Isopod Environment Preference - Dark vs Light

Arjun Talapatra

11/14/23

L10

Lab partners: Alan Lin, Rachel Riddle, Jiang Yiting

Instructor Name: Sydney John

Introduction

Michael R. Warburg. Behavioral Adaptations of Terrestrial Isopods

This article discusses how when isopods move from sea to shore (like the Ligia species), they go from having a preference for light to avoiding it. For isopods who live in relatively moist environments (such as Oniscus, Porcellio, and Armadillidium), their reaction to light is less important than isopods species who live on the shore, and specifically in the case of Armadillidium, they become attracted to light. Similarly, in the case of Porcellio, they become attracted to light at higher temperatures.

In the "Responses to Light" experiment the researchers performed, they found that terrestrial isopods (isopods that live on land), tend to have a strong reaction to avoid light as opposed to aquatic isopods. Furthermore, isopods that live in the littoral zone and in moderately moist environments tend to avoid light, but they could move towards light if they dehydrate or if the temperature increases. Additionally, Isopods that live in grasslands or forests are often more photopositive (preferring light) then isopods that live in the desert (such as Venezillo Arizonicus and Armadillo Albomarginatus), as they can control their water levels better (Warburg 2015). Overall, isopods' reaction and preference towards light or dark can depend on various factors such as their species, their location (sea/shore/desert), and temperature.

Influence of Light of Very Low Intensity on Phototropic Reactions of Animals

This article seeks to determine the threshold of light intensity for which a reaction occurs in isopods. Essentially, the authors determined that the isopods tested exhibited strong negative phototropism, meaning that they avoided light. In their experiment, they created a consistent illumination field using their light source and adjusted the light intensity without changing the light's distance from the isopods. Essentially, the authors concluded that this threshold is at a very low intensity (Hartline 1923). Overall, the article was beneficial in shedding light on how isopods react to different light intensities and gave me a better idea of their behavior in the presence of light.

Purpose/objective: To determine whether isopods have a preference for dark vs light environments

Experiment 1 Rational: The first part of the experiment was the control, where we seeked to demonstrate that isopods didn't have a preference for going to the right or left side of the dish without us controlling light.

Experiment 2 Rational: This is the main experiment, where we performed three trials seeking to determine whether the isopods had a preference for light or dark.

Experiment 3 Rational: We did this part of the experiment as another form of a control, where we directly looked at the isopod's movement *during* a specified time, compared to in part 1 where we only looked at which side they ended up in after the 30 minutes.

Hypothesis: Isopods will have a preference for dark environments over light ones

Experiment 1 prediction: Isopods would not have a statistically significant preference for going to the right or left side of the dish.

Experiment 2 prediction: Isopods will have a statistically significant preference to go to the covered side of the chamber over the exposed side.

Experiment 3 prediction: The number of isopods with an initial instinct to go right will be roughly equal to the number of isopods with an initial instinct to go left. Moreover, the majority of isopods will not have a preference to go either right or left, and will instead spend a roughly equal amount of time going in both directions.

Methods

For experiment 1, we first transferred 16 isopods from the terrarium provided to a petri dish using a plastic spoon. We then set up a large choice chamber kit and transferred the 16 isopods to the center of the choice chamber. Note we didn't add additional light nor removed any light and simply used the ambient room light for this part of the experiment. We then removed the two "gates" in the chamber, allowing the isopods to move freely throughout the chamber, and started a timer. After 5 minute, 10 minute, 20 minute, and 30 minute intervals, we took pictures of the apparatus. We then recorded the number of isopods on each side of the apparatus (for isopods in the middle, we recorded them in "other") for each interval and put our data on excel. We then returned to isopods and began the next part of the experiment.

For experiment 2, we performed three trials where we seeked to determine whether the isopods had a preference for light or dark. Here, we transferred another 16 isopods from the terrarium to the choice chamber kit using the same method explained in the first part. In this part, we added two flood lights (85W incandescent) to one side of the choice chamber and covered the

top of the other with 3 sheets of paper stacked on top of each other. We then removed the two "gates" in the chamber and took pictures of the apparatus after 5 minute, 10 minute, 20 minute, and 30 minute intervals. Then, we recorded the number of isopods on each side of the apparatus similar to how we did in the first part, and returned the isopods. We repeated these steps 2 more times using 16 different isopods each time. Here, our independent variables are light and dark, and our dependent variable is the number of isopods that go on each side.

For experiment 3, we performed a choice experiment. We first collected a choice-tube chamber, then obtained two small Q-tips and measured their L × W × H dimensions with a Vernier caliper. They should be similar to each other. Then, we placed one Q-tip in a stopper at each end of the choice tube. Then we placed an isopod at the tube chamber release site, set a 30 second timer, and analyzed the isopods movement. We recorded the isopod's *initial instinct* (which direction they go in first) and whether they had a preference for going right or left (for instance, if the isopods moved in the right direction for the majority of the 30 seconds, they would have a preference for going right). We repeated this for 16 isopods. Note we did not add any additional light or remove light - we just used the ambient room light. We used the activity 2b procedure from lab 7.2 to help us with this procedure.

For experiment 1, we used a chi-square analysis to first show that the difference between our observed and the expected data for the control is merely due to chance. For experiment 2, we used a chi-square analysis seeking to show that for each trial in the experiment, the difference between observed and the expected data is NOT due to chance and that isopods have a statistically significant preference to dark. Note we would only use the data where the isopods were on either the left or right side - we wouldn't use the isopods in the "other" category. Our null hypothesis is that there isn't a statistically significant relationship between light and where isopods place themselves. Our alternate hypothesis is that isopods have a preference to go to the dark over light. To do this, we used the formula

$$\lambda^2 = \sum \frac{(observed-expected)}{expected}$$
 to calculate the chi-squared statistic for each of the 3 trials. Then, we

calculated the probability level (P value) using the Chi Square Table and 1 degree of freedom (since there were only two groups we used - the exposed and non-exposed). Lastly, for experiment 3, we compared the number of isopods with an initial instinct to go right vs left, and checked to see if the majority of isopods had a preference for going right or left, or if they had no preference.

Results

For experiment 1, we found that 7 isopods went to the left side, 8 went to the right, and 1 was in the middle after 30 minutes. To show this isn't a statistically significant difference, we

could use a chi-squared analysis. The chi-squared statistic was

 $\lambda^2 = \frac{(7-7.5)^2}{7.5} + \frac{(8-7.5)^2}{7.5} = 0.0667$. The P-value is slightly less than 0.8 (from using the chi-squared table). This would support the fact that there isn't a significant difference between the right and left side in the absence of adding or removing light. Table 1 shows the data we collected for experiment 1 below.

(Table 1 Comparing isopods' tendency to go to the left or right side of the chamber in ambient light)

O /				
Control group (no addition light				
Time interval	Left	Right	Other	
5 mins	6	9	1	Ī
10 mins	9	7	0	
20 mins	8	7	1	
30 mins	7	8	1	Ī

For experiment 2, in trial 1, our expected value after 30 minutes was 7 on each side. The chi-squared statistic was $\lambda^2 = \frac{(6-7)^2}{7} + \frac{(8-7)^2}{7} = 0.286$. The P-value is between 0.1 and 0.05. Here, we would fail to reject our null hypothesis, as this value is too high to reject the null hypothesis at an α level of 0.05.

For trial 2, our expected value was 7.5 on each side (after 30 minutes). The chi-squared statistic was $\lambda^2 = \frac{(3-7.5)^2}{7.5} + \frac{(12-7.5)^2}{7.5} = 5.4$. The P-value is between 0.01 and 0.05. Here, at an α level of 0.05, we would reject our null hypothesis because the P-value is less than 0.05.

For trial 3, our expected value was 7 on each side. The chi-squared statistic was

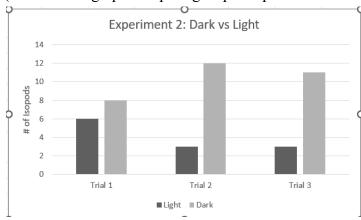
 $\lambda^2 = \frac{(3-7)^2}{7} + \frac{(11-7)^2}{7} = 4.6$. The P-value is between 0.01 and 0.05. Here, at an α level of 0.05, we would reject our null hypothesis because the P-value is less than 0.05. Table 2 shows the data we collected for experiment 2 below.

(Table 2 Comparing isopods' preferences for light vs dark)

	_		_		_	
Trial 1						
Time						
interval	Light		Dark		Other	
5 mins		5		9		2
10 mins		1		10		2 5 3 2
20 mins		3		10		3
30 mins		6		8		2
Trial 2						
Time						
interval	Light		Dark		Other	
5 mins		12		4		0
10 mins		3		13		0
20 mins		3		10		3
30 mins		3		12		1
Trial 3						
Time						
interval	Light		Dark		Other	
5 mins		6		9		1
10 mins		1		12		3
20 mins		4		11		1
30 mins		3		11		2

The figure below is a bar graph that visually shows these results.

(Table 3 Bar graph comparing isopods' preferences for light vs dark)



As we can see, we are categorically separating the 3 trials (using the data after 30 minutes) to show that for each of the trials, more isopods go to the dark vs the light. However, for trial 1, there isn't much of a difference between the number of isopods that go to the dark vs light, whereas for trials 2 and 3, there is a significant difference.

For experiment 3, we found that out of the 16 isopods, 14 of them did not have a preference for moving left or right (they would spend a roughly equal amount going right and left). Since the vast majority of the isopods didn't have a preference for a particular side, we can conclude that the isopods overall didn't have a significant preference. Moreover, 7 out of the 16 had an initial instinct for going right, while the remaining 9 had an initial preference for going left. Since these two values are relatively close to each other, we can conclude that the isopods

overall did not have a significant initial instinct for going either right or left. Figure 4 shows the data we collected for experiment 3 below.

(Table 4 Looking at isopods' initial instinct to go right or left and which direction they spend the majority of time moving in)

Initial instinct	Preference?	
R	No	
L	No	
L	Yes	
L	No	
R	No	
L	No	
L	No	
L	No	
R	No	
L	No	
R	No	
L	No	
R	Yes	
R	No	
R	No	
L	No	
	Initial instinct R L L L R L L R L R R L R L R L R L R	

We didn't collect any qualitative data, but all of the data collected, first in the control, then in the three trials of the experiment, and lastly instinct and preference, are all quantitative, as they can all be objectively measured.

Discussion

Overall, for our control experiments (experiments 1 and 3), they both ran as expected, and the isopods overall didn't have a preference in the absence of controlling light. This increases the validity of our results in experiment 2 by showing that the results weren't caused by factors outside of our independent variable - the presence of light.

In experiment 2, we found that for two out of the three trials we performed, the number of isopods that went to the dark side was significantly greater than the number that went to the exposed side. However, the result in trial 1 was unexpected and inconsistent with the rest of trials. A possible reason for this was due to experimental error. There is a possibility that for trial 1, we didn't place the flood lights high enough to surround the entirety of the exposed-chamber. We made sure to be more careful for this in trials 2 and 3, but this is something that could have affected our trial 1 results. We should perform more trials to determine if trial 1 was a type II error, or if trials 2 and 3 were both type I errors. If trial 1 was a type II, we would have failed to reject the actually-false null hypothesis in trial 1. If trials 2 and 3 were type I errors, we would

have rejected the actually-true null hypothesis. Performing more trials would be beneficial because increasing sample size is a good way to decrease the probability of both type 1 and type 2 errors. This would decrease uncertainty in our findings. However, for now, we can't confidently make a claim of whether or not we can reject the null hypothesis, because we were only able to do so for 2 trials. If by performing more trials, we come to the conclusion that isopods do indeed have a preference for dark over light, that would be consistent with previous findings. According to the article Influence of Light of Very Low Intensity on Phototropic Reactions of Animals, at even a relatively low light intensity, isopods tend to move away from the light and prefer the dark (Hartline 1923). The article states that isopods were "equally negative in turning away from a light source in illuminations ranging from several hundred meter candles to 0.01 meter candle". This clearly shows that isopods tend to avoid light, even when the intensity is low.

In our experiments, we didn't control for species, but that is something else we could have done to improve upon our experiment. According to the article_Behavioral Adaptations of Terrestrial Isopods (Warburg 2015), isopods' preference for light or dark depends heavily on their species. Something scientists could do in future experiments is perform isopods' preferences after separating species and comparing each of their preferences. Additionally, scientists could use multiple intensities of light rather than just one. Overall, our experiment was beneficial in better understanding the behavior of isopods and their behavior in different conditions and environments.

References:

Warburg MR. 2015. Behavioral adaptations of terrestrial isopods. In: American Zoologist, Volume 8, Issue 3. pp. 545–559

Hartline HK. 1923. Influence of light of very low intensity on phototrophic reactions of animals. Biological Laboratory of Lafayette College. pp. 137-152