A Comparative Study of the Effects of Various Repellants on Freshwater Leeches

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EEB 381: General Ecology
15 August 2016
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Abstract

While leeches provide many valuable ecosystem services, they are also a pest species that may spread pathogens to humans and animals. We tested four different home repellents (DEET, lemon-eucalyptus oil, tobacco, and vinegar) to determine which were successful at repelling a species of freshwater leech, *E. obscura*. We applied each repellent to a piece of beef liver, which was suspended in a tank of leeches for 30 minutes, during which the number of leeches attaching to the liver were recorded in ten-minute intervals. We also recorded the pH of each treatment to compare the effects of acidity on leeches. We found that the percent of leeches attached for DEET, tobacco, and lemon-eucalyptus oil were significantly different than both the control and vinegar; there was no difference between the vinegar and the control ($F = 43.671$, $df = 4$, $p < 0.000$). We also found the lemon-eucalyptus oil was quantitatively the most successful over time with 0 total attachments. We did not find a correlation between acidity and number of attachments. This knowledge may be useful in the protection against potential pathogens for people who spend time in freshwater ecosystems.

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Introduction

Leeches, a highly-specialized group of annelids that comprise the subclass Hirudinea, are notorious for their ectoparasitic blood-sucking habits. However, with nearly 700 species of leeches covering every continent except Antarctica, there is vast variety in both the ecological preferences and importance of leeches (Sket & Trontelj 2007). The majority of leeches are found in freshwater habitats, although around 15% are marine and fewer than that are terrestrial. While there are many species which are blood-sucking on vertebrates and invertebrates, the rest are mostly predators, with some species of scavengers. The blood-sucking hematophagous leeches use their razor-like teeth to attach to their prey and release once they are full of blood, while the

other predator species feast on tissue or whole invertebrates (Sket & Trontelj; Mooser et. al. 2009).

Leeches play many important roles in ecosystems. In North America, leeches help control macroinvertebrate populations while also serving as prey for many wetland species (Mooser et. al. 2009). They have also been used by humans for both medicinal purposes, such as therapy and bloodletting, and as fishing bait (Sket & Trontelj). As one of the most acid-sensitive freshwater organisms, leeches may serve as potential indicators of acidification and overall aquatic health (Schindler et. al. 1989). Despite their benefits, leeches are considered a pest due to the anticoagulant properties of their bites, which can cause open sores that bleed for long periods of time (Mooser et. al.). Their status as a pest often leads to leeches being killed when found rather than returned to their habitat, where they provide important functions.

There are many home-remedies recommended to prevent leeches from attaching to humans. One such remedy is insect repellent with N,N-Diethyl-meta-toluamide (DEET), which insects have been shown to avoid by smell (Syed & Leal 2008). Tobacco is also thought to be a repellent of slugs, and it is hypothesized that the intoxicating effects of nicotine may inhibit the ability of leeches to find food (Lee et. al. 2009; Kirton 2005). Extracts from lemon-eucalyptus plants has been shown to effectively prevent terrestrial leech bites in the field in tropical areas (Kirton). Household vinegar is also thought to be a useful repellent of leeches due to their sensitivity to acidity. While these potential repellents have been studied in the context of other invertebrates, including insects or terrestrial leeches, little is known about their comparative effects on freshwater leeches.

Our aim in this study is to determine the effectiveness of multiple proposed home repellants on the freshwater leech species *Erpobdella obscura*, which is abundant in the streams

and lakes surrounding the University of Michigan Biological Station property in Pellston, MI. We selected repellents that can be safely applied to human skin and are non-toxic to aquatic ecosystems and applied it to beef liver, which was placed in a tank with the leeches. The number of leeches that attached to the beef liver was recorded to measure the success of each repellent. We hypothesize that the repellents will cause fewer leeches to attach to the beef liver than to the untreated liver. We also predict that due to the aversion of leeches to acidity, we will see an inverse relationship between the number of leech attachments on each treatment and the acidity of that treatment.

Methods

We purchased 120 leeches purchased from Young's Bait Shop in Alanson, MI (45.443661, -84.786969) which we identified as *Erpobdella obscura*. The *E. obscura* leeches were wild caught from the Crooked River, also located in Alanson. We divided the leeches evenly into five treatment groups: REPEL lemon-infused eucalyptus oil (pH = 7.18), REPEL insect repellent with 40% DEET (pH = 8.34), Copenhagen snuff tobacco (diluted with water in 1:1 ratio; pH = 6.73), Great Value 5% distilled white vinegar (pH = 2.73), and control (water with pH = 7.05). Each group of twenty-four leeches was then divided into groups of six individuals, which allowed for four repetitions of each treatment. Each leech was subjected to only one treatment to eliminate variability due to multiple exposures. We ran all treatments simultaneously using twenty 10-gallon aquariums to avoid cross contamination of treatments.

Before being treated, all of the leeches were left unfed for one week to ensure that they would be hungry enough to feed. For each of the treatments, we used four five-gallon tanks filled with three gallons of water from Douglas Lake, MI, which was refrigerated at a constant temperature (Note: Temp. measurement will be added). The night before the treatment, we

placed the leeches in the tanks in which they would be tested to allow them to acclimate to the new conditions.

We applied each repellent to our bait, a room-temperature 4-ounce piece of Skylark beef liver, in a way that mimics application to human skin, i.e. the insect repellent was sprayed on the liver, the eucalyptus oil and vinegar were spread onto it. We soaked 4 tbsp. of the tobacco leaves in 4 tbsp. of water overnight to get a concentrated liquid which was also spread onto a piece of liver.

Once the deterrent was applied to the bait, the liver was hung from the side of the tank opposite of where the leeches were nested. We simultaneously observed the four tanks of the treatment for 30 minutes divided into three intervals (1-10min, 11-20min, and 21-30min) and recorded the number of individuals that attached to the liver during each interval.

We made several assumptions in this study, the foremost being that E. obscura is representative of other freshwater species of leech in terms of hunting mechanisms and ability to be deterred. We also assumed that beef liver was a suitable representation of human flesh and that our species of leech was identified properly. We used an ANOVA statistical analysis to compare the number of leeches attached among the various treatments.

Results

We first calculated the total percent of leeches attached and found that Control had the highest proportion of attachment (91.7%), followed by vinegar (66.7%), DEET (12.5%), tobacco (4.2%) and lemon-eucalyptus oil, which had zero attachments. We used an ANOVA analysis of the percent attached among each treatment group and found a significant difference between two groups: Group A (control and vinegar) and Group B (DEET, tobacco, and lemon eucalyptus) ($F = 43.671$, $df = 4$, $p < 0.000$; Figure 1). There was no significant difference within the groups.

We did not observe an inverse relationship between pH of treatment and percent attachment (Figure 2).

We compared the time intervals of each treatment group to measure the effectiveness of each repellent over time. We found that both Control (73.9%; Figure 3) and vinegar (33%; Figure 4) had the largest proportion of attachment in the first ten minutes, while both DEET (8.3%; Figure 5) and tobacco;s (4.2%; Figure 6). largest proportion of attachment occurred from 21-30 minutes. Lemon-eucalyptus oil had no attachments (Figure 7).

Discussion

We found significant evidence that three of the repellents tested were effective at repelling freshwater leeches (DEET, tobacco, & lemon-eucalyptus oil). Vinegar did not differ significantly from the control and is therefore ruled out as an effective leech repellent. These findings support our hypothesis that the repellents would lower the number of leech attachments. We did not find a relationship between the acidity of the treatment and the number of attachment to support our second hypothesis; rather, the most acidic treatment was the least effective at repelling leeches. We believe that this could be due to the dilution of the vinegar in the water. Dilution could further explain the increasing proportion of attachments as time went on for both DEET and tobacco. Neither the DEET nor tobacco were waterproof and may have worn off over time, allowing more leeches to attach. The hydrophobic properties of lemon-eucalyptus oil, which was quantitatively most effective, may have allowed it to last thirty minutes without leech attachment.

Aside from pH, there are several alternative explanations for the success of each repellent. DEET has been found to physically interfere with the smell receptors on insects antennae (Leal 2014). It is possible that DEET also affected the leech’s smell receptors,

inhibiting them from detecting the beef liver. Kirton (2005) found lemon-eucalyptus oil to be irritating and even toxic to Malaysian terrestrial leeches. While we did not report an casualties from the lemon-eucalyptus treatment, we did anecdotally observe the leeches approaching and then quickly retreating after contacting the beef liver. Nicotine has been shown to bind to insect acetylcholine receptors and cause muscle weakness (Bahmani et.al. 2012). It can also intoxicate leeches, making it difficult to detect prey (Bahmani 2014). We observed multiple leeches approach the beef liver but retreat, often spasming and then becoming lethargic, which may suggest either muscle weakness or intoxication.

This study had several limitations. We were unable to attain large quantities of hematophagous leeches and therefore had to use a predatory leech (*E. obscura*) instead. We worked under the assumption that the repellents will have similar effects on related freshwater species that are hematophagous. We also had some leech casualties and were forced to introduce several new leeches before the experiment; these new leeches were randomly distributed amongst the old ones in the treatment groups. Lastly, we had relatively small sample sizes, which we attempted to rectify by doing four repetitions of each treatment.

Leeches serve an ecologically important role in their ecosystem as prey for larger animals, predators of invertebrates, and indicators of aquatic health. However, they can be pests, causing bloody sores that bleed for hours. They may also be a vector for both animal and human pathogens. When leeches are removed by squeezing or salting, they regurgitate their stomach contents into the wound. Leeches stomachs have been found to contain pathogens that can survive for at least 6 months inside of the leech (Nehili et. al. 1994). However, further research is needed to determine the roles that leeches may play in the spread of disease and parasites. We determined that lemon-eucalyptus oil is an effective repellent of leeches for up to 30 minutes,

while DEET and tobacco are effective only for short amounts of exposure to water. This information could be used by the general public in order to prevent leech bites and the potential spread of pathogens, as well as the unnecessary death of an ecologically important species.

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Tables & Figures

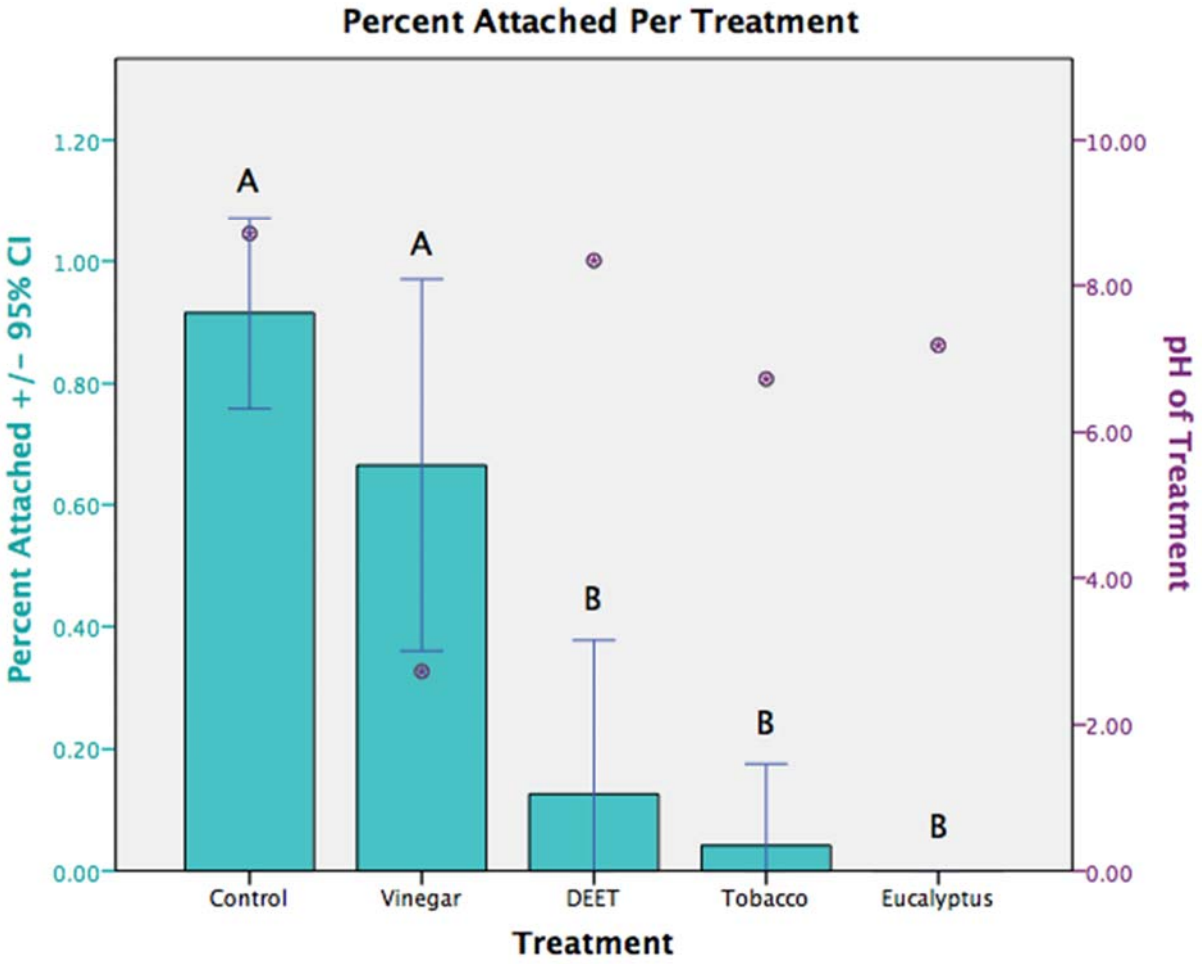

Figure 1. Percent of leeches attached within thirty minutes by treatment. Purple dots represent pH of treatments. We found statistically significant differences between treatment groups A and B (ANOVA: $F = 2.748$, $df = 4$, $p < 0.00$).

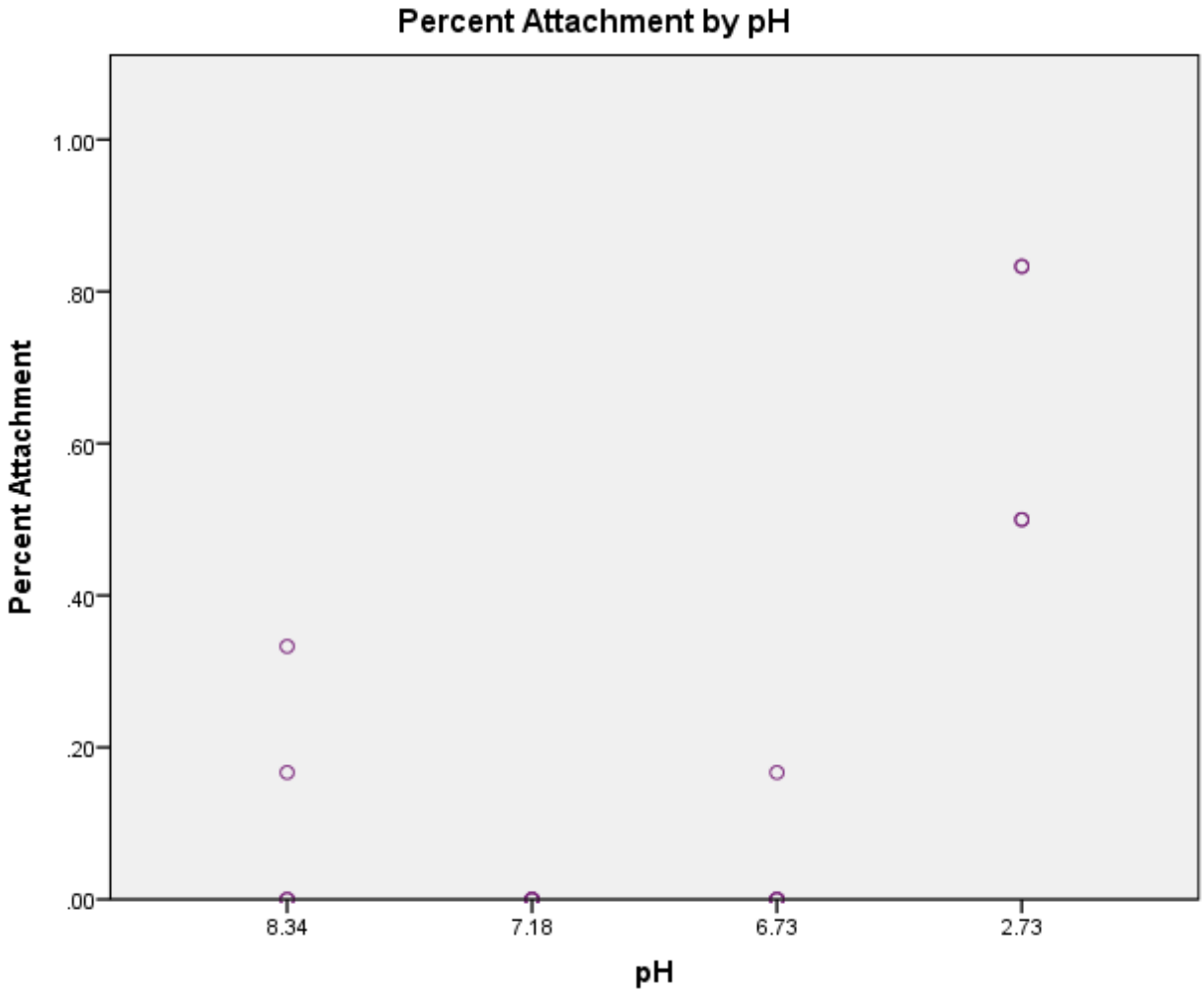

Figure 2. Percent of attached leeches by pH. We observed no relationship between acidity and attachment of leeches.

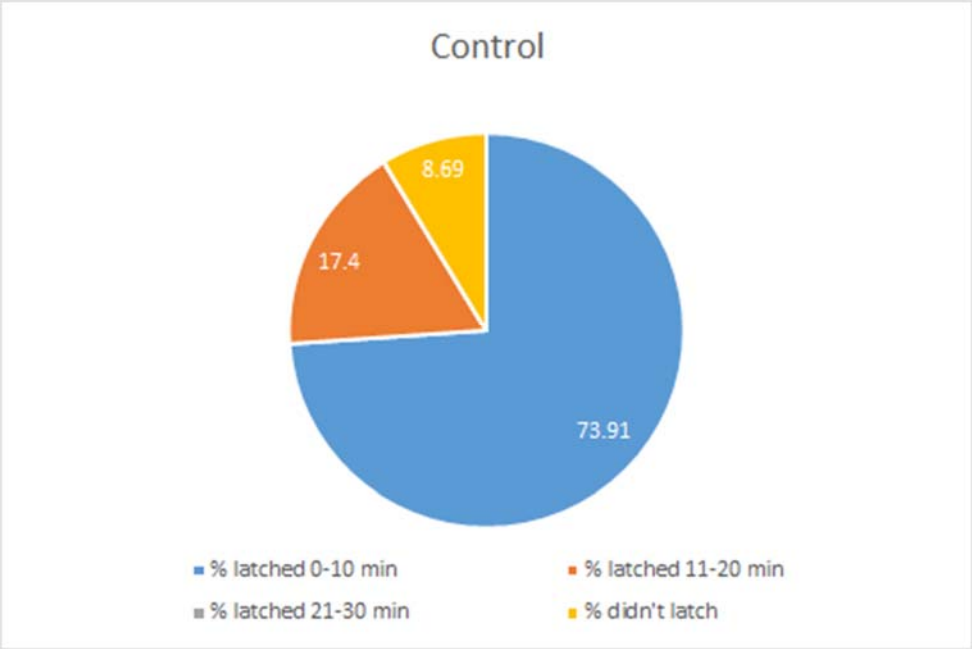

Figure 3. Proportion of leeches attached per time interval for control group.

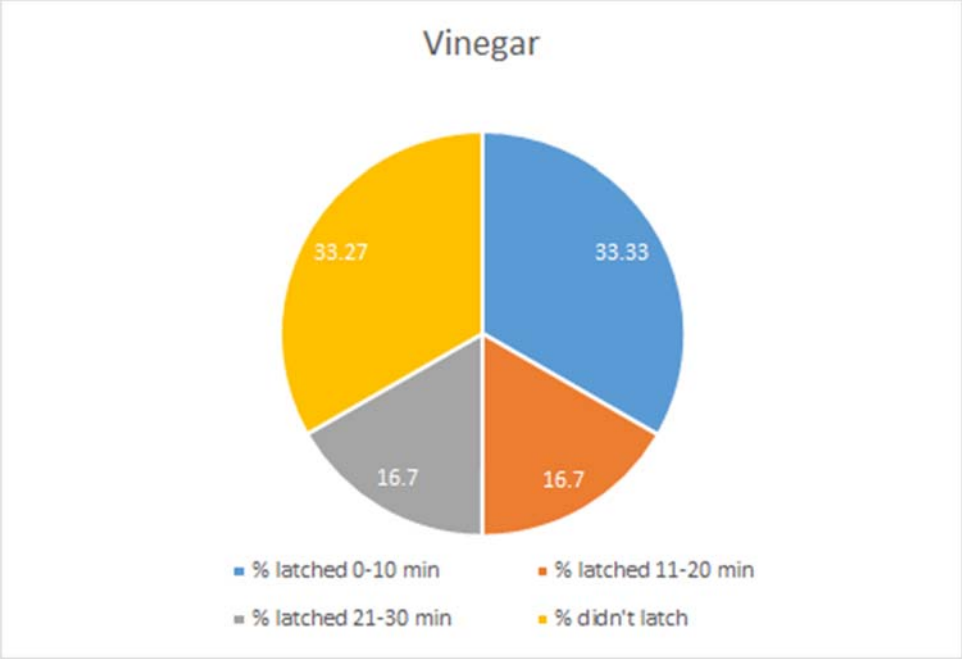

Figure 4. Proportion of leeches attached per time interval for vinegar.

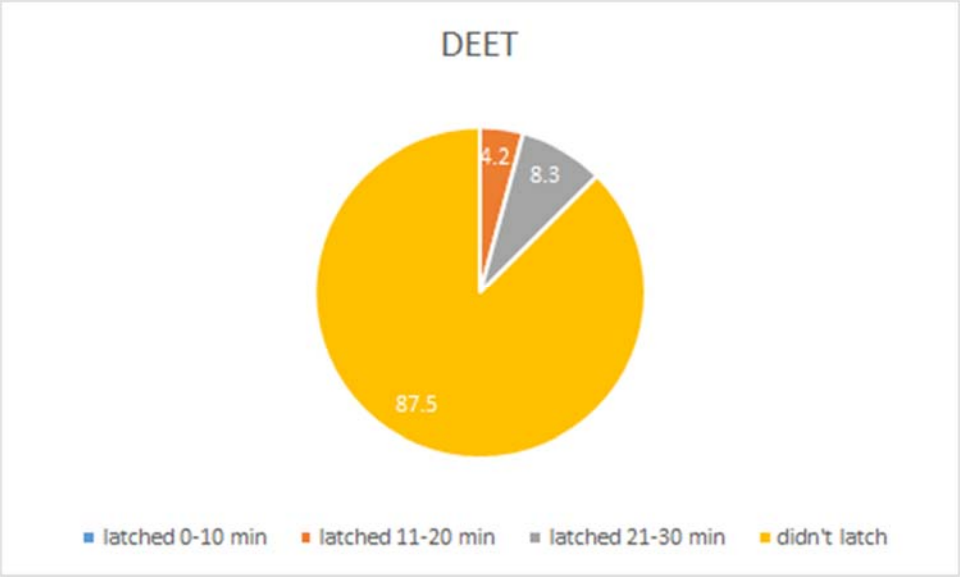

Figure 5. Proportion of leeches attached per time interval for DEET.

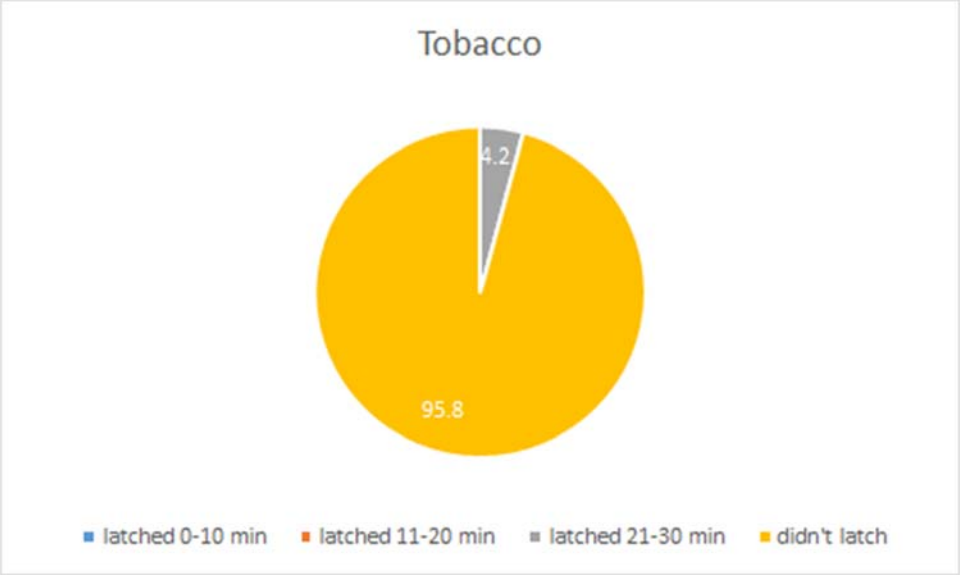

Figure 6. Proportion of leeches attached per time interval for tobacco.

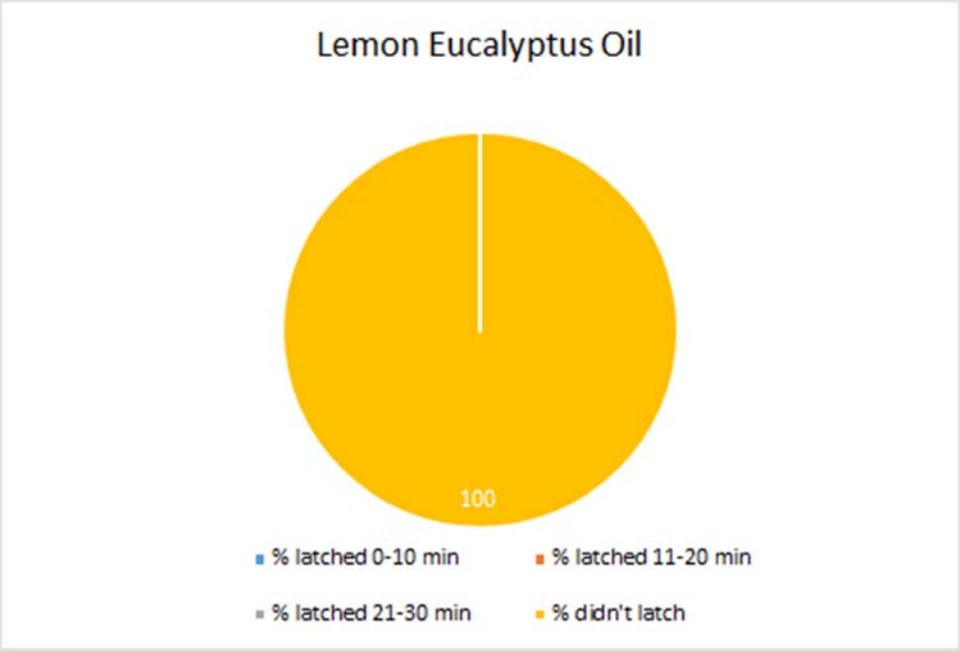

Figure 7. Proportion of leeches attached per time interval for lemon-eucalyptus oil.