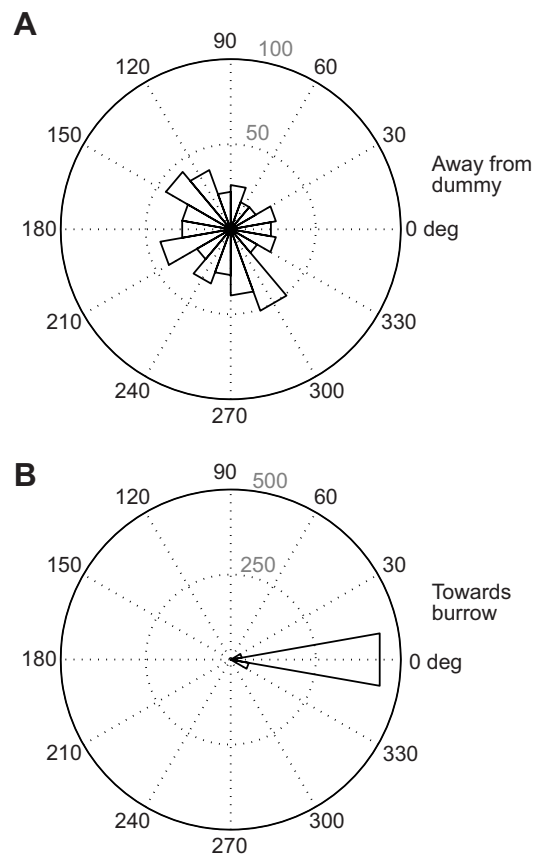


**Fig. 2. *Neohelice granulata* run directly towards their burrow rather than away from the dummy.** (A) The direction of escape relative to the direction of the approaching dummy (180 deg) is random. (B) The direction of escape relative to the direction of the crab's burrow location (0 deg). The approach direction of the dummy is clearly ignored and crabs run directly towards the safety of the burrow. Note the difference in scale for the two histograms; the inner circle represents 50 responses in A and 250 responses in B.

**Table 1. Final statistical models for the decision to run home**

| Model | Significant model terms (5%) | P-value |
|---|---|---|
| Distance (at response) | Crab–burrow distance | $P \ll 0.001$ |
| | Dummy size | $P = 0.051$ |
| | Dummy speed | $P = 0.38$ |
| Angular speed (at response) | Crab–burrow distance | $P \ll 0.001$ |
| | Crab–track distance | $P < 0.001$ |
| | Dummy speed | $P \ll 0.001$ |
| | Simulated dummy speed[1] | $P = 0.39$ |
| Angular size (at response) | Crab–burrow distance | $P \ll 0.001$ |
| | Dummy size | $P \ll 0.001$ |
| | Simulated dummy size[2] | $P = 0.054$ |

Terms included in the final statistical model are underlined. In each case there were $N = 593$ interactions and 54 crabs contributed to the data.
[1]Simulation: angular speed was calculated as if all dummies were moving at the slower speed.
[2]Simulation: angular size was calculated as if all dummies were of the smaller size.

(crosses) in Fig. 3A; at these censor points, individual crab–dummy interactions were removed from further analysis because the dummy had reached its closest possible point to the crab without evoking a response. The response probability for crabs that were approached more directly by the dummy is therefore best estimated by the end point of the survival analysis (95.1%; Fig. 3A, end point of line). Crabs always responded by running towards the entrance of their burrow where they would often stop before finally retreating underground or resuming other activities. Crabs that were further away from their refuge (more than the median crab–burrow

distance) at the start of the dummy's approach responded earlier, indicating that they were sensitive to the risk of being caught outside the burrow (Fig. 3B, Table 1; $N = 593$, 54 crabs, $P \ll 0.001$). The probability of crabs retreating underground was very low overall (35.8%, $N = 606$), which is a direct result of the fact that only crabs that are approached very closely retreat underground.

Burrow descent does not usually follow the run to the burrow immediately, suggesting that each crab makes a second decision whether or not to disappear underground. This was indicated by the large difference in response distance between the run home and the retreat underground (compare Fig. 3A and C) and by the fact that there was no difference in the response timing between crabs that were already close to home at the start of the experiment and those that were further away Fig. 3C.

As a consequence of the smaller crab–dummy distance, the dummy's angular size at the time of the response was much larger for the burrow-descent than for the run home response (Fig. 3D). The average angular size of the dummy at the time the crabs decided to run home was 1.9±0.04 deg (mean±s.e.m.) whereas the angular size at the time the crabs decided to escape underground was 4.6± 0.12 deg.

### Decision criteria – run home
What characteristics of the approaching dummy triggered the crabs' run towards their burrow? One way to establish this is to examine how the response timing varies relative to perceived dummy characteristics. For instance, if the crabs were measuring the angular size of the dummy to determine when to respond, then we would
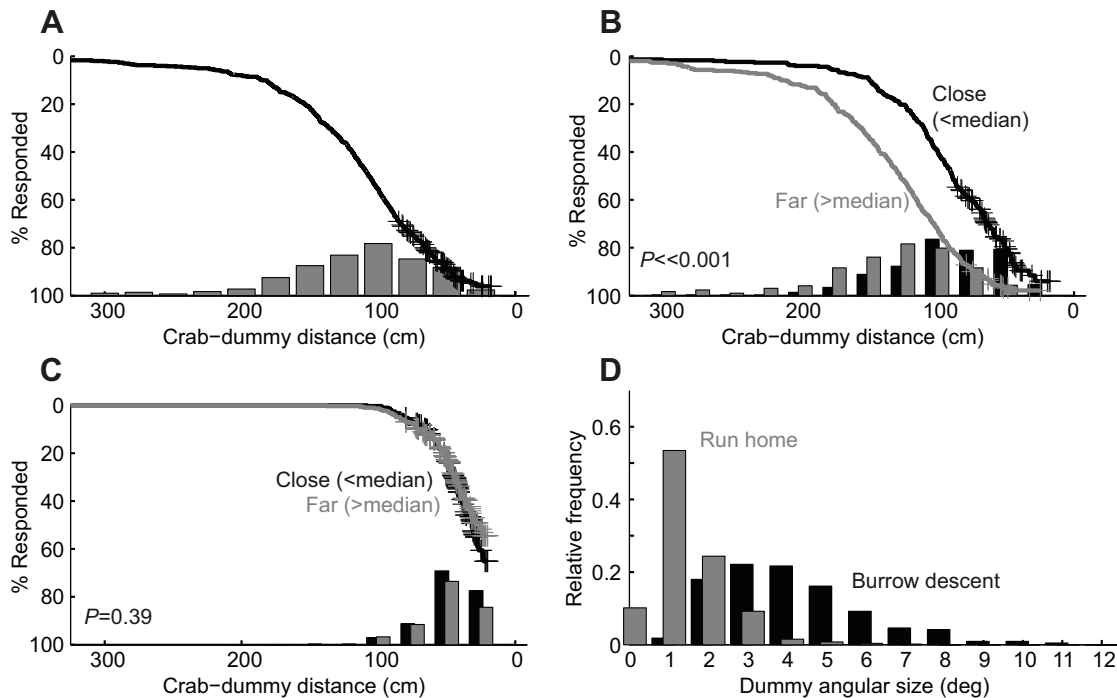


**Fig. 3. The characteristics of the responses of *N. granulata* towards approaching dummy predators.** (A) 95% of crabs respond to the dummy and over 50% of home runs are triggered before the dummy has reached a distance of 1 m to the crab. The solid line shows an inverted cumulative distribution function, based on a Kaplan–Meier survival curve (Kaplan and Meier, 1958). This curve shows the percentage of crabs that responded before the dummy reached a certain distance to the crab. Crosses show censor points where a dummy reached its closest possible distance to the crab without eliciting a response and therefore could not contribute to the overall estimate of response probability at shorter crab–dummy distances. The histogram shows the probability that a crab responded while the dummy moved through a given distance bin. The histogram should have its own *y*-scale in units of percentage change over distance; we have omitted the extra scale in order to reduce clutter. (B) Same as in A, but the data have been divided into runs where the crab was closer or further away from its burrow than the median crab–burrow distance (13.8 cm) at the start of the run. (C) Same as in B, but for the decision to retreat underground. Close and far are again based on the crab's position at the start of the run. (D) Histogram of the angular size of the dummy as seen from the crab's perspective for the run home and the burrow-descent decision.
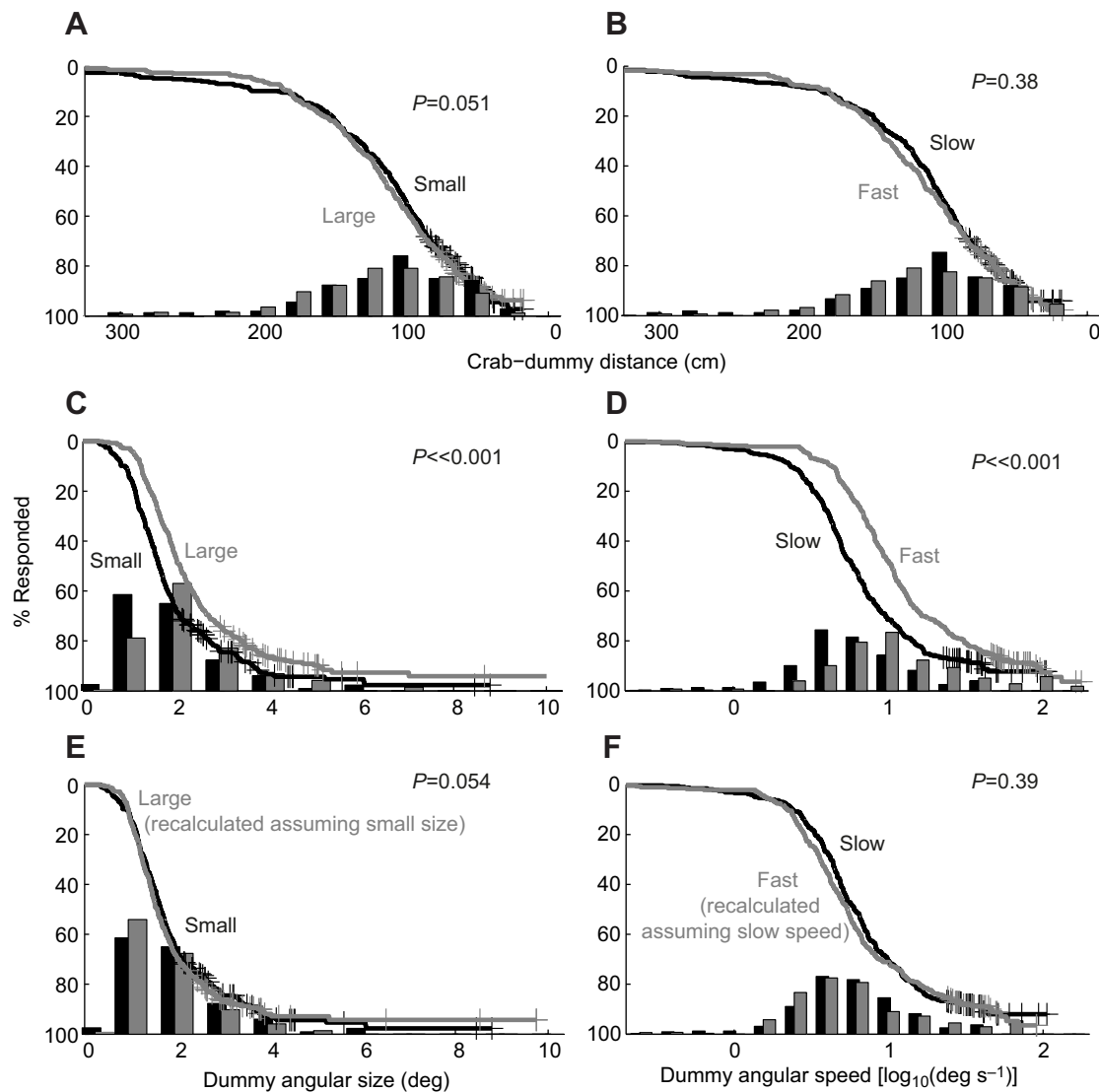
**Fig. 4. The timing of the escape run (run home).** (A) Dummy size also has no effect on the timing or probability of the decision to run home. There is no difference in response distance for large (grey) or small (black) dummies. (B) Crabs run towards home at the same crab–dummy distance for both fast (grey) and slow (black) dummies. (C) The perceived angular size at the time of the response differs for dummies of different sizes: large (grey), small (black). The shift between the two curves is exactly predicted by the response distance (A). The difference in angular size disappears when the calculations are done assuming that all dummies are small (E). (D) The perceived angular speed of the dummy at the time of the response differs for fast and slow dummies. This is a consequence of the fact that the crabs respond to slow and fast dummies at the same distance (B). This difference is removed when we calculate angular speed assuming all dummies moved slowly (F), indicating that the difference is purely a distance effect. Conventions as in Fig. 3.

expect that the response distance should increase with dummy size so that the apparent size of the dummy at the time of the response remains constant. The same argument can be made for other variables such as dummy speed.

**Dummy size**
Regardless of the dummy's actual size, the crabs responded at the same distance to the dummy. While there was a small, statistically almost significant, effect of dummy size on the response distance (Fig. 4A, Table 1; $N$=593, 54 crabs, $P$=0.051), the effect size was so small that it is biologically probably irrelevant. The fact that the crabs responded to the two different dummy sizes at the same distance means that the angular size of the dummy, from the crab's point of view, was clearly different at the time of the response (Fig. 4C, Table 1; $N$=593, 54 crabs, $P \ll 0.001$). This indicates that angular size was not used as a response criterion. If we recalculate

apparent size assuming that the larger dummy was the same size as the smaller dummy, the difference between the two angular size distributions becomes negligible again (Fig. 4E, Table 1; $N$=593, 54 crabs, $P$=0.054), i.e. crabs responded at the same distance.

**Dummy speed**
To our surprise, and in stark contrast to previous results in fiddler crabs, the dummy's speed did not affect *N. granulata*'s decision to run home. The response distance was unaffected by the approach speed of the dummy (Fig. 4B, Table 1; $N$=593, 54 crabs, $P$=0.38), even though the faster dummy moved on average twice as fast as the slow dummy (64.4 versus 32.8 cm s$^{-1}$; Fig. 1B). Because perceived angular speed depends on the dummy's real speed, this result indicates that the crabs did not use angular speed as a criterion to initiate their escape (Fig. 4D, Table 1; $N$=593, 54 crabs, $P \ll 0.001$). The observed difference between the angular speed of fast and slow

dummies at the time of the response is predicted by their actual speed. This can be shown by recalculating the angular speed of the fast dummy as if it were moving at the slower speed. After this recalculation, the distribution of responses to the fast dummy matches that of the slow dummy (Fig. 4F, Table 1; $N$=593, 54 crabs, $P$=0.39), which is a direct consequence of the fact that response distances were equivalent for the two dummy types (Fig. 4B).

### Decision criteria – retreat underground

At the response stage when the crabs decide whether to retreat underground, the results show a clear effect of both dummy size and dummy speed on response distance (Fig. 5A,B).

### Dummy size

When confronted with a larger dummy (4 cm rather than 3 cm), the crabs responded at a greater distance (Fig. 5A, Table 2; $N$=606, 54

crabs, $P \ll 0.001$), but at the same angular size (Fig. 5C, Table 2; $N$=606, 54 crabs, $P$=0.40). This indicates that angular size, or a fixed increase in angular size (Oliva and Tomsic, 2012), played a major role in the crabs' decision to retreat underground. Predictably, if we recalculate the angular size of the large dummy at the time of the response as if it was the same size as the small dummy, the two survival curves, plotted against angular size, clearly separate again (Fig. 5E, Table 2; $N$=606, 54 crabs, $P \ll 0.001$).

### Dummy speed

Surprisingly, the crabs let the faster dummy approach closer before they responded (Fig. 5B, Table 2; $N$=606, 54 crabs, $P \ll 0.001$). It is not clear why the crabs did this, but it is clearly not because they use angular speed as a decision criterion. The differences between slow and fast dummies were even more pronounced when plotted over angular speed (Fig. 5D, Table 2; $N$=606, 54 crabs, $P \ll 0.001$). The
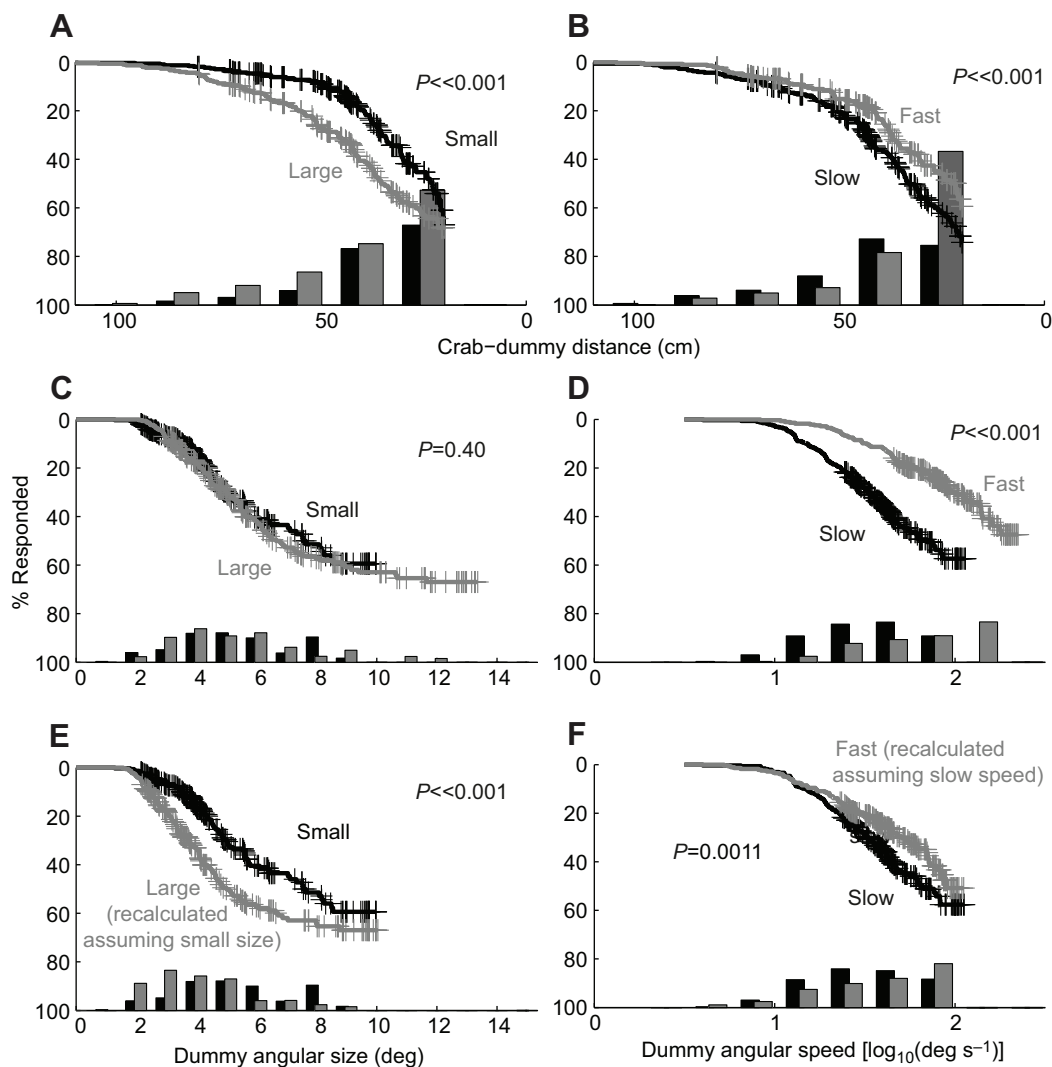


**Fig. 5. The timing of the retreat underground.** (A) Larger dummies (grey) trigger earlier burrow-descent responses (i.e. at larger distances) than smaller dummies (black). (B) Dummy speed has a small effect on the timing of the decision to retreat underground. Faster dummies (grey) are responded to slightly later than slower dummies (black). (C) Perceived angular size at the time of the underground retreat does not differ between responses to dummies of differing sizes, suggesting that crabs may use angular size or the increase in angular size as a criterion to time their escape decision. The difference in apparent size reappears if we calculate angular size assuming all dummies are small (E) – indicating the analysis is sensitive enough to show relevant differences. (D) The perceived angular speed of the dummy predator at the time of the response differs strongly for fast and slow dummies, indicating that speed is not used as a decision criterion. The difference in responses towards fast and slow dummies remains even when calculating angular speed assuming all dummies are slow (F; see Results for details). Conventions as in Fig. 3.

**Table 2. Final statistical models for the decision to retreat underground**

| Model | Significant model terms (5%) | P-value |
|---|---|---|
| Distance (at response) | Dummy size | P≪0.001 |
| | Dummy speed | P≪0.001 |
| | Crab–track distance | P=0.038 |
| | Crab–burrow distance | P=0.39 |
| Angular speed (at response) | Dummy size | P≪0.001 |
| | Dummy speed | P≪0.001 |
| | Crab–track distance | P=0.034 |
| | Simulated dummy speed[1] | P=0.0011 |
| Angular size (at response) | Dummy speed | P≪0.001 |
| | Crab–track distance | P=0.24 |
| | Dummy size | P=0.40 |
| | Crab–burrow distance | P=0.38 |
| | Simulated dummy size[2] | P≪0.001 |

Terms included in the final statistical model are underlined. In each case there were $N$=606 interactions and 54 crabs contributed to the data.
[1]Simulation: angular speed was calculated as if all dummies were moving at the slower speed.
[2]Simulation: angular size was calculated as if all dummies were of the smaller size.

difference between the two distributions was larger than predicted by the travelling speed of the dummies alone (Fig. 5F, Table 2; $N$=606, 54 crabs, $P$=0.0011).

The relative position of the two curves in Fig. 5B depends to some extent on our definition of the delay between the response decision and the behavioural measure of the point in time when crabs disappeared below ground. Faster dummies cover more ground during this time and are therefore more affected by this assumption. However, even extending the assumed time between the decision and behavioural execution from 3 to 6 frames (960 ms) does not merge the two curves, but makes them cross over in the middle (data not shown).

Note, unlike the estimation of angular dummy speed, the accuracy with which we can predict the exact time point at which the crabs made their escape decision affects both small and large dummies equally, as both dummies travel on average at the same speed.

## DISCUSSION
To allow comparisons of laboratory and field results with regard to the neurobiological control of escape responses in the crab *N. granulata*, we evoked escape responses in this crab under naturalistic conditions in the field. The aim was to find out what criteria the crabs use to make their escape decisions and compare those criteria with results obtained from previous behavioural and neurophysiological experiments (e.g. Berón de Astrada et al., 2013; Oliva et al., 2007; Sztarker and Tomsic, 2008, 2011). In order to make the results comparable to previous results from another grapsid species, *U. vomeris*, we used the exact same setup to test *N. granulata* predator avoidance responses that was previously used in these fiddler crabs (Hemmi, 2005a; Hemmi and Pfeil, 2010). These two species live in similar habitats, and have similar social and similar eye structures (Berón de Astrada et al., 2012; Smolka and Hemmi, 2009; Zeil et al., 1986).

The results for the crabs' decision to run home show that *N. granulata* crabs respond well and consistently to the dummy predators. The overall response probability was close to 90%. The crabs also clearly indicated that they are sensitive to the risk associated with the approaching dummy predator, running home earlier if they were further away from their refuge (Fig. 3B), a result found consistently also in fiddler crabs (e.g. Hemmi, 2005a).

Like fiddler crabs (Hemmi and Zeil, 2005), *N. granulata* responded to the incoming dummy in stages. There was a large difference in response distance between the decision to run home and the decision to retreat underground. On average, the difference between the decision to run home and the decision to retreat underground was separated by about 5 s for the fast-moving dummy and 10 s for the slow-moving dummy. With a median crab–burrow distance of just under 14 cm, crabs have enough time to run home first, then wait at the burrow entrance to make a second independent decision on whether to retreat underground. The response probability for retreating underground was much lower than for the run home. This is mostly due to the fact that the crabs retreated underground only when the dummy approached much closer.

### Response criteria
The decision to run home of *N. granulata* was not triggered by retinal speed or by retinal size (Fig. 4). Although it is surprising that the crabs ignored both the speed and the size of the approaching object, the results are clear and show that regardless of the dummy's speed or size, the crabs responded when the dummy approached to within a certain distance (the median distance was 1.13 m).

This result does not necessarily indicate that the crabs are able to measure distance, however. As the experiment kept the height of the dummy above the ground constant, for a horizontally moving dummy, distance and retinal elevation perfectly predict each other. In other words, the crabs could have simply measured the retinal elevation of the approaching dummy.

It is highly unlikely on theoretical grounds that the crabs are able to measure the distance to an object above the horizon (Collett and Harkness, 1982; Hemmi, 2005b). It has also been shown that *U. vomeris* does not measure the distance to the dummy in a predation context (Hemmi, 2005a,b), but does so in a social context, when they can use elevation in the visual field as an approximation of distance (Hemmi and Zeil, 2003b,c).

*Neohelice granulata*, like fiddler crabs (Nalbach et al., 1989), keep their eyes aligned with the visual horizon. Therefore, each row of ommatidia is aligned with a particular elevation, making it easy to estimate elevation. This suggests strongly that retinal elevation rather than distance triggers the home run. From the median response distance and the height of our dummy (20 cm), the median elevation of the dummy when the crabs ran home was 10.0 deg.

Optimal escape theory (Kramer and Bonenfant, 1997; Ydenberg and Dill, 1986) predicts that the prey should measure the distance between the predator and its burrow in order to decide when to run home. For an animal that has a large response distance relative to its own distance from the refuge, as is the case for *N. granulata*, simply measuring their own distance to the predator might be an acceptable and easier alternative. In a flat environment, retinal elevation would make a very simple mechanism by which an animal that does not have the required depth perception could make such a judgement. If birds were to change height during their approach flight, this would interfere with the distance judgement, but it would be very difficult for a large bird to approach without significantly moving up in the crab's visual field (i.e. gaining elevation). Future experiments will have to test the elevation hypothesis more directly.

### Escape responses: laboratory versus field experiments
In the laboratory, the escape response in *N. granulata* is initiated when the stimulus angular size has increased by a fixed amount (Oliva and Tomsic, 2012, 2014). In this context, the crabs run directly away from the approaching stimulus (Medan et al., 2015). In contrast, in the field context, the timing of the initial response is

size independent and the crabs run towards their burrow. The decision to escape underground, however, does depend on angular size or the increase in angular size. The median angular size increase at the time the crabs retreated underground was 4.0 deg, which is substantially smaller than the 7 deg measured in the laboratory for the initiation of the escape response (Oliva and Tomsic, 2012).

The difference in the initial 'run' response between the laboratory and field appears to suggest that we are observing different response stages. In the absence of a refuge, the laboratory crabs may supress the run home and remain frozen for a longer period, only responding to the stimulus when a higher level criterion is triggered (equivalent to that eliciting the burrow-descent response in the field). The same argument could also explain the larger angular size when the response is finally triggered in the laboratory. A crab without a refuge will never be able to outrun a bird. It would make sense for such a crab to delay any response until it is absolutely clear that it is in fact the target of the approaching bird.

In the laboratory, it has been shown that the escape response of *N. granulata* is not ballistic. The crabs adjust their escape speed and direction according to the looming characteristics of the stimulus (Oliva and Tomsic, 2012, 2014). Whether the same is true for the earlier run home performed in the field, or whether the run home is ballistic, is still unclear.

### Comparison of *N. granulata* and fiddler crabs

While there are clear differences in the responses of *N. granulata* in the laboratory and the field, their responses in the field also contrast with those of fiddler crabs. Surprisingly, the response criterion used by *N. granulata* to decide when to run home appears to be dummy elevation. Fiddler crabs use a less-specific response criterion for deciding when to run home. In fiddler crabs, most motion and even flicker stimuli above the horizon produce escape responses (e.g. Hemmi and Pfeil, 2010; Smolka et al., 2012, 2011).

However, retinal elevation, the criterion used by *N. granulata* for the run home response, is consistent with the criterion used by fiddler crabs when they decide when to disappear underground (Hemmi and Pfeil, 2010). As the environment of the two species is very similar, it is not clear at this point why the species use different decision criteria.

A reason for the difference in the timing of the run response between the two species, which is more delayed in *N. granulata* and more similar to the fiddler crab's 'disappear underground' response, may be that given their larger size and the two robust claws (fiddler crabs have only one large claw), *N. granulata* can better defend itself from predators. This may allow them to delay their escape until the predator is closer, allowing them to employ more specific response criteria (Hemmi and Pfeil, 2010).

A difference in the reliability of refuges is another potential hypothesis that could explain the difference in the criteria used to trigger escape responses between the two species. *Uca vomeris* has frequently been seen to block other conspecifics from entering their burrow in the event of a sudden threat (J.M.H., personal observation). This leaves the blocked crab stranded at the top of the burrow entrance, exposed to predation. If fiddler crabs are less sure about the availability of their refuge as a result of conspecific competition, they may be forced to return to the burrow earlier to make sure it is still free. Because of their smaller size, but similar foraging range, fiddler crabs may be more vulnerable to an intruder entering their burrow undetected while they are out foraging. It is also important to note that in the *N. granulata* field study sites, there were many more empty, and therefore available, burrows for

emergency refuge than in the fiddler crab study sites. In this study, *N. granulata* were regularly seen using more than one burrow and many burrows had multiple entrances.

In conclusion, there are commonalities as well as some clear differences between the escape responses of *N. granulata* when tested in the laboratory and the field. The response criteria and behaviours vary consistently and logically, suggesting that what we are observing are contextual adaptations to the availability of a refuge rather than experimental artefacts. The results highlight the adaptability and flexibility of the crabs' escape behaviour and the need to study such behaviours at a number of different levels of abstraction in order to obtain a true and composite picture of the decision-making process.

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