Can habitat preferences of ground-dwelling insects be a good indicator for terrestrial ecosystem recovery after an oil-spill?

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Abstract

Although oil spills are a major source of global pollution, little is known about the effects of oil spills on animals in terrestrial ecosystems. We investigated two oil spills that occurred in 1975 and 2014 in a nature reserve within a hyper-arid desert, aiming at determining the long- and short-term effects of oil spills on the habitat selection behavior of desert insects, as a possible behavioral indicator for restoration success. We performed habitat selection experiments under lab conditions, giving ground-dwelling *Mesostena angustata* beetles and *Myrmeleon hyalinus* antlion larvae choice trials between various combinations of clean and oil-contaminated soils from the new and old oil spills sites. When given a choice between clean and 2014 contaminated soil, beetles and antlions selected the clean soil. Moreover, antlions in contaminated soil from 2014 reduced their movements and did not dig pit-traps. Surprisingly, both beetles and antlions selected the 1975 contaminated soil over other soil types, and antlions dug many pit-traps in the 1975 contaminated soil.

**Implications for insect conservation:** The antlions' behavior reflects the status of its population in the field and can be used as an indicator for habitat restoration. In contrast, the beetle's behavior may not be a reliable behavioral indicator. Beetles were scarce in 1975 contaminated areas in the field. The disparity between individual behavior of beetles in the lab and field population distribution raised the question: Can old oil spills become an ecological trap for these species? This remains an open question for further research.

Introduction

In our modern era, species extinction is expedited by exposure of species and habitats to human-induced rapid environmental changes (HIREC; Sih et al. 2011), including habitat change (e.g., habitat loss, habitat degradation, fragmentation, and urbanization), invasive and exotic species, overharvesting, climate change and pollution (Horváth and Herczeg 2013; Berger-Tal and Saltz 2016). A major worldwide source of pollution is contamination emanating from oil spills that can cause widespread and massive damage to species and ecosystems. The immediate effects of oil contamination are manifested by mortality in some individuals, and direct impacts on other individuals' physiology, behavior, and ultimately, fitness. In addition, long term impacts of oil-contamination are manifested through chronic exposure of individuals to the polluted area (Hunt et al. 1973; Freedman and Hutchinson 1976; Wells et al. 1995; Al-Hashem et al. 2007; Nothers et al. 2017).

Two of the largest oil spills in the history of Israel occurred in the hyper-arid region of the southern 'Arava Valley, both within the boundaries of the protected 'Avrona Nature Reserve. In 1975 and 2014, approximately 5 and 10 million liters (respectively) of crude oil breached a degraded oil pipe and spilled into numerous ravines within the alluvial fan and the margins of 'Avrona Salt Flat (Brester 1975; Groner et al. 2015; Nothers et al. 2017). To better understand the distribution and dispersal patterns of insects in the 'Avrona Nature Reserve following the oil spills, we examined the habitat selection behavior of
individuals of two focal species: the darkling beetle (*Mesostena angustata*) and antlion larva (*Myrmeleon hyalinus*) under controlled conditions.

Darkling beetles were chosen because beetles (Coleoptera) account for ~20% of the total worldwide diversity of arthropods, and they have a significant role in maintaining soil quality, regulating populations of other invertebrates, and energy flow. Beetles from the order Coleoptera have been used as biological indicators of pollution from oil and from other components, such as sulfur, herbicides, CO$_2$, insecticides, and radioactive phosphorus (Da Rocha et al. 2010).

In general, ground beetles are in direct and constant contact with the ground and their mobility is limited to a habitat scale (i.e., they are not migrating species). They are considered excellent bioindicators for habitat perturbations such as nutrient enrichment, as some species are sensitive to pollutants and most species carefully select the habitat they occupy (Abdel-Dayem et al. 2007). Selective pressures for choosing an adequate habitat are expected to be strong in sessile, trap building, organisms. Thus, antlion larvae are an ideal subject for studying habitat selection (Orians and Wittenberger 1991; Farji-Brener 2003) and the effects of physical and microclimatological factors on foraging characteristics (Scharf et al. 2011).

We hypothesized that both beetles and antlions will distinguish between clean and contaminated soils and will prefer the clean soil. We further hypothesized that avoidance of contaminated soils will diminish over time, as the contaminating chemicals may decompose. Therefore, we predicted that the preference for clean soil will be stronger in animals choosing between clean soil and 2014 contaminated soil than the preference between clean soil and contaminated soil from 1975. Lastly, we hypothesized that antlions will consider soil consistency in addition to chemical properties when choosing a habitat. Thus, we predicted that antlions would prefer to dig their traps in sifted soils, regardless of their origin.

**Methods**

**Soil properties**

Soil samples were collected from the 'Avrona Nature Reserve in the southern 'Arava Rift Valley, Israel. The area is part of a hyper-arid desert with low annual precipitation (15–50 mm/yr.), intense solar radiation, and hot temperatures reaching up to 48°C in summer and resulting in high potential evaporation (~3000–3,500 mm/yr; Nothers et al. 2017). Shortly after the 2014 oil spill event, we collected newly contaminated soil from the dry ravines as well as soil from the ravines that were contaminated during the 1975 oil spills. We also collected clean soil from nearby dry ravines that were not contaminated. We removed large objects (stones, sticks, etc.) and determined the sediment color according to the Munsell Soil Color Charts to be "Pale Brown" for both of the controls, "Dark Brown" for the 1975 spill, and "Almost Black" for the 2014 spill (Munsell 1994). The soil texture of the 1975 and 2014 uncontaminated soils was characterized by different percentages of sand, silt, and clay (ordered from high to low). The soil at the 1975 site was classified as loamy fine sand, characterized by a somewhat higher percentage of the
coarse fraction. The soil at the 2014 site was slightly more fine-textured and was classified as sandy loam (Gordon et al. 2018).

**Study species and habitat of origin**

**Beetles** - *Mesostena angustata* *(Fabricius, 1775)*

*M. angustata* (Tenebrionidae), a darkling beetle in the subfamily Pimeliinae, is a 2 cm long, elongated black beetle. It was the most common beetle found in the ‘Avrona Nature Reserve field surveys conducted in May and September 2016–2019 (Segev et al. 2021). The species of the genus *Mesostena* are widely distributed in North Africa and the Arabian Peninsula (Bouchard et al. 2005). In Israel, it was reported in the Negev desert (Ayal and Merkl 1994; Krasnov and Mazor 2001; Groner et al. 2012; Renan and Reichmann 2015) and the ‘Arava, where it was strongly associated with semi-stable sands occasionally mixed with gravel, and with *Haloxylon persicum* and *Calligonum comosum* shrubs (Shanas 2016). Despite the general abundance and widespread distribution of *M. angustata*, scant information has been published on its ecology and behavior (Dewanand Makhan et al. 2011), and only a few previous studies have used *Mesostena* as a bioindicator. Krasnov and Mazor (2001), for example, used the relative abundance and composition of Tenebrionid species for environmental monitoring and used the *Mesostena* species as an indicator of particular environmental parameters; *M. angustata* had a negative correlation with shrub crown volume, with abundance of succulent and aphyllous shrubs, as well as with primary production levels (Krasnov and Mazor 2001).

For the habitat selection experiments, we collected 80 individuals of *M. angustata* beetles using pitfall traps from uncontaminated areas near Kibbutz Ketura, ~40 km north of ‘Avrona (29.973076/35.068996). The beetles were kept on clean local soil under lab conditions for six weeks prior to the experiments.

**Antlions** - *Myrmeleon hyalinus* *(Olivier 1811)*

In contrast to the limited knowledge on *Mesostena*, several previous habitat selection studies have been conducted on antlion larvae (e.g., Scharf & Ovadia 2006; Scharf et al. 2008b; Alcalay et al. 2015). In their larval stages, antlions are partially sessile, sit and wait predators (Arnett and Gotelli 2001). They can move up to a few meters just below the soil surface to relocate their pit. The timing of such relocations is not fixed and can fluctuate between every 5 days, up to every 70 days (Farji-Brener 2003). Myrmeleonidae are common in the ‘Avrona Nature Reserve and are represented by several species. We chose to work on the most abundant pit-building species in Israel, *Myrmeleon hyalinus* (Simon 1988), whose ecology and habitat preferences are well known (Loria et al. 2008; Scharf et al. 2008a, b; Barkae et al. 2010, 2014).

We collected 80 larvae of the pit-building *M. hyalinus* antlion from a shaded sandy area near Kibbutz Yotvata, ~30 km north of ‘Avrona (29.878334/35.075725). We measured the larva’s body length and included only individuals larger than 5mm. Prior to the experiments, we fed each antlion larva a small (<1cm) flour beetle (*Tribolium castaneum*) larva and left it on clean local soil for two days in order to standardize hunger level.
Experimental system and study design

The purpose of this experiment was to assess insect microhabitat selection among three types of soil: 2014 contaminated, 1975 contaminated, and uncontaminated control. We also used artificially colored dark and light sand to examine whether color preference influences habitat selection. Another aspect that was examined is whether soil texture influences microhabitat selection.

Paired habitat selection experiments - Beetles

To assess habitat selection in beetles, we used 80 rectangular aluminum arenas (40cm X 80cm), divided to two sections (40cm X 40cm) each filled with one liter of sieved soil (through 3 mm mesh, to create uniformity) according to the designed paired treatments (Fig. 1a):

- Clean soil and contaminated soil from the 2014 oil spill.
- Clean sand: artificially colored pale yellow, and dark brown.
- Contaminated 2014 soil and contaminated 1975 soil.
- Clean soil and contaminated soil from the 1975 oil spill.

We repeated each treatment in 20 arenas. We introduced a single *M. angustata* beetle into each arena, at the center of one of its sides (half of the beetles entered on one side and the other half on the other). The beetles were able to move freely throughout the arena. After two hours, we placed two identical shelters in the arenas – one on each substrate. We examined the beetles' location after 2, 24, 48, and 72 hours, during daytime, to see where these nocturnal beetles prefer to hide. The beetles were not fed during the experiment and were released back to their capture sites after completion of the experiment. The experiment was conducted in indoor ambient light (windows) conditions with night/day photoperiod of 11/13, and an average room temperature of 29.5°C day/24.4°C night.

Paired habitat selection experiments – Antlions

The general experimental paired design (Fig. 1b) was similar to that of the beetles with a few modifications to assess antlion habitat selection. A single *M. hyalinus* antlion larva was introduced into each arena. Each experiment lasted one week to ensure that the antlions were hungry enough to dig hunting pits. In addition to the daily intervals, at the final observation, 168 hours after the start of the experiment, we documented the antlions' locations, the number of pits and their locations and photographed a scaled picture of each arena. We then digitized the pictures with ImageJ software (Abramoff et al. 2004) and marked the tracks left by the antlions during the experiment to quantify the antlions' movement. We also measured the circle area of each pit. The average room temperature was 33.4°C day/22.2°C night.

Soil texture selection experiment - Antlions

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To evaluate the effect of soil texture on antlions' ability to dig their hunting pits, we conducted another experiment using soils from the 2014 spill site (oil and control) but with the addition of sifted/un-sifted treatments. In order to standardize particle size distribution, we sifted both the 2014 contaminated soil and clean soil using a 3mm sieve. Every treatment contained two different soil types (Fig. 1c):

- 2014 oil-contaminated soil versus clean soil, both sifted.
- 2014 oil-contaminated soil versus clean soil, both not sifted.
- Sifted versus not sifted 2014 oil-contaminated soil.

Here too, every treatment was repeated in 20 arenas. We introduced a single *M. hyalinus* antlion larva into each arena, in the center of one of the sides of the arena (6 cm from the interface between the soils), and examined the antlions' location, number of pits, and travel distance after 2, 24, 48, 72 and 168 hours, during daytime (as detailed above). The average room temperature was 33.5°C day/25.8°C night, with 11/13 light regime.

### Triple habitat selection experiment - Beetles

In order to better understand the soil preferences of the beetles, we conducted an additional microhabitat selection experiments in which we expanded the habitat choice from two to three options. We used 60 circular aluminum arenas (26 cm diameter), divided each arena into three equal parts, and placed 100 ml of three types of sifted soil (3 mm mesh) in each part: contaminated soil from the 2014 spill site, contaminated soil from the 1975 spill site, and clean soil from both sites mixed together (Fig. 1d). Afterwards, we randomly placed a single *M. angustata* in every arena, with an indoor fluorescent light regime of 12/12, and an average room temperature of 26.4°C. Prior to the beginning of the experiment, we left the beetles undisturbed for one week, allowing them to adjust to the light regime. Since *M. angustata* are nocturnal, during four dark hours of each experiment, we used a red torch to observe the beetles' position in the arena every 20 minutes, without interfering with their activity. We did not supply food or shelter during the experiment.

We repeated this experiment three times with the same beetles randomly redistributed in the arenas. The second experiment was conducted 29 days after the first one was completed, and the third experiment was conducted 10 days after the second one was completed. Between the experiments, we transferred the beetles to a clean substrate and provided food.

### Data analysis

To assert the habitat selection of each tested individual for all paired microhabitat selection experiments (for both beetles and antlions), we ran a One-proportion Z-test. This assessment is used to compare an observed proportion to a theoretical one, when there are only two categories, with a 95% confidence level. Cases in which individuals were found on the border between two soil types were excluded from the analysis.
For the triple microhabitat selection experiment, we used the Permutational Multivariate Analysis of Variance (Permanova, 999 permutations) (McArdle and Anderson 2001), followed by Tukey's Honestly Significant Difference (HSD) Test (Tukey 1949), with 95% family-wise confidence level.

To analyze the antlions' movement paths, we used a paired t-test to examine whether antlions moved longer or shorter distances in the different soil types within each tray.

**Results**

**Paired microhabitat selection experiments**

Both beetles and antlions preferred the **control over 2014 oil** at the end of the experiment (after 72 hours for beetles, after one week for antlions) (Figs. 2a,e). The beetles initial choice was the dark, 2014 contaminated microhabitat. However, after two hours, when hiding shelters were supplied, the trend reversed, and eventually, more beetles were present in the control side of the arena (NS) (Fig. 2a). Antlions gradually moved away from contaminated soil to clean soil, with 85% of them found in the control soil after one week ($P = 0.0025$) (Fig. 2e). Moreover, the antlions did not dig any hunting pits in 2014 oil, compared to 32 pits that they dug in the control, by the end of the week (Fig. 2e).

The choice between **artificially colored light and dark sand** combination (which differs only in color) was different for beetles and antlions (Figs. 2b,f). Beetles showed a non-significant preference for dark soil (up to 70% were found on the dark side after 48 hours, $P = 0.1153$). However, after 72 hours, an equal number of beetles was found in each side (50%-50%, $P=1$) (Fig. 2b). (We omitted 5 out of 80 individuals from the data analysis, as they were found exactly on the border between the two types of soil). However, antlions did not show any preference to either dark nor light sand, and the experiment ended with almost similar number of pits and antlions in both sides of the arena (NS, $P=0.8238$) (Fig. 2f).

The habitat selection results for both organisms were similar when placed in **1975 oil versus 2014 oil**, as well as **1975 oil versus control**, with a significant preference for 1975 oil in all cases (Figs. 2c,d,g,h): When given the choice between **1975 and 2014 contaminated soils**, beetles gradually shifted from the new oil (2014) to the old oil (1975); after 48 hours, 85% of individuals were in the 1975 contaminated side ($P = 0.0025$) (Fig. 2c). Similarly, the antlions preferred 1975 oil during the entire experiment; their numbers gradually increased, and at the end of the week 75% of the antlions were on the 1975 contaminated side ($P = 0.041$). The antlions did not dig any pits in soil contaminated in 2014, while they dug 13 pits in soil contaminated in 1975 (Fig. 2g).

In the **1975 oil versus control** treatment, beetles consistently preferred 1975 oil soil over control throughout the entire experiment, with high significance two hours after commencing the experiment ($P = 0.0025$) (Fig. 2d). For antlions, there was no preference during the first two days. Then the preference for 1975 oil over control gradually increased and became statistically significant by the end of the experiment with 85% of the antlions in the 1975 contaminated soil ($P = 0.0025$), and 41 pits in the 1975 oil, compared to 18 pits in the control (Fig. 2h).
Hunting pits' surface area

The pit's surface area in all the arenas shows a high negative correlation with the total number of pits, for each type of paired soils. The smallest total pit surface area is found together with the highest number of pit relocations in the arenas of 1975 soil paired vs. control. In the 1975 vs. 2014 arenas, only few large pits were found. The 2014 vs. control level was intermediate due to numerous pits with diverse sizes in control and scant pits in oil soil (Fig. 3).

Movement distances

An antlion's movement indicates its effort to find an appropriate place for digging its sit-and-wait hunting pit. In all cases, the distance traveled was relatively longer in lighter soils, whether it was control, 1975 oil, or artificial sand. The difference was only significant in the 2014 oil versus control trial ($P = 0.0092$) (Fig. 4). The short distances measured in 2014 oil may be biased: due to the dense texture of this soil, the antlions may have been forced to walk above the sediment, where they leave fewer path marks (Fig. 3, Fig. 4).

Soil texture effect

The soil texture affected the antlions' microhabitat selection and their ability to dig pits. However, antlions preferred clean soil to contaminated soil, regardless of sieving.

Similar to the paired microhabitat selection experiments, the options of control and 2014 oil revealed a high preference for staying on clean soil, both in the sifted and unsifted pair ($P = 0.0072$ Fig. 5a and $P = 0.0017$ Fig. 5b, respectively). Several pits were dug in both control soils with a slight preference for the sifted control (16 pits in unsifted and 24 pits in sifted soil), while hardly any pits were dug in 2014 oil, regardless of soil texture (Figs. 5a,b). When given the choice between sifted and unsifted 2014 contaminated soil, antlions significantly preferred the sifted texture ($P = 0.0253$). Although 75% of the antlions were present in the sifted 2014 oil soil, they did not dig a single pit, regardless of soil texture (Fig. 5c).

Triple microhabitat selection experiment

When the beetles were given the opportunity to choose between three types of soil, they clearly preferred dark, oil-contaminated soils, and avoided the clean control soil. Preference for both types of contaminated soil was significantly higher than the control ($P < 0.001$), but not different from each other ($P = 0.158$) (Fig. 6).

Discussion

Most of the methods humans use to determine habitat quality (e.g., following habitat deterioration, such as in the case of oil pollution) are based on structural components, such as geographic (e.g., geology, soil...
morphology, landscape altitude), climatic (e.g., temperature, water regime), or biotic (mostly vegetation cover) parameters. But to better understand the habitat, especially under major disturbances, we need to assess its functional components. The distribution and abundance of organisms can also reveal habitat quality, through patterns of habitat selection (Johnson 2007). Such an approach may detect conservation opportunities and assist in habitat restoration for endangered species (Van Dyck 2012). However, tracking distribution and population dynamics of species may provide trailing indicators, i.e., by the time a demographic change to the population is discernible, the habitat's condition may have already changed drastically, and the population decline may be irreversible (Kotler et al. 2016). Mobile animals can improve their condition by choosing the habitat that provides them with the best biotic and abiotic conditions. Habitat selection is an almost universal behavior among animals, affecting nearly all of the individual's subsequent choices (Orians and Wittenberger 1991). Since choosing the wrong habitat may significantly reduce survival or prevent breeding, the selection pressures on habitat selection behaviors are strong. Therefore, this behavior may provide us with a leading indicator of change. Understanding animal behavioral responses may serve as an early warning for the impacts of anthropogenic disturbances and evaluate the effectiveness of environmental management programs at their early stages (Kotler et al. 2016). In our study system, the two oil spills at the Avrona Reserve instantly altered an entire ecosystem and, in a matter of hours, created a novel and unfamiliar environment for the local flora and fauna, in general, and for ground-dwelling organisms in particular. The chemical properties (e.g., carbon contents, toxic fractions, and odor) and physical characteristics of the soil (e.g., color, viscosity, and hydrophobicity) drastically changed (Gordon et al. 2018).

As far as we know, this is the first study showing behavioral responses to oil-polluted habitats in pit-building antlion larvae and darkling beetles.

As predicted, both organisms selected clean soil when faced with a choice between clean soil and the 2014 oil-spill contaminated soil. This preference was only statistically significant for the antlions, in all the tested parameters (i.e., location, number of pits, and movement distance). But surprisingly, both species showed a clear preference for the soil contaminated by the 1975 oil spill.

The dependence of sit-and-wait predators on their immediate surroundings for successful hunting means that exogenous factors largely dictate crucial behaviors, such as foraging and habitat selection (Bruce et al. 2004; Scharf and Ovadia 2006). In this study, the antlion's behavior was examined both for foraging, signaled by pit construction, and for habitat selection, indicated by relocation distances and habitat choice (Alcalay et al. 2014). As predicted, all these behaviors showed the antlions' clear preference for clean over 2014 contaminated soil, indicating that the antlions probably considered the clean soil as a superior habitat for pit construction. Another sit-and-wait predator, the Sahastata aravaensis spider, was also recently reported to prefer clean soil over the 2014 oil soil (Gavish-Regev et al. 2022).

The beetles showed an initial attraction to dark soils in most treatments, including the artificial sand treatment. This preference may derive from two parsimonious explanations: First – the nocturnal Mesostena hide in dark places (e.g., under stones or wood) during the day, hence dark environments are
associated with safety. Because the experiment started during the day, the beetles chose the dark substrate to hide from the light. When shelters were added on both sides of the arena, this preference declined. Second - the dark soil may be a better camouflage environment for the black _Mesostena_. Consequently, this would be their initial choice, until they recognize other important components in their habitat selection. Unlike the beetles, when introduced to artificial sand, the antlions did not show any preference for color, not in the number and location of pits, nor their movement distances. The parsimonious explanation, in this case, is the fact that antlions mainly move beneath the surface. Therefore, visual predators do not see them, and camouflage is not a consideration for their safety. In nature, dark soils may change temperature and become warmer microhabitats, which may influence substrate selection in antlions (Devetak et al. 2012). However, the experiment was conducted under ambient room temperatures and the fluorescent ceiling lights do not affect soil temperature. Thus, soil color did not play any role in the antlions' habitat choice.

Another result that confirmed our predictions was that when choosing between 1975 and 2014 oil-spill contaminated soils, both studied species preferred the 1975 soil, which is lighter in color and less contaminated than the 2014 soil (Munsell 1994; Yakir and Mandelbaum 2015; Coifman 2020). Moreover, the antlions dug numerous pits in the 1975 soil, as opposed to no pits at all in the 2014 soil. _M. hyalinus_ inhabits a variety of soil types but occurs mainly in sandy soils (Barkae et al. 2012). Therefore, 'Avrona Reserve is a suitable habitat, as its soil contains 73–80% sand, 11–20% silt, and 3.5-6% clay (Gordon et al. 2018). Fine-grained sand enables the construction of large and efficient antlion pits (Farji-Brener 2003; Devetak et al. 2005). Thus, one might expect that if given a choice, antlions would prefer the 2014-control soil, which is slightly less coarse than the 1975-control soil (Gordon et al. 2018). However, the 2014 oil spill made this soil a dense and viscous environment that is very difficult to move under and dig in. In contrast, the beetles did not seem to have any problem moving on this medium.

Foraging effort among trap-building predators is reflected mainly in the amount of energy invested in the process of trap construction and maintenance (Uetz 1992; Eltz 1997). The antlion larva must decide which trap modifications to employ, whether to stay and improve the trap or relocate and construct a new pit. Because trap reconstruction is likely less costly than trap relocation (Scharf and Ovadia 2006) and relocation involves additional uncertainty including predation risk by scorpions as well as other predators (Segev et al. 2019), an alternative strategy is to enlarge trap size (Lomáscolo and Farji-Brener 2001).

Pit surface area may be an indication of several different factors: physical soil structure (e.g., grain size, density), size of the individual antlion, or its hunger level – the antlions increase their foraging effort by enlarging the pit's dimensions (Scharf et al. 2008b). In this study, hunger level and larval size were standardized before the beginning of each experiment, therefore, we assume that the differences in pit sizes and relocations result from the soil structure. We found a strong negative correlation (R = 0.9935) between pit size and the number of relocations, indicating that antlions indeed choose one of the two options mentioned above – when they chose to relocate often, their pits were small and vise-versa. Most relocations occurred in the 1975-control habitat, where the pit size was significantly smaller than in the other habitats. The surface area of pits in the 1975 spill soil did not differ from the control. This outcome
indicates similar foraging effort in old oil and control. In the 2014 vs. control trial, the pits were larger and concentrated on the control side of the arena only, and the same goes for 2014 vs. 1975, where almost no foraging efforts occurred on the 2014 oil soil, while the 1975 oil soil served the antlions the same as the control.

The results of the sieving experiment strengthen the claim that the number of pits constructed is negatively correlated with substrate density (Devetak et al. 2012). In the current experiment, sieving minimized the structural and textural difference between oil and control soils. The antlions preferred sieved 1975 contaminated soil to the original texture and managed to dig more pits in control sifted soil than in original texture control, while hardly any pits were dug in 2014 contaminated soil, regardless of whether it was sieved or not. This may indicate that sieving the contaminated soil did not render it suitable for pit digging (i.e., the grains were still too dense, too sticky or too big for digging), but it enabled more movement than unsieved contaminated soil. These results are in line with a field population survey conducted in 'Avrona (Segev et al. 2021) in which no pits were found in the first year after the 2014 oil spill. Pits were constructed only after comprehensive tillage of the contaminated ravines, and even more pits were documented after a second tillage, four years after the oil spill.

Surprisingly, both beetles and antlions preferred the 1975 contaminated soil even when the alternative choice was clean soil. For antlions, this result agrees with a population field survey (Segev et al. 2021), which showed several pits in the 1975 oil plots, compared to few in the 1975 control. Moreover, ants, which are the major food source for the antlions (Simon 1988) were also abundant in 1975 oil-spill plots, in comparison to their adjacent clean control plots.

Not only did the beetles in the lab prefer 1975 contaminated soil, but the beetles subjected to the triple choice lab experiment spent significantly less time in the control, compared to both contaminated soil options. However, this was not the case for the beetles in the field; _M. angustata_ was least abundant in the 1975 oil plot compared to all other plots (clean or contaminated 2014). The fact that our lab experiment findings contradict the field observation for this species may suggest that the beetles' behavior pushes them into an ecological trap (Robertson and Hutto 2006; Robertson et al. 2013). The beetles are attracted to the 1975 oil-contaminated area, although their survival rate on this soil is lower (maybe because the food supply is insufficient, or because the soil is not appropriate for laying eggs or for larval development). In the experiment, we did not check for the fitness consequences of choosing a particular soil, therefore, the question of whether or not the beetles' preference represents an ecological trap requires further in-depth examination.

The attraction of beetles and antlions to the 1975 contaminated soil is difficult to explain based on soil characteristics. When comparing the 1975 contaminated soil to the corresponding control soil, the pH, conductivity values, and bacterial content are not significantly different (Girsowicz et al. 2018; Angel 2019). Moreover, the mean values of water capacity of all sediments, including 1975 oil soil, are very low (below 3%) (Stavi and Rosenzweig 2020). Possibly, properties of the 1975 contaminated soil enable antlions to construct their pitfall traps more efficiently. For example, maybe this moderately contaminated
soil is better at supporting the pitfall traps because of strong particle adherence. This direction needs further investigation. The non-contaminated soils are purely hydrophilic, and both contaminated soils are very hydrophobic underground, but since the contaminated 1975 soil in the field is covered by a thin layer of clean soil that was deposited by wind and floods over the last decades, the 1975 surface hydrophilic measurements are more similar to the control (Gordon et al. 2018). In the Avrona habitat, organisms experience a hyper-arid climate, a large temperature fluctuation between day and night, and extreme temperatures during summertime. Soil surface temperature can reach a maximum of 70°C (Goldreich and Karni 2001), while darker soils are expected to be even hotter. However, under lab conditions, the temperatures of the different soils are comparable and should not affect the habitat selection of beetles and antlions. Another cautious hypothesis is that the animals may be attracted to the 1975 spill substrate because of the oil itself. Many insects have extremely developed olfactory senses, used not only for foraging, but also for breeding, navigation, communication, and alarm (Karlson 1959; Zhang et al. 2015; Aldrich and Zhang 2016). Even though the 1975 contamination is more than 40 years old and most of the original volatile fractions of the crude oil have already disappeared, a small quantity of volatiles are constantly created by biodegrading bacteria (Girsowicz et al. 2018), and the smell of oil is still noticeable even by humans (personal observation). These contaminated sediments also contain a high concentration of total organic carbons (TOC), mainly long and heavy fractions (Yakir and Mandelbaum 2015). The presence of the carbonate fraction may provide supplementary or surrogate information when antlions consider sediment density, but little is known about the mechanism of how the antlion distinguishes between different substrate densities (Devetak et al. 2012), and even less is known about the reaction of beetles to soil densities or the smell of oil. Further research is needed on these questions.

Behavioral indicators can be a valuable tool in active conservation management (Berger-Tal et al. 2011), helping to efficiently determine the ecological status of an ecosystem and assist in future remediation attempts of disturbed areas. However, the behavior exhibited by individuals of the focal species must reliably represent the changes found in the field. Thus, according to the results of this habitat selection experiments, the antlions' behavior reflects the status of its population in the field. In addition, the antlions' behavior also fits the distribution of its most common prey – ants - and can therefore be considered a good indicator for system recovery (provided there was no surface intervention, such as tillage). In contrast, Mesostena's behavior may not be a reliable behavioral indicator. The disparity between the individual's behavior in the lab and field population distribution raised the question: Is this situation indicative of an ecological trap? This remains an open question for further research.

Declarations

Funding

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None conflict of interests

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Figures
Figure 1

The experimental design, details of the four lab experiments, including the different soil combinations in the arenas, and the studied species; Mesostena angustata beetle, and Myrmeleon hyalinus antlion larva.
Figure 2

The experimental design, details of the four lab experiments, including the different soil combinations in the arenas, and the studied species; Mesostena angustata beetle, and Myrmeleon hyalinus antlion larva.
Figure 3

Left: Digitized photo of an arena, demonstrating the pits' surface area and one movement trail of an antlion. Right: The average pit surface area (cm) vs. the total number of pit relocations that were counted and measured during the experiment in three different microhabitats: 1975 oil vs. 1975 control (yellow), 2014 oil vs. 2014 control (red), and 2014 oil vs. 1975 oil (blue). Arenas yielding zero pits are excluded. Trend line $R^2 = 0.9935$. Error bars are SE.

Figure 4

The average distance antlions travelled in the different microhabitat combinations. Dark or light soil is relative to the paired soil (i.e., 1975 soil is light in comparison to 2014 soil, but dark relative to control); means ± SE are presented. (T-test, $P<0.01$ represented by two asterisks.)
Figure 5

The location of the antlions in the arena throughout the experiment. Each chart represents a different microhabitat combination: (a) 2014 oil versus control, both soils are unsifted, (b) 2014 oil versus control, both soils are sifted, (c, photo) sifted 2014 oil versus unsifted 2014 oil. The numbers surrounded by circles near the relevant microhabitat line represent the number of pits after one week. (Fisher's exact test, P<0.05 denoted by one asterisk)

Figure 6

Left: Photo of a single arena, containing the three soil types and a single beetle (on control soil). Right: The proportion of beetles choosing the favorable microhabitat when given a choice between three soil types: 2014 oil, 1975 oil, and clean control; A and B express significant difference between microhabitats. Data aggregates four-hour experiments repeated three times, in separate days