

RESEARCH ARTICLE

Habituation under natural conditions: model predators are distinguished by approach direction

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SUMMARY

Habituation is an active process that allows animals to learn to identify repeated, harmless events, and so could help individuals deal with the trade-off between reducing the risk of predation and minimizing escape costs. Safe habituation requires an accurate distinction between dangerous and harmless events, but in natural environments such an assessment is challenging because sensory information is often noisy and limited. What, then, comprises the information animals use to recognize objects that they have previously learned to be harmless? We tested whether the fiddler crab *Uca vomeris* distinguishes objects purely by their sensory signature or whether identification also involves more complex attributes such as the direction from which an object approaches. We found that crabs habituated their escape responses after repeated presentations of a dummy predator consistently approaching from the same compass direction. Females habituated both movement towards the burrow and descent into the burrow, whereas males only habituated descent into the burrow. The crabs were more likely to respond again when a physically identical dummy approached them from a new compass direction. The crabs distinguished between the two dummies even though both dummies were visible for the entire duration of the experiment and there was no difference in the timing of the dummies' movements. Thus, the position or approach direction of a dummy encodes important information that allows animals to identify an event and habituate to it. These results argue against the traditional notion that habituation is a simple, non-associative learning process, and instead suggest that habituation is very selective and uses information to distinguish between objects that is not available from the sensory signature of the object itself.

Key words: habituation, fiddler crab, learning, predator avoidance, limited information, sex difference.

INTRODUCTION

Habituation occurs in virtually all animal taxa and is characterized by a progressive decrease in responsiveness to a repeated event that has, over repeated encounters, proven to be irrelevant (Thorpe, 1969; Hemmi, 2005a; Glaudas et al., 2006; Coleman et al., 2008; Hemmi and Merkle, 2009; Rankin et al., 2009). It allows animals to adapt to a dynamic and constantly changing environment and helps them to focus on important information. In contrast to other forms of response decrements such as muscle fatigue or sensory adaptation, habituation is an active learning process, where animals receive the sensation but choose not to respond (Domjan, 2003).

Habituation is important in an anti-predator context because it helps animals to minimize false alarms to harmless events (Thorpe, 1969; Dacier et al., 2006; Glaudas et al., 2006). Under conditions where an object cannot be classified *a priori* as either harmless or dangerous, animals need to learn whether the object can be safely ignored. Habituation is one mechanism that allows animals to do so. In order to be safe, however, such habituation must be highly selective to harmless events as any mistake could be disastrous. If habituation is too selective, though, animals will flee from most harmless objects, thus wasting time and energy. During habituation, animals should identify each event either as one that has previously been harmless or one that is unknown and potentially dangerous.

Such an assessment is particularly challenging under natural conditions, where sensory input is noisy and objects are never perceived in exactly the same way. For instance, a subtle change in weather and light conditions or a change in orientation can substantially change an object's appearance. In addition, such judgements often need to be made at large distances, where animals have limited visual information on distance, size, shape and the direction of movement of potential threats.

Animals can learn to recognize different events or objects based on stimulus parameters such as the object's colour, movement pattern, position in the visual field, or by its context (Collett et al., 1997; Pereyra et al., 2000). Learning can be non-associative, if it reflects the properties of a single stimulus (e.g. Kandel et al., 2000; Rankin et al., 2009), or associative, if it is based on an association between the stimulus and its surroundings or the relationship between two stimuli (Kandel et al., 2000). Habituation is generally classified as non-associative (e.g. Thorpe, 1969; Kandel et al., 2000; Rankin, 2009), but previous studies have suggested that habituation can be influenced by the relationship between the stimulus and its context, suggesting that it could be associative (Shalter, 1978; Lozada et al., 1990; Tomsic et al., 1998; Shettleworth, 2010; Sztarker and Tomsic, 2011). Indeed, common definitions of habituation do not specify the learning mechanism: "Habituation is defined as a

behavioral response decrement that results from repeated stimulation and that does not involve sensory adaptation/sensory fatigue or motor fatigue” [p. 136 in Rankin et al. (Rankin et al., 2009)].

The way animals achieve the necessary stimulus selectivity during habituation remains largely unknown (Giles and Rankin, 2009; Thompson, 2009). Most research in this field focuses at the cellular and molecular level and is conducted under strictly controlled and simplified laboratory conditions. In contrast, there are relatively few studies on habituation conducted in the natural environment in which this process has evolved (see Petrinoich and Peeke, 1973; Brooks and Falls, 1975; Krebs, 1976; Fathala et al., 2010a; Fathala et al., 2010b). The natural environment is complex, noisy and unpredictable. In order to understand the mechanisms of habituation, it is therefore important to discover how animals that operate under such dynamic conditions categorize objects and what rules they use to identify events they can learn to ignore.

We investigate here whether the fiddler crab *Uca vomeris* is able to identify and thereby habituate to objects in their natural surroundings by using information that is not directly available from their instantaneous sensory signatures, such as the compass direction from which an object approaches. Previous work has shown that these crabs habituate their home run and burrow descent responses after repeated presentations of a dummy predator under natural conditions (Hemmi and Merkle, 2009). This species therefore provides a good model to investigate the rules and mechanisms underlying habituation under natural conditions, and in a species with limited visual acuity. Our results show that approach direction encodes essential information on an object’s identity, which strongly suggests that habituation under natural conditions is associative.

MATERIALS AND METHODS

Study site and species

All experiments were conducted in the crabs’ natural habitat on an intertidal mudflat near Cungulla, Queensland, Australia (19°24’S, 147°07’E) in April 2010. This species of fiddler crab (*Uca vomeris* McNeill 1920; Ocipodidae: Brachyura: Decapoda) lives in mixed-sex colonies of different age classes. Each crab occupies its own burrow from which it ventures onto the mudflat for short feeding excursions during low tide. The burrow is an essential refuge for all crabs (Crane, 1975). When threatened, individual fiddler crabs escape towards their own burrow irrespective of whether it is the closest refuge, and may then descend into the burrow.

Apparatus and procedure

Each experiment consisted of a habituation phase and a test phase. During the habituation phase, the dummy predator repeatedly approached a group of approximately four to 10 resident crabs from one compass direction, to allow them to habituate to this dummy. During the test phase, a second physically identical dummy approached the crabs from a new direction. Crab responses were recorded with two video cameras (Sony HDR-CX550, Kensington, VIC, Australia) fixed onto a steel pole 1.6 m above the ground. Each camera filmed an area of approximately 2 m² (Fig. 1).

Black plastic spheres of 4 cm in diameter were used as dummy predators. The dummies were attached to fishing line (dummy pulling line) such that they could be remotely moved towards and away from the crabs at a known speed (mean \pm s.d.) of 37.9 \pm 3.3 cm s⁻¹ using a cordless screwdriver (see Hemmi and Merkle, 2009). It has previously been shown that these dummies can be used to model the hunting behaviour of gull-billed terns

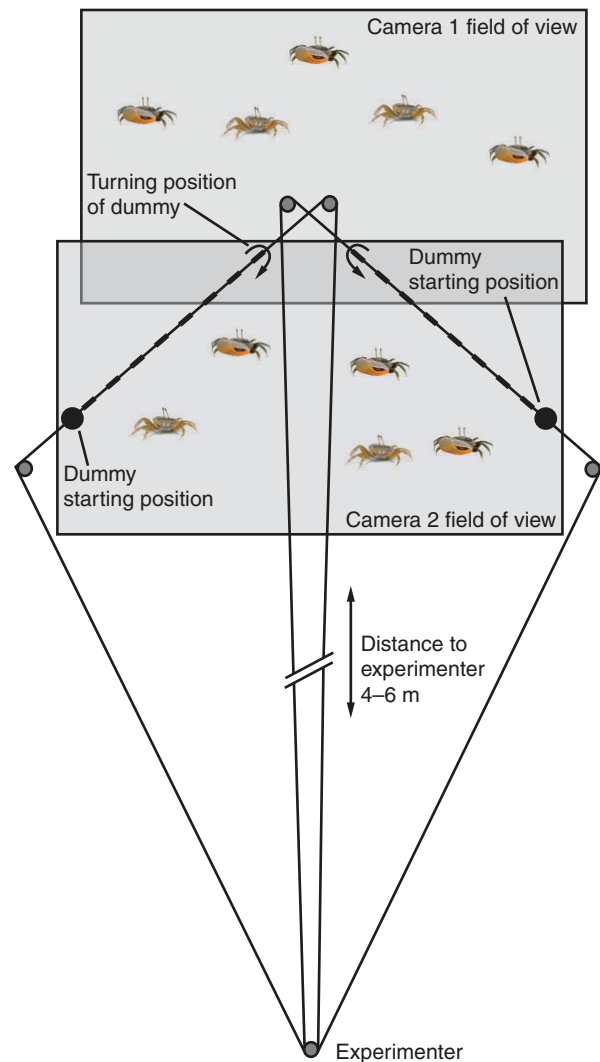


Fig. 1. Experimental setup showing the relationship between the experimenter, the two dummy tracks and the fields of view of the two video cameras recording crab responses. The dashed lines show the track the dummies followed.

(*Gelochelidon nilotica*), the crabs’ main natural predator at the field site (Hemmi, 2005a).

Each experiment consisted of a total of 32 dummy predator runs at intervals of approximately 2 min (119.9 \pm 6.0 s). A 2-min interval allowed the crabs to resume their normal activity before the next run. The first 26 runs were presentations of the habituation stimulus, runs 27 and 28 were presentations of the test stimulus followed by runs 29–32, where the original habituation stimulus approached the crabs again. During a run, one dummy approached over a distance of approximately 1.5 m, moving at least partly across the two cameras’ fields of view, before returning to its starting position (Fig. 1). The stretch of the dummy line over which the dummy moved is referred to as the dummy track (Fig. 1, black dashed line). Both dummies remained visible to the crabs at all times during and between the approaches, but only one dummy moved at any given time.

The data were collected from six experimental setups. A block design was used to determine which approach direction the crabs were habituated to for each setup. A block consisted of one left and one right habituation approach direction. These two approach directions, however, do not correspond to a particular physical

direction in the environment. The filming areas and their aspect were solely chosen based on the burrow distribution of crabs. The order of presentation within a block was randomized. In total we completed three blocks.

After the cameras and dummy system were set up, the crabs were given approximately 10 min to resume normal foraging before the experiment was initiated through the first dummy movement. Each experiment ran over a period of approximately 70 min, during which we noted whether any birds or insects flew nearby.

Video analysis and response measures

At the beginning of all video recordings, the two cameras were synchronized with an external clock and calibrated using a checkerboard standard to correct for optical lens distortion and perspective distortion (http://www.vision.caltech.edu/bouguetj/calib_doc/). Because the two cameras were recording adjacent patches of crab colonies with a small overlap between the two fields of view, it was possible to spatially align the two cameras such that dummy movements across cameras could be faithfully tracked. All video recordings were down-sampled to 6.25 frames⁻¹ (every fourth frame) before data analysis started. This allowed us to track crab and dummy positions with an accuracy of within 160 ms using a custom-made, automated video analysis program written in MATLAB (MathWorks, Natick, MA, USA). Each crab could be assigned to its individual burrow. The combined information allowed us to reconstruct the precise distances between the crabs, their burrows, the dummy track and the dummy at any time during the experiment.

We monitored the home run and burrow descent responses of the crabs, which are the two most costly of the six stages of their escape response (Hemmi and Zeil, 2005). A home run was defined as a fast movement of at least 2 cm by a crab towards its burrow within a three-frame (480 ms) period (Hemmi and Merkle, 2009; Hemmi and Pfeil, 2010). A burrow descent was defined as when a crab had entered its burrow to the extent that it was no longer visible.

Analysis and statistics

We analysed the response probabilities for each crab that met specific criteria. Home run responses were measured only while the dummy moved from its starting position towards the crabs. Furthermore, responses were excluded from the analysis whenever: (1) a crab had already begun walking towards its burrow before the dummy started to move; (2) crabs sat within 5 cm of their burrow at the start of the run; (3) crabs were outside the filming area at the start of the dummy run; (4) crabs were involved in a physical interaction with another crab; or (5) crabs responded to any outside

disturbance, such as a flying bird. In addition, the statistical model we used to analyse the data (see below) controlled for the response influence of individual crabs on others. If crabs had responded strongly to each other, we should have found a large amount of variation across setups and little variation across crabs, but in fact the opposite was found (see Tables 1–4).

To test for habituation (decline in response probability), we measured the response probabilities to dummy runs in sequence as observed by individual crabs rather than actual dummy run sequence because individual crabs spent some time during the experiment in their burrow and did therefore not see every dummy run. In contrast, to test for the effect of change in direction on crab response probability, we compared the response probabilities of crabs to the two habituation dummy runs preceding and following the two test runs with the response probabilities of the two test runs, irrespective of how many times the crabs had sighted the dummy. This was necessary to obtain meaningful sample sizes because not many crabs saw equal numbers of dummy runs prior to the test runs.

Data were analysed using a generalized linear mixed model in R 2.9 (R Development Core Team, 2011), using the glmer function of the lme4 package (link function=logit, family=binomial). We used separate statistical models for the home run and burrow descent responses. Explanatory variables included the direction of approach and sequence of presentation. We treated crabs, setups and dummy runs nested within setups as random factors. This accounts for variance and possible biases due to response differences between crabs and setups, as well as changes in the environment that might have correlated with particular dummy approaches. We used the distance of crabs to the dummy track and to their burrow as covariates, as each is likely to affect the probability of response (Hemmi and Pfeil, 2010). The statistical model was determined by sequentially adding parameters of interest to the model and only keeping those that had a significant effect at a 5% probability level. All variables were retested against the final model and only those that remained significant were kept.

RESULTS

Home run

Fiddler crabs responded to the dummy predator in the same manner that they respond to real predatory birds in flight (Smolka et al., 2011). During the habituation phase, crabs ran towards their burrow with a mean probability of 74.7% over the 32 runs. In 82% of the elicited home runs, crabs covered more than 90% of the way home, which means that they were able to touch the burrow entrance with their legs. Both sexes were more likely to run towards home when they were closer to rather than farther from the dummy track

Table 1. Probability of the home run response in the fiddler crab *Uca vomeris*

Fixed effect	Estimate	d.f.	χ^2	P
a) Crab–track distance	-0.002	1	5.31	0.021
b) Sex (males) ^a	2.56	1	–	–
c) Dummy run encounters	-0.12	1	–	–
d) Crab–burrow distance	0.009	1	–	–
e) Crab–burrow distance : sex (males)	-0.009	1	12.39	<0.001
f) Dummy encounters : sex (males)	0.13	1	5.49	0.019
g) Crab–dummy rail distance : sex (males)	–	1	0.13	0.72
h) Crab size	–	1	0.016	0.90

Results of the generalized linear mixed model analysis: N=711; random effects (s.d.): crab (2.5) + setup (0.9)/dummy run (1.3). Final model: logit(P)=2.42 + crab–track distance + sex × (dummy encounters + crab–burrow distance).

^aThe estimate given in the table indicates the difference between males and females. To estimate response probabilities for males, the estimate has to be added to the model. The estimate for females is already incorporated in the intercept.

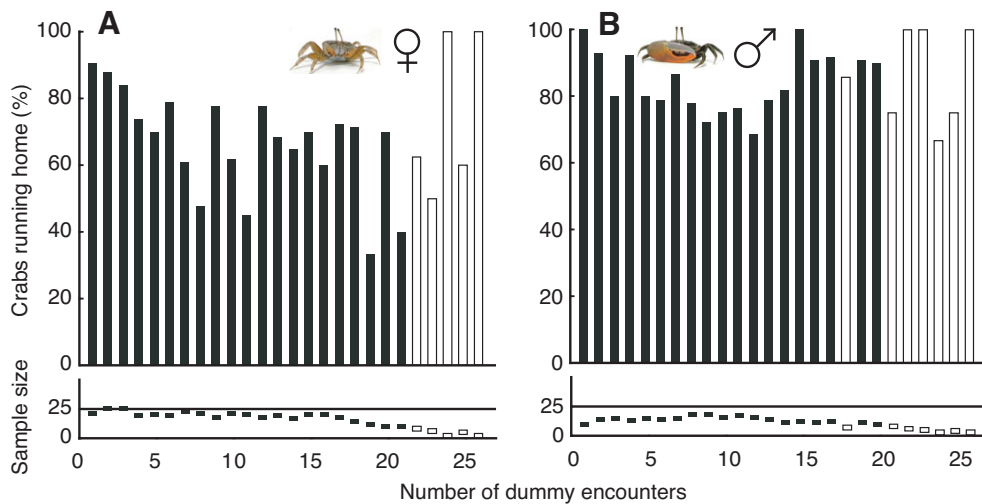


Fig. 2. Home run. The percentage of (A) female and (B) male crabs escaping towards home as a function of repeated dummy encounters differs (interaction between sex and sequence of encounters $P < 0.019$, Table 1, fixed effect f). Although the response probability of female crabs running home decreased with repeated dummy encounters, it did not decrease for males. The x-axis shows the number of runs individual crabs have seen up to that point of the experiment. Sample sizes on a per run basis are shown in the lower pane of each figure. Open bars in both figures indicate that less than 10 crabs contributed to the mean.

($P = 0.021$; Table 1, fixed effects a and g), but only female crabs were more likely to respond when they were further from their refuge ($P < 0.001$; Table 1, fixed effects d and e).

Female crabs habituated to the dummy predator whereas males continued to respond strongly throughout the experiment (Fig. 2A,B). Approximately 90% of female crabs responded the first time the dummy approached and their probability of response decreased to approximately 33% over the course of the experiment (Fig. 2A). Males were overall more likely to run home than females and their responsiveness did not decrease during the habituation phase (interaction between dummy encounters and sex: $P = 0.019$; Table 1, fixed effects b and f; Fig. 2B). In fact, the logit of response probability (log of odds) for females decreased by 0.12 for every dummy encountered (Table 1, fixed effect c), whereas the males' coefficient was almost zero (0.01; sum of estimates: Table 1, fixed effects c and f). The habituation effect for the females is unlikely to be due to sub-sampling crabs, because the reduction in response probability occurred well before the decline in the numbers of crabs that contributed to a particular response bar (Fig. 2A). The decrease in sample size reflects the fact that not all crabs saw all dummy approaches, rather than a drop in crab numbers during the experiment.

Burrow descent

Both sexes showed statistically significant habituation of the burrow descent response after repeated encounters with a dummy. The probability to descend into the burrow declined from 33% to 10% during the habituation phase ($P = 0.0011$; Fig. 3, Table 2, fixed effect a), and there was no difference between the sexes ($P = 0.45$; Table 2, fixed effect e). Compared with the home run, a substantially smaller proportion of crabs retreated into their burrow in response to the dummy. The crabs' probability of response was unaffected by the distance between crab and dummy track ($P = 0.19$; Table 2, fixed effect b).

Effects of a change in dummy approach direction

Crabs were more likely to respond to the approaches of the physically identical test dummy than to the habituation dummy following the habituation phase. The reduction in response probability during the habituation phase is therefore due to habituation – an active suppression of response – and not simply sensory adaptation or motor fatigue. This held true for both the home run ($P = 0.018$; Fig. 4A, Table 3, fixed effect a) and the burrow descent

($P = 0.027$; Fig. 4B, Table 4, fixed effect a). There was no sex difference for either of these responses (home run $P = 0.68$, Table 3, fixed effect h; burrow descent $P = 0.90$, Table 4, fixed effect e). We recognize that for the home run response this result seems contradictory because males did not habituate. An investigation of the figures showing response probabilities of males and females separately (data not shown), however, shows a clear response recovery for females only. This suggests that the statistical model failed to pick up a sex effect possibly because the number of females generally exceeded the number of males.

The final statistical models examining response probability to the test dummy accounted for possible effects of distance between the crab and the dummy track on both the home run and burrow descent. We added a factor 'close' (Table 3, fixed effect e; Table 4, fixed effect d) to the model, which measured whether the crabs were closer to the habituation track or the test track. Even though this term was clearly not significant for either the home run ($P = 0.27$, Table 3, fixed effect e) or the burrow descent response ($P = 0.80$, Table 4, fixed effect d), we kept it in the final model to adjust for any non-significant effects of relative crab–dummy track distance. This ensured that crabs that were sitting closer to

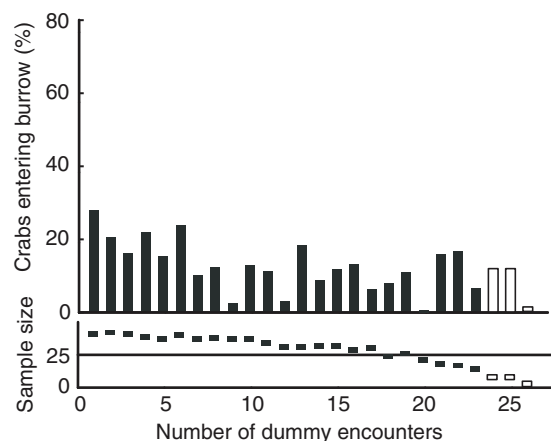


Fig. 3. Burrow descent. The percentage of crabs descending into their burrows decreased with repeated dummy encounters for both females and males (sequence of encounters $P = 0.0011$, Table 2, fixed effect a; interaction with sex $P = 0.45$, Table 2, fixed effect e). Sample sizes are shown in the lower pane and conventions follow Fig. 2.

Table 2. Probability of the burrow descent response in the fiddler crab *Uca vomeris*

Fixed effect	Estimate	d.f.	χ^2	P
a) Dummy encounters	-0.065	1	10.7	0.0011
b) Crab-track distance	-0.0002	1	1.74	0.19
c) Sex (males)	-	1	10.69	0.45
d) Crab size	-	1	1.59	0.21
e) Dummy encounters : sex (males)	-	1	0.56	0.45

Results of the generalized linear mixed model analysis: N=747, random effects (s.d.): crab (1.4) + setup (0.7)/dummy run (0). Final model: $\text{logit}(P) = -1.04 + \text{dummy encounters} + \text{crab-track distance}$.

the test track did not bias the results by being more likely to respond to the test dummy simply because they were approached more closely.

DISCUSSION

Fiddler crabs habituated to repeated presentations of a dummy even though it was initially treated as a potential predator. The degree to which fiddler crabs habituated their response stages differed between the sexes, with females less responsive overall and more sensitive to variation in risk. Female fiddler crabs habituated both the home run and the burrow descent response, and were more likely to respond when further away from their burrow. By contrast, males were more responsive overall, only habituated the burrow descent response, and did not vary home run probability according to the distance from their burrow.

Stimulus identification under natural conditions

Both males and females responded more strongly to a physically identical dummy that approached them from a new compass direction. The direction from which an object approaches is thus an essential part of the information that defines events in a predation context.

It is unlikely that retinal position could be used to distinguish the habituation dummy from the test dummy. In a previous study on the same species, the orientation of fiddler crabs varied widely between dummy runs and there was no correlation between the response probability of individual crabs and the changes in direction in which they saw successive dummy runs (Hemmi and Merkle, 2009). Thus, the response decline to the habituation dummy could not be a result of retinotopic habituation. Similarly, in the laboratory the brachyuran crab *Chasmagnathus granulatus* can recognize a learned stimulus independently of its retinal position (Sztarker and Tomsic, 2011). Because the crabs in the present study constantly change orientation, they would not have been able to reference the dummy with respect to their own body direction, but as an absolute compass direction.

The increased response to the test dummy is unlikely to have resulted from a non-associative learning process alone. The crabs might have responded more strongly to the test dummy because it looked slightly different when viewed from a different angle relative to the sun. However, outdoors, where crabs move around on the mudflat and see objects from different orientations all the time and where clouds regularly change lighting conditions, object or event identification should clearly be independent of shading and illumination to be effective under natural conditions (Li et al., 2009). Moreover, crabs also moved around their burrows during habituation and thus also saw the habituation dummy from different directions and against different backgrounds, and yet still habituated to it.

We suggest that associative learning allowed fiddler crabs to discriminate between the habituation and test dummies by using

contextual information. They could have associated each dummy either with its respective compass direction or with the region of the panorama against which they saw it, and so discriminated dummies as physically discrete objects.

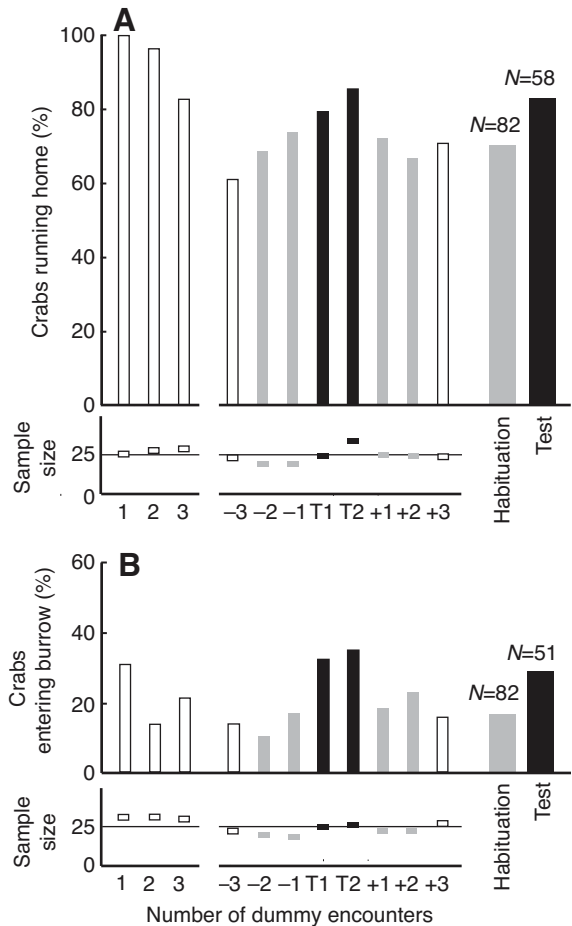


Fig. 4. Following the habituation phase, crabs were more likely to respond to a dummy that approached from a different compass direction. (A) Home run (sequence of dummy runs $P=0.018$, Table 3, fixed effect a; interaction with sex $P=0.68$, Table 3, fixed effect h). The x-axis shows the number of dummy runs in the sequence of actual dummy runs rather than dummy encounters as in previous figures. We show the first three dummy runs of the experiment (runs 1–3), the three runs preceding the tests (-3 to -1), the two test runs (black bars, T1, T2) and the three runs following the tests (+1 to +3). The grey bars were used in the statistical comparison. The two larger bars on the far right show the mean response probabilities for all test runs (black) and all habituation runs (grey) used in the analysis. (B) Burrow descent ($P=0.027$, Table 4, fixed effect a). Conventions as in A. Sample sizes are shown in the lower pane of each figure.

Table 3. Probability of the home run during the testing phase in the fiddler crab *Uca vomeris*

Fixed effects	Estimate	d.f.	χ^2	P
a) Test runs	2.2	1	5.16	0.018
b) Crab-track distance	-0.003	1	5.02	0.025
c) Crab-burrow distance	0.02	1	–	–
d) Sex (males)	6.5	1	1.16	0.070
e) Close	-1.3	1	1.23	0.27
f) Crab-burrow distance : sex (males) ^a	-0.02	2	9.45	0.009
g) Sex (males) : close	–	1	0.15	0.70
h) Sex (males) : test runs	–	1	0.30	0.68

Results of the generalized linear mixed model analysis: $N=145$, random effects (s.d.): crab (2.3) + setup (1.9)/dummy run (1.2). Final model: $\text{logit}(P)=-0.42 + \text{test runs} + \text{crab-track distance} + \text{crab-burrow distance} \times \text{sex} + \text{close}$.

^aThe interaction with crab-burrow distance was tested with 2 d.f. because the main effect was insignificant.

Is habituation associative?

Associations between a habituation stimulus and its context have been shown previously. In a field study on pied flycatchers, the initial mobbing response strength was evoked by changing the location of a model predator, to which the birds had habituated (Shalter, 1978). Furthermore, habituation has clearly been shown to be context specific in laboratory studies using the worm *Caenorhabditis elegans* (Rose and Rankin, 2001) as well as in *C. granulatus* (Tomsic et al., 1998; Sztarker and Tomsic, 2011). These studies suggest that habituation can be facilitated by associative processes. Our study does not specifically address the context specificity of habituation, as the experiments were conducted using two physically identical dummies in different parts of the crabs' surroundings rather than a single stimulus within a changing context. Nonetheless, the crabs clearly distinguished the two physically identical objects, which is most likely accomplished by associating them with contextual information.

In an earlier study, fiddler crabs were habituated to a dummy, which repeatedly approached the crabs and remained visible to them during and between approaches (Hemmi and Merkle, 2009), like the habituation dummy in our experiment. Crabs, however, responded more strongly when the same dummy subsequently approached them along the same track but from a greater distance, as if they no longer recognized it as the same object that was previously safe to ignore. It is possible that the crabs associated the local dummy's identity with its position in the environment or that the distant dummy, which would have been very difficult to see at its starting position, might have been treated as a new object every time it appeared. Alternatively, integrating neurons may have simply responded more strongly to a dummy approaching from a greater distance because of the object's greater increase in apparent size. In the present study, the effect of stimulus position in the surroundings has been isolated from the potentially confounding effects of the continuing visibility of the dummy throughout the experiment. Furthermore, both the habituation and test dummies approached over the same distance

and duration, ruling out integration as an alternative explanation to association.

The classification of habituation

Despite clear evidence that some forms of habituation are context specific and therefore associative (Shalter, 1978; Tomsic et al., 1998; Rose and Rankin, 2001; Sztarker and Tomsic, 2011) (present study), most textbooks still classify habituation as a simple, non-associative form of learning (Kandel et al., 2000; Bear et al., 2007; Bouton, 2007). This classification has been unaltered since the earliest studies on habituation, even though there are, as far as we know, no studies providing clear evidence to this effect. In contrast to its classification, the definitions of habituation (e.g. Thompson and Spencer, 1966; Bear et al., 2007; Rankin et al., 2009), make no assumptions about the underlying mechanism, but simply stress that habituation should be distinguished from sensory adaptation or motor fatigue.

The mechanisms underlying habituation remain largely unidentified (e.g. Bear et al., 2007), and it is likely that no single mechanism accounts fully for all forms of behavioural habituation (Rankin et al., 2009). In the absence of a clear mechanism, it seems reasonable to retain 'habituation' as a descriptive term for the time course of a behavioural learning phenomenon, rather than one that implies the mechanism.

From an ecological point of view, we predict that habituation of most behaviours, including simple escape responses, must be highly stimulus specific and context dependent to allow animals to predict and learn about biologically relevant events. Thus, for habituation to work safely and efficiently under dynamic and complex natural conditions, it should be associative. Moreover, it has recently been shown in one of the best-known behavioural paradigms of habituation, the tail-flick escape reflex in crayfish, that the establishment of habituation depends on input from higher-level ganglia, which appear to reconfigure the lower-level control circuits (Shirinyan et al., 2006). This clearly provides a pathway for higher-level processes to contribute associative information.

Table 4. Probability of burrow descent during the testing phase in the fiddler crab *Uca vomeris*

Fixed effects	Estimate	d.f.	χ^2	P
a) Test runs	1.56	1	4.88	0.027
b) Crab-track distance	-0.009	1	12.75	<0.001
c) Crab-burrow distance	–	1	1.39	0.24
d) Close	0.24	1	0.07	0.80
e) Sex (males)	–	1	0.002	0.90

Results of the generalized linear mixed model analysis: $N=133$, random effects (s.d.): crab (1.6) + setup (0.9)/dummy run (0). Final model: $\text{logit}(P)=0.49 + \text{test runs} + \text{crab-track distance} + \text{crab-burrow distance} + \text{close}$.

The importance of movement and context in habituation

The crabs appeared to classify objects by their behaviour rather than simply their continued presence. The crabs saw both the test and the habituation dummies at their starting position during the entire habituation phase, but they habituated only to the dummy that repeatedly moved during the habituation phase. The continued visibility of the test dummy during the habituation phase alone did not lead to habituation. We suggest that for an animal with limited visual resolution, a change in behaviour is probably more easily recognized than the object itself and might imply a change in intention by real predators.

The fiddler crabs' habituation strategy appears to reflect their sensory constraints and the predators to which they are vulnerable. Fiddler crabs habituate to repeated movements in the same location, but do not transfer habituation to movement from a different location (present study) or to objects arriving from a distance (Hemmi, 2005a; Hemmi, 2005b; Hemmi and Merkle, 2009). We suggest that habituation to repeated movements in a predictable location is likely to allow individuals to ignore harmless events, such as a mangrove branch moving in the wind (Tomsic et al., 1993). By contrast, terns, which are the main predator of *U. vomeris*, can approach from any direction and do so almost always from far away. Therefore, it makes adaptive sense for these crabs to not transfer habituation to objects that approach from different directions or to objects that suddenly appear, even if they approach from the same direction.

The effects of a contextual change on an animal's behaviour are likely to depend on its evolutionary history as well as its current situation. It is therefore necessary to consider what a meaningful context constitutes. A recent laboratory study of the crab *C. granulatus*, for instance, found a transfer of habituation to a visual danger stimulus from one location to another (Sztarker and Tomsic, 2011), in contrast to the results of our study. This could represent a difference in learning between species, or a difference in experimental setting. It is possible that the change in starting position and approach direction in a natural landscape provided stronger contextual cues in our study than in the laboratory study of *C. granulatus*. Comparative studies using similar methods would help resolve differences among species, and place habituation into an ecological and evolutionary context.

Sex differences in habituation

Although female fiddler crabs clearly habituated both their home run and burrow descent responses, males only habituated their burrow descent response. Sex differences in habituation have previously been found in the domestic Aylesbury duck, where females habituated faster to a novel stimulus in their pen than males (Desforges and Wood-Gush, 1975), and in yellow-eyed penguins (*Megadyptes antipodes*), where females showed a higher habituation potential to repeated approaches by a single person (Ellenberg et al., 2009). More generally, sex differences in learning are not unusual, and are often explained by motivational differences between the sexes because of different requirements for reproductive success (Reader and Laland, 2000; Lonsdorf et al., 2004; Lonsdorf, 2005).

We suggest that sex differences in habituation in fiddler crabs could be due to either differences in probability of attack or access to burrows. First, predators may preferentially attack males because their large, brightly coloured claws make them more conspicuous than females or a more rewarding meal (for details, see Montague, 1980; Koga et al., 2001). Our results show that males are more likely to run towards their burrow than females and respond equally

strongly irrespective of their distance to the burrow. Although these differences are consistent with a greater probability of attack, there were no differences between the sexes in probabilities of burrow descent, which might also be expected if males were targeted more by predators. Second, males might be more likely to be excluded from their own burrow than females, and therefore need to move to the burrow more quickly. Fiddler crabs are at a constant risk of losing their burrow to conspecifics (Hemmi and Zeil, 2003), but the risk is minimal when the owner is at its burrow entrance. In an emergency, burrowless crabs often enter an already occupied burrow, whereas at other times crabs actively block the burrow entrance against intruders (C.A.R. and J.M.H., personal observations). We suggest that females are more likely to be allowed into their own usurped burrow or another occupied burrow than are males, as they would be easier to evict again afterwards. Hence, the sex differences could be driven by burrow defence mechanisms rather than a differential vulnerability to predators. Sex differences in habituation of the home run but not burrow descent are consistent with this hypothesis.

Conclusions

Despite their poor visual acuity and almost complete lack of depth perception (Smolka and Hemmi, 2009), this study shows that, even in a natural and dynamic environment, fiddler crabs have an extremely specific and selective habituation response. The evidence argues against the notion that habituation is a simple, non-associative learning mechanism. Instead, habituation is finely tuned to the crabs' circumstances. It differs between the sexes and, for males, it differs between the two main stages of their escape response. Regardless of the definition of habituation, our study suggests that *U. vomeris* individuals have learning rules appropriate to their natural environment, allowing crabs to reduce false alarms while still retaining responses to potentially threatening stimuli.

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