Choosy Crustaceans: Habitat preference of the terrestrial isopod, *Armadillidium vulgare* (Isopoda: Oniscidea)

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ABSTRACT

The introduction of exotic species, such as *Eucalyptus globulus*, or Blue Gum, to California alters the species composition of the present community. To assess ecosystem degradation, researchers have suggested use of detritivores such as terrestrial isopods for use as bioindicators. With deeper understanding of their ecology, the terrestrial isopod *Armadillidium vulgare* may serve as a suitable indicator of ecosystem health in the future. In this study, *A. vulgare* underwent two habitat selection experiments to determine their habitat preferences. The objectives were to determine whether 1) habitat selection varies between species of leaf litter, 2) the chosen leaf species is associated with the sex of isopods, and 3) habitat selection varies between leaf litter of different water contents. The first experiment presented *A. vulgare* with a choice between two species of leaf litter: *E. globulus* and *Quercus agrifolia,* or Coast Live Oak, a California native. Chi-square tests determined that isopod choice of oak over eucalyptus was statistically significant and that the species of leaf litter selected was not associated with sex of isopods. A Chi-square test for the water content experiment showed that selection for water content of 0.39 over 0.53 in *Q. agrifolia* leaves was significant, suggesting optimal water content is closer to 0.39 than 0.53. The results may be explained by factors not controlled in this study, including diet, chemistry, or behavioral tendencies. Further studies on terrestrial isopods can provide better information on their habitat preference and the effect of exotic plants on their distribution and abundance.

KEYWORDS

exotic species, indicator species, habitat selection, water content, leaf litter

INTRODUCTION

The introduction of exotic species can heavily influence the structure of native ecosystems. Exotic species can alter community composition, sometimes leading to extinctions of native species (Seabloom et al., 2006). In some cases, the introduction of exotic species negatively alters various aspects of their ecosystem (Simberloff, 2011). Exotic plant species are ubiquitous in California, and disturb and threaten various ecosystems in the state (Seabloom et al., 2006).

A common exotic tree in California, *Eucalyptus globulus*, or Tasmanian Blue Gum, has detrimental effects on the biota of ecosystems where the tree has been introduced by humans. *E. globulus* increases the potential for fire damage because, as a physiological adaptation to resist fire, the flammable bark leads fire to quickly reach the canopy (Louppe, Oteng-Amoako, & Brink, 2008). Its oils, which contain phenol and terpene compounds, are allelopathic to other plants, reducing their germination and growth rates (Louppe, et al., 2008; Singh, Kohli, & Saxena, 1991). The effects of *E. globulus* on biological communities may be better understood through the study of indicator species and their population dynamics.

The small-scale changes in the soil environment caused by *E. globulus* may be revealed through the study of soil-dwelling macroinvertebrates. Detritivores like terrestrial isopods have been suggested for use as indicators of ecosystem degradation (Paoletti et al., 2007). Several species of terrestrial isopods live in various ecosystems in California, which has a characteristically Mediterranean climate (Paris, 1963). Although many species are European natives, isopods live intimately with soils and their population dynamics can indicate changes in the soil environment (Jass & Klausmeier, 2000; Paoletti et al., 2007). Researchers have experimented with various aspects of the ecology of terrestrial isopods, including the abundant species *Armadillidium vulgare*, commonly known as woodlice or pillbugs, investigating responses to and competition over food resources, optimal foraging over a heterogeneous landscape, and responses to humidity (Grosholz, 1992; Grosholz, 1993; Rushton & Hassall, 1983; Miller 1938). More research on the ecology of terrestrial isopods is necessary in order for them to become suitable bioindicators. With a greater wealth of knowledge on habitat needs and preferences in the future, researchers can accurately interpret their population dynamics as responses to environmental changes.

This study investigated the habitat preference of *A. vulgare* in forest stands of Berkeley, CA. In order to determine habitat preferences of *A. vulgare*, the isopods underwent two choice experiments. The first experiment presented *A. vulgare* with a choice between two species of leaf litter: *E. globulus* and *Quercus agrifolia*, commonly called Coast Live Oak, a native to California. I also investigate the possibility that sex is a potential confounding factor in habitat preference. Because eucalyptus tends to inflict toxic chemicals on soils, I hypothesized that isopods prefer to inhabit *Q. agrifolia* leaf litter rather than *E. globulus* leaf litter. The second experiment presented a choice between leaf litter of two different water contents. I predicted that the isopods would have a preference of one water content over the other, assuming that the isopods have a water content that is optimal for their physiology.

METHODS

Pilot Study

Study Sites and Isopod Sample

Wild terrestrial isopods were collected from two sites in Northern California: Strawberry Canyon in Alameda County and Tilden Regional Park in Alameda and Contra Costa Counties. This region of Northern California has Mediterranean climate, characterized by a distinct rainy season and a drought season (Paris, 1963). From October 21 to December 17, 2011, preliminary observations in the field showed *A. vulgare* to occur in Strawberry Canyon in high abundance relative to other species of terrestrial isopod.

I collected wild isopods from both Strawberry Canyon and Tilden Regional Park from October 21 to December 17, 2011, during the wet season in coastal California when *A. vulgare* is more active and abundant (Paris, 1963). In Strawberry Canyon, I collected on the slopes adjacent to the firetrails. Collections took place typically after rain events because *A. vulgare* is active closer to the surface when humidity is high (Paris, 1963), and thus are easier to collect in these conditions. I determined species and sex of individuals, according to an identification guide and dichotomous key (Brusca, Coelho, & Taiti, 2001; Miller, 1938).

I collected leaves for the experiment from two locations on the University of California, Berkeley campus. I collected *E. globulus* leaves from the Eucalyptus Grove on the western side of campus near West Circle, and *Q. agrifolia* leaves from an oak-canopy area south of the Valley Life Sciences Building, along south fork of Strawberry Creek. I removed trash from the collected leaf litter but kept large twigs and bark to have a sample representative of a real habitat. I used newly collected leaves for each trial day.

Trials to determine feasibility and length of experiment

Using wild isopods, I conducted a pilot study with two objectives: 1) to test whether isopods would choose one species of leaf litter as habitat over another, and 2) to determine what length of time to allow for the isopods to select a habitat. The choice of leaf litter was between two species: *Q. agrifolia* and *E. globulus*. The experimental design was identical to the design described below. I conducted five trials with varying lengths of time: 2-hr, 4-hr, 6-hr, 12-hr, 24-hr.

Habitat selection experiments

Isopod sample

The subjects for the habitat selection experiment were *A. vulgare* purchased from Connecticut Valley Biological Supply Company. These isopods were bred in a stock supply. Purchase from a supply company was necessary because I collected an insufficient number of isopods from Strawberry Canyon and Tilden for the desired amount of replication. I separated the isopods by sex to determine the sex ratio of the purchased isopods. I then created nine sets of ten isopods with identical sex ratios by randomly distributing individuals from each sex category. Throughout the duration of the experiment, each set was kept separately in its own container.

Leaf litter selection experiment

I designed an experiment to determine which habitat isopods will select when given a choice of two species of leaf litter. Trials took place within plastic, lidded terrariums (13.5cm x 8cm x 8cm). I placed a divider in the terrarium, and then placed *E. globulus* leaves on one side and *Q. agrifolia* leaves on the other side. When the divider was removed, there was no gap between the *Q. agrifolia* and *E. globulus* in order to prevent a gap from potentially deterring the isopods from moving from one to the other. The 6 hours began when one set of isopods was placed in the very center of the terrarium. After 6 hours, I located and counted the isopods on each side of the terrarium. I also determined the sex ratio for each side of the terrarium.

On March 12, 14 and 16, 2011, I conducted all trials between the hours of 11am and 7pm. This eight-hour window was a safeguard against possible behavioral differences at different times of day, because isopods exhibit diel pattern of behavior (Paris, 1963). Each set of ten isopods underwent three trials. For each trial and each species of leaf litter, I recorded the total number of individuals present, and the sex ratio. On each of the three trial days, I additionally calculated the gravimetric water content of the oak leaves and eucalyptus leaves separately. I used a drying oven to dry each sample to determine the mass of water. The formula for gravimetric water content is: $u=m_w/m_b$, which is the mass of the water divided by bulk mass. Water content was an uncontrolled condition that later could potentially describe peculiarities in the data.

Water content experiment

To assess a second environmental condition, I conducted an experiment on water content of the leaf litter. The layout of the terrarium was identical to the leaf litter species selection experiment. Using only the leaves of *Q. agrifolia*, I tested isopod selection of oak leaf litter with varying moisture contents. Following collection of fresh-fallen oak leaves at the study site, a small portion of the collected leaves was weighed then placed in the drying oven for one hour. From the recorded masses, I calculated the water content of the collected leaves, and then calculated the additional amount of water necessary to achieve these two different water contents: 0.39 and 0.53. After adding the water, I allowed the leaves to rest overnight to absorb the water. In the morning, the actual water content was calculated again, and the choice experiment was set up similarly to the leaf litter species experiment. The isopods, placed in the center of the terrarium, then had a choice between two habitats of different moisture levels. Isopods stayed in the terrariums for 6-hrs, after which they were located and counted.

Data Analysis

The R Commander package (Fox, J., et al., 2009) in the statistical software R (R Development Core Team, 2009) was used to calculate means and standard deviations, and to conduct Chi-square tests of independence. Chi-square tests for goodness of fit were conducted according to Quinn and Keough (2002).

For the leaf litter species experiment, I calculated the mean and standard deviation of the proportion of each set of ten isopods to choose oak or eucalyptus, and determined the significance of the difference using a Chi-square test for goodness of fit, comparing observed frequencies to expected frequencies if oak and eucalyptus were each selected by 50% of isopods. A Chi-squared test of independence determined whether males or females are more likely to choose one species of leaf litter than the other.

For the water content experiment, I conducted a similar analysis. For each option of water content, I calculated the mean and standard deviation of the fraction of each set to choose the leaves with that water content. A Chi-squared test determined whether males or females are more likely to choose leaf litter of one water content than the other.

RESULTS

Pilot Study

In the pilot study, the majority of isopods chose oak over eucalyptus. Pooling all trials, the average fraction of isopods per set to choose oak over eucalyptus was 0.68 ($n = 5$, $SD = 0.13$). The isopods consistently chose oak over eucalyptus, with only minor differences at various time intervals (Table 1). Therefore, I chose 6 hours to be the length of time used in the experiment.

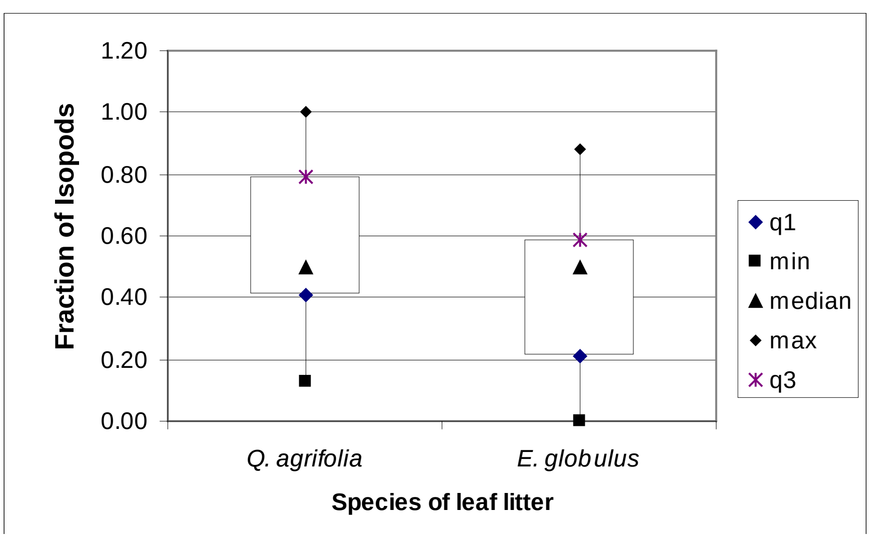
Table 1. Pilot Study Results. After each trial, I counted the number of isopods that chose each leaf litter species, and then calculated the fraction out of ten that went to oak.

Trial Length	Fraction to Oak
2-hr	0.7
4-hr	0.6
6-hr	0.8
12-hr	0.9
24-hr	0.6

Isopod selection of species of leaf litter

Overall, the isopods mostly chose oak over eucalyptus in most trials. Pooling all 27 trials, 58% of isopods selected oak for cover (n = 255). Across trials, the average fraction of isopods to choose oak for cover was 0.572 (n = 27, SD = 0.234), while the average fraction of isopods to choose eucalyptus was 0.428 (n = 27, SD = 0.234). These data are presented in the boxplot in Figure 1. I conducted a Chi-square test in order to determine whether isopod choice of oak over eucalyptus was statistically significant. Pooling all trials, the isopods' choice of habitat did differ by species of leaf litter χ^2 (1, N = 252) = 7.68, $p < 0.01$.

Figure 1. Fraction of isopods to select each species of leaf litter. Each fraction is the proportion of the number of isopods alive at the end of the trial. The median fraction for both *E. globulus* and *Q. agrifolia* was 0.5.



The initial sex ratio on Trial Day 1 was 2M:8F, and I intended for the sex ratio to remain the same through the entire experiment. However, some isopods died during the rest periods between the three trial days, causing the initial sex ratios for trials two and three to vary. Pooling all trials, the sex ratio in the oak leaves was 1M:3.77F, compared to the sex ratio of 1M:3.71F in the eucalyptus leaves. I tested for independence of the sex of the isopods and the species of leaf litter that they chose. The choice of species of leaf litter did not differ by sex χ^2 (1, N = 252) = 0.0002, p = 0.99.

Throughout all trials, moisture content remained fairly consistent for each respective leaf litter species (Table 2). New leaves were used on each trial day. The average moisture content for eucalyptus was 0.53 (n = 3, SD = 0.04), and that of oak was 0.65 (n = 3, SD = 0.03).

Table 2. Gravimetric water content. For the leaf litter selection experiment, I calculated gravimetric water content of the leaf litter used in each day of trials. The formula is as follows: $u=m_w/m_b$.

	Eucalyptus	Oak
Trial 1 – March 14	0.53	0.62
Trial 2 – March 16	0.49	0.67
Trial 3 – March 18	0.57	0.67
Average	0.53±0.04	0.65±0.03

Isopod selection of water content

In the water content experiment, the isopods had a choice between moist leaf litter with water content of 0.39 and wetter leaf litter with water content of 0.53. Most isopods chose to take cover amongst moist leaves over wetter leaves. Pooling all sets from one trial day, 70% of isopods selected moist leaves for cover (n = 40). Across trials, the average fraction of isopods to choose moist leaves for cover was 0.7 (n = 4, SD = 0.216), while the average fraction of isopods to choose the wetter leaves was 0.3 (n = 4, SD = 0.216). Similar to the first experiment, I conducted a Chi-square test for goodness of fit in order to determine whether isopod choice of moist leaves over wetter leaves was statistically significant. Pooling all sets from the single trial day, the choice of habitat did differ by species of leaf litter but the difference was statistically significant, χ^2 (1, N = 40) = 6.4, p = 0.02.

DISCUSSION

I investigated two factors that influence the choice of habitat by *A. vulgare*: litter species and water content. My results suggest that *A. vulgare* prefer *Q. agrifolia* leaf litter to *E. globulus* leaf litter. I found a significant difference in the proportion of isopods to choose leaves with a water content of 0.39 over leaves with a water content of 0.53.

Leaf litter species experiment

My results indicate that the species of leaf litter chosen is not associated with sex, and overall, a habitat consisting of *Q. agrifolia* leaves is preferable to one consisting of *E. globulus* leaves. The compounds of *E. globulus* that are allelopathic to other plant species may contribute to this preference by creating soil conditions that are adverse to isopods (Louppe, et al., 2008; Singh et al., 1991).

The quality of the leaves as food resources may have differed between the leaf litter samples, and therefore may also contribute to the trend to choose *Q. agrifolia* over *E. globulus*. A study on *A. vulgare* found a preference for fresh-fallen leaves over slightly decayed leaves, and that fresh-fallen leaves increased survivorship and growth rate (Rushton & Hassall, 1987). The *E. globulus* leaves may have been less fresh-fallen than the *Q. agrifolia*. Decay also affects the digestibility of leaves, changing the appeal of food sources over varying levels of decay (Rushton & Hassall, 1983). Plant species provided as habitat to *A. vulgare* can alter the growth rates and fecundity of individuals (Miller & Cameron, 1983). The composition of the leaves also varies, such that one species of leaf may be more or less nutritious, or simply less toxic than another species of leaf.

As I counted the isopods after the six-hour trials, I observed isopods located in loose aggregation within the leaf litter in several of the terrariums. Behavioral tendencies toward aggregation may explain the unanticipated observation. Although aggregations are not considered a social behavior, this tendency to cluster together reduces the amount of moisture lost by each individual, and is a beneficial adaptation to life on land (Allee, 1926; Brockett & Hassall, 2005). When density is low and thereby competition for resources is low, the benefit of spending more time searching for others with which to aggregate outweighs the cost of spending less time searching for food and of expending energy to walk (Brockett & Hassall, 2005). Aggregation behavior may have influenced habitat selection, making individual decisions partially dependent on whether they sought or encountered other individuals.

Water content experiment

The results of the water content experiment confound the results of the leaf litter species experiment. The water content experiment suggests that *A. vulgare* has a preference for leaf litter with water content of 0.39 over that of 0.53. Moisture content is highly variable in the laboratory as it is in the field. It is perhaps one of the strongest influences on soil microbial and invertebrate communities (Sylvia, Fuhrmann, Hartel & Zuberer, 2004). The water content may vary with the amount of decay the leaves have undergone, which correlates to the digestibility of those leaves (Rushton & Hassall, 1983). Unfortunately the low replication of this experiment gives the results low statistical power, and thus higher replication in future studies is necessary to confirm whether water content actually confounds isopod selection of leaf litter species.

Limitations

Factors not controlled in this study, including diet, chemistry, or behavioral tendencies, may help explain the results. The time constraint severely limited the scope of this study. While numerous factors influence the habitat preference of an animal, I could only explore two during the allotted time.

Low abundance of isopods during collection caused a diminished number of trials, which therefore caused lower statistical power. It also necessitated the purchase of isopods from a supply company. Using subjects bred in a stock supply may have caused unseen problems due to potential behavioral differences between wild-caught isopods and purchased isopods.

Pseudo-replication was inherent in the experiment design. Each isopod should not be counted as an independent trial because the set of ten was the single experimental replicate to receive a treatment. Pseudo-replication was necessitated by the low number of wild-collected isopods and my choice to better simulate the wild by entering the isopods in higher density into each terrarium. Despite resulting in statistically significant differences, both experiments have low statistical power due to low replication, especially in the water content experiment, for which only one trial day was conducted.

A. vulgare are most active at night (Paris, 1963), and the diel pattern of activity is regulated by light, which inhibits their activity (Refinetti, 2000). The trials in this study were conducted during daylight hours, and the isopods were under the natural light regime. However, the light regime can be reversed in the laboratory to allow observations during the day when the isopods in their most active hours for foraging (Brockett & Hassall, 2005).

Future Research

Further studies on terrestrial isopods can provide better information on their habitat preference and the effect of exotic plants on their distribution and abundance. Future isopod researchers should investigate the response of *A. vulgare* to changes in the chemistry of the leaf litter and soil, including changes in the pH, nitrogen content, and amount of allelopathic compounds such as phenols and terpenes. Field studies on *A. vulgare* are important to corroborate any future laboratory research.

Broader Implications

The intimate dependence of terrestrial isopods on leaf litter as a quality food resource and on a healthy soil environment makes terrestrial isopods a potentially strong indicator species of changes in the soil environment. Although they are exotic, terrestrial isopods functioning as decomposers are very important in nutrient cycling, releasing nutrients from dead organic material via digestion. Monitoring their population dynamics can indicate degradation caused by disturbances such as planting of exotics such as *E. globulus*, and other species. After an adequate amount of research is conducted to fully understand their ecology, *A. vulgare* may serve as suitable and accurate indicators of the health of their ecosystem in the future.

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