To escape or to pursue: opposite decision-making concerning a single moving object is influenced by starvation and sex

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SUMMARY STATEMENT

Incompatible decisions between prey catching or predator escape responses are elicited by the same moving object. The behavioural choice depends on starvation level and sex.

ABSTRACT

Decision-making processes in the context of prey-predator interactions are studied from the side of the prey or the predator. Thus, prey capture and escape behaviours are researched separately, using different stimuli in different species. The crab *Neohelice* preys upon individuals of its own species, hence, it behaves as prey as well as predator. These two innate opposite behaviours can be elicited by the same object moving on the ground. Here we studied how the decision to perform avoidance, predatory, or freezing responses to a moving dummy depends on sex and starvation level. In the first experiment, we assessed the probability of each response type in unfed crabs for 22 days. Males showed a higher predatory response probability than females. When starvation increased, the predatory response increased, while avoidance and freezing declined, but this only occurred in

males. In the second experiment, we compared regularly fed and unfed males for 17 days. While fed crabs did not change their behaviour throughout the experiment, unfed crabs significantly intensified their predatory responses, displayed different exploratory activities and pursued earlier than fed crabs. Our results show the unusual situation of an animal that, to deal with a single stimulus, has to choose between opposite innate behaviours. Our results show that this is a value-based decision since it is affected by factors other than the stimulus itself.

INTRODUCTION

The ability to make behavioural decisions is a biological property of most active moving animals, from leeches to humans (Palmer and Kristan, 2011; Orsini et al., 2015). Decision-making processes have been extensively studied in the context of prey-predator interactions. Most studies have researched decisions made by animals behaving either as prey, i.e. displaying avoidance behaviours from threatening stimuli (Lima and Dill, 1990; Herberholz and Marquart, 2012; Römer and Holderied, 2020), or as predators, i.e., displaying prey capture behaviours towards appetitive stimuli (Lin and Leonardo, 2017; Farhadinia et al., 2020). However, to our knowledge, no study has investigated the choice between escaping or chasing made by an animal in response to the same stimulus. Such a situation is observed when investigating the decision-making processes of the crab *Neohelice*. This crab inhabits a mudflat where it is preyed upon by seagulls, but also by larger individuals of its own species (Daleo et al., 2003; Bas et al., 2019). Thus, the crab may behave as prey as well as predator (Tomsic et al., 2017).

Active foraging animals face a trade-off between the need to eat and the risk of being eaten. Therefore, the selection of the most convenient behaviour requires an evaluation of the costs and benefits associated with different actions, a concept usually referred to as value-based decision-making (Schreiner et al., 2021; Villar et al., 2022). Animals experiencing food shortages must respond to avoid starvation. Consequently, foraging animals must accurately measure predation risk and weigh this risk against their nutritional state (Lima, 1998; Liden et al., 2010). In crayfish, for example, starvation affects the type of anti-predatory decisions made by the animal (Schadegg and Herberholz, 2017).

The role of hunger level in crab's predatory response has been studied only in aquatic crabs that feed on sessile prey such as clams or mussels (e.g. Mascaró and Seed, 2001; Smallegange et al., 2008; Nadeau et al., 2009; Wong et al., 2010; Sun et al., 2015), but not on moving prey. Interestingly, the prey capture and escape behaviour of the crab *Neohelice* are both elicited and guided by visual moving stimuli (Tomsic et al., 2017). Capturing a moving prey entails rapid decisions that can be affected by starvation differently than those associated with foraging immobile prey (Gancedo et al., 2020). Therefore, studies involving *Neohelice* may contribute to understanding how hunger affects quick decision-making processes.

Behavioural studies often exclude female animals with the argument that hormonal cycles increase response variability. On the other hand, the numerous studies that include both sexes do not analyse them separately (Beery and Zucker, 2011). Many studies have recognized these limitations and have begun to emphasise the need to distinguish the effect of sex in behavioural studies (Orsini and Setlow, 2017; Palanza and Parmigiani, 2017). In the context of predatory and escape behaviours, sexual differences in size, strength or defensive weapons can affect decisions on how to deal with prey or predators. Thus, further research into the decisions made by male and female crabs separately is required.

The predatory and escape behaviours of *Neohelice* can be investigated in the field and in the laboratory using moving dummies and computer-generated stimuli (e.g. Tomsic et al., 2017; Gancedo et al., 2020; Hemmi and Tomsic, 2015; Oliva and Tomsic, 2016). An ethogram built on *Neohelice* responses to dummies of different sizes moving on the ground at different speeds revealed three distinct behavioural responses: a predatory response (PR), a freezing response (FR), and an avoidance response (AR) (Gancedo et al., 2020). The probability of eliciting each one of these responses proved to be independent of the dummy position across the azimuthal visual field of the animal (the monocular field of vision of *Neohelice* encompasses 360 deg (De Astrada et al., 2012)) and of whether the dummy was approaching or retreating from the crab. However, response probabilities were shown to diminish after 10 trials of repeated stimulation (Gancedo et al., 2020). Stimulus parameters that elicit a predatory response and an avoidance response in male crabs have been identified. A moving object on the ground slightly larger than the crab mostly triggers avoidance responses, whereas an object considerably smaller than the crab mainly elicits pursuing behaviour. However, an intermediate-sized object can bring about

either response (Gancedo et al., 2020). This begs the question, "Can crab decisions to undertake the prey or the predator behaviour be determined by factors other than those related to the stimulus?"

We hypothesise that starvation affects the decision to pursue or escape from a moving target. We found that starving male but not female crabs diminished the frequency of the avoidance response and increased that of the predatory response. Starved males began to pursue the dummy earlier (at a longer distance) than well fed animals. Our results demonstrate that a single moving target can represent either a prey or a predator for crabs, which compels the animal to quickly select between two opposite innate behaviours: to run after a meal or to run away from being eaten. We found that this decision is strongly affected by the crab's feeding state and sex.

MATERIALS AND METHODS

Study species and specimen collecting

Adult male and female crabs, *Neohelice granulata,* were collected from narrow coastal inlets of San Clemente del Tuyú, Argentina. Females and males included in this study had similar sizes of carapace width (range 2.7-3.1 cm) with a mean ± S.E.M. of 2.77 ± 0.02 and 2.8 ± 0.01 cm, respectively. Females were not sexually receptive (Sal Moyano et al., 2012). Crabs were individually kept in glass jars filled with 2 cm of artificial seawater prepared using hw-Marinex (Winex, Hamburg, Germany), with a salinity 10-14 ‰, a pH of 7.4-7.6, and were maintained at 22-25°C. The holding and experimental rooms were illuminated by natural light and the experiments were conducted between 9:00 AM and 5:00 PM from day one up to a maximum of twenty-two days after the animals arrived at the laboratory. The animals had not been fed since they arrived, unless stated otherwise.

Experimental set up and recording procedures

The experimental arena comprised a rectangular plastic box (80 cm long x 40 cm wide x 40 cm high), with a 5 cm floor layer of mud from the crab's natural environment. A fishing line passing through two vertical plastic pipes located on the opposite sides of the arena allowed a dummy attached to the line to be pulled at ground level. The line emerging from the top of the pipes was associated with a hand wheel used to move the dummy in either direction (Fig. 1A). Dummy movements and

crab behaviours were recorded during trials using a video camera with a frame rate of 60 Hz (Sony Handycam HDR-CX440) located 110 cm above the arena. Crab and dummy positions were calculated at 16.7 ms intervals from calibrated video information. Video footage analysis and lens distortion calibration were made using the free software Tracker.

Visual stimulus and protocols

The visual stimulus consisted of a black plastic sphere of two sizes, a small dummy (SD: 1 cm) or a medium dummy (MD: 1.8 cm), moved at ground level at a rather constant speed of 18.5 ± 0.4 cm/s, a velocity encompassed by *Neohelice*'s speed range, which can run twice as fast (Fathala and Maldonado, 2011; Oliva and Tomsic, 2012). Although the effect of dummy size has already been investigated in a previous study (Gancedo et al., 2020), that study only included male crabs. As the present study contains females, whose object size preferences are unknown, we decided to include two dummy sizes.

A test session included two stages (Fig. 1B). The first stage involved the crab's adaptation, in which the animal was placed at a random location to explore the arena for 10 minutes (Fig. 1C). During the second stage, the crab was stimulated with the dummy throughout five trials with an inter-trial interval of 3 minutes (Fig. 1B). The criteria to initiate a trial (i.e. to begin moving the dummy) were twofold. First, we waited for 3 minutes following the conclusion of the previous trial. Second, we checked whether the crab's position was at least 5 cm away from the tracking line. If not, we waited until this critical distance was exceeded. The crab's behaviour was observed throughout the session by a mobile phone connected to the video camera, which was also used to turn recording mode on and off during each trial. Each trial always began with a dummy departing from one side and finished when it reached the opposite side. When the crab captured the moving stimulus, we stopped pulling the dummy until the crab gave up trying to break it and moved away, which usually occurred in less than one minute. We alternated dummy direction between trials. Each crab was evaluated with a single dummy size in one test session only. We adopted this experimental design, instead of evaluating each crab's response to both dummies on different days, to prevent potential effects associated with recalling the arena, since crabs have been proven to possess enduring contextual memories that modify their responses to stimuli (Tomsic et al., 1998; Pedreira and Maldonado,

2003). Experiments with the two dummy sizes were balanced across sex and study days (Fig. 1D).

We conducted two experiments. Experiment I involved unfed animals of both sexes (111 males: 55 tested with the small and 56 with the medium dummy; 102 females: 51 with the small and 51 with the medium dummy). The experiment began the day after the animals arrived at the laboratory and lasted for 22 days, thus including individuals with different starvation levels (Fig. 1D). On average we tested 10 crabs (ranging from 8 to 12) each day. For the purpose of our analysis, the tested animals were separated according to the number of days of starvation in two groups designated: 'early days' (ED), which included crabs tested between days 1 and 10; and 'late days' (LD), which included crabs tested between days 11 and 22.

The second experiment involved male crabs exposed to one of two different feeding conditions for a period of 17 days (Fig. 1E). One group, named 'unfed crabs' (UC), was never fed (30 crabs: 16 tested with the small and 14 with the medium dummy). The other group, named 'fed crabs' (FC), was fed every other day with 3 rabbit pellets in the mornings and allowed to eat for 2 h (28 crabs: 14 tested with the small and 14 with the medium dummy). After each meal we changed the water of the jar housing each fed or unfed crab. Fed crabs were tested between 6 hours and 30 hours after having been fed. In Experiment II, the tests began on the third day after the animals' arrival, so that unfed crabs had been starved for at least 48 hours. We tested 3 to 4 crabs per day, attempting to include one fed and one unfed animal for each dummy size. As in the previous experiment, the animals were separated into two groups: 'early days' (ED), which included crabs tested between days 3 and 9 (35 crabs: 18 unfed and 17 fed); and 'late days' (LD), which included the crabs tested between days 10 and 17 (23 crabs: 12 unfed 11 fed).

Response criteria and measures

In a previous study (Gancedo et al., 2020), the behaviours displayed by crabs to dummy stimulation were categorised into four mutually-exclusive response types: a predatory response (PR: pursuing the dummy); an avoidance response (AR: running away from the dummy or defending itself by raising its claws against it); a freezing response (FR: stopping walking and remaining still for the whole trial); and no response (NR: ignoring the dummy, exhibiting no behavioural changes). In the current study, we used slightly different criteria, disregarding no response as a

behavioural response. Thus, a response was considered to have occurred whenever a crab changed its behaviour in response to the visual stimulus. We termed this general response assessment Responsiveness (R), which was calculated as the number of trials when a predatory, an avoidance, or a freezing response was elicited over the total number of trials. The probability of each particular behaviour was calculated for each crab as the number of trials in which such a response was performed over the 5 trials of the test session, excluding trials with no response. The individual probabilities were then averaged across all crabs.

We evaluated other behavioural parameters, such as the crab-dummy distance at the time of response and the level of exploratory activity. The crab-dummy distance was measured offline, as previously described (Gancedo et al., 2020). Each crab's exploratory activity was computed online by manually drawing the locomotor path of the crab during the 10 minutes of adaptation to the arena on a scale graph paper (Fig. 1C). The grid represented the arena divided in 10 x 10 cm square sectors.

Statistical analyses

To evaluate whether sex, stimulus size or starvation affect crab responses to the dummy, response probabilities were compared using a Wilcoxon signed-rank test with Bonferroni corrections. Response probabilities of fed and unfed crabs were compared using the same test. Comparisons of crab-dummy response distances and exploratory behaviour were conducted using Mann-Whitney and Wilcoxon signed-rank tests with Bonferroni corrections. We utilised non-parametric tests due to the lack of normality of the data. All statistical analyses were performed with the software R.

The probability of a predatory response among fed and unfed crabs (coded as 1 and 0 for pursue and not pursue respectively) was modelled using generalised linear mixed models (GLMMs) assuming binomial error distributions and logit link functions. The models were developed to determine if crabs' predatory response (dependent variable) varied by treatment (fed or unfed), tracking line distance (TLD), starvation level (early or late days), exploratory activity level, and dummy size. These variables were incorporated in the model as fixed factors and the crab number was considered as a random factor. We tested a complete model, encompassing all possible interactions, before choosing a final simplified model according to Akaike

information criterion [AIC (Akaike, 1973)], using the R function "drop1" and selecting the model with the lowest AIC. We verified the homogeneity of variance and the lack of patterns in the residual values using graphic methods. All statistical analyses were performed using R software. Finally, we utilized packages stats (R Core Team, 2022), lme4 (Bates et al., 2015), lsmeans (Lenth, 2016), and MuMIn (Barton, 2009) for tests and comparisons.

RESULTS

Experiment I: sex, dummy size and starvation level

In this experiment, individual crabs accumulated different days of starvation, from 1 to 22 days, both in males and females (Fig. 1D). The overall response probability (R) that accounts for the proportion of trials when a predatory, an avoidance, or a freezing response was elicited (see video in supplementary material), was near 90%, showing that the dummy represented a highly relevant stimulus for the crabs. No difference was observed between responsiveness values of females and males (Fig. 2A, grey bars). However, the analysis of specific behavioural probabilities revealed clear sex differences. Males showed a higher predatory probability than females, while females presented higher avoidance and freezing response probabilities than males (Fig. 2A, coloured bars; AR: $p < 0.001$; FR: $p < 0.05$; PR: $p < 0.001$). An evaluation of possible interactions between dummy size and starvation level revealed no statistical differences ($p = 0.584$), which led us to analyse the effect of these two variables separately.

Figure 2B shows the effect of dummy size on the behavioural responses of females and males. The responsiveness of females and males was not affected by stimulus size (grey bars). However, the probability of particular behaviour was highly influenced. The effect of dummy size was similar in males and females. In both sexes, the small dummy elicited a significantly higher predatory response probability than the medium dummy and significantly fewer avoidance and freezing responses (coloured bars; females AR: $p < 0.001$; FR: $p < 0.05$; PR: $p < 0.001$. males AR: $p < 0.001$; FR: $p < 0.01$; PR: $p < 0.001$).

To analyse the effects of starvation level, we separated the animals in two groups, which included less starved animals tested in the early days of the experiment and more starved animals tested in the later days of the experiment (see

Materials and Methods). Figure 2C shows that starvation level did not affect the general response of males or females (grey bars). However, the difference in days of starvation between the early and late groups strongly affected each particular type of behaviour, but these effects were only observed in males. Indeed, no differences were found between females tested in the early and the late days (left panel, coloured bars). In contrast, males tested in the late days showed a significantly higher probability of predatory response than those tested in the early days, as well as lower avoidance and freezing response probabilities (right panel, coloured bars; AR: $p < 0.05$; FR: $p < 0.01$; PR: $p < 0.001$).

Studies in different species have shown that food shortage affects searching activities in different forms (Scharf, 2016). We examined the exploratory activity displayed by female and male crabs of the early and late days groups during the 10 minutes that preceded the sequence of stimulus trials. The amount of walking was measured as the number of crossed square sectors defined in the arena (Fig. 1C). Figure 2D shows that the exploratory activity of females and males was very similar and did not change between early and late days groups ($p = 0.938$).

Given the clear differences observed in the behavioural probabilities between male and females (Fig. 2A), we speculated as to whether sex differences would be reflected in other response parameters. In a previous study, we have shown that crabs initiate pursuit when a dummy is at a fixed distance, regardless of the size of the dummy (Gancedo et al., 2020). We then analysed the crab-dummy distances for avoidance, freezing, and predatory responses of female and male crabs (Fig. 2E). Crabs initiated avoidance responses at a distance significantly longer than freezing and predatory responses, while freezing responses occurred at a larger distance than predatory ones ($p < 0.001$ for all comparisons). Results showed no sex differences for freezing and avoidance responses (AR: $p = 0.83$; FR: $p = 0.33$), but a significant difference for predatory responses, with males starting pursuit at a greater distance than females (PR: $p < 0.001$).

Experiment II: food shortage

The previous experiment indicated that the level of starvation strongly affected the decision of male crabs to run after or away from the dummy, irrespective of whether it was small or medium size. Although unlikely, the behavioural change could have been caused by uncontrolled factors other than the food shortage, such

as the time of isolation from the beginning of the experiment. Therefore, we performed an experiment over 17 days using two groups, one of unfed crabs (UC) and one of regularly fed crabs (FC), such that the only difference between the two groups is their feeding state. As in our previous experiment, we separated the unfed and fed crabs into two groups that included animals tested in the early days or later days of the experiment (Materials and Methods and Fig. 1E).

Figure 3A shows the response probabilities of fed and unfed male crabs of the early and late days groups. The responsiveness of fed and unfed animals was similar in both the early and the late days groups (grey bars). In the early days groups, the probability of fed and unfed crabs for each specific behaviour was identical (coloured bars, left panel). In contrast, clear-cut differences between fed and unfed crabs emerged in the late days groups, where unfed crabs displayed a significantly higher predatory response probability than fed crabs and significantly lower avoidance and freezing responses (coloured bars, right panel; AR: $p < 0.05$; FR: $p < 0.01$; PR: $p < 0.001$). We further analysed these data by comparing the probabilities of early and late days groups in fed and unfed crabs. There were no statistical differences in the AR ($p = 0.22$), the FR ($p = 0.42$) and the PR ($p = 0.16$) between the groups of fed crabs or in the AR of unfed crabs ($p = 0.48$), but significant differences in the FR ($p < 0.05$) and the PR ($p < 0.05$) between the early and late days groups of unfed crabs. These analyses confirm that the behavioural change between early and late days groups occurred only in unfed crab.

Figure 3B shows that in the early days of the experiment, unfed crabs displayed significantly lower exploratory activity than fed crabs ($p < 0.001$). No such difference occurred between late days groups. Yet, between unfed animals, the late days group (more starved) displayed a slightly, but statistically significant, higher exploratory activity than the early days group ($p < 0.05$). However, a comparison between the equivalent groups of fed crabs showed no statistical difference ($p = 0.23$).

Given the strong differences observed in the behavioural probabilities between the fed and unfed crabs comprising the late days groups, we analysed their crab-dummy distances for avoidance, freezing, and predatory responses (Fig. 3C). The distances at which crabs initiated avoidance and freezing were similar between unfed and fed crabs. However, the distance at which unfed crabs initiated dummy pursuit was significantly greater than that of fed crabs ($p < 0.01$).

To further investigate the weight of the factors that affected the crabs' decisions to undertake pursuing behaviours, we performed a GLMM analysis on the data of Experiment II. Previous results from a similar analysis revealed that the predatory response probability strongly depends on the distance of the crab to the tracking line (crab-tracking line distance) at the time of response initiation (Gancedo et al., 2020). We selected the model that best fit our data based upon interactions that, although not statistically significant, dropping them from the model would decrease its prediction power considerably. The final simplified model was built based upon the effect of tracking line distance and interactions between treatments (fed and unfed) using three different parameters: starvation level, exploratory intensity, and dummy size. Table 1 shows the parameters estimates for the GLMM predicting the PR probability in our study. As expected, the graphic representation of the model illustrated in Figure 4 shows that unfed crabs pursued the dummy with a higher probability than fed crabs. However, the model provides additional relevant information, as it shows that the difference between unfed and fed crabs becomes more pronounced at longer tracking line distances. In fact, an analysis of the speed of change of the curve of fed crabs shows that the response probability starts to quickly fall at tracking-line distances beyond 10 cm and is almost negligible (below 0.2) at distances longer than 15 cm, while the probability of unfed crabs continues to be high (above 0.7) even at the maximum possible tracking-line distance of our experimental set up.

Individual response differences

The analyses of mean probabilities shown in figures 2 and 3 disclosed the effects of sex and starvation level on the different types of responses to the dummy. However, mean values do not enable the visualisation of the composition of individual performances. For example, a mean predatory response probability of 50% could result from a situation where 100% of tested animals displayed a predatory response in 50% of the trials, while performed an avoidance response in the other 50% of the trials. However, a mean predatory response of 50% could also be accounted for by a situation where 50% of the animals showed a predatory response in all trials and the other 50% of animals performed an avoidance response in all trials. The first situation would signify that each particular crab always responded in the same way to the dummy, whereas the second situation would

indicate that each crab displays both responses alternately. In order to investigate whether the decision to execute a predatory response or an avoidance response remains consistent for each animal across trials or if it changes from trial to trial, we analysed the number of trials in which each animal displayed predatory or avoidance responses. We performed this analysis on male and female crabs of the early days groups in Experiment I because in the late days groups males were strongly affected by starvation and, hence, were heavily inclined towards a predatory response. We found that 51 out of the 58 females tested and 55 out of the 62 males tested, exhibited predatory or avoidance responses in at least one trial (Fig. 5). Among females, 17 displayed a variable number of avoidance responses, but never predatory responses (red bars), 13 displayed both predatory and avoidance responses (green bars), and 21 displayed only predatory responses, never avoidance responses (blue bars). Among males 11 displayed avoidance responses, but never a predatory response (red bars), 14 displayed both predatory and avoidance responses (green bars), and 31 displayed only predatory responses, never avoidance responses (blue bars).

DISCUSSION

Because *Neohelice* respond to a dummy moved on the ground with either chasing or avoidance behaviours, this crab offers uncommon opportunities for studying the decisions made by an animal between two innate opposing behaviours elicited by the same visual stimulus. In addition to running away or running after the dummy, a crab can simply freeze. Among *Neohelice*, the probability of performing a predatory, an avoidance, or a freezing response partially depends on the information provided by the stimulus target (Gancedo et al., 2020). Here, we show that the behavioural decision-making after detecting a moving dummy depends on additional factors, such as the sex and starvation level.

Predatory, avoidance and freezing responses

Predatory and avoidance responses have unambiguous purposes, i.e., to get food and to evade a threat, respectively. Freezing, on the other hand, can serve a variety of purposes including: a) to gather more reliable information about a moving object by eliminating self-induced image motion (Hemmi and Pfeil, 2010; Procacci et

al., 2020); b) to accumulate enough information to decide whether to approach or avoid a stimulus (Livermore et al., 2021); c) as a defensive strategy to be undetected by the predator (Blanchard et al., 2011); d) to improve action preparation in response to potentially appetitive objects (Procacci et al., 2020); e) to save time, energy, and opportunities in comparison to other more conspicuous avoidance responses (Liden et al., 2010). Among crabs, freezing responses can be transitory, preceding avoidance (Hemmi and Tomsic, 2012) or predatory responses (Gancedo et al., 2020). Conversely, they can be more sustained (Gancedo et al., 2020), as assessed by the present study (see methods). Thus, in the context of our experiments freezing likely complies with more than one of the functions described above.

Sex differences

Male and female crabs are both highly responsive to dummy movements. However, they display significant differences in their probabilities for specific predatory, avoidance, or freezing responses. Females exhibited significantly higher avoidance and freezing response probabilities than males, while males showed a higher predatory response probability than females (Fig. 2A). These differences could be related to the fact that males have bigger claws than females, which may encourage them to attack more and retreat less than females. Both females and males were significantly more likely to display predatory response to the small dummy than to the medium one, in addition to significantly lower probabilities of freezing and avoidance (Fig. 2B).

Studies on prey size selection in crabs are abundant, but all were conducted using solely male crabs (e.g. Mascaró and Seed, 2001; Smallegange et al., 2008; Wong et al., 2010). Furthermore, previous studies were performed using aquatic crabs that feed on sessile prey such as clams or mussels, rather than on moving prey. According to these studies, the maximum size of shelled prey that can be eaten by crab predators is related to the size and biomechanics of the predator's claws (Elner and Hughes, 1978; Ap Rheinallt, 1986). Our results show that females pursued the small moving dummy with a probability similar to which males pursued the medium dummy. Thus, small and medium-sized stimuli might represent comparable prey sizes for the claws of female and male crabs, respectively. On the other hand, crab preferences for small over larger immobile prey (clams) is well documented and is related to the fact that the cost of breaking larger prey's shells is

greater than the meat reward (e.g. Brousseau et al., 2001; Micheli, 1995). For *Neohelice*, breaking the carapace of small prey crabs would be easier than that of medium crabs. Additionally, small crabs would be less likely to retaliate than medium ones. Current experiments aimed at assessing responses to dummy sizes of crabs with different body sizes will shed light on this issue.

We found that starvation clearly affected the behavioural decisions of male crabs. Animals tested in the later days of the experiment exhibited a significantly higher predatory response probability than crabs tested in the early days of the experiment, in addition to showing significantly lower avoidance and freezing responses (Fig. 2C, right panel). Surprisingly, such starvation effects were not observed among females (Fig. 2C, left panel). These sex differences might be explained by a difference in starvation tolerance between males and females, as has been found in other species (Finiguerra et al., 2013; Gilad et al., 2018). It could also be related to the energetic cost of exploratory intensity, as has been shown to occur in other species (Hutchings and Gerber, 2002; King et al., 2005), although our assessment of exploratory activity among female and male crabs did not uncover any differences (Fig. 2D). Thus, future studies that examine metabolic measurements would be needed to better address this subject.

Starvation affects the response probabilities of male crabs

In a previous study, we found no evidence of changes in the avoidance, freezing, and predatory response probabilities throughout 10 days of starvation (Gancedo et al., 2020). In agreement with this, in the present experiments, we did not observe an obvious starvation effect among crabs tested during the first 10 days (early days groups). However, we found that male crabs starved for more than 10 days (late days groups) behaved noticeably differently than less starved ones (early days groups). In Experiment I and II, unfed males from the late days group showed a significantly higher predatory response probability and lower avoidance and freezing response probabilities than the corresponding early days group (Fig. 2C, right panel). In Experiment II, comparisons of fed and unfed crabs' behaviour exhibited compatible results, displaying no observable effects in the early days tested crabs (Fig. 3A, left panel) and a clear-cut increase in predatory response probability and reductions of avoidance, and freezing probabilities in the late days group of unfed crabs (Fig. 3A, right panel). These results indicate that unfed animals were far more

motivated to undertake prey capture behaviours and less willing to avoid or to freeze than fed crabs. This indicates that crabs integrate hunger levels in their decision-making process regarding how to respond to the dummy.

An extensive series of studies have proven that *Neohelice* is a convenient animal model for investigating neurophysiological mechanisms underlying visually guided behaviours (for a review see Tomsic, 2016). In particular, several classes of giant neurons involved in visuomotor transformation underlying the escape from visual danger stimuli have been characterized (i.e. Medan et al., 2007; Oliva and Tomsic, 2016; for review see Tomsic and Sztarker, 2019). Although much less characterized, we also found neurons that specifically respond to small targets moved at ground level, which are thought to be involved in prey capture behaviour (Tomsic et al., 2017). Our current results show that the decision between escaping or pursuing a moving object is strongly modulated by starvation level. This raises the possibility of investigating whether starvation-related effects can be detected at the level of these central identified neurons.

Exploratory activity

To further investigate the starvation-related effects, we analysed the exploratory activity displayed by crabs. In the first experiment, the exploratory activity of females and males were very similar, both for the groups tested on the early days as well as for those tested on the late days (Fig. 2D). On the other hand, the second experiment revealed differences in exploratory activity between fed and unfed crabs (Fig. 3B). It is well established that animals cope with food shortage in different ways, including suppressing exploratory activities to save energy or exploring more actively in search of food (e.g. Weiss et al., 2014; Gutman et al., 2007). Moreover, the level of exploratory intensity can shift, from being reduced, while there are still enough body reserves, to be later increased, when intense starvation occurs and finding food becomes decisive (Wang et al., 2006). The results of our second experiment align well with this description since between the early days groups, unfed crabs explored less than fed ones, and unfed crabs of the late days group explored slightly more than unfed crabs of the early days group. This result between unfed groups somehow contradicts the result of the first experiment, where no statistical difference between unfed animals of the early and late days was disclosed.

This discrepancy could be due to the fact that increasing starvation levels appear to have only a mild effect on exploratory activity.

Predatory response distance increases with hunger

In a previous study, we showed that male crabs initiate predatory responses to small and medium dummies at equal distances, signifying that the decision regarding when to begin pursuit does not depend on the size of the object, but on its distance (crab-dummy distance) (Gancedo et al., 2020). We then investigated if starvation affected the distances at which crabs initiated avoidance, freezing, and predatory responses. In the first experiment, our results showed that crabs initiated avoidance response at greater distance from the dummy than freezing and the predatory response (Fig. 2D). It is important to note that, as described in the methods section, the freezing response analysed here last the entire trial and, therefore, does not include the transitory freezing responses that usually precedes predatory and avoidance responses (for further details see Gancedo et al., 2020). The fact that the avoidance response occurs at a longer crab-dummy distance than the predatory response is not surprising, since a stimulus judged to be threatening represents a mortal risk and, consequently, requires a more rapid response than a stimulus judged to be prey. Interestingly, males initiated pursuit at a larger distance than females (Fig. 2E), which may partially explain their higher predatory response probability in comparison with females.

The second experiment revealed that starved male crabs began pursuing the dummy at a mean distance significantly larger than that of fed crabs (Fig. 3C). This result suggests that starved male crabs might be more aware of prey and detect it sooner or that they had become less hesitant to undertake chasing behaviours than satiated crabs.

A model built on these data to better portray the differences in predatory response distances between unfed and fed crabs confirmed previous results, showing that the predatory response probability highly depends on the distance of the crab to the tracking line (Gancedo et al., 2020). The model (Fig. 4; Table 1), shows that unfed crabs pursued the dummy with a higher probability than fed crabs, in addition to indicating that differences in this probability increased with the crab-tracking line distance. In other words, a crab close to the dummy's trajectory is likely to display a predatory response regardless of whether it is well fed or starved. In

contrast, a crab that is far away from the dummy's trajectory will likely only undertake a predatory response when it is starved. A logical interpretation of these findings is that when a crab is close to a potential prey's path, the effort invested in capture is low and is permissible, even if the individual is not hungry. On the other hand, when a crab is far away from a possible prey's path, capture effort increases, and would be justified only if the animal is starved enough.

Value-based decisions in crayfish and crabs

The ability to choose among options that differ in their rewards and costs (value-based decision making) has long been a topic of interest for neuroscientists, psychologists and economists alike. This is likely because this is a cognitive process in which all animals (including humans) engage on a daily basis (Orsini et al. 2019). Thus, decision making processes is an area of intensive investigation encompassing studies in vertebrate and invertebrate animals, with approaches that range from analyses of behaviour to neuronal mechanisms (Crossley et al., 2016; Juavinett et al., 2018).

Juvenile crayfish have been shown to respond to a threatening shadow with one of two incompatible behaviours, they either freeze or perform a tail-flip that thrust the animal away from the potential danger (Liden et al., 2010). When facing the same visual danger stimulus in the presence of a food odour plume, starved animals performed more freezing and less tail-flip than satiated ones. This has been interpreted as a value-based decision made by hungry crayfish to remain near a food source, since the tail-flip has the cost of moving the crayfish away from it. Increasing the concentration of food odorant did not significantly change the frequency of tail-flips and freezing. However, the authors argued that this lack of an effect could have been due to the relatively mild change in food odorant concentration and the relatively short period of starvation they used in their study (Schadegg and Herberholz, 2017).

These studies with crayfish share many features with our studies with crabs. This is exemplified by the fact that both animals respond to a single stimulus (a moving shadow or a moving dummy) by choosing among incompatible behaviours, freezing or tail-flip in crayfish and freezing, pursuing, or escaping in crabs. Furthermore, starvation affects the decision-making of both animals, further facilitating opportunities to obtain food, shown by the fact that crayfish were more

likely to freeze to remain near a food odour source and crabs were less likely to escape and more likely to pursue in an attempt to capture potential prey. Moreover, in crayfish there are indications that an increase of odour concentration, assumed to represent a greater food reward, favours the freezing decision, while in crabs, a small dummy assumed to represent a preferred prey size, increases chasing behaviour (Fig. 2B). Thus, intrinsic physiological conditions such as starvation level and extrinsic conditions, such as reward value, indicate that crayfish and crabs undertake value-based decisions.

Despite these similarities, studies in crayfish differ in many aspects from our studies in the crabs. Some important differences are: a) the decision made by the crayfish involved two defensive strategies to avoid a visual danger stimulus, while the decision made by the crabs was about to avoid or to pursue a visual stimulus, i.e. about considering it a predator or a prey; b) the starvation period used in crayfish was relatively short (2 to 7 days) compared to the one used in crabs (1 to 22 days); c) crayfish were tested in a single trail, while crabs were tested in 5 consecutive trials, which allowed us to evaluate the consistency of the decisions made by each individual. Finally, Schadegg and Herberholz (2017) identified the crayfish sex, but did not conduct an analysis of behavioural sex differences, perhaps because they were juveniles (i.e. not sexually mature). Given the similarities observed in the decision-making processes of crayfish and crabs, it would be worth further researching if the sex differences that we found in crabs are also present in crayfish.

Deciding between innate opposite behaviours

Escaping from a moving object or running after it are two innate opposite behaviours. When faced with the exact same stimulus, what compels an animal to behave in one way or the other? In other words, what does an animal take into account when classifying the same stimulus as prey or predator? Surely there is not a single cause that guide such a decision. Here we show that sex and starvation level play key roles in this decision-making process. Yet, individuals of the same sex and the same experimentally-induced starvation level displayed both avoidance and predatory responses to the dummy. One possibility is that genetic differences or lived experiences influence individual personalities, rendering some crabs more prone to escape and others more likely to pursue the same moving object. Over the

last two decades, scientists have documented personalities, that is, consistent behaviours, such as boldness, shyness, sociability, or aggressiveness, in a range of invertebrate species, from octopuses to water striders, cockroaches, and damselflies (Kralj-Fiser and Schuett, 2014; Golab et. al, 2021). Consistent behaviours should be evaluated both across various contexts and throughout time (Tremmel and Muller, 2013). Although our experiments were not planned to research crab personalities, the individual performances observed during five trials hints at the possibility that there may be consistent response differences among individual crabs. In fact, certain crabs exclusively displayed avoidance responses while others only displayed predatory responses. There were also crabs that displayed both responses throughout the five trails (Fig. 5). Although rather speculative, these findings may suggest the existence of three personality categories: 1) aggressive crabs, more likely to exhibit predatory behaviours; 2) wary crabs, more disposed to respond as prey; 3) indecisive crabs, which alternate between prey and predatory behaviours. In order to investigate whether an individual's behavioural preferences are sustained and, hence, could be considered personality traits, future experiments including repeated evaluations of the same individual across several days and in distinct experimental contexts are required.

List of Symbols and Abbreviations

AR	avoidance response
CDD	crab-dummy distance
ED	early days
EI	exploratory intensity
FC	fed crabs
FR	freezing response
LD	late days
MD	medium dummy
R	responsiveness
PR	predatory response
SD	small dummy
TLD	tracking-line distance
UC	unfed crabs

Conflict of interest declaration

We declare we have no competing interests.

Authors' contributions

C.A.S.: data curation, methodology, software, formal analysis, investigation, visualisation, writing - original draft, writing - review and editing; B.J.G.: data curation, methodology, software, formal analysis, investigation, visualisation, writing - original draft, writing - review and editing; D.T.: conceptualization, methodology, validation, formal analysis, investigation, resources, writing - original draft, writing - review and editing, visualisation, supervision, project administration, funding acquisition.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Data availability

The dataset, supplementary video and codes supporting this article have been deposited in FigShare Collection at: **https://doi.org/10.6084/m9.figshare.c.6267846.v3**.

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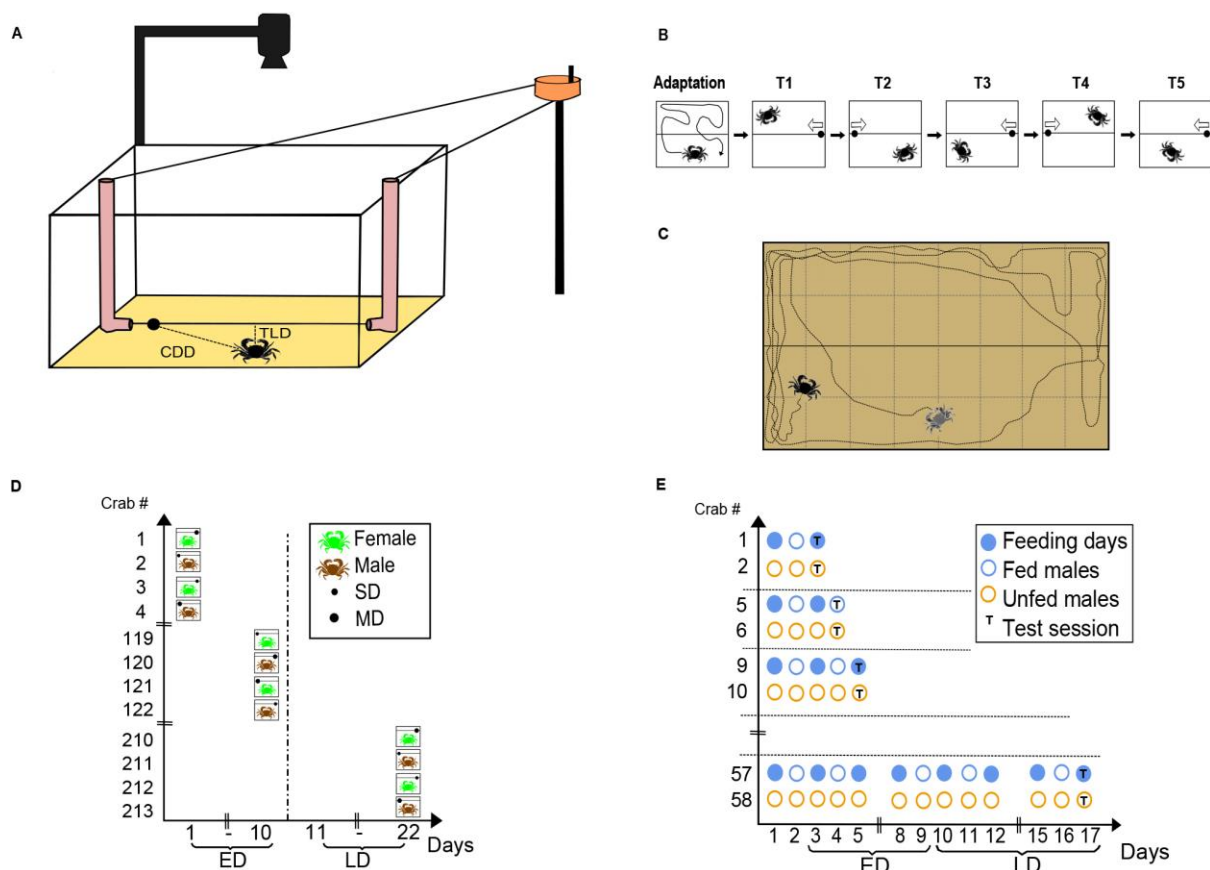
Figures

Fig. 1. Experimental arena and protocols. A. Experiments were run in a rectangular arena with a mud covered floor. A fishing line passing through two vertical pipes at the sides of the arena was used to pull an attached dummy at ground level. A video camera located above was used to record the crab's behaviour and dummy movement. TLD: tracking-line distance, CDD: crab-dummy distance. B. Each animal was evaluated in a single session that included an adaptation and a stimulation phase. During adaptation, the crab was allowed to explore the arena for 10 minutes. Then, it was stimulated with the dummy in 5 trials, with inter-trial intervals of 3 minutes. All trials began with the dummy departing from one side and ended when it reached the opposite. C. Example of exploratory intensity of a crab during the 10 minutes adaptation in the arena. Crabs' initial and final positions are represented by the grey and black silhouette, respectively. D. **Experiment I.** protocol

included different sexes, dummy sizes, and starvation periods. Female (n=102) and male (n=111) crabs with 1 to 22 days of starvation were tested with a medium (MD) or a small (SD) dummy in a balanced design. Crabs were separated in two groups, which included less starved animals tested in the early days (ED: days 1 and 10) of the experiment and more starved animals tested in the late days (LD: days 11 and 22). E. **Experiment II.** Protocol included male regularly fed crabs (FC, n=28) and unfed crabs (UC, n=30). Crabs evaluated between day 3 and 9 were designated as early days (ED) and those evaluated between days 10 and 17 as late days (LD).
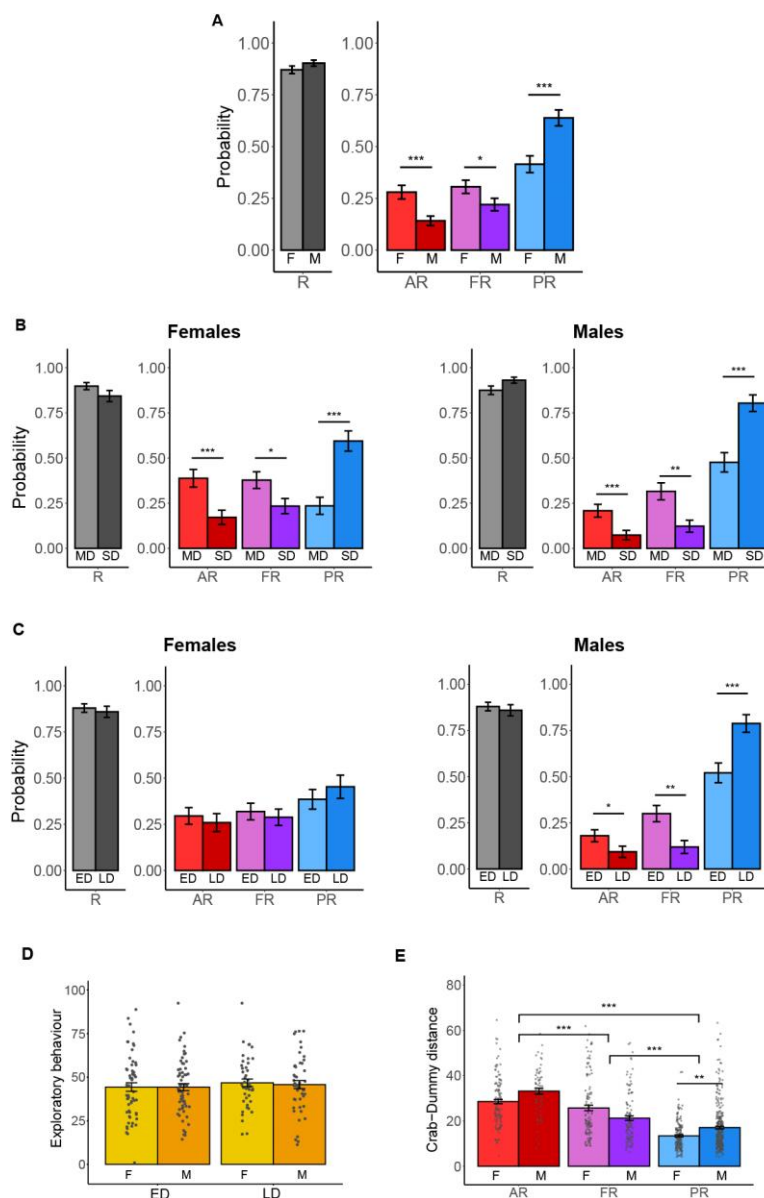
Fig. 2. Experiment I. A. Mean probabilities of general responsiveness (R: grey bars) and of specific avoidance, freezing, and predatory responses (AR, FR, and PR, respectively; coloured bars) of female and male crabs. B. Comparisons of R (grey bars) and of AR, FR, and PR (coloured bars) to the medium dummy (MD) and small dummy (SD) of females and males. C. Comparisons of R (grey bars) and of AR, FR, and PR (coloured bars) between early days (ED) and late days (LD) groups of female and male crabs. D. Mean exploratory behaviour of females and males between ED and LD groups. E. Mean crab-dummy response distance of female and male crabs at the time of AR, FR and PR initiation in the ED and LD groups. Statistical significance was evaluated using the Wilcoxon test. *: p < 0.05; **: p < 0.01; and ***: p < 0.001. Bars show means ± s.e.m.
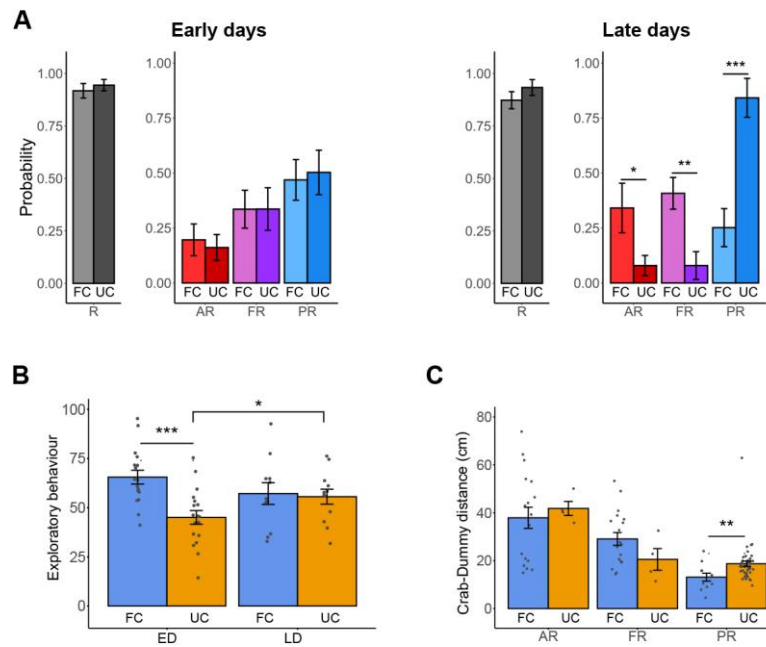
Fig. 3. Experiment II. A. Mean probabilities of general responsiveness (R: grey bars) and specific avoidance, freezing, and predatory responses (AR, FR, and PR, respectively; coloured bars) of fed crabs (FC) and unfed crabs (UC) of the early days (ED) and late days (LD) groups of animals. B. Mean exploratory activity of FC and UC of the ED and LD groups. C. Mean crab-dummy distances at the time of AR, FR, and PR initiation of FC and UC in the LD groups. Statistical significance was evaluated using the Wilcoxon test. *: p < 0.05; **: p < 0.01; and ***: p < 0.001. Bars show means ± s.e.m.
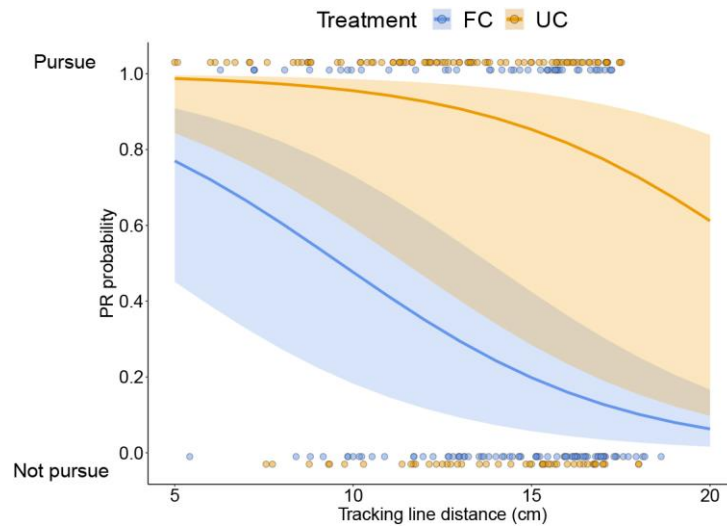
Fig. 4. Probability of predatory response of unfed and fed crabs predicted by the statistical model. Responses were coded 1 and 0 for pursue and not pursue, respectively. Dots represent individual data on which the model was based. Solid lines represent the probability of response of each treatment (FC and UC) as a function of the tracking line distance at the time of PR initiation. Shaded areas represent interquartile ranges. Further details are in the text and in Table 1.
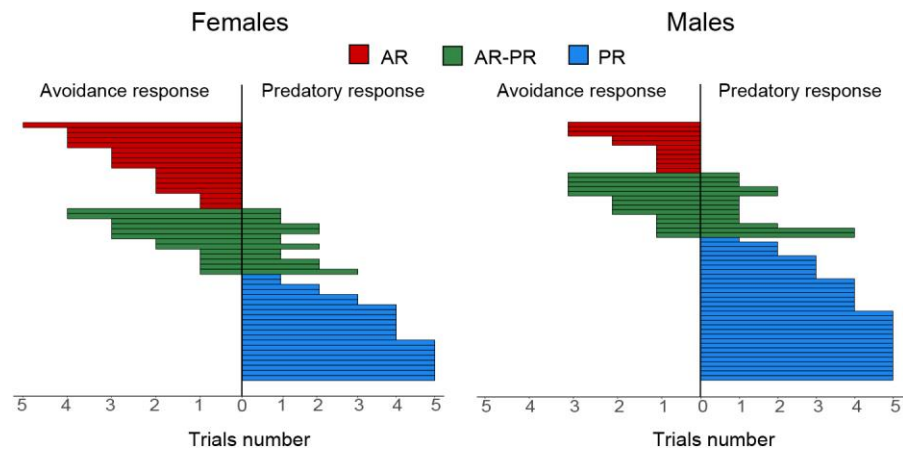
Fig. 5. Number of avoidance and predatory responses displayed by each individual on the 5-trials testing session. Data correspond to crabs of the early days group of Experiment I. Each horizontal bar corresponds to a single crab. Data distinguishes three categories: crabs that performed avoidance but never predatory responses (red bars), crabs that performed predatory but never avoidance responses (blue bars), and crabs that alternatively performed both responses (green bars).

Table1. Parameter estimates for the GLMM predicting PR probability

	Estimate	±s.e.m.	Z-value	Pr(>|z|)
PR probability				
Intercept	**6.29901**	**2.99674**	**2.102**	**0.03**
TLD	**-0.26071**	**0.07835**	**-3.328**	**0.0001**
Treatment UC	-6.33214	3.56496	-1.776	0.07
LD	**-3.00460**	**1.31278**	**-2.289**	**0.03**
SD	0.52855	1.25291	0.422	0.67
EI	-0.07211	0.07148	-1.009	0.31
Treatment UC:LD	**5.36392**	**1.92590**	**2.785**	**0.005**
Treatment UC:SD	1.97391	1.73112	1.140	0.25
Treatment UC:EI	0.15520	0.09680	1.603	0.11

Random effect (variance, s.d.); crab number (6.07, 2.46)

GLMM, generalized linear mixed model; PR, predatory response; LD, late-days tested crabs; Treatment: UC, unfed crabs; EI, exploratory intensity; TLD, tracking line distance; SD, small dummy. Significant results with |z|>2.0 are shown in bold.
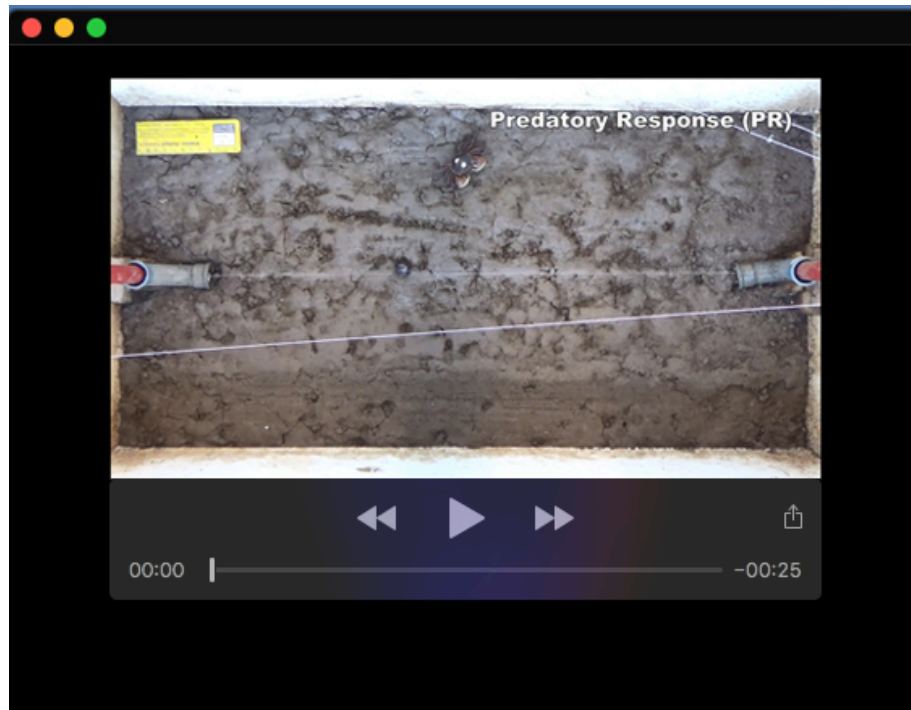
Movie 1. Three examples of crab responses to a moving dummy.
This video illustrates a predatory response (PR), an avoidance response (AR) and a freezing response (FR), elicited within the experimental arena by a medium dummy moved on the ground. For further experimental details, see the main text.