Testing and comparing the effectiveness of different leech repellents

Zoë Goodrow

University of Michigan Biological Station
EEB 381 General Ecology
August 18, 2016
Dr. Shannon Pelini

We studied the difference in effectiveness among different leech repellents, including eucalyptus oil, insect repellent with DEET, household vinegar, and tobacco leaves. Specifically, we analyzed the differences in percentage of individual *Erpobdella ocura* attachment and the time distribution of attachments. We recorded the number of attachments to a food source covered in a deterrent and categorized attachments by three equal time intervals for 30 minutes. There was a significant difference in percent attachment between the control and three of the deterrents: eucalyptus oil, tobacco leaves, and insect repellent with DEET ($p<0.0001$). There was no significant difference in deterrence among these three effective treatments. There was a difference in time distribution of attachments among ineffective and effective treatments. These results show a significant difference in the effectiveness of *E. obscura* deterrence of suggested repellents. The knowledge obtained from these results could be used to draw conclusions on the mechanisms this species uses to hunt prey and could be useful for future ecological studies.

Testing and comparing the effectiveness of different leech repellents

Zoe Goodrow

zgoodrow@umich.edu

University of Michigan

Dr. Shannon Pelini

12 August 2016

Abstract

We studied the difference in effectiveness among different leech repellents, including eucalyptus oil, insect repellent with DEET, household vinegar, and tobacco leaves. Specifically, we analyzed the differences in percentage of individual *Erpobdella ocura* attachment and the time distribution of attachments. We recorded the number of attachments to a food source covered in a deterrent and categorized attachments by three equal time intervals for 30 minutes. There was a significant difference in percent attachment between the control and three of the deterrents: eucalyptus oil, tobacco leaves, and insect repellent with DEET ($p<0.0001$). There was no significant difference in deterrence among these three effective treatments. There was a difference in time distribution of attachments among ineffective and effective treatments. These results show a significant difference in the effectiveness of *E. obscura* deterrence of suggested repellents. The knowledge obtained from these results could be used to draw conclusions on the mechanisms this species uses to hunt prey and could be useful for future ecological studies.

Keywords:
DEET, deterrence, *Erpobdella ocura,* eucalyptus oil, repellent, tobacco, vinegar

Introduction

Among the 600 species of leeches (*Hirudinea)* that are found worldwide, approximately 40 are found in Michigan (Mooser et al., 2009). Most species of leeches are found in fresh water habitats, but some inhabit terrestrial or salt water environments. A majority of leeches feed on the blood of other animals, which makes them "hematophagous". These leeches latch onto their prey with their razor-like teeth and fill a reservoir in their body full of blood, releasing itself when it is full. Other species are not blood-suckers, and eat either invertebrate tissue or invertebrates whole (Mooser et al., 2009). While leeches can be a pest to humans, they are ecologically valuable. They serve as indicators to the chemistry, biodiversity, and overall health of aquatic systems (Mooser, et al., 2009). They help control macroinvertebrate populations and leeches are preyed on by most wetland omnivores, including fishes, turtles, and birds. Traditionally leeches have been used medicinally, for therapy and bloodletting (Whitaker et al., 2004). Humans also catch and sell leeches, typically *Erpobdella obscura,* for fishing bait. Contrary to popular belief, leeches cannot transmit parasites to humans. However, because of the anticoagulant leeches use to prey, their bites leave open sores that can bleed for hours (Mooser, et al., 2009). Unfortunately, how to repel these creatures is less understood than their ecological and economical significance.

Many methods have been tested to deter leeches from latching onto humans. Among these repellents are eucalyptus oil, insect repellent with *N, N diethyl-3-methylbenzamide* (DEET), tobacco leaves, and vinegar. Eucalyptus has been studied as a leech repellent because insects do not like the smell of the oil (Kirton, 2005). Secondly, insect repellents with DEET repel leeches because of the way the chemicals physically interfere with the receptors on insects' antennae (Leal, 2014). Tobacco leaves have been studied as a deterrent because of the way the nicotine intoxicates leeches, making it difficult for them to hunt (Bahmani, 2014). Because leeches thrive in neutral pH levels, the acidity of household vinegar is

said to be a useful repellent (Mills & Schindler, 1986). While these remedies have been recommended and somewhat studied, they have not been comparatively studied.

The purpose of this study is to compare the four previously mentioned suggested leech repellents; insect repellent with DEET, tobacco leaves, eucalyptus oil, and household vinegar, and determine which is most effective at deterring the species *Erpobdella obscura.* *E. obscura* is a common leech found in rivers surrounding the University of Michigan Biological Station, near Pellston, Michigan. While the hunting mechanism of this species is unknown, most leech species hunt their prey primarily by detecting blood with its chemoreceptors and others hunt by sensing water vibrations (Ginsburg, 1998). We hypothesize that if *E. obscura* detect prey their chemoreceptors, then insect repellent with DEET will be the most effective deterrent. We predict, assuming that *E. obscura* hunt in the same mechanism that most leeches do, that the chemical effects of the DEET will interfere with the way the chemoreceptors detect blood.

Materials and Methods

We purchased 100 leeches from Young's Bait Shop in Alanson, MI (45.443661 -84.786969). These leeches, which we identified as *Erpobdella obscura,* were wild-caught from the Crooked River, also located in Alanson. We divided the 100 leeches evenly between the five treatments; REPEL lemon infused eucalyptus oil, REPEL insect repellent (40% DEET), Copenhagen tobacco leaves, Great Value distilled white vinegar, and control; and divided the 24 leeches per treatment into groups of six. This allowed us to do four repetitions of five leeches each per treatment, with each individual leech being subjected to a single treatment only one time. This experimental design allowed us to account for variance among individuals and eliminate the variable of multiple exposures.

For each treatment, we had six leeches in each of the twenty 10-liter tanks filled with 3 liters of water from Douglas Lake. We stored the leeches in the same manner the bait shop did: refrigerated in the 8

oz. containers we bought them in. We began testing the repellents on the leeches one week after we bought them, to ensure they were hungry enough to feed. The night before we exposed them to their treatments, we put the leeches in their room temperature testing tanks to allow them to properly acclimate. Roughly half of the leeches did not survive the week, so we purchased more the night before testing to replace those we lost. We evenly distributed the new leeches with the week-old ones.

We used a 4-ounce piece of Skylark beef liver, at room temperature, for each test. Our applications of the deterrents were done to mimic the way they would be applied to human skin. We sprayed the insect repellent and eucalyptus oil and rubbed the vinegar and tobacco mixture directly onto the liver. Our tobacco mixture was a 1:1 ratio of tobacco leaves and water soaked overnight to get a concentrated liquid application.

After applying the deterrent to the beef liver, we hooked the meat with a paperclip and hung it directly in the middle of the tank, suspended from a string stretched across the top of the tank. We observed the four tanks of a single treatment simultaneously for 30 minutes, recording the number of individuals that attached to the meat and in what time interval the attachment occurred (0-10 min, 11-20 min, 21-30 min).

We used an ANOVA analysis on SPSS to compare the percentage of attachments among the different treatments. We made pie charts to depict the distribution of attachments in terms of time per treatment.

Results

Percent attachment. The results of our ANOVA test showed a statistically significant difference in percentage of attachment among the different treatments ($F=43.671$, $df=4$, $p<0.0001$). The differences among the control and eucalyptus oil, tobacco, and insect repellent with DEET were significant (Fig. 1).

There was no statistical difference between attachment percentage between vinegar and the control and among eucalyptus oil, insect repellent with DEET, and tobacco (Fig. 1).

Time distribution of attachments. The attachment percentage of the 24 *E. obscura* tested with insect repellent with 40% DEET was distributed as: 4.2% 11-20 min, 12.5% 21-20 min, and 83.3% no attachment (Fig. 2). Of the 24 *E. obscura* tested with lemon-eucalyptus oil, 100% did not attach (Fig. 3). 83.3% of the 24 *E. obscura* tested with tobacco leaves did not attach and the 16.7% that did attach did so within 21-30 mins (Fig. 4). The attachment percentage of the 24 *E. obscura* tested with household distilled vinegar was distributed as: 16.7% 0-10 min, 16.7% 11-20 min, 33.33% 21-30 min, and 33.27% no attachment (Fig. 5). The attachment percentage of the 23 *E. obscura* control group was distributed as: 73.91% 0-10 min, 17.4% 11-20 min, and 8.69% no attachment (Fig. 6).

Discussion

While there was a statistically significant difference in the effectiveness of each treatment we cannot say that insect repellent with DEET was statistically the most effective. According to the error bars on our ANOVA graph (Fig. 1), tobacco, eucalyptus oil, and insect repellent with DEET were equally effective as leech repellents. Our data do not exclusively support our hypothesis that insect repellent with DEET would be the most effective repellent, but it does show that this deterrent is one of the most effective repellents. There were apparent differences in the time distribution of attachments. The only attachments that occurred with the three most effective deterrents happened after the first 10 minutes (for DEET) or after the first 20 minutes (for tobacco leaves). We presume this is because the application may have washed off, becoming less effective. We also predict that the 0% attachment with the eucalyptus treatment was because the application was in oil form, making it difficult to wash off. Hindering the chemoreception mechanism and ability of *E. obscura* to detect water vibrations was an effective way to repel the leech, which is also supported by other research (Bahmani, 2014 & Leal,

2014). However, our data also suggests that there are other possible of mechanisms, other than the ones we hypothesized, to repel leeches. An additional mechanism could be through sense of smell, similar to findings of other studies on the effectiveness of eucalyptus oil as a leech deterrent (Kirton, 2005). Our results contradict with research on the effectiveness of vinegar on repelling leeches, as we found no statistical difference in attachment percentages between the control and vinegar groups (Mills & Schindler, 1986).

The basis of this study was done on the assumption that the hunting mechanism of *E. obscura* is an apt representation of the hunting mechanism of all leeches. If this assumption is incorrect, then we cannot draw conclusions on the reasons for why and how each effective repellent works or that these repellents would be effective against all leeches. Future studies could test this assumption and compare the effectiveness of these repellents across different species. We also assumed that beef liver we used as a food source was an apt substitution for human flesh. Regardless of whether or not these repellents are effective across all species of leeches, we found multiple effective repellents against *E. obscura.* This information could be useful for individuals wanting to avoid being latched onto by this particular species.

The knowledge obtained from these results could be used to draw conclusions on the variety of mechanisms leeches use to hunt. Additionally, this study has created new avenues of research in terms of leech deterrents. Manufacturers could use this information to craft a repellent specific to leeches. More generally, this experimental design could be replicated for testing the effectiveness of any product on similar species.

Figures

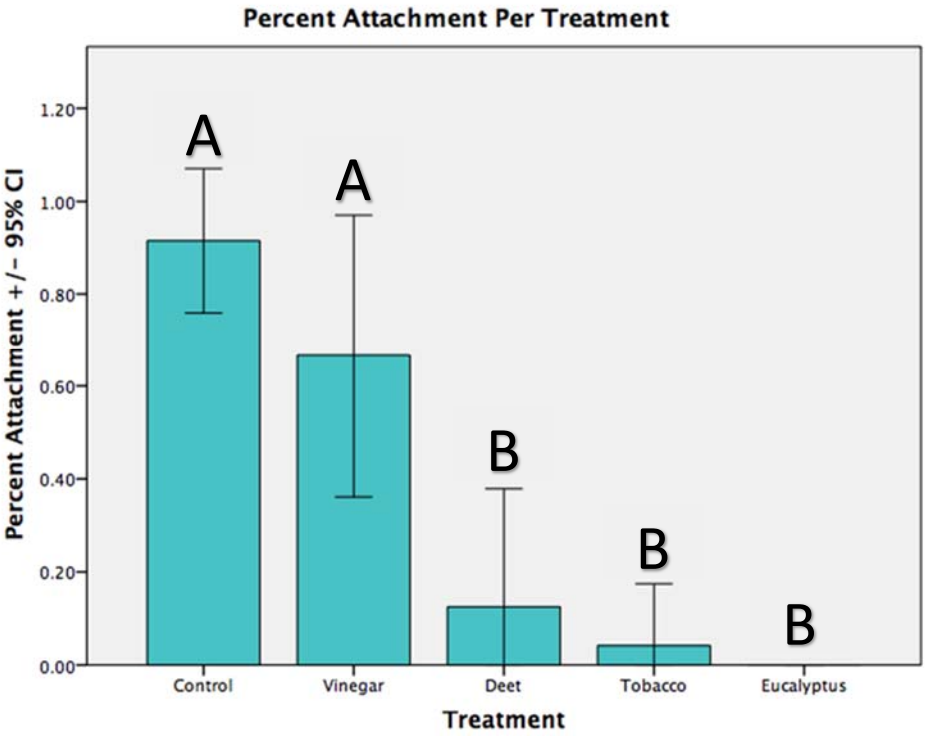

Figure 1. ANOVA analysis shows a significant difference between the ineffective deterrents (A) and the effective deterrents (B). Analysis shows no difference in effectiveness of vinegar and control (A) or among DEET, tobacco leaves, and eucalyptus oil (B).

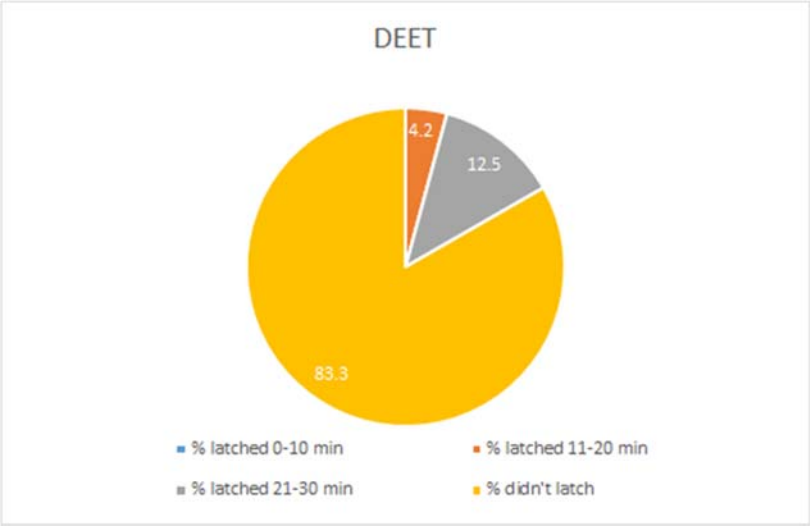

Figure 2. Insect repellent with DEET had no attachments in the first ten minutes and 83.3% of leeches did not attach.

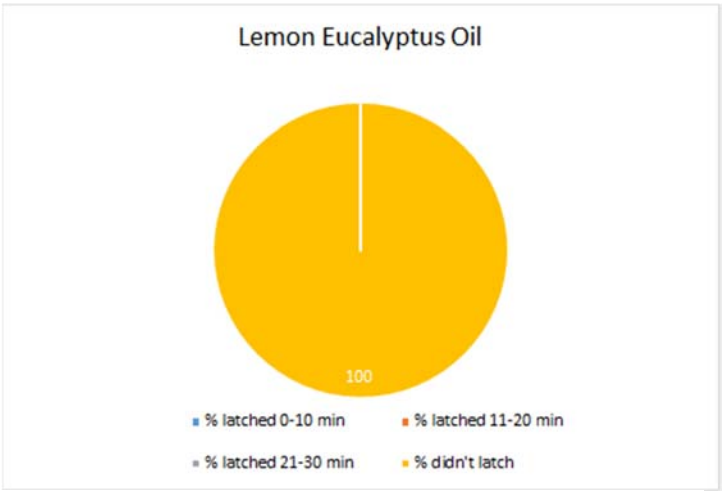

Figure 3. Lemon eucalyptus oil had 0% attachment.

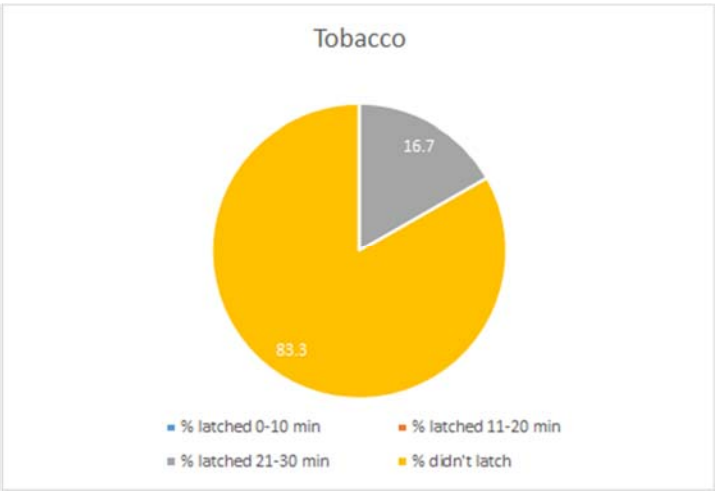

Figure 4. Tobacco had no attachments in the first 20 minutes and 83.3% no attachment overall.

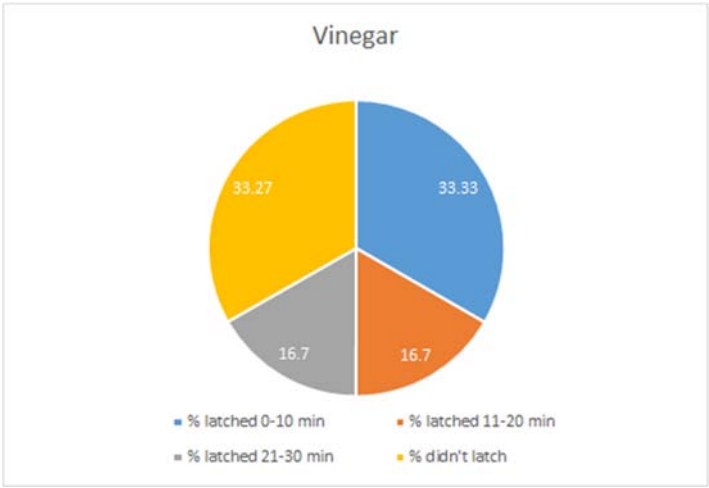

Figure 5. Vinegar had 50% of attachments in the first 20 minutes and only 33.27% did not attach.

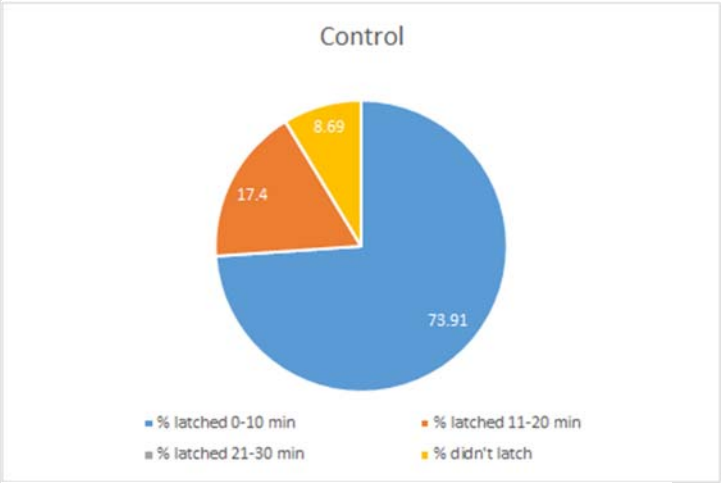

Figure 6. The control group had 73.9% of attachments in the first 10 minutes and only 8.69% did not attach.

Resources

Bahmani, M., Rafieian-kopaei, M. (2014). Medicinal plants and secondary metabolites for leech control. *Asian Pacific Journal of Tropical Disease,* 4(4): 315-316. doi:10.1016/S2222-1808(14)60580-6

Ginsberg, D. (1998). Natural History of Leeches (Annelida: Hirudinea). University of Michigan Biological Station Archives. http://archive.is/iLM9f

Kirton, L. G. (2005). Laboratory and field tests of the effectiveness of the lemon-eucalyptus extract, Citridiol, as a repellent against land leeches of the genus Haemadipsa (haemadipsidae). *Annals of Tropical Medicine & Parasitology,* 99(7): 695-714. doi:10.1179/136485905X51517

Leal, W. S. (2014). The enigmatic reception of DEET – the gold standard of insect repellents. *Current Opinion in Insect Science,* 6: 93-98. doi:10.1016/j.cois.2014.10.007

Mills, K.H. & Schindler, D.W. (1986). Biological indicators of lake acidification*. Water Air Soil Pollution*, 30: 779. doi:10.1007/BF00303344

Mooser, W. E., Govedich, F. R., & Klemm, D. (2009). Annelida, Euhirudinea (leeches). In *Encyclopedia of Inland Waters.* (Vol. 2, 116-123). Oxford, UK: Elsevier Ltd.

Whitaker, I. S., Rao, J., Izadi, D., Butler, P. E. (2004). Historical Article: *Hirudo medicinalis:* ancient origins of, and trends in the use of medicinal leeches throughout history. *British Journal of Oral and Maxillofacial Surgery.* 42(2): 133-137. doi:10.1016/S0266-4356(03)00242-0