

# Effect of spatial pattern and microhabitat on pit construction and relocation in *Myrmeleon hyalinus* (Neuroptera: Myrmeleontidae) larvae

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**Abstract.** 1. In two sets of enclosure experiments, we studied the spatial pattern, relocation rates, pit construction rates, and microhabitat preference of *Myrmeleon hyalinus* larvae.

2. We showed that *M. hyalinus* larvae actively prefer shady sites and often relocate to shady areas when exposed to the sun. This behaviour may constitute a life-saving strategy in desert environments.

3. The initial spatial pattern in a cluster of antlion larvae did not affect the final pattern, relocation rate, or the pathway moved while relocating. We interpret this finding to mean that the spatial pattern of *M. hyalinus* larvae is mainly influenced by factors operating at large time-scales, such as exploitation competition and physical-microclimatological constraints, rather than those operating at shorter time-scales, such as interference competition.

4. In contrast, the likelihood of pit construction was positively correlated with nearest neighbour distance, possibly as a result of interference competition.

5. Pit construction rates were constant throughout the experiment, while relocation rates decreased with time.

6. Contrary to previous studies, we found no correlation between body mass and spatial position inside the cluster, suggesting that these differences are the products of slower processes (e.g. exploitation and body condition).

**Key words.** Antlions, habitat selection, null model, sit-and-wait predators, spatial pattern.

## Introduction

Microclimatological and habitat constraints dictate that pit-building antlion larvae usually live in dense clusters. These adequate habitats, known as *antlion zones* (Gotelli, 1993), are characterised by a specific combination of sand particle size, density of plants, shade, and shelter. In light of the high densities of larvae in antlion clusters and their restriction to these areas, density-dependent factors may have a considerable influence on antlion fitness. Similar to many other animal groups, antlions compete for resources but may also enjoy the benefits of living close together, such as a decrease in predation risk (Hamilton,

1971; Krause & Ruxton, 2002). In such animals, the benefits and costs of different spatial positions may vary within the groups. For example, peripheral positions in stationary groups and front positions in moving groups have higher prey capture rates (e.g. Rayor & Uetz, 1990; Gotelli, 1997; Krause & Ruxton, 2002, p. 77). Indeed, previous studies on antlion spatial patterns suggest that *shadow competition* – when an antlion closer to the edge catches moving prey before it encounters other antlions – has important effects on the positions preferred by antlions within the cluster (Wilson, 1974). Wilson (1974) proposed that an adequate response would be for the larvae to occupy only the antlion zone edges, thus creating a *doughnut-shaped* cluster. Wilson's suggestion was, however, rejected by other investigators (e.g. McClure, 1976; Matura & Takano, 1989), who claimed that interference or other types of exploitation competition dominate in the process of spatial pattern formation, and therefore, antlion larvae will distribute themselves uniformly at

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high densities and randomly at low densities. In addition, in some groups central positions may be safer than peripheral ones (e.g. Rayor & Uetz, 1990; Gotelli, 1997; Krause & Ruxton, 2002, p. 81). As there is little information on antlion predators, however, it is difficult to determine the extent to which the risk of predation affects antlion positioning.

The spatial pattern of an antlion cluster is formed by each individual's decision to relocate its ambush site. The act of relocation itself may be far more costly for a sit-and-wait predator than for individuals in a moving group. For example, relocation entails a corresponding increase in metabolic rates while moving or building a new trap (antlions: Lucas, 1985; spiders: Tanaka, 1989), and it exposes the predator to cannibalism and intra-guild predation (Griffiths, 1991). The decision to relocate may also be influenced by the type of habitat variation, that is whether a shortage in prey arrivals is temporal or spatial (Scharf & Ovadia, 2006). Consequently, despite a lack of prey for an extended period of time, the best response may be for the antlion to stay in the same position, even when prey does not arrive for a long period of time (e.g. Matura, 1987; Tanaka, 1989).

The spatial positioning inside the cluster is affected by a combination of several factors that vary in relative importance (Scharf & Ovadia, 2006). In our opinion, the most important biotic factors are exploitation competition, interference competition, risk of cannibalism, risk of predation, and the metabolic costs of movement. Clearly, exploitation and interference should act to increase dispersion, but at high densities antlions face the threat of cannibalism (e.g. Griffiths, 1991, 1992), which may reduce the propensity to move. Metabolic cost should be negatively correlated with body condition (i.e. relocation for an exhausted larva may be very expensive). Risk of predation, as a function of dispersion, may increase or decrease, depending on the type of predator. Cluster aggregation and the reduction of inter-individual distances may provide some protection (safety in numbers, Hamilton, 1971) against small predators that consume only a small number of antlions, such as small scorpions or predatory ground beetles. On the other hand, larger predators, such as birds, are capable of consuming entire clusters of antlions, and thus a survival strategy would be dispersal to reduce the signal that enables predators to locate the antlions (Taylor, 1976).

To evaluate the effects of antlion spatial patterns and habitat preferences on pit-related behavioural processes, we focused on how interference competition, together with a microclimatic factor (sun vs shade), affected antlion spatial patterns, pit construction, and pit relocations over short time-scales. By combining data on initial spatial patterns and microclimatic factors, we examined the interaction between biotic and abiotic factors and their relative importance in pit relocation. We also calculated pit construction and relocation rates in an attempt to improve the temporal resolution of past studies. Previous studies on antlion larvae indicate that after a disturbance they reconstruct pits on either the same or the next day (e.g. Eltz, 1997; Botz *et al.*, 2003), but our protocol enabled us to test pit construction on a finer scale comprised of the first 3–4 h after disturbances. Finally, we also analysed the relationship between body mass and three parameters: the pathway travelled by the larvae; the distance from the cluster centre to a larval pit; and the distance between a larva and its nearest neighbour, that

is nearest neighbour distance (NND). Although Griffiths (1993) and Prado *et al.* (1993) suggested that larger antlion larvae are likely to move less than smaller ones and several studies have dealt with differences in spatial positioning with respect to antlion mass (e.g. McClure, 1976; Griffiths, 1992), there is no consensus regarding the preferred positions within the cluster occupied by the larger antlion larvae (cluster centre: McClure, 1976; cluster periphery: Griffiths, 1992).

## Methods

### *Study species and habitat of origin*

*Myrmeleon hyalinus* (Neuroptera: Myrmeleontidae) is the most abundant pit-building antlion in Israel. The larvae are about 10 mm long (Simon, 1988), and may attain body weights of up to 0.06 g before pupating (personal observations). *M. hyalinus* larvae are sit-and-wait predators that lie at the bottom of funnel-shaped pits with only their heads protruding, waiting for small arthropods to step over the pit margins. Their physiology dictates that *M. hyalinus* larvae occupy shaded areas under trees, bushes, and rocks. The larvae are capable of inhabiting different types of soils (Simon, 1988).

Field observations on antlion spatial patterns were conducted under 10 tamarisk trees in Nahal Secher (31°06'N, 34°49'E). This sandy area, about 15 km south of the city of Beer-Sheva, Israel, is an extension of the sand belt of northern Sinai. Nahal Secher receives about 150 mm of rainfall per year, and can usually be divided into areas of shifting/mobile sand, semi-stabilised sand, and stabilised sand. During the 1960s tamarisk trees were planted in the area, and a gradual process of sand stabilisation began (Danin, 1978). Antlions are found mainly in clumps under the tamarisk trees and under smaller bushes. We recorded antlion densities and spatial patterns under 10 tamarisk trees by measuring the Euclidean distances from the tree to the pits and the angle of deviation from the north using a protractor compass.

### *Experimental system and study design*

We collected about 150 antlion larvae (of which about 90% were second and third instar larvae) from Nahal Secher (median mass of 0.0088 g with inter-quartile range (25–75%) of 0.0047–0.0164 g). As antlion zones in the field averaged about 6 m<sup>2</sup>, depending on tree size, we constructed three enclosures of similar size (2 × 3 m). Enclosures were filled with sand (10 cm depth) brought from the habitat of origin. The study comprised two enclosure experiments conducted under semi-field conditions. One experiment tested the effect of initial spatial pattern on both antlion behaviour and final spatial pattern, and the other, a microhabitat preference experiment, investigated how exposure to the sun affected pit construction and relocation propensity. Prior to each experiment, antlions were fed ad libitum, starved for 2 weeks and then stocked into the enclosures. Each individual in our antlion pool was used several times (three to four) during the different experiments. To avoid dependence of the data, individuals were randomly assigned to different experimental

treatments and only their aggregate or mean responses were analysed.

### Spatial pattern experiment

The experimental set-up comprised three enclosures, one for each of the initial spatial patterns: random, regular, or clumped. The patterning was achieved by positioning a  $100 \times 53$  cm Perspex plate with holes on the sand in the centre of the enclosure. Antlions (12–16 individuals) were randomly assigned to the holes in the plate in the late evening, and then the plate was removed (Fig. 1). We followed the positions of each individual antlion for 3–4 h thereafter because our preliminary observations indicated that most activities, such as pit location and construction, occurred during this period of the night and that there was little or no change in antlion positions during the next 2 days. Pit locations were obtained by measuring the distance from the enclosure edges to the pits (two measurements per pit with precision of 0.5 cm). This protocol was replicated 10 times with an interval of 1–2 days between replications, except for the regular pattern which was replicated 11 times (3 enclosures  $\times$  10 nights). During the experimental period the mean ( $\pm 1$  SD) air temperature at 20:00 was  $25.9 \pm 1.0$  °C, and there were only minor temperature fluctuations across nights (range 24.3–28.9 °C).

### Microhabitat preference experiment

*Experiment I.* Half of each enclosure was covered with a 0.8 m high dark shade net ( $1 \times 1.5$  m), blocking 90% of the direct sun radiation (Fig. 1). The antlions were separated into

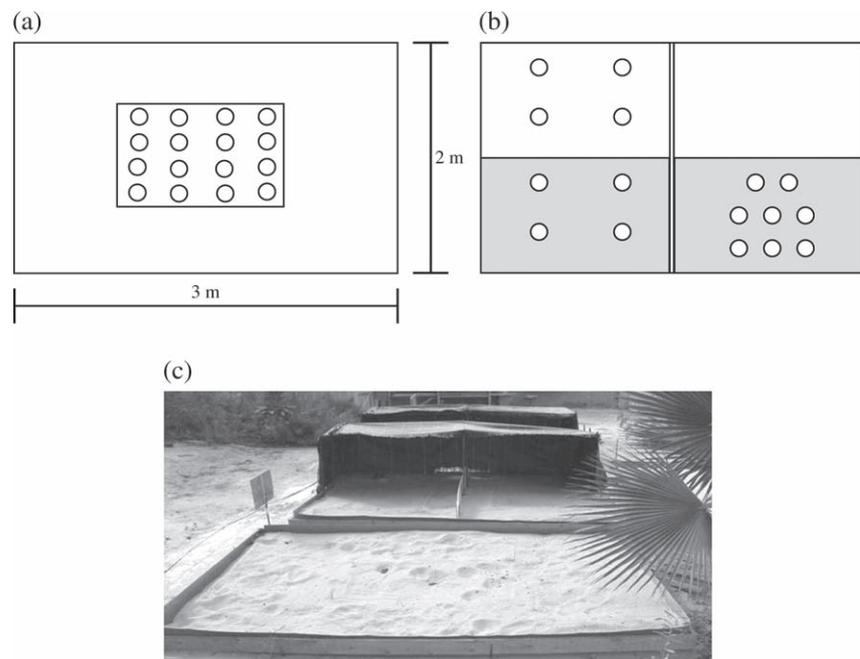
groups of eight, and all were placed either on the sunny or shaded sides of the enclosure, in a clumped pattern (mean NND = 3.3 cm). This protocol was replicated 24 times with an interval of 1–2 days between replications (four enclosures  $\times$  six nights). Each replicate continued from the evening until the next morning (about 15 h). Final antlion positions were recorded (with precision of 0.5 cm) and rates of pit construction were calculated.

*Experiment II.* The experiment was similar to the previous experiment (Fig. 1), except that four antlions were placed simultaneously in the sunny and shaded sides of the enclosure and that the mean NND was larger than that in the previous experiment (12 cm), giving a less aggregated pattern. This protocol was replicated 20 times with an interval of 1–2 days between replications (four enclosures  $\times$  five nights). Each replicate continued from the evening until the next morning (about 15 h).

### Calculating antlion spatial patterns

Perry *et al.* (2002) suggested using more than a single method to analyse spatial patterns, because no single method can identify all of the spatial characteristics in the data. We therefore used the following indices and methods to estimate antlion spatial patterns:

**1** NNI – nearest neighbour index (Krebs, 1999, pp. 192–195, after Clark & Evans, 1954): A conventional method to estimate spatial patterns, NNI equals the ratio between the mean NNDs of all individuals and the expected NNDs calculated assuming random patterns. Thus, values smaller or larger than one indicate clumped or uniform patterns, respectively.



**Fig. 1.** Two schemes and a photo of an enclosure. (a) A scheme presenting the spatial pattern experiment. Twelve to 16 antlions were placed in three different spatial patterns using a Perspex plate ( $100 \times 53$  cm, inner square). The plate was then removed and antlion behaviour was documented. (b) Each enclosure was divided into two equal size enclosures (vertical line). Then half of each of these two enclosures was covered with a dark shade net. In the first microhabitat experiment, eight antlions were placed either in the shaded or sunny sides (right part); in the second experiment, four antlions were placed in each side (shaded and sunny, left part). (c) A photo of an enclosure. The entire enclosure was used during the spatial pattern experiment but was divided into two equal size enclosures during the microhabitat experiment.

- 2  $I_E$  – Eberhardt index (Krebs, 1999, p. 211, after Eberhardt, 1967): Based on the variance:mean ratio,  $I_E$  incorporates the Poisson distribution assumption that dictates that for a random pattern the mean equals the variance. This index uses the mean and standard deviation of the distances between random points in the enclosure and their nearest observed points. Small ratio values (smaller than 1.27) suggest regular patterns and higher values suggest clumped ones.
- 3  $I_G$  – Green's index (Krebs, 1999, pp. 215–216, after Green, 1966): Also based on the variance/mean ratio,  $I_G$  is calculated by dividing the enclosure into sampling units or quadrats (49 in our case), and counting the abundance of points in each quadrat. The mean and variance of this set of abundances are used to calculate  $I_G$ .
- 4 CS – centroid size (Zelditch *et al.*, 2004): Used in morphometrics to measure the size of a sample, CS is calculated according to the following formula:

$$CS = \sqrt{\sum_{i=1}^n (x_i - \bar{x})^2 + (y_i - \bar{y})^2}$$

where  $n$  is the number of points (antlions, in our case),  $x$  and  $y$  are the point coordinates, and  $\bar{x}$  and  $\bar{y}$  are mean  $x$  and  $y$  coordinates of all the points. We use CS as a simple index of dispersion or cluster size and not of spatial pattern.

#### Data analysis

*Spatial pattern experiment.* We estimated final spatial patterns and cluster dispersion using the four indices described above. Our purpose was not only to compare among the final patterns across treatments, but also to determine whether the observed differences could be randomly obtained. In other words, we asked whether antlion larvae movement in our experiment was random (based on some distribution of movement directions and length) and uninfluenced by biotic interactions; alternatively, final patterns are the outcome of the combined effects of initial patterns and biotic interactions (i.e. interference). We could not, therefore, use ordinary statistical tests, because even a significant difference among the aggregation indices does not imply biotic interactions but may instead be a direct product of the initial spatial pattern. Therefore, we developed a null model (Gotelli & Graves, 1996) in which new final positions for the larvae were calculated for each of the 31 replications.

Starting with the same initial positions, we assigned to each individual a random direction and a distance randomly selected from a pool of the original distances. For each replication we evaluated the following parameters: CS, NND, NNI,  $I_E$ , and  $I_G$ . Next we calculated the mean for each spatial pattern separately and the absolute differences between the means (Random–Regular, Random–Clumped, and Regular–Clumped). We repeated this procedure 2000 times and estimated  $P$  values by comparing the observed results with the simulation results. The proportion in which the simulation results were equal to or greater than the original results is considered to be the  $P$  value for each parameter (this procedure is similar to a bootstrap test,

Manly, 1997, p. 34). In addition, because the data were not normally distributed we compared between the frequency of pit construction, the frequency of relocations, and the path moved in each treatment using Kruskal–Wallis tests (Zar, 1999, p. 197). Finally, to test for a relationship between NNDs and pit construction we used a logistic regression (Neter *et al.*, 1996, p. 567), the results of which are presented using the following formula:

$$P_{\text{pit construction}} = \frac{1}{1 + e^{-(a+bx \times \text{NND})}}$$

$P_{\text{pit construction}}$  is the probability (0–1) of pit construction relative to the NND. Additionally, we calculated the odds ratio =  $P/1 - P$ , which shows how a variation of one unit in the distance affects  $P_{\text{pit construction}}$ .

*Pit construction rate analysis.* Using the time to pit construction in random and regular patterns we constructed Kaplan–Meier survival curves to represent the rate of pit construction over time (Kleinbaum & Klein, 2005, p. 45) (pit construction was taken as analogous to survival, as antlions almost never relocated after constructing pits during the first 3–4 h of the experiment). We plotted the natural logarithm of each curve versus time to construction, using linear regression to estimate the slope.

*Antlion movement rate analysis.* First, we tested whether the pathways moved by the antlions depend on body mass and whether larger antlions are not evenly distributed within the cluster. We used linear regression analysis to test for the relationship between body mass and each of the following response variables: total pathway moved, distance from cluster centre, and NNDs. Second, we examined whether antlions tend to move at constant rates throughout the experiment and whether the tendency to move at a particular point in time was affected by the path taken in previous movements (moving in time  $t + 1$  is affected by movement in time  $t$ ) or by the duration of the experiment. Therefore, we analysed the time intervals between movements in random and regular patterns using survival analysis. We used the Cox regression model (Kleinbaum & Klein, 2005, p. 83) to evaluate to what extent the rate of movement is affected by the following five covariates: (i) cumulative path moved until the present movement; (ii) total time from the beginning of the experiment until the present movement; (iii) experiment duration; (iv) the path previously moved; and (v) spatial pattern, random or regular. We considered the possible correlation between antlions in the same enclosure and used a robust jackknife variance estimator grouped by observations per plot (Lin & Wei, 1989).

*Microhabitat preference experiment.* We used log-likelihood ratio tests (G tests, Zar, 1999, p. 505) to compare the relative frequencies of pit construction under shady and sunny conditions and the tendency to relocate from one region to the other. We used a mixed ANOVA model to compare between the pathways moved by individuals originally located in the exposed and shaded parts of the enclosure (initial position–fixed effect) while also testing for possible time effects (night–random effect). Note that (i) only relocating individuals were included in this analysis, and (ii) when testing for the effect of initial

position on the pathways, we had to use the mean sum of squares of the interaction term (initial position  $\times$  night) and its degrees of freedom (rather than those of the error term) in the calculation of the  $F$  statistics and significance level (Zar, 1999, p. 243).

## Results

### Field observations

Antlion spatial patterns under 10 different trees were estimated using three spatial indices, NNI,  $I_E$ , and  $I_G$ , and the  $z$  statistics for the NNI were used to determine the pattern type. In most cases ( $n = 6$ ) the antlion cluster spatial patterns were consistent with a random pattern (aggregation index according to NNI,  $R = 1.03 \pm 1.80$  & 1.00 (mean  $\pm$  1 SD & median),  $|z| < 1.96$ ). Two cases exhibited clumped patterns [ $R = 0.69 \pm 0.03$  (mean or median, as there are only two data points  $\pm$  1 SD),  $z < -1.96$ ] and in another two the patterns were regular [ $R = 1.42 \pm 0.06$  (mean or median  $\pm$  1 SD),  $z > 1.96$ ]. Median (inter-quartile range) CS and NND under the 10 trees were 340 (239–395) and 18.5 (13.5–30.5) cm, respectively. Median CS after the 4 h experiment were similar in all treatments to the values observed in the field (Kruskal–Wallis test,  $P = 0.302$ ,  $\chi^2 = 3.645$ ).

Median NNDs (inter-quartile range) measured in the random and regular final patterns of the experiment were 20.5 (16.5–23.0) and 26.5 (17.5–30.0), respectively, and were similar to those observed in the field; however, the NNDs in the clumped treatment (18.5; 14.0–19.5) were significantly smaller than those observed in the field (Kruskal–Wallis test,  $P = 0.012$ ,  $\chi^2 = 10.976$ ).

### Spatial pattern experiment

In all treatments there was a tendency to decrease aggregation during the experiment (Fig. 2). The null model estimated the range of differences among treatments in the aggregation indices obtainable by examining the combined effects of initial pattern and random movement. Therefore, we could not refute the null hypothesis each time the observed values fell within this range.

Our null model suggested that there should be no significant differences in NND among the three final patterns, and it was

consistent with the observed results (Table 1a, second row, NS). CS was smaller in the clumped treatment than in random and regular treatments (Kruskal–Wallis test,  $P = 0.045$ ,  $\chi^2 = 6.2$ ), but this difference can be explained by the null model, and thus could be a direct result of initial conditions and random movement (Table 1a, first row, NS). As predicted by the null model, NNI and  $I_E$  did not differ across treatments (Table 1a, third row and fourth row, respectively, NS). Only  $I_G$  (Table 1a, fifth row) measurements were inconsistent between the null model and the observed results. The difference between the clumped treatment and the two other treatments was smaller in the observed than in the simulation results indicating that when clumped, individuals tended to over-disperse and aggregate to a lesser degree. Application of the Kruskal–Wallis test to study the differences in  $I_G$  revealed the opposite pattern. The final patterns of the clumped treatment showed greater aggregation than those of other treatments ( $P = 0.0004$ ,  $\chi^2 = 15.85$ ). This result is counterintuitive when comparing the null model and the Kruskal–Wallis test, as described in the discussion.

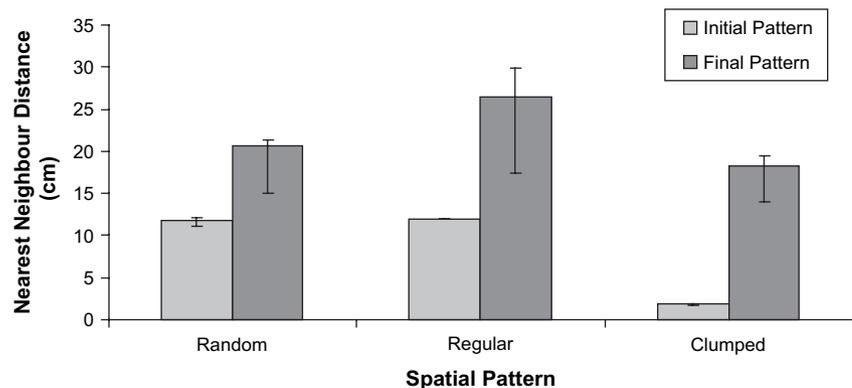
The pathway moved and frequency of relocations did not differ significantly among the three treatments ( $P = 0.6$ ,  $\chi^2 = 0.7412$ ;  $P = 0.2751$ ,  $\chi^2 = 2.58$ , respectively; Table 1b). The frequency of pit construction was significantly lower in the clumped treatment than that of the two other treatments ( $P = 0.0125$ ,  $\chi^2 = 8.77$ ; Table 1b). Logistic regression analysis showed a positive correlation between NND and the likelihood to construct a pit ( $P = 0.015$  for the NND,  $P = 0.041$  for the constant; Fig. 3). In this case, the odds ratio = 1.033, meaning that an increase of 1 cm in the NND increased the probability to construct a pit by 3.3%.

### Pit construction rate analysis

A linear regression analysis of the natural logarithm of the Kaplan–Meier curve vs the time to pit construction (Fig. 4;  $F_{[1,30]} = 2188$ ,  $R^2 = 0.9865$ ,  $P < 0.001$ ) indicated that the pit construction rate was constant during the experiment.

### Antlion movement rate analysis

Linear regression analysis was used to test how body mass affects total pathway moved, distance from cluster centre,



**Fig. 2.** Differences in the NNDs (median and inter-quartile range) between the beginning and the end of the experiment (bright vs dark columns) and among treatments (left vs right columns).

**Table 1.** (a) Results of the null model. Using a simulation, each antlion in the original data set was assigned a random direction and distance relative to its initial position. These new randomly generated coordinates were used to calculate the spatial pattern indices for the three different treatments. The absolute difference between the observed and random patterns was then calculated and a distribution of these values was generated by repeating this process 2000 times. In this test, the null hypothesis (that the final spatial patterns in the original data set could be obtained from a random movement in combination with the initial pattern) is rejected if less than 5% of the randomised samples show an absolute difference equal to, or greater than that of the original pattern.

	Random–Regular		Random–Clumped		Regular–Clumped	
	Mean & median	<i>P</i>	Mean & median	<i>P</i>	Mean & median	<i>P</i>
CS	17.62 & 15.00	0.628	46.39 & 46.37	0.682	57.26 & 57.26	0.821
NND (cm)	2.16 & 1.87	0.639	3.43 & 3.13	0.532	4.13 & 3.93	0.757
NNI	0.070 & 0.061	0.653	0.145 & 0.140	0.500	0.173 & 0.171	0.721
$I_E$	0.0799 & 0.0652	0.184	0.0825 & 0.0683	0.261	0.0795 & 0.0660	0.092
$I_G$	0.0129 & 0.0115	0.890	0.1907 & 0.1884	0.031	0.2009 & 0.1984	0.070

(b) Kruskal–Wallis results of comparisons between the pathway moved, frequency of pit construction and frequency of relocation in the three treatments.

	Random median (inter-quartile range)	Regular median (inter-quartile range)	Clumped median (inter-quartile range)	Kruskal– Wallis [ $\chi^2$ , <i>P</i> ]
Sample size ( <i>n</i> )	10	11	9	
Pathway (cm)	75.0 (59.0–79.0)	82.0 (58.0–87.5)	79.5 (68.5–92.0)	[0.6, 0.7412]
Frequency of pit construction	0.813 (0.657–0.875)	0.875 (0.735–0.934)	0.615 (0.600–0.729)	[8.77, 0.0125]
Frequency of relocation	0.688 (0.646–0.782)	0.688 (0.475–0.735)	0.600 (0.425–0.667)	[2.58, 0.2751]

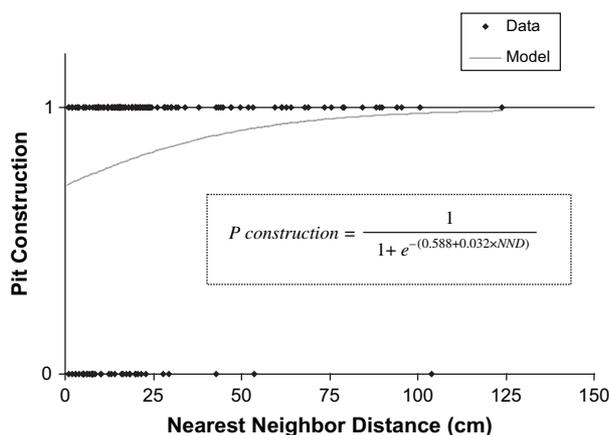
and NND. The regressions were not significant ( $R^2=0$ ,  $F_{[1,163]}=0.002$ ,  $P=0.963$ ;  $R^2=0$ ,  $F_{[1,163]}=0.005$ ,  $P=0.0942$ ;  $R^2=0$ ,  $F_{[1,163]}=0.001$ ,  $P=0.971$ , respectively). Using power analyses, we found that the chance of committing a type II error with respect to the effect of body mass on these three measurements was of the order of 6.8–14.7%. We thus concluded that antlion mass had no effect on the above three parameters.

Using Cox regression we found that cumulative pathway moved by the larva was positively correlated with its tendency to move (i.e. shorter inter-movement intervals), indicating that antlions that already moved had a higher tendency to move again. The cumulative

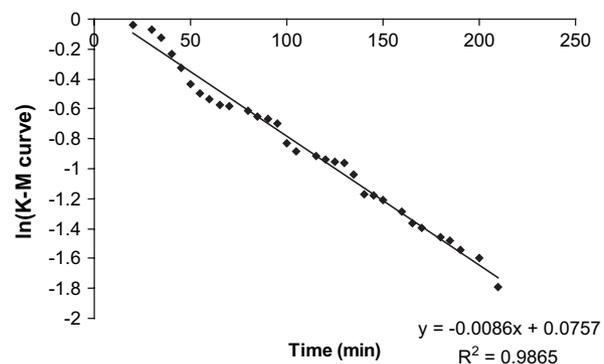
time from the experiment beginning and the experiment duration were both negatively correlated with the tendency to move. Neither the previous movement distance nor the initial spatial pattern had any significant influence on the tendency to move (Table 2).

#### Microhabitat preference experiment

*First experiment.* We tested whether the tendency to relocate differed between two microhabitats, exposed and shaded, by comparing the frequency of individuals relocating from one



**Fig. 3.** NNDs were positively correlated with the probability to construct a pit. This model presents the probability to construct a pit as a function of increasing NNDs.



**Fig. 4.** Results of Kaplan–Meier survival analysis, presented in natural logarithm. The regression slope is highly significant indicating that pits are constructed at a constant rate.

**Table 2.** The effect of different factors on the relocation rate. The coefficient ( $\beta$ ) for each factor was estimated using a Cox proportional hazard model and tested for its significance. The expected change in the time to relocation per one unit change in each of these covariates was predicted using the exponent coefficient ( $e^\beta$ ). For example, a change of 1 cm in the cumulative path moved so far increased its probability of relocation by a factor of 0.896.

Cumulative path (cm)		Cumulative time (min)		Experiment duration (min)		Path (previously moved) (cm)		Spatial pattern (0 = random, 1 = regular)		Model statistics
$e^\beta$	Statistics	$e^\beta$	Statistics	$e^\beta$	Statistics	$e^\beta$	Statistics	$e^\beta$	Statistics	
1.065	$z = 4.790$ , $P < 0.0001$	0.896	$z = -3.842$ , $P = 0.0001$	0.985	$z = -2.146$ , $P = 0.032$	—	NS	—	NS	LRT = 718, d.f. = 5, $P < 0.0001$

position in the enclosure to another (i.e. the frequency that a larva did not remain in the microhabitat in which it was initially placed). The frequency of individuals relocating from the exposed to the shaded microhabitat was significantly higher than that in the opposite direction ( $G$  test;  $G = 23.892$ ,  $P < 0.001$ ). A comparison of the pathways moved by antlions from their initial positions showed that antlions initially placed in the sun moved greater distances than those initially placed in a shaded microhabitat (mixed ANOVA model;  $F_{[1,5]} = 31.09$ ,  $P = 0.003$ ). There was no significant time effect (i.e. night;  $F_{[5,122]} = 1.18$ ,  $P = 0.33$ ), nor a significant initial position  $\times$  night interaction ( $F_{[5,122]} = 1.15$ ,  $P = 0.34$ ). Finally, the two microhabitats had similar frequencies of pit construction ( $G = 0.7891$ , NS), although fewer antlions remained exposed to sun.

**Second experiment.** The average number of relocations between microhabitats in each replication was compared using a  $G$ -test to determine whether the tendency to relocate differed between the two microhabitats. Antlions that were initially placed in the shade never moved to the exposed microhabitat, whereas  $72 \pm 24\%$  (mean  $\pm 1$  SD) moved from the exposed to the shaded microhabitat ( $G = 115.443$ ,  $P < 0.001$ ). The pathway moved while relocating was significantly larger for the antlions that were initially placed in the sun (mixed ANOVA model;  $F_{[1,4]} = 69.98$ ,  $P = 0.001$ ). There was no significant time effect (i.e. night;  $F_{[4,100]} = 1.49$ ,  $P = 0.21$ ), nor a significant initial position  $\times$  night interaction ( $F_{[4,100]} = 0.86$ ,  $P = 0.49$ ). The frequency of pit construction was slightly higher under shade ( $G = 4.090$ ,  $P < 0.05$ ), where antlions were more abundant.

Finally, the frequency of microhabitat change was slightly higher in the second experiment, where antlions were placed simultaneously in both sides of the enclosure at higher NNDs (12 compared with 3.3 cm,  $G = 4.604$ ,  $P < 0.05$ ).

## Discussion

Antlion larvae are strongly influenced by the physical characteristics of the environment; consequently, they live in clusters, and the spatial pattern inside each cluster is determined mainly by biotic interactions such as interference and exploitation. Our experiments were designed to test the effects of initial spatial pattern and exposure to sun on relocation rate, pit construction, and final spatial pattern. We varied the initial spatial pattern because in the short term, it can affect the strength of biotic interactions, such as interference and risk of cannibalism, and in the

long term it can induce different levels of exploitation competition, ultimately leading to different individual growth rates. Our short-term experiments allowed for the isolation and estimation of the effect of short-term interference on the spatial pattern, thus distinguishing its effects from those of exploitation. By separating between such short- and long-term effects we can arrive at a better understanding of the mechanisms of competition among antlion larvae, and the role each one of them play in determining antlion behaviour (i.e. relocation and pit construction) and spatial pattern.

We found that the initial spatial pattern affected neither the tendency to relocate nor the pathway moved while relocating. The null model showed that random movements of individual antlions, as measured by most of our indices, can generate similar final patterns. In other words, the combination of the initial positions and the random movements could explain the final patterns, irrespective of possible responses to biotic interactions. Based on the fact that in all three treatments antlions organised themselves in similar final patterns, the logical conclusion would be that antlions relocate to increase the inter-individual distances to an *optimal level*, thus reducing the strength of biotic interactions. This apparently reasonable hypothesis, however, is rejected in favour of the null model, which shows that such patterns can also result from random relocation.

We do not argue that there is no density effect on relocation, but this effect is probably evident only at larger time-scales. Interference competition in this experiment was not the trigger for pit relocation events. As previous studies found a positive correlation between the density or NNDs and the frequency of relocation (Griffiths, 1992; Day & Zalucki, 2000), we suggest that other types of competition, especially exploitation, affect the frequency of relocation.

CS was smaller in the clumped treatment, but this finding indicates nothing more than random movement, and can be randomly obtained by the null model (Table 1a, first row). In other words, it is expected and understandable that the antlions that began the experiment in a more clumped pattern and moved in random directions would finally be a little bit less dispersed than the antlions that began the experiment in random or regular pattern. The only significant result in the null model was the difference between clumped and other treatments in relation to  $I_G$ . A Kruskal–Wallis test showed that in the clumped treatment the larvae ended more aggregated. The expected difference in the null model, however, was significantly higher than the observed

difference, indicating that the degree of dispersal was greater when the antlions started from a clumped pattern. Although this conclusion contradicts that deducible from the Kruskal–Wallis test, it may suggest that mutual disturbance triggers dispersion when NNDs are smaller. Findings for  $I_G$  support this conclusion; however, it is not supported by either the other indices or by the path measurements, so it should be treated with caution.

The frequency of pit construction seemed to be the most important parameter that varied among treatments in this experiment. Animals in the clumped treatment constructed fewer pits than those in the two other treatments. Similar results were obtained from the logistic regression analysis, when the relationship between pit construction and NNDs was examined. Our results supported the previous conclusion that reduced inter-individual distances, which are derived from density, decrease both the frequency of pit construction (e.g. McClure, 1976; Matsura & Takano, 1989) and the average pit diameter (as shown elsewhere, e.g. Day & Zalucki, 2000; Devetak, 2000). On the other hand, Matsura and Takano (1989) found in *Myrmeleon bore* that pit size was independent of density. In conclusion, we suggest that interference plays an important, but indirect, role in shaping spatial patterns. As high antlion larvae densities are negatively correlated with the distance between antlion pits, fewer pits are constructed, and as a result, fewer prey items are caught. The consequent hunger usually induces additional relocation events, further dispersing the antlions, and finally results in a regular pattern to maximise distances among individuals.

Antlions respond strongly to physical properties of their habitat, and they actively choose the preferred microhabitat, often in relation to sand particle size (e.g. Farji-Brener, 2003; Devetak *et al.*, 2005; Matsura *et al.*, 2005). Although *M. hyalinus* is known to inhabit only shaded microhabitats (under trees or bushes), it is unclear whether this microhabitat selection is determined by the behaviour of the ovipositing adult females or whether it can be changed by the larvae. Our study showed that *M. hyalinus* larvae are indeed sensitive to exposure to sun and actively select the preferred shaded microhabitats: relocations were more frequent from the exposed to the shaded part of the enclosures, where more pits were constructed. A comparison of the two similar experiments in which the initial NND varied showed that the trend for habitat change was positively correlated with inter-individual distances. This result is counterintuitive and difficult to explain, as we expected that the relocation rate would rise as crowding increased.

Previous studies describing the importance of pit construction noted that post-disturbance pit reconstruction took place on the same or on the next day (e.g. Eltz, 1997; Botz *et al.*, 2003). Our data enabled us to estimate the pit construction rate on a finer scale. Construction occurred at a constant rate of 0.86% per minute, suggesting that pit construction occurs gradually. Some antlions, however, refrained from immediate pit reconstruction, preferring first either to wait or to relocate. This behaviour probably represents an estimation measure of the antlion's current site. An additional estimation measure involves constructing a small pit and only enlarging it after assessing the current site (e.g. Scharf & Ovadia, 2006).

The analysis of relocation rate revealed a number of important findings. First, in contrast to pit construction, relocation occurred more frequently at the beginning of the experiment and

then declined with time. This pattern is consistent with our initial observation that after deciding on a pit site, an antlion rarely tends to move. Second, our analysis revealed that the tendency to relocate once again is higher among antlions that had already moved large distances. This behaviour may represent two opposite strategies, depending on whether the antlions had already relocated, and it is possible that the tendency to relocate is correlated with other characters that together form a behavioural syndrome, but additional research is needed.

Some studies suggest a negative correlation between body mass and the relocation frequency (Griffiths, 1993; Prado *et al.*, 1993), but our study found no such correlation. We suggest that the explanation for the contradiction lies in differences in hunger levels. Larger antlions can exploit a wider spectrum of prey sizes and usually suffer less from hunger. Consequently, this difference should be easily observed in an experimental set-up, during which mostly larger antlions catch prey. We could not detect differences in the spatial positions of larger versus smaller antlions, in contrast to previous suggestions (McClure, 1976; Griffiths, 1992). It is possible that the spatial patterns observed, in which larger antlions inhabit the peripheries of antlion zone, emerge from differences in prey arrivals at different spatial positions in a cluster (peripheral antlions catch more prey and consequently grow faster).

In conclusion, we emphasise the importance of using null models in behavioural ecology. If regular statistics alone are applied, a simple explanation of random results could be mistakenly rejected in favour of a more complex, but erroneous explanation involving various biological interactions. Although null models are commonly applied in community ecology (Gotelli & Graves, 1996), they are not widely implemented in behavioural ecology (but see, e.g. Hein *et al.*, 2005). We suggest that antlion larvae actively select their microhabitats and relocate when exposed to unsuitable conditions. Additional research is thus needed on the oviposition behaviour of the adult females (e.g. Matsura *et al.*, 2005), as they surely have dominant roles in larval habitat selection. Finally, additional studies are needed of inter-individual differences (such as variance in the propensity to relocate). Some strategies may be correlated and therefore should be studied together as a behavioural syndrome.

## Acknowledgements

We would like to thank Matan Golan for his help in the field. The research was supported by the Israel Science Foundation Grant 1084/05 (to O.O.).

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Accepted 19 October 2007

First published online 8 February 2008