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Costs and benefits of freezing behaviour in the harvestman *Eumesosoma roeweri* (Arachnida, Opiliones)

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ABSTRACT

Animals present an enormous variety of behavioural defensive mechanisms, which increase their survival, but often at a cost. Several animal taxa reduce their chances of being detected and/or recognized as prey items by freezing (remaining completely motionless) in the presence of a predator. We studied costs and benefits of freezing in immature *Eumesosoma roeweri* (Opiliones, Sclerosomatidae). Preliminary observations showed that these individuals often freeze in the presence of the syntopic predatory spider *Schizocosa ocreata* (Araneae, Lycosidae). We verified that harvestmen paired with predators spent more time freezing than when alone or when paired with a conspecific. Then, we determined that predator chemical cues alone did not elicit freezing behaviour. Next, we examined predator behaviour towards moving/non-moving prey and found that spiders attacked moving prey significantly more, suggesting an advantage of freezing in the presence of a predator. Finally, as measure of the foraging costs of freezing, we found that individuals paired with a predator for 2 h gained significantly less weight than individuals paired with a conspecific or left alone. Taken together, our results suggest that freezing may protect *E. roeweri* harvestmen from predatory attacks by wolf spiders, but at the cost of reduced food and/or water intake.

1. Introduction

Selection on prey to evade predation has resulted in a tremendous diversity of antipredator traits and/or behaviours. Prey animals can increase their probability of survival through defensive mechanisms such as morphological traits or behaviours that decrease their probability of being detected, attacked or killed by a predator (Lind and Cresswell, 2005). Since predation risk is a function of both attack frequency and probability of being caught when attacked, defensive behaviours of prey are often classified into two categories: primary and secondary defences (Edmunds, 1974). While primary defences act to decrease the likelihood of an encounter with a predator, secondary defences increase the likelihood of survival given an encounter (Edmunds, 1974). Secondary defences may be triggered by direct or indirect contact with a predator (Kats and Dill, 1998) and are usually not cost free (Persons et al., 2002).

Costs of secondary defences are often related to a differential allocation of time to vital activities in situations where the risk of predation is high. In such high predation risk situations, animals usually shift from higher activity levels spent acquiring resources (e.g. food, mates, etc.) to other behaviours that might protect them against attack from a predator (Dicke and Grostal, 2001). This shift can lead to costs that may include reduced foraging efficiency, impaired and/or delayed reproduction, and/or compromised growth and/or development (see Persons et al., 2002; Stoks et al., 2003 and references therein). For example, reducing activity when a threat is imminent might minimize the likelihood that prey will be detected by a predator, but also minimizes its foraging return (Stoks et al., 2003). In order to optimize the tradeoff between defensive behaviour and other activities (like foraging), it is expected that prey assess the degree of threat related to a predator and adjust their antipredator behaviour accordingly (Kusch et al., 2004). The threat sensitive predator avoidance hypothesis predicts that sensitivity to different degrees of threat is favoured by selection, since it can reduce the costs of antipredator strategies without influencing their efficiency (Helfman, 1989).

A secondary defensive strategy is efficient if it allows animals to increase their chances of survival or reduce their chances of attack and subsequent injury in the direct or indirect presence of a predator. One way to achieve this purpose is by behaving in a way that diminishes the likelihood of being detected and/or recognized as

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a prey item. In order to do so, several taxa are known to adopt a state of complete immobility when in the presence of a threat. Such a cessation of all movement except that associated with respiration and vision is referred to as 'freezing behaviour' (Misslin, 2003). Unlike true thanatosis (death feigning behaviour), freezing is not always associated with the adoption of a stereotyped posture (Misslin, 2003; Honma et al., 2006). In addition, while true thanatosis generally reduces an animal's responsiveness to external stimuli, animals engaged in freezing behaviour are alert and physiologically unchanged (Gallup, 1974; Misslin, 2003). Freezing often represents an initial response to danger and is typically triggered immediately upon predator detection (Misslin, 2003; Caro and Girling, 2005). This defensive mechanism seems particularly important for animals whose predators rely mainly on substrate borne vibrations or visual cues for prey detection (Caro and Girling, 2005), since cessation of movement would remove or diminish these cues.

Spiders (Order Araneae) are common predatory arthropods that hunt primarily via tactile and vibratory cues, often relying on motion even when using vision for prey capture (Uetz, 1992; Barth, 2002). The wolf spider *Schizocosa ocreata* (Araneae, Lycosidae) in particular is a generalist 'sit and wait' predator (Cady, 1984; Persons and Uetz, 1999) that relies on visual and substrate borne cues to locate and attack prey. In the mixed leaf litter habitats of southeastern Nebraska, *S. ocreata* is very abundant and is found syntopically with the equally abundant harvestman *Eumesosoma roeweri* (Opiliones, Sclerosomatidae). Although the spiders do not appear to consume the harvestmen, they will attack them and cause serious injury (MCC, pers. obs.). Preliminary observations indicated that immature harvestman (*E. roeweri*) engage in a freezing behaviour in the presence of the predatory spiders (*S. ocreata*) (i.e. they stood absolutely motionless for several minutes, while harvestmen alone tend to wander and explore more often. No stereotyped posture was associated with this cessation of movement).

We tested the hypothesis that freezing is indeed an effective defensive behaviour of the harvestman, *E. roeweri*, and present costs related to foraging. Harvestmen (Order Opiliones) are known to exhibit several types of behavioural, morphological and chemical defences (see review by Gnaspini and Hara, 2007). However, their best known mechanism of defence is the use of chemical secretions. Regarding other secondary defences, members of six families, belonging to two of the three sub-orders of Opiliones (Laniatores and Dyspnoi), are known to exhibit thanatosis. In these groups, thanatosis is usually defined as the retraction of the legs over the body or as the extension of the legs in a characteristic fashion (Gnaspini and Hara, 2007; Machado and Pomini, 2008). Species that engage in thanatosis or other alternative defensive mechanisms tend to release chemical secretions less often than species that present only chemical defences (Machado and Pomini, 2008). To our knowledge, there is no current record of freezing as an antipredator behaviour in harvestmen and even in studies where thanatosis has been described, none have elucidated its potential costs and/or benefits. Here, we aim to first document freezing behaviour in the harvestman *E. roeweri* and then examine potential benefits as well as costs of this defensive behaviour with respect to a common syntopic predatory wolf spider.

2. Material and methods

2.1. Collection and maintenance of harvestmen and spiders

Immature individuals of *E. roeweri* and of *S. ocreata* were collected in December 2006, in leaf litter at Wilderness Park, Lincoln, NE, USA. Both harvestmen and spiders were maintained in the laboratory in individual plastic boxes (5 cm × 5 cm × 10 cm) with two climbable sides and free access to water, in a room with a 12:12 h light:dark cycle and controlled temperature (25 °C). The harvestmen were fed twice a week with dead crickets (*Acheta domesticus*) or drosophilid flies (*Drosophila* sp.) and the spiders were fed once a week with live 1-week-old crickets (Bassetts Cricket Farm). All the experiments were conducted at room temperature (25° C), between 09:00 and 18:00 h. The harvestmen used in all the experiments had dorsal scute lengths measuring between 2.45 and 4.99 mm (mean ± SD = 4.16 mm ± 0.34 mm), and dorsal scute widths measuring between 2.57 and 3.44 mm (mean ± SD = 2.94 mm ± 0.21 mm). The spiders used in all the experiments had carapace lengths varying from 5.30 to 7.30 mm (mean ± SD = 6.3 mm ± 0.06 mm), and total body lengths (when satiated) varying from 1.16 to 1.58 cm (mean ± SD = 1.36 mm ± 0.12 mm). Different focal individuals were used in each experiment. All statistical tests were performed with Sigmastat software, with $\alpha = 0.05$.

2.2. Experiment 1: Influence of context on freezing behaviour

In order to examine whether freezing in immature *E. roeweri* was dependent on the presence of another individual, we asked, specifically, whether harvestmen engaged in freezing behaviour for longer periods in the presence of a predator than in the presence of a conspecific or alone. Individual harvestmen were placed in arenas either (1) with a spider, (2) with a conspecific, or (3) alone. Arenas housing the focal individuals were 9 cm in diameter and the bottom surface was covered with clean filter paper. Stimulus individuals (spider or other harvestmen) were introduced to the arena under a 2.5 cm diameter glass vial and allowed to acclimate for 3 min. Focal harvestmen were introduced using the same method. The vials covering both individuals were removed simultaneously at the start of a trial. All trials lasted 10 min and were videotaped from above using a Sony Handycam DCR-HC65. Videotapes were later scored in the following manner: beginning when individuals first touched each other (or just after the acclimation period for the 'alone' treatment), we quantified the percentage of time that the focal harvestman spent (1) 'freezing' (absolutely motionless), (2) 'stand waving' (stationary but leg waving, grooming, pivoting or displaying other movements that did not involve displacement of the body), and (3) 'walking' (displacing around the arena, speed less than 3 cm/s). We also quantified the number of times each individual was seen (4) 'running' (walking very quickly away from the other individual, strikingly different gait from the 'walking' pattern, speed more than 6 cm/s) and (5) 'trembling' (when in 'stand waving' or 'freezing', slightly moving the body off the substrate two or three times, in a jerky manner). None of the animals moved around the arena with speed between 4 and 5 cm/s, what allowed us to discriminate walking from running with precision. We used a one-way ANOVA to compare the percentage of time spent freezing, stand waving and walking among treatments. A Chi-square test was used to compare the number of running and trembling events among treatments. Seventeen animals were tested per treatment and animals were never used more than once. We ran five or six trials of each treatment (i.e. with spider, with conspecific, and alone) per day, in a randomly defined sequence. All 51 trials were run over 3 consecutive days. After each trial, the glass vials and the arena were cleaned with 70% alcohol and allowed to dry before the following trial.

2.3. Experiment 2: Influence of predator chemical cues on freezing behaviour

In order to test whether *E. roeweri* individuals would engage in freezing behaviour upon contact with chemical cues of predators (silk and feaces) in the absence of such a predator, we placed a harvestman in an arena containing a piece of filter paper either: (1) impregnated with chemicals from *S. ocreata*, (2) impregnated with

chemicals from a conspecific, or (3) clean. We used 22 individuals of similar size in this experiment in a repeated measures design. All individuals were tested in all three treatments, on three different days. Seven or eight individuals were tested per treatment, per day, in a randomly defined sequence.

We impregnated filter paper with spider or conspecific chemicals by placing stimulus individuals (either spider or harvestman) in a 9 cm diameter covered arena lined with filter paper for 24 h. During trials, the focal harvestman was introduced in the center of this arena. They were introduced immediately upon removal of the stimulus individual, under a 2.5 cm diameter glass vial and then allowed to acclimate under this vial for 3 min. To avoid early contact with the chemical cues left by the stimulus individuals and consequent stress, the inverted vial where focal harvestmen acclimated was placed on a 3 cm of diameter piece of clean filter paper, in the center of the arena. Upon removal of the vial, we videotaped the focal individual for 10 min. The videotapes were then scored in a blind fashion. For each trial, we quantified the percentage of time that the focal harvestman spent freezing, stand waving and walking, and compared it among treatments through a one-way repeated measures ANOVA. We also quantified the number of times each individual ran or trembled and compared it among treatments using a Chi-square test. After each trial both the arena and the glass vials used for acclimation were cleaned with 70% alcohol and allowed to dry before the following trial.

2.4. Experiment 3: Potential benefits of freezing behaviour

In order to address whether freezing behaviour could decrease the likelihood of an attack by a spider, we asked if spiders attack moving prey more often than motionless prey. We used 13 spiders that had been starved for 6 days. Each spider was run twice in random order with either (1) a motionless, or (2) a moving cricket (weight range = 0.0022–0.004 g, randomly assigned for each treatment). Half of the spiders (six or seven individuals), randomly chosen, were first paired with the live cricket and, after 32 days, with the dead crickets. The other half was first tested with dead crickets and, after 32 days, with live crickets. No significant difference was found between the animals tested before and after the 32 days ($p > 0.8$). Crickets were chosen as a prey model since they are readily eaten by *S. ocreata*, allowing us to consider the absence of an attack as resulting from the experimental manipulations and not to any structural or behavioural characteristic of the prey item. Predation trials were run in 9 cm of diameter covered arenas lined with filter paper. For the motionless prey treatment, prey crickets were frozen to death immediately before the trial, left at room temperature for 3 min (time enough for them to thaw and return to room temperature) and placed in the arena during the spider's acclimation period. Live crickets were used as moving prey. The spiders were introduced in the arena in 2.5 cm diameter inverted glass vials and were left to acclimate for 3 min. Live crickets were introduced in the same manner and all individuals were released simultaneously and videotaped for 15 min. We compared the number of crickets eaten among treatments using a Fisher's Exact test. We also compared the time to predation among treatments using a one-way repeated measures ANOVA. As a motivational control, we offered live crickets to any spider that did not prey upon the dead cricket during the trial. After each test, both the arena and the glass vials used for acclimation were cleaned with 70% alcohol and allowed to dry before the following test.

2.5. Experiment 4: Potential costs of freezing behaviour

In order to explore one potential cost of harvestmen freezing – time lost to foraging during freezing, we indirectly measured foraging rate under different treatment by weighing individuals before and after trials. Sixteen harvestmen starved for 2 days were paired for 2 h with either (1) spiders, (2) conspecifics, or (3) not paired (i.e. left alone). A total of 48 harvestmen were only used once. Trials were run in 9 cm diameter covered arenas, lined with filter paper. Within each arena, we placed four pieces of wet bread (2 mm × 2 mm × 2 mm), evenly spaced, against the arena's wall. We chose bread as our food source because it is eaten by harvestmen but not spiders. The focal individuals were introduced in the arena inside an uncovered vial of 4 cm of diameter. Stimulus individuals (spiders and harvestmen) were left to acclimate for 3 min in the arena in a 2.5 cm diameter inverted glass vial placed inside the 4 cm diameter vial used for the focal individuals' acclimation. After the acclimation time, the 2.5 cm diameter glass vial was removed and the stimulus individuals were released inside the 4 cm diameter vial with the focal harvestmen for three more minutes, in order to maximize the chances that the individuals would touch each other before the beginning of the trial. For the 'harvestman alone' treatment we followed the same procedure, leaving an empty 2.5 cm diameter vial inside the uncovered 4 cm of diameter one, for 3 min. The stimulus individuals (spiders and harvestmen) were satiated with crickets prior to the start of the experiment. Focal individuals were weighed immediately before and after the experiment with an OHaus Adventurer Pro Scale (AV64, .0000 g precision). We compared the weight of the individuals before and after the experiment within each treatment using a paired *t*-test. We also compared the differences in weight variation between the treatments, using a one-way ANOVA. The 48 harvestmen were tested the same day, in three series of five or six individuals each, in a randomly distributed sequence.

3. Results

3.1. Experiment 1: Context and freezing behaviour

Harvestmen paired with spiders spent more time freezing than harvestmen paired with a conspecific or those alone (Kruskal–Wallis test: $H_2 = 9.578$, $p = 0.008$; Fig. 1). They also spent more time stand waving (ANOVA: $F = 7.991$, d.f. = 49, $p = 0.001$) than individuals in the two other treatments, but there was no difference in time spent stand waving between harvestmen paired with a conspecific versus those alone (SNK post-hoc test: $q = 2.789$, $p > 0.05$). Harvestmen paired with spiders spent less time walking than harvestmen alone (ANOVA: $F = 4.695$, d.f. = 49, $p = 0.014$, SNK post-hoc test: $q = 4.301$, $p < 0.05$), but not than harvestmen paired with a conspecific (SNK post-hoc test: $q = 1.757$, $p > 0.05$) and there was no difference in time spent walking between harvestmen alone and

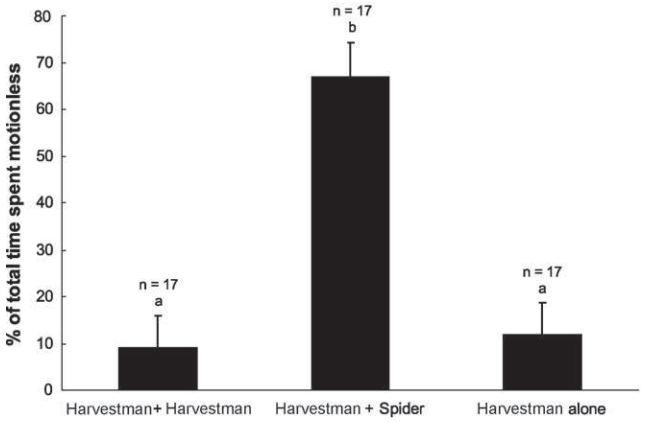

Fig. 1. Context dependent freezing behaviour in *E. roeweri*. The percentage of the total time harvestmen spent freezing in the presence of different individuals (median values and standard errors). Different letters indicate significant differences.

harvestmen paired with a conspecific (SNK post-hoc test: $q = 2.583$, $p > 0.05$). Harvestmen paired with spiders ran more than harvestmen alone or harvestmen paired with another harvestmen: 14 of the 16 harvestmen paired with spiders ran at least once during the 10 min recording, versus three of the 17 harvestmen paired with another harvestman and two of the 17 harvestmen left alone (Chi-square test: $\chi^2 = 13.427$, d.f. = 1, $p < 0.001$ for harvestmen paired with spider versus harvestmen paired with harvestmen; Chi-square test: $\chi^2_1 = 18.976$, $p < 0.001$ for harvestmen paired with spider versus harvestmen alone). No significant difference was found between the two other treatments (Chi-square test: $\chi^2_1 = 0.283$, $p = 0.595$). In 71.4% of all cases, the running events performed by the harvestmen paired with spiders were immediately preceded by a contact between the individual (all individuals combined). The trembling behaviour was observed only in harvestmen paired with spiders, being displayed by nine of the 16 harvestmen tested in this treatment (Fisher's Exact test: $p < 0.001$). The total number of 'trembling' events was 19, 12 of which followed an attack by the spider upon the harvestman and six followed apparently accidental contacts between spider and harvestman. Trembling was followed by the spider moving away from the harvestmen.

3.2. Experiment 2: Predator chemical cues and freezing behaviour

Although harvestmen on filter paper impregnated by spiders spent slightly more time freezing than those of the other treatments, we found no significant differences between the treatments in the percentage of time spent freezing, stand waving or walking (repeated measures ANOVA: $F_{65} = 0.731$, $p = 0.488$, for freezing; Friedman ANOVA: $p = 0.280$ for stand waving and $p = 0.195$ for walking; Fig. 2). However, there was a significant difference between the three treatments in the number of individuals that ran. Nine of the 22 harvestmen paired with spider impregnated filter paper ran, versus none of the harvestmen paired with harvestmen impregnated paper and one of the harvestmen on clean filter paper (Fisher's Exact test: $p = 0.001$).

3.3. Experiment 3: Benefits of freezing behaviour

Spiders were more likely to prey on mobile crickets than on immobile crickets (Fisher's Exact test: $p = 0.011$). Twelve out of the 13 spiders preyed upon the live cricket, versus five out of 13 upon the dead one. Among the eight spiders that never ate the dead cricket, seven readily preyed upon a live cricket offered immediately after the end of the trial. Spiders tended to prey upon live crickets faster than upon dead crickets, but no significant differences were found between the treatments (repeated measures ANOVA: $F_{16} = 4.764$, $p = 0.117$) (Fig. 3). Nine of the 12 spiders that preyed upon the live cricket detected it before contact, moving towards the cricket and attacking it from distances ranging from 4.33 to 0.31 cm (mean ± SD = 1.81 cm ± 1.55 cm, shortest distance between the two bodies, including legs). None of the spiders walked towards the cricket before it moved. The five spiders that preyed upon the dead cricket captured the prey immediately after touching it.

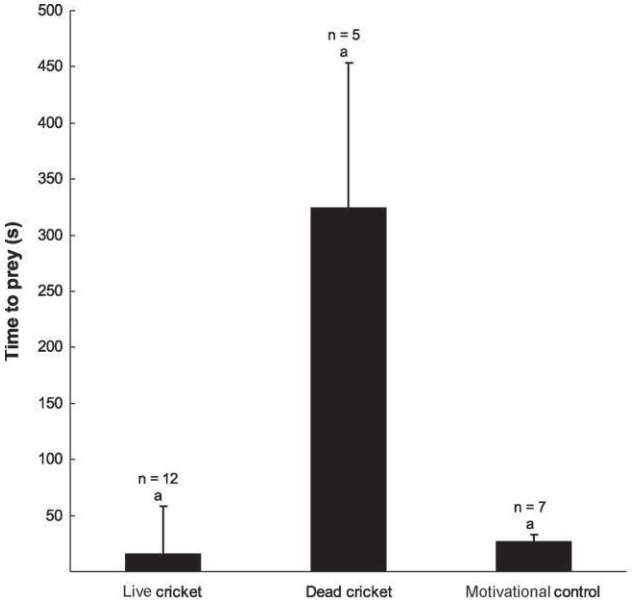

Fig. 3. Motion and spider foraging behaviour. The time to spider attack for live crickets (moving prey) and dead crickets (motionless prey) (median and standard errors). The motivational control indicates the latency to spider attacks of live crickets for spiders previously exposed to only dead crickets.

3.4. Experiment 4: Potential costs of freezing behaviour

Harvestmen gained significantly less weight in the spider treatment than in the other two treatments (one-way ANOVA: $F = 3.78$, d.f. = 2, $p = 0.031$). The weight of the individuals paired with spiders did not change after the experiment (paired t-test: $t = 1.54$, d.f. = 13, $p = 0.147$), but the weight of the individuals paired with other harvestmen or left alone increased (paired t-test: $t = 5.69$, d.f. = 15, $p < 0.001$ and $t = 4.27$, d.f. = 15, $p < 0.001$, respectively) (Fig. 4).

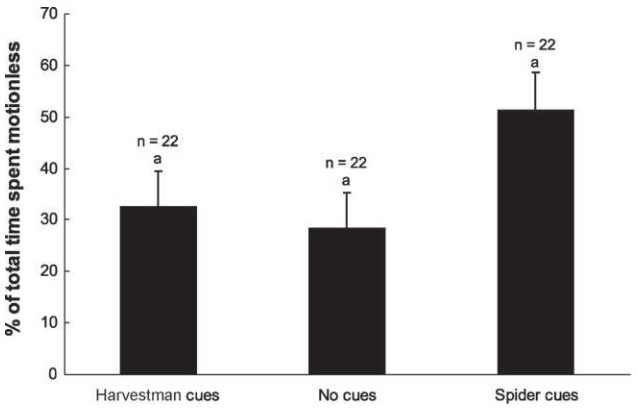

Fig. 2. Freezing in response to predator chemical cues. The percentage of the total time harvestmen spent freezing in the presence of chemical cues from different organisms (median values and standard errors).

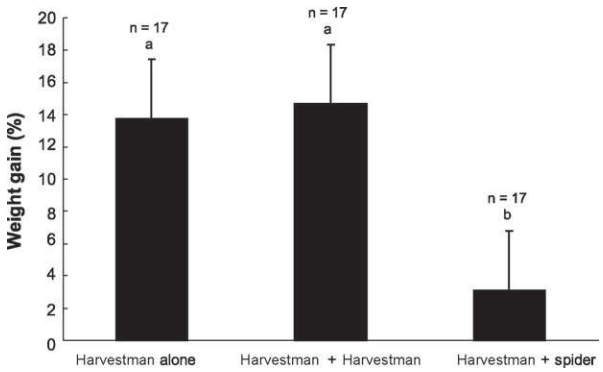

Fig. 4. The relationship between freezing and foraging behaviour. The weight gain of harvestmen in the presence of other individuals (median and standard error). Different letters indicate significant differences.

4. Discussion

Our results demonstrate that the freezing behaviour exhibited by immature harvestmen *E. roeweri* likely decreases the chances of being attacked and thus injured by the syntopic predatory wolf spider, *S. ocreata*, at the cost of decreased food and water intake. We first confirmed that freezing is context specific as it was elicited only in the presence of the predatory spider. Furthermore, we demonstrated that spider chemical cues are not sufficient to elicit this antipredator behaviour. Next, we demonstrate that spiders are more likely to attack moving versus motionless prey, signifying an advantage to motionless (i.e. in freezing behaviour) harvestmen in the presence of the spider predator. Finally, we show that harvestmen housed with food in the presence of a spider gain less weight than those housed with a conspecific or those housed alone – suggesting an energy/water acquisition cost to freezing behaviour.

Freezing is a common secondary defence observed across numerous taxonomic groups including insects (Kohler and McPeek, 1989; Civantos et al., 2004), spiders (Persons et al., 2001, 2002; Wilder and Rypstra, 2004; Bell et al., 2006), fishes (Kusch et al., 2004) and fish larvae (Williams and Brown, 1991), amphibians (Epp and Gabor, 2008), reptiles (Eifler et al., 2008), birds and mammals (see review by Caro and Girling, 2005). Our first experiment allowed us to determine that the harvestman *E. roeweri* freezes in the presence of a syntopic predatory spider, *S. ocreata*. In fact, although harvestmen of the three treatments spent some time freezing, those paired with spiders did it significantly longer than those of the other treatments, suggesting that freezing is indeed a defensive behaviour. In a similar predator–prey system, the wolf spider *Pardosa milvina* exhibits a similar antipredator behaviour, reducing movement, when paired to the larger predatory wolf spider *Hogna helluo* (Persons et al., 2001, 2002). Freezing when in the presence of a predatory spider likely relates to the fact that substrate borne vibrations and visually detected motion are important cues for spiders foraging (Lizotte and Rovner, 1988; Barth, 2002; Persons and Uetz, 1997, 1999).

In addition to freezing, harvestmen running behaviour was also context dependent, being far more frequent in the spider treatment than in the other two. Running is a common tactic of escape for many animals (Edmunds, 1974), and is frequently presented combined with, or as an alternative to freezing (see Eilam et al., 1999; Caro and Girling, 2005). Triggered by contact with the predator, running might allow the harvestmen to get out of the immediate reach of the predator. This has been observed in the harvestman *Mischonyx cuspidatus* when briefly touched by the spider *Enoploctenus cyclothorax* (Willemart and Pellegatti-Franco, 2006). Finally, trembling behaviour was also found to be context dependent – occurring more frequently in spider treatments. This behaviour was triggered mostly by spider attacks or spider contact, and differs from "bobbing", another defensive behaviour seen in harvestmen (Gnaspini and Hara, 2007). We hypothesize that the trembling that we observed is a chemical (associated with an unnoticed secretion release) and/or a mechanical defence (trembling might produce vibrations that somehow frightens or confuses the spider). We also suggest that this might be one of the reasons why the spiders never actually consumed the harvestmen, despite the numerous attacks we observed.

Although *E. roeweri* used freezing behaviour as a defensive mechanism against the wolf spider, they did not freeze when in the presence of spider chemical cues only. Cues concerning the presence of natural enemies play a crucial role in animal survival, and it is expected that antipredator decisions reflect the relative risk levels to which these animals are being exposed (Dicke and Grostal, 2001). The adaptiveness of behavioural flexibility in prey towards different levels of relative risk or magnitudes of the threat is known as the threat sensitivity hypothesis (Gyssels and Stocks, 2005). For example, the presence of predator chemical cues may inform the prey that an area was, at some point, risky, but that risk may no longer exist. The confirmed presence, however, of a predator, indicates immediate high risk, justifying an enhanced defensive behaviour (Kats and Dill, 1998). Plasticity of antipredator behaviour in the harvestmen based upon perceived threat may help to explain the presence versus absence of defensive behaviours in the presence versus absence of the actual predator. Specifically, in our first experiment (spider present), harvestmen displayed two defensive behaviours (namely running and freezing) while they only displayed one (running) in the presence of spider chemical cues only. Harvestmen exposed to spider chemical cues did spend more time freezing and stand waving than those of the other two treatments, but these differences were not significant (Fig. 2). We discard the possibility that the harvestman did not detect the chemicals based on evidences from previous papers (Willemart and Chelini, 2007; Willemart et al., 2009). We also discard the possibility that experiment 1 results were related to spider movement and not to spider chemicals based on the fact that harvestmen do not detect substrate borne vibrations produced by small animals like arthropods (Willemart et al., 2009). In previous studies with arachnids, the spider *P. milvina* was seen reducing its movement rate significantly in the presence of predatory spider cues only (Persons and Rypstra, 2001; Persons et al., 2001, 2002; Wilder and Rypstra, 2004; Folz et al., 2006). Considering that both *P. milvina* and *E. roeweri* were able to detect the predator chemical cues, the different levels of response found in these two systems might be due to the different degree of threat inflicted on these individuals by their respective predators. For example, the predatory organisms used in the *P. milvina* trials, the spider *H. helluo* and the mantid *Tenodera aridifolia sinensis*, regularly kill and consume individuals of *P. milvina* (Persons et al., 2001; Wilder and Rypstra, 2004). In contrast, although some of our harvestmen were severely injured during spider attacks, we never witnessed *S. ocreata* kill and consume a harvestmen. As such, the potential costs of predator detection for *E. roeweri* and *P. milvina* are extremely different. For *E. roeweri*, in low risk situations such as predator cues only, the costs associated with antipredator behaviour might be greater than its benefits (Epp and Gabor, 2008).

Our third experiment pointed out a potential benefit of freezing behaviour. In fact, spiders preyed upon moving prey (i.e. live crickets) faster (although not significantly) and more frequently than upon motionless prey (i.e. dead crickets). Since spiders ate dead crickets both in this experiment and in their maintenance boxes (MCC, pers. obs.), we can assume that the difference in the number of live versus dead crickets attacked is due to the fact that dead crickets were not detected (or at least not recognized as a prey item) without contact. Considering that some spiders never touched the dead (and thus motionless) cricket in our arenas, being motionless (i.e. exhibiting freezing behaviour) likely translates into a reduced risk of spider attack in the field. The results of this experiment, in addition to our knowledge of spider hunting behaviour, highlights one of the benefits of freezing behaviour – decreased likelihood of attack.

Although freezing behaviour appears to be an effective secondary defence, it also seems to present costs related to food and/or water intake. The results of our fourth experiment show that the presence of a spider interferes with harvestmen foraging behaviour. Individuals paired with spiders gained less weight than harvestmen from the other two treatments. Correlating these results with those of experiment 2, this reduced food and/or water ingestion is probably due to the fact that these spider paired individuals spent more time freezing than individuals of the other treatments. A similar behaviour was observed in the wolf spider *P. milvina*, which kills and consumes less prey in the presence of a predator or of its cues than in presence of conspecific, conspecific cues or blank controls (Persons et al., 2002; Wilder and Rypstra, 2004; Folz et al.,

2006). Less closely related taxa such as chironomid larvae (Hölker and Stief, 2005), jumpfish larvae (Williams and Brown, 1991), and rodents (Eilam et al., 1999) also present a similar tradeoff, foraging less and freezing more when in presence of a predator. It is actually expected that prey animals allocate more antipredator effort to high risk situations, often stopping feeding completely (if the periods of high risk are brief), adopting a state of heightened antipredator behaviour, and leaving the foraging behaviour for the low risk periods (Lima and Bednekoff, 1999). This tradeoff between freezing and foraging might be especially costly for animals that rely on active search to forage, like harvestmen. Indeed, harvestmen consume not only live prey but also motionless items (dead prey, vegetal matter, fungus, etc.), which require environment exploration to be found (Acosta and Machado, 2007; Willemart et al., 2007, 2009). Being so, harvestmen exhibiting freezing behaviour frequently and/or for long periods of time would not be able to forage optimally.

According to our results, freezing behaviour protects harvestmen from wolf spiders attacks, but it is probably not the only mechanism that allows these organisms to avoid been predated by wolf spiders. In fact, none of the spiders preyed upon the harvestmen, even when paired with them for 2 days, but some harvestmen presented severe injuries and autotomized several legs after this experiment (unpublished data). Spiders usually attacked harvestmen and retreated, similarly to what was described by Eisner et al. (2004) and Willemart and Pellegatti-Franco (2006) in interactions between harvestmen and spiders. In flour beetles, chemical defence followed by immobility increases the survival rate after jumping spider attacks (Miyatake et al., 2004). In this study, jumping spiders usually retreated after the first attack due to the beetle's chemical defences, and they did not attack again if the beetle remained motionless – however, the spiders kept attacking and frequently killed the beetles if they moved or attempted to flee (Miyatake et al., 2004). In *E. roeweri*, freezing in the presence of a predator seems to reduce not only its chances of being detected and recognized as a prey item, but also the chances of being attacked and thus injured.

We were able to show in this study that the defensive behaviour exhibited by *E. roeweri* when experimentally paired with *S. ocreata* constitutes an effective protection against attacks, at the cost of less effective foraging behaviour. The foraging cost related to the freezing behaviour may be weaker in natural conditions than in our experiments since, in nature, spiders wander in an area infinitely larger than our arenas. However, because both immature *E. roeweri* and *S. ocreata* are extremely dense where they were collected and inhabit exactly the same microhabitat (leaf litter), we expect these harvestmen to encounter not only silk but also actual spiders quite often in the wild. Our laboratory results might therefore offer a fairly decent picture of what actually happens in nature.

Investigating both the potential costs and benefits of a specific behaviour is important in order to understand the pressures involved in its evolution. In our study, we not only determined cost and benefits of a specific defensive behaviour but also found evidence for the threat sensitivity predator avoidance hypothesis (Helfman, 1989): *E. roeweri* might assess the predation risk present in different situations and modulates its defensive behaviour accordingly. We used for the first time a harvestman as a model organism for such a study, revealing an interesting taxon that could be widely used in the future for studies on prey predator interactions.

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