Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 09, pp.73541-73544, September, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

THE ROLE OF OLFACTORY CUES IN SETTLING BEHAVIOR OF THE ISOPOD *ARMADILLIDIUM VULGARE* (ISOPODA: ARMADILLIDIIDAE)

[1*]Dillan Brown and [2,*]Marianne W. Robertson

[1]Bachelor of Science, Department of Biology, Natural Science and Mathematics, Division, Millikin University
[2]Professor of Biology, Department of Biology, Natural Science and Mathematics Division, Millikin University

ARTICLE INFO

Article History:
Received 09th June, 2018
Received in revised form
24th July, 2018
Accepted 15th August, 2018
Published online 30th September, 2018

Key Words:
Olfaction, Pheromones,
Settling Behavior,
Aggregation, Isopods,
Armadillidium vulgare.

ABSTRACT

The aggregation behavior of isopods like *Armadillidium vulgare* functions to limit desiccation and reduce metabolic rates. Aggregation may also function as a cooperative form of communication enabling *A . vulgare* to relay habitat quality to conspecifics. Grouping is stimulated by social interactions and physical cues and aggregation pheromones in feces. This study examines whether chemical cues, in the absence of social or physical cues, will influence settling behavior in *A. vulgare*. We determined whether a lone conspecific would spend more time on the side of an arena with no previous isopod cues versus the side that previously held conspecifics but was absent any discriminating physical cues, such as disturbed earth or exuviae. We also determined whether the number of previous occupants influenced settling behavior.A Chi-Square Binomial test, and an ANOVA followed by a Tukey's Post Hoc Test were used for data analyses. Isopods spent more time on the side where five or ten previous conspecifics had been housed but not a single conspecific. Lone isopod movements across a median line decreased in arenas that previously held occupants. Thus, olfactory cues from conspecifics can stimulate settling behavior, and lone conspecifics prefer an area recently abandoned by a grouping of conspecifics versus a previously unoccupied area. Settling behavior appears to be stimulated by chemo-sensitive stimuli from conspecifics in *A. vulgare* even in the absence of conspecifics or physical cues.

Citation: *Dillan Brown and Marianne W. Robertson.* 2018. "The role of olfactory cues in settling behavior of the isopod *Armadillidium vulgare* (Isopoda: Armadillidiidae)", *International Journal of Current Research*, 10, (09), 73541-73544.

INTRODUCTION

Isopods have a role in decomposition of organic matter such as leaf litter, decayed wood, fungi, and bacteria (Broly *et al*., 2012; Broly and Deneubourg, 2015; Devigne *et al*., 2011). They spread microbiota and create habitats for microbiotic populations via their feces (Broly *et al*., 2012). Many isopods, such as *Armadillidium vulgare*,are gregarious and prefer to settle in groups (Beauché and Richard, 2013; Broly and Deneubourg, 2015). Gregarious isopod species aggregate by using social and physical interactions/cues (Broly *et al*., 2012; Devigne *et al*., 2011). Cohesion is also influenced by aggregation pheromones thought to be present in isopod feces (Beauché and Richard, 2013; Broly *et al*., 2012; Devigne *et al*., 2011). Crustaceans detect olfactory cues, such as aggregation pheromones, using chemoreceptors in their antennulae that excite neuromuscular interactions in the olfactory lobes and cause the release of gamma-aminobutyric acid (Smigel and Gibbs, 2008; Blinova and Cherkashin, 2012; Corey *et al*., 2013;). Terrestrial isopods have two pairs of antennae - small vestigial primary antennae and large functioning secondary antennae (Kenning and Harzsch, 2013). The reduced neural connectivity between the chemo-sensitive hairs (sensilla) and the deutocerebrum suggests functional loss and is hypothesized to result from the evolutionary transition from water to land (Kenning and Harzsch, 2013).

***Corresponding author:* Dillan Brown**
Bachelor of Science, Department of Biology, Natural Science and Mathematics, Division, Millikin University
DOI: https://doi.org/10.24941/ijcr.31476.09.2018

Aggregation is a social adaptation useful for terrestrial isopods in preventing desiccation, which can be a primary evolutionary challenge when transitioning from aquatic to terrestrial life (Beauché and Richard, 2013; Rojas *et al*., 2014). However, *A. vulgare* is well adapted to terrestrial life having evolved a thicker cuticle and the ability to conglobate; thus, limiting desiccation is unlikely to be the main selective pressure acting to maintain aggregative behaviors (Beauché and Richard, 2013; Smigel and Gibbs, 2008). Aggregation may be maintained in *A. vulgare* due to reproductive benefits. Aggregation quickens vitellogenesis, aids in synchronizing female molting cycles, and may decrease time required for mate location (Beauché and Richard, 2013).

Grouping is also an effective way of establishing habitat quality. *A. vulgare* prefer to settle in areas currently or recently inhabited by conspecifics (Robinson *et al*., 2011). *A. vulgare* are attracted to chemical and physical signs of previous occupancy, such as feces or molted carapace fragments. This preference is hypothesized to involve the sharing of information among conspecifics regarding the presence of food and mates, high humidity, moderate temperatures, and low light levels (Devigne *et al*., 2011; Robinson *et al*., 2011). Animals benefit from detecting quality habitat patches prior to exhaustion of resources through energy and water loss (Zollner and Lima, 1999). Groups do not always remain together, and dispersal from aggregate groups is common. Individual dispersal from an aggregated group can be stimulated by numerous external and/or internal factors such as feeding efficiency, predatory stimuli, and desiccation avoidance (Broly *et al*., 2012; Broly and Deneubourg, 2015). Dispersal, especially over long distances, is a high cost decision among terrestrial animals (Zollner and Lima, 1999). Settling behavior of isopods is thought to be influenced by the same chemical cues involved in group cohesion, specifically aggregation pheromones (Robinson *et al*., 2011).

Examining the factors involved in aggregation and settling behaviors in isopods improves our understanding of the roles these detritivores have on the ecosystem, and their potential use as bio-indicators for monitoring the accumulation of pollutants (Broly *et al*., 2012; Devigne *et al*., 2011). Parasitic infection in *A. vulgare* can disrupt gregarious behavior by infecting the brain and nerve chain (Temple and Richard, 2015). For example, infection by *Wolbachia* bacteria, or *Toxoplasma gondii* protozoa results in behavioral alterations in crustaceans' ability to perceive chemoreceptive stimuli. This is of specific importance because invertebrates infected with *Wolbachia* can be intermediate hosts of vertebrate infections, which hold broader ecological and evolutionary consequences post-infection (Temple and Richard, 2015). The stability of aggregate groups remains a poorly understood phenomenon, and this study serves to investigate the relationships between physical and chemical cues involved in stimulating settling behavior.

The settling of *A. vulgare* was observed in the absence of any discriminating physical stimuli to determine whether physical cues, such as disturbed soil or exuviae, were necessary to invoke settling behavior or whether olfactory stimuli alone might stimulate settling. Since isopods prefer areas containing conspecific cues (Robinson *et al*., 2011), it was hypothesized that individuals placed in control arenas with no previous occupants would not exhibit any directional bias, whereas individuals placed in the experimental arenas which had previously housed conspecifics would favor the side of the arena that previously contained conspecifics. Thus, as time spent on a particular side of an arena increases and settling occurs, the number of crosses over a median line should decrease. Therefore, individuals placed in a previously unoccupied arena should display more crosses between sides than individuals placed in an arena that previously contained conspecific(s). Juvenile isopods did not react differently to areas that varied in conspecific abundance (Robinson *et al*., 2011). Therefore, it was also hypothesized that there would be no significant difference in the number of crosses over the midline between individuals placed in arenas containing one conspecific, five conspecifics, or ten conspecifics.

MATERIALS AND METHODS

Approximately 200 *A. vulgare* were obtained from the Carolina Biological Supply Company. Upon arrival, individuals were placed in a 20 cm W x 33 cm L x 10 cm H plastic housing container with approximately 300 g of Jobes natural and organic seed starting potting mix moistened with distilled water. Isopods were fed carrot slices *Ad libitum*. Isopods were not screened based on sex, but individuals between 0.7 cm - 1.0 cm were used because this indicates sexual maturity (Beauché and Richard, 2013). Only individuals who possessed both secondary antennae were used. We were not concerned with the primary antennae since they are vestigial (Kenning and Harzsch, 2013). Research was conducted in the Animal Behavior Laboratory, Millikin University, Decatur, IL, and all trials were conducted at random between 0900 and 1900. Although IACUC protocol does not apply to invertebrates, we followed Animal Care and Use Guidelines required by IACUC for vertebrates.

Control isopods were tested by adding approximately 7.0 g of soil to a 14.5 cm diameter x 1.0 cm H petri dish arena with a lid. The same brand of soil, moistened with distilled water, was used in housing and testing arenas. The testing arena was placed on paper with a circular outline and a line extending outward past the edges of the dish to denote the midline of the arena. An isopod was removed from its housing container and placed in the center of the testing arena oriented parallel with the median line to control for possible situational bias. The time that passed before the isopod crossed the median line and the side it crossed to were recorded. These data were recorded for 1.0 h. After 1.0 h, the total number of crosses were counted, and the total time spent on each side of the arena was calculated. To control for fatigue, isopods were never tested more than once per day. The dish and lid were washed with warm water and the soil was replaced between each trial to remove any residual contamination. Control trials (n = 30) had no previous occupants; the isopod being tested was the only one in the arena.

There were three experimental groups. Thirty trials were conducted in arenas that previously contained one isopod, 30 trials were conducted in arenas that previously contained five isopods, and 30 trials were conducted in arenas that previously contained ten isopods. For all experimental groups, occupant(s) were added to the testing arena, which contained 7.0 g of moistened, soil and were isolated beneath a 6 cm diameter x 7.3 cm h sterilized plastic cup for 24 h. A 3.0 mm hole was made in the top of each cup for oxygen flow, and clear tape was used to secure the cup firmly to the bottom of the petri dish, thus ensuring the isopods could not exit underneath the cup. The position of the cup in the arena (left versus right of midline) was randomized for each trial. Isopods remained isolated for 24 h. After 24 h, the cup and isopods were removed from the arena and the disturbed soil was flattened to make it physically indistinguishable from the rest of the soil. Special care was taken not to mix the soil exposed to previous isopods with unexposed soil in the arena. Finally, a novel isopod was placed in the center of the petri dish, and isopod movements across the median line were recorded and timed following the same procedures used for control isopods.

For each control and experimental trial, the time (sec) the isopod spent on each side of the arena and the number of crossings over the midline were recorded. The total amount of time spent on the left side versus the right side were then used to calculate the percentage of time spent on each side. A Chi-square binomial test was used to determine whether there was a bias in which side isopods spent the majority of their time. Additionally, an ANOVA followed by a Tukey's Post Hoc Test was used to analyze the number of times isopods crossed over the median line to determine whether there was a difference in the number of crosses between groups.

RESULTS

Isopods placed in control arenas with no previous conspecifics did not have a bias towards a particular side; rather, they were statistically just as likely to spend the majority of their time on either side of the arena ($p = 0.858$) (Fig. 1). Isopods placed in arenas that previously held one isopod were also statistically as likely to spend the majority of their time on either side ($p = 0.585$) (Fig. 1). Isopods placed in arenas that previously held five preoccupants statistically favored the side of the arena that held previous isopods ($p = 0.043$) (Fig. 1). Also, isopods placed in arenas that previously held ten preoccupants statistically favored the side of the arena that held previous isopods ($p = 0.001$) (Fig. 1).

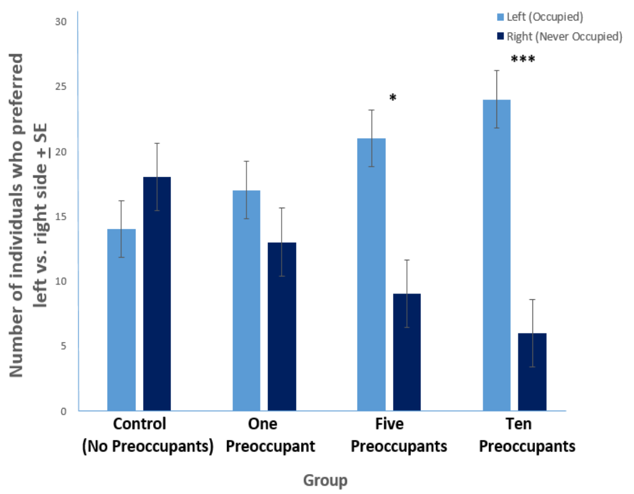

Figure 1. Number of isopods (*Armadillidium vulgare*) that preferred the left side versus the right side of the arena tested using a Chi-square binomial test.

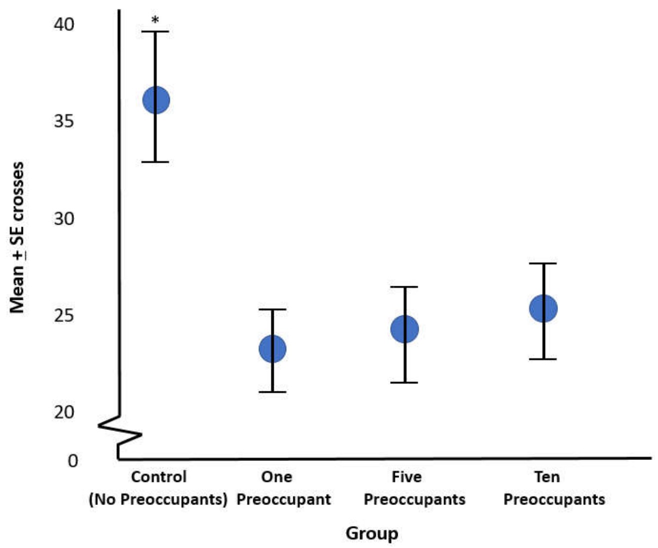

Figure 2. Mean difference in the number of crosses over the median line of the arena by isopods (*Armadillidium vulgare*) tested using an ANOVA followed by Tukey's post hoc test.

Insert Figure 1 Here: Isopods placed in the control arenas crossed over the median line significantly more frequently than isopods placed in arenas containing one, five, or ten preoccupants ($p< 0.05$) (Fig. 2). The number of crosses did not differ significantly between the three experimental groups (one, five, and ten preoccupants) ($p> 0.05$) (Fig. 2).

DISCUSSION

The hypothesis that individuals placed in control arenas with no previous occupants would not show bias towards any particular side, left or right, was supported. The hypothesis that isopods placed in arenas with either one, five, or ten previous occupants would prefer the side of the preoccupants was partially supported. Isopods placed in arenas with one preoccupant did not show a preference for the side that held the conspecific. However, a significant preference was observed for the side that had recently held conspecifics in arenas that contained five or ten preoccupants.

This difference in behavior between individuals placed in arenas with one preoccupant versus five or ten cannot be explained by the limited amount of olfactory stimuli one isopod could produce. While the effectiveness of perception of chemical stimuli is affected by intensity (Blinova and Cherkashin, 2012), it cannot be said that the amount of stimuli present affected the results due to the statistical similarity between all three experimental groups concerning the number of crosses over the median line. It is possible that the difference in preference between isopods placed in arenas with one versus five and ten preoccupants is due to not sexing the isopods. Isopods prefer areas that contain a conspecific of the opposite sex, but show no preference for areas that contain a conspecific of the same sex (Beauché and Richard, 2013). In arenas with 5 and 10 preoccupants, the odds of having conspecifics of the opposite sex present on the side of preoccupancy increase compared to a single preoccupant.

The hypothesis that individuals would cross over the median line more often when placed in control arenas versus arenas which contained preoccupants was supported. The hypothesis that no significant difference in the number of crosses would be observed between individuals placed in arenas with one, five, or ten preoccupants was also supported. Since no physical cues were present, this provides evidence that olfactory cues stimulated settling behavior in experimental groups.

Isopods represent a sophisticated transition from aquatic to terrestrial environments (Broly *et al*., 2012). The brood pouch in Oniscidae, conglobation in Armadillidiidae, and potentially co-opted aggregation pheromones to relay habitat quality to conspecifics are all adaptations by isopods for resisting desiccation (Robinson *et al*., 2011; Smigel and Gibbs, 2008; Wolff, 2009). The main evolutionary challenge for isopods transitioning from water to land was preventing desiccation (Beauché and Richard, 2013; Devigne *et al*., 2011; Rojas *et al*., 2014). The ability to detect aggregation pheromones could benefit individuals by indicating the presence of mates or cooperatively communicating prime habitat to conspecifics (Beauché and Richard, 2013; Robinson *et al*., 2011).

Conclusion

The isopod *A. vulgare* responded to olfactory cues, in the absence of possible physical cure, left by conspecifics which suggests important, previously unexplored behavior involved in their settling behavior. This research presents empirical evidence of chemo-stimulated settling in *A. vulgare* which further supports the relationship between aggregation behavior and olfactory cues between conspecifics.

Acknowledgment

The authors thank Dr. Travis Wilcoxen for statistical analyses and Sue James for clerical assistance.

Conflict of Interest Statement: The authors have no conflict of interest involved in the submission of this manuscript to IJCR.

Funding Statement: The authors are grateful to the Millikin University Biology Department for funding this research.

REFERENCES

Beauché, F. and Richard, F.J. 2013. The best timing of mate search in *Armadillidium vulgare* (Isopoda, Oniscidea). *PLoS ONE*. 8, 1-9.

Blinova, N.K. and Cherkashin, S.A. 2012. The olfactory system of crustaceans as a model for ecologo-toxicological studies. *Journal of Evolutionary Biochemistry and Physiology*. 48, 155-165.

Broly, P. and Deneubourg, J.L. 2015. Behavioral contagion explains group cohesion in a social crustacean. *PLoS Computation Biology*. 11, 1-18.

Broly, P., Mullier, R., Deneubourg, J.L. and Devigne, C. 2012. Aggregation in woodlice: social interaction and density effects. *Zoo Keys*. 176, 133-144.

Corey, E.A., Bobkov, Y., Ukhanov, K. and Ache, B.W. 2013. Ionotropic crustacean olfactory receptors. *PLoS ONE*. 8, 1-10.

Devigne, C., Broly, P. and Deneubourg, J.L. 2011. Individual preferences and social interactions determine the aggregation of woodlice. *PLoS ONE*. 6, 1-14.

Kenning, M. and Harzsch, S. 2013. Brain anatomy of the marine isopod *Saduria entomon* Linnaeus, 1758 (Valvifera, Isopoda) with special emphasis on the olfactory pathway. *Frontiers in Neuroanatomy*. 7, 1-14.

Robinson, B.G., Larsen, K.W. and Kerr, H.J. 2011. Natal experience and conspecifics influence the settling behavior of the juvenile terrestrial isopod *Armadillidium vulgare. Canadian Journal of Zoology*. 89: 661-667.

Rojas, J.M., Castillo, S.B., Folguera, G., Abades, S. and Bozinovic, F. 2014. Coping with daily thermal variability: behavioral performance of an ectotherm model in a warming world. *PLo S ONE*. 9, 1-9.

Smigel, J.T. and Gibbs, A.G. 2008. Conglobation in the pill bug, *Armadillidium vulgare*, as a water conservation mechanism. *Journal of Insect Science*. 8, 1536-2442

Templé, N. and Richard, F.J. 2015. Intra-cellular bacterial infections affect learning and memory capacities of an invertebrate. *Frontiers in Zoology*. 12, 1-6.

Wolff, C. 2009. The embryonic development of the malacostracan crustacean *Porcellio scaber* (Isopoda, Oniscidea). *Dev Genes Evol*. 219, 545-564.

Zollner, P.A. and Lima, S.L. 1999. Search strategies for landscape-level interpatch movements. *Ecology*. 80, 1019-1030.

****