Marking Terrestrial Isopods for Recapture: Not as Easy as You Might Think

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INTRODUCTION

In order to study the metapopulation dynamics for the terrestrial isopod *Armadillidium vulgare*, commonly known as roly polies, across the campus of Grossmont College, we wanted to use mark-recapture techniques. However, previous work ruled out nail polish and paint pen.[1, 2] Other marking methods used on insects[3] are unsuitable due to the small size, porous exoskeleton, and lifelong molting patterns of these isopods, which are crustaceans. It is crucial that a marking method retain fidelity (the mark stays on the individual) and does not affect survival or behavior. We are interested in a mark that lasts months, as questions about which individuals migrate between what types of habitat patches and why they do could require longer-term identification. A recent publication estimated potential dispersal of *A. vulgare* around buildings at a university[4]. They found that individuals went as far as 50m in 8 days. They used paint pen marks, however, so dispersal was underestimated and their work did not investigate the types of habitats that terrestrial isopods may preferentially move into or out of.

→ At right is a campus map of Grossmont College, showing the pattern of landscaping areas separated by concrete pathways and buildings. Some of our previous work has shown that isopods could cross campus in about 11 hours if they walked continuously. Image from Google maps.

MARKING METHODS TESTED

Nail Polish and Paint

Nail polish marks were difficult to apply and displayed poor retention (<25% over one month) as well as immediately modifying behavior (individuals froze and remained immobile). Sharpie™ marker was toxic within minutes, but using a paint pen to carefully place a dot on the exoskeleton seemed feasible.

Color-dyed food

Potato slices were dyed with green food coloring. *A. vulgare* eating the food were easily detected by eye due to the color. However, after eating other foods the color was lost after one to two weeks. Roly polies refused to eat other potentially staining foods such as beet and turmeric.

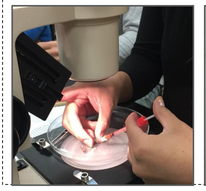
We attempted multiple potential marking methods and results showed that only leg removal and paint marks demonstrated potential for being a suitable method.

Leg Removal

Leg removal was easily detectable as a mark and has the possibility to identify 14 different groups (7 pairs of legs). In addition, after the first molt the removed leg regrew shorter and slightly white as compared to a normal leg so the mark was still detectable.

Latex Injection

Injection of red visible implant elastomer (Northwest Marine Technologies, Inc) into *Armadillidium vulgare* was unsuccessful with 50% mortality rate after one week.



ABSTRACT

The terrestrial isopod species *Armadillidium vulgare* is native to Europe but has naturalized globally. We were interested in investigating their dispersal ability in a fragmented urban landscape where suitable habitat patches are separated by roads, paths and sidewalks. Mark-recapture methods are commonly used in such studies, however no suitable marking method has been published for these small crustaceans in part because the exoskeleton is regularly molted. A marking method needs to be both effective and safe for the focal species. We piloted multiple marking methods which included marking the exoskeleton with paint, feeding the isopods with color-dyed food, latex injection, and removing one leg. Preliminary results showed that marking the exoskeleton with paint and leg removal showed promise, therefore, we performed a replicated controlled experiment testing these two methods. Leg removal was 100% detectable through week six, whereas only 20% of the paint marks were detectable by week four. Both methods also showed significantly higher mortality rates than the control. We concluded that neither method is an appropriate marking method for our mark-recapture study. Therefore, further experiments are planned.

METHODS

- Each of 10 10x5x4 cm plastic containers with lids were drilled on four sides with 1mm holes. 10g sterile potting soil was added and spritzed with water to moisten. Ample slices of carrot were added to containers for food and replaced weekly during the experiment. Moisture levels were kept high enough to allow condensation to form inside the container. The containers were kept on a shaded bench in a greenhouse under natural day/night conditions. Locations of containers on the experimental tray were randomized weekly.
- Three adult *A. vulgare* that had been raised in captivity were randomly assigned to control, paint, or leg removal treatments for each container. Each container had only male or only female individuals with five replicates of each for a total of 30 individuals.
- To establish the leg removal treatments, the fourth leg of *A. vulgare* on its right side was pulled off carefully at the base with forceps. To establish paint treatments, the dorsal exoskeleton of *A. vulgare* was marked in the center with one dot using a white fine-point Sharpie™ oil-based opaque paint marker. Control isopods were handled and manipulated in a similar manner to the treatment individuals prior to being added to the containers.
- Fidelity of marks and survival rate were monitored weekly for seven weeks starting 7/7/18.

RESULTS

Mark Fidelity

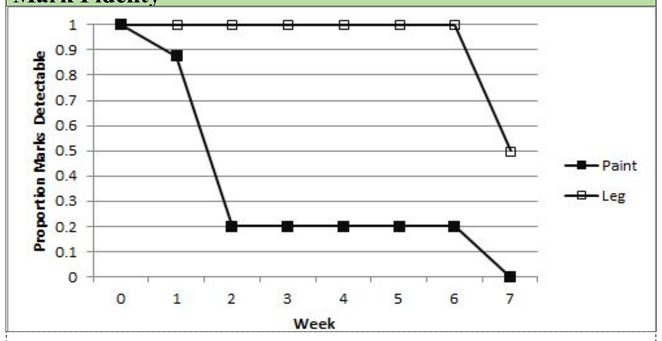


Fig. 1 Mark fidelity for leg removal and paint was observed over the span of seven weeks for *A. vulgare*. Leg removal was 100% detectable through week six (open symbols), whereas only 20% of paint marks were detectable through week four (filled symbols).

Survival

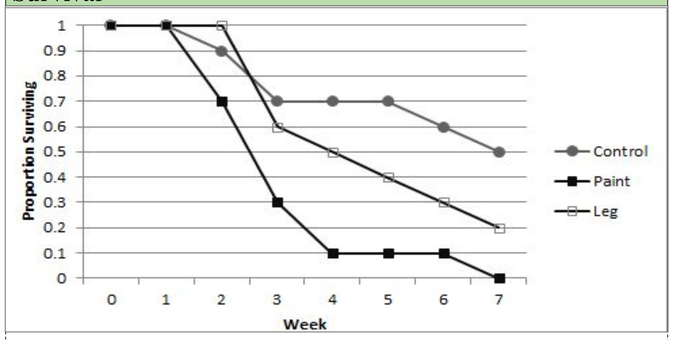


Fig. 2 Mortality rates for *A. vulgare* was observed over the span of seven weeks. Mortality rates for both marking methods (square symbols) revealed to be significantly different (X^2 test for goodness of fit, $X^2 = 6.8$, $p = 0.0009$) than the control group (circular symbols).

CONCLUSIONS

- Leg removal demonstrated 100% of mark fidelity through week six with individuals still identifiable after one molt, while paint marks showed 80% loss by week two.
- The number of survivors for both leg removal and paint marks was significantly lower compared to the control.
- We conclude that neither method is suitable for a mark-recapture experiment due to the negative effects on survival or lack of mark fidelity.

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REFERENCES

1. Tuf, I. H. et al. (2013) Suitability of nail polish for marking the common rough woodlouse, *Porcellio scaber* (Oniscidea). Acta. Soc. Zool. Bohem. 77: 159-163.
2. Drahokoupilová, T., & Tuf, I. H. (2012). The effect of external marking on the behaviour of the common pill woodlouse *Armadillidium vulgare*. ZooKeys: 145–154.
3. Hagler, J. R. & Jackson, C. J. (2001) Methods for marking insects: current techniques and future prospects. Ann. Rev. Entomol. 46: 511-43.
4. Furukawa, T. et al. (2017) Estimation of the walking ability of an exotic terrestrial isopod *Armadillidium vulgare* Latreille by field and laboratory measurements. Edaphologia 101: 27-32.