# Effects of Nutrient Deprivation in Drosophila: Locomotor Activity, Climbing and Mating

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## Introduction

Nutrition and environmental conditions during early development play a critical role in shaping an organism's physiology, behavior, and reproductive success. Drosophila melanogaster provides an excellent system for exploring how environmental stressors, such as malnutrition affect various behaviors of an organism. In natural and laboratory settings, fruit flies often encounter variable food availability and population densities, which can lead to competition for resources and developmental stress.

The behaviors we study in this experiment are as follows-

**1. Mating behavior:** It is widely accepted that hunger state and food availability affect mating behavior. Mating behaviors may exhibit trade-offs with survival because the energy allocated to survival may reduce the energy available for mating behaviors that are important for reproduction. In this study, we aim to find out the effect malnutrition on the productive behavior of Drosophila.

**2.Locomotor activity:** Every animal expends a considerable part of its energy for locomotion. Using drosophila as a model, we aim to understand how an organism adapts to limited energy resources and adjusts its energy budget for locomotion. We also aim to develop an understanding of how physiological differences in males and females, affect their response to starvation.

**3. Climbing assay:** Negative geotaxis is Drosophila is a well-established behavior. In this assay, we aim to study the effect of malnutrition on this behavior. Since climbing requires active force generation, this assay can also act as a proxy for the effect of nutrient malnutrition on the muscle strength of drosophila.

### *(I) Drosophila Activity Monitor(DAM) Assay*

## Methodology

The DAM Drosophila Activity Monitor measures the locomotor activity of 32 individual flies, each in a separate tube. As a fly walks back and forth within its tube, it interrupts an infrared beam that crosses the tube at its midpoint, and this interruption, detected by the onboard electronics, is added to the tube's activity count as a measure of fly activity. Insert the tubes through the 32 holes in the monitor, and leave them centered so the detection beam will bisect the tube. If captivation of the tubes is necessary to prevent sliding, use rubber bands, stretch first over the 4 corner tubes of 2 adjacent rows, and press up against the monitor surface and then in a criss-cross pattern.

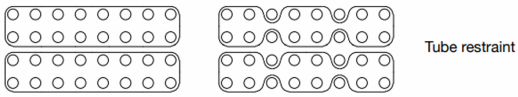


Figure 1: With the tubes installed, the monitor may be plugged into the DAM system network

The monitor will accumulate activity counts for as long as it has operating power, and will uplink its accumulated counts (and then reset to 0) whenever commanded to do so by the host computer. Counts will be accumulated as the flies are active in both total darkness and bright ambient light.

## Analysis

### Part 1

Questions to be answered

1. Is there a significant difference between the activity of control and treatment groups(irrespective of male and female)?
2. Is there a significant difference between the activity of males and females?
3. Is there a significant difference between the resting proportion of control and treatment groups(irrespective of male and female)?
4. Is there a significant difference between the resting proportion of males and females?

We apply a T-test for the difference of means between control and treatment in order to understand whether the difference in activity between various groups is significant. We are applying a Z-test for resting proportions hypothesis testing. ( for proportions we do not use T-test) We employ a significance value of 0.05 for testing the hypothesis. Our null hypothesis for all the above questions is that there is no significant difference between the two groups being compared. Our alternative hypothesis for 1,3 is that the application of treatment(i.e.

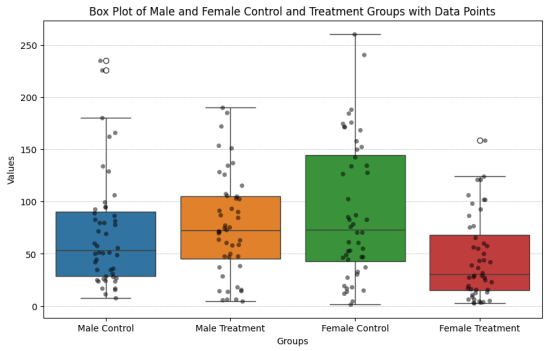


Figure 2: Box plot for Activity per hour of different groups

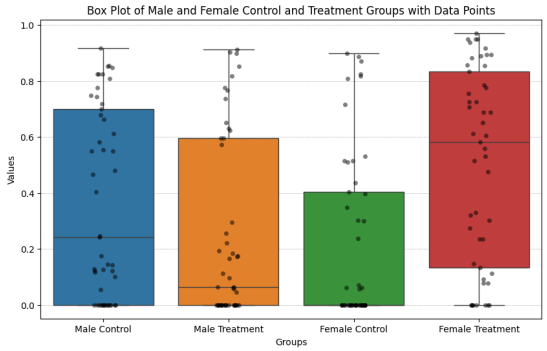


Figure 3: Box plot for Resting Proportion of different groups

lack of nutrition) reduces the activity per hour compared to the control.

Our alternative hypothesis for 2,4 is that the application of treatment(I.e lack of nutrition) increases resting proportion compared to control.The reasoning behind these alternative hypotheses is that lack of nutrition reduces the rate of metabolism, and thus energy is available for activity.

## Part 2

Calculation of activity/hr-

We did a summation of the number of times the fly crosses the beam every minute for all time recorded and divided it with total time. Calculation of resting bout-

To calculate the resting bout and resting proportion, we employed a method where we start counting zeros only if they occur greater than or equal to 5 times consecutively. Thus 1,2,3 or 4 successive zeros are ignored and not used for resting proportion count. A discrepancy was observed between our method and the method employed by the other two groups at this stage described above, we considered all zeros greater than or equal to 5 in calculations, so if 7 consecutive zeros were observed, considered them as 7 minutes of rest activity. The other two groups considered any sequence of consecutive zeros greater than equal to 5 as a rest bout of "only 5" minutes.

Table 1: Significant Test(Yes/No): For activity/hour-Welch's T-Test

| **Type** | **T statistic** | **P-Value** | **Yes or No?** |
|---|---|---|---|
| Group 1-Male treatment vs male control-33,34 | -0.247 | 0.807 | No |
| Group 1-Female treatment vs female control | 2.379 | 0.027 | Yes |
| Group 1-Treatment vs control | 1.598 | 0.116 | No |
| Group 2-Male treatment vs male control-37,38 | -1.507 | 0.142 | No |
| Group 2-Female treatment vs female control | 1.536 | 0.138 | No |
| Group 2-Treatment vs control | 0.221 | 0.826 | No |
| Group 3-Male treatment vs male control-35,36 | 0.525 | 0.603 | No |
| Group 3-Female treatment vs female control | 4.249 | 0.00021 | Yes |
| Group 3-Treatment vs control | 2.937 | 0.0047 | Yes |
| All groups-Male treatment vs male control | 0.6332 | 0.5281 | No |
| All groups-Female treatment vs female control | 4.0184 | 0.0001 | Yes |
| All groups-Treatment vs control | 2.4306 | 0.0161 | Yes |

## Discussion

The results of hypothesis testing vary from group to group and also along with the sex of drosophila being considered. No clear trend is observed for any of the questions put forth. Probably the errors involved in the experiment were significantly high and thus negatively affected the assay.

However some observations are useful to note-

1. In all three groups and in their summation, the assay for activity/hr, as well as the resting proportion, indicates that there is no significant difference in activity of drosophila males between treatment and control groups, which implies that drosophila males are more resistant and less affected by food unavailability(the data obtained is very small and not sufficient to prove this proposition), however, this observation is also supported by a previous study which quotes-"Males dispersed at a higher rate and was more active than females when food was unavailable, but tended to stay longer in environments containing food than did females"( statement from the abstract of research paper-Simon, J.C., Dickson, W.B. Dickinson, M.H. Prior Mating Experience Modulates the Dispersal of Drosophila in Males More Than in Females. Behav Genet 41, 754–767 (2011). https://doi.org/10.1007/s10519-011-9470-5)

Table 2: Significant Test(Yes/No): For resting proportion-Z test

| Type | Z statistic | P-Value | Yes or No? |
|---|---|---|---|
| Group 1-Male treatment vs male control-33,34 | 0.7545 | 0.4505 | No |
| Group 1-Female treatment vs female control | -2.8025 | 0.0373 | Yes |
| Group 1-Treatment vs control | -1.0775 | 0.2813 | No |
| Group 2-Male treatment vs male control-37,38 | 1.1944 | 0.2323 | No |
| Group 2-Female treatment vs female control-37,38 | -0.574 | 0.566 | No |
| Group 2-Treatment vs control-37,38 | 0.4353 | 0.6633 | No |
| Group 3-Male treatment vs male control-35,36 | 0.0612 | 0.9512 | No |
| Group 3-Female treatment vs female control-35,36 | -2.1474 | 0.0318 | Yes |
| Group 3-Treatment vs control-35,36 | -1.4732 | 0.1407 | No |
| All groups-Male treatment vs male control | 1.1443 | 0.2525 | No |
| All groups-Female treatment vs female control | -2.77 | 0.0057 | Yes |
| All groups-Treatment vs control | -1.2004 | 0.23014 | No |

2. In all three groups as well as in their summation, the assay for resting proportion showed no statistical difference. This is counter-intuitive and probably is an erroneous outcome.
3. If we consider the cumulative results of all groups, we observe that for females, both the activity per hour and resting proportions is statistically different. This aligns with the thought described in 1. Furthermore, for all groups except group 2 in both assays, we observe a significant difference. However, due to a smaller sample size and one group not showing the result, this is not considered a general conclusion.

### Limitations of the assay

1. We considered 5 minutes of no activity as a rest bout and only counted zeros if they occurred successively greater than or equal to two 5 times. However it is possible that during these 5 minutes, the drosophila is moving in only one part of the vial, without crossing the infrared beam. This is a limitation of the instrument and hence cannot be controlled. We took the above consideration to reduce this error, however it does not ensure 100% accuracy.
2. The Welch's T-test employed for analysis assumes that both groups are normally distributed. Since our sample size is <=32, this assumption has certain errors associated with it. However, for the same reason, we are using a T-test, and not Z statistic for activity assay analysis.
3. We did not put all the vials together inside the monitor, hence the initial movement of some insects was not recorded. There is a possibility that after the initial bust of movement, due to exhaustion, drosophila shows lesser movement. Hence an uncontrolled variable was introduced in our assay. We cannot quantify the error associated with this.
4. Handling of insects while preparing for assay might have also affected there activity recorded.

## Conclusion

To conclude, we cannot generate a general conclusion for any of the hypothesis put forth in this study. However, physiologically, it is still considerable to assume that if the experiment is performed with all precautions, treatment group should show a lower activity than the control group.

## *(II) RING(Rapid Iterative Negative Geotaxis) Assay*

## Methodology

The RING(Rapid Iterative Negative Geotaxis) assay is a widely used method to study climbing behavior in Drosophila melanogaster (fruit flies). It is particularly useful for assessing locomotor function, which can be influenced by factors such as aging, genetic mutations, neurodegenerative diseases, or pharmacological treatments.
With treatment being the larval stage crowding and random sampling taken by the PhD students of male and female from control and treatment, with all being separated in the chamber of fixed length area width so volume.
We are assessing the impact at the level of behavior in the condition of random fluctuations(non-controlled conditions) of light, sound, temperature, and positioning of the RING setup (since it was done multiple times on the same apparatus and we assume that previous imprints do not impact the current data collection) for the duration of the 2 minutes in a group. We did not check the reproducibility of the experiment by performing it again hence. Since the number of individuals is in the same amount hence, we choose to consider the number of drosophila the same since the error bar is plus minus 1 after repetitive counting in slow and steady manner.

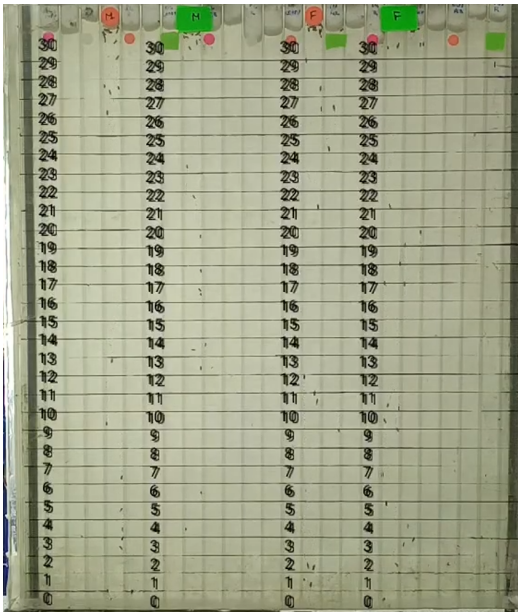

Figure 4: Method 2: Video Analysis

### Method 1

In the method, we are taking a screenshot every 15-second interval and trying to find the average distance of the flies by the location of those flies who are still in between 0 to 29 bins. By this method, we are only able to see whether they prefer either lower or higher in the column by the average distance (only for those who are in 0 to 29 bins).

### Method 2

In this case, we are trying to use a different approach. We used OpenCV to analyze the change in the position of the Drosophila. With the help of this, we tracked the positions of the flies and summed over the distance traveled. We tried to find out which group is more active and can easily distinguish whether they are showing negative or positive movement by their movement observed.

### Logic used for simulation:

Each Drosophila is counted as a new individual when movement is detected. Movement is recorded based on changes in pixels, and individual identification is not possible with this approach. Additionally, detection is only feasible for sparse distributions and not for dense clusters. Continuous time duration is considered in which total distance traveled is observed,as well as the movement of individuals can be identified within the 30 bins also. However, this poses a challenge because the distance traveled in a short time may differ between treatment and control groups.

## Analysis

Question to be answered

1.How is the behavior different in control total vs. treatment total?

2.Evaluation of the fact that can we assess the impact of different treatments from the above methodology on the climbing behavior.

### By Method 1:

For Males:

The treatment group shows a constant average distance because once they reach the 30th bin, they do not exhibit significant positive geotaxis, resulting in a relatively higher and stable average distance over time. From the data, it can be inferred that the average distance traveled by the male treatment group remains consistent, whereas the male control group reaches the 30th bin, but some individuals continue to show positive geotaxis, causing a decrease in the average distance over time. This suggests that the male control group exhibits more anxious behavior; even after reaching the 30th bin, some individuals display positive geotropism, leading to a reduction in their average distance. In contrast, the male treatment group stabilizes once they reach the 30th bin, showing minimal movement and maintaining a consistent average distance.

For Females Only: Female controls show a slightly higher average distance compared to female treatment groups, and this trend is mostly consistent. The behavior of the control and treatment groups is almost similar.

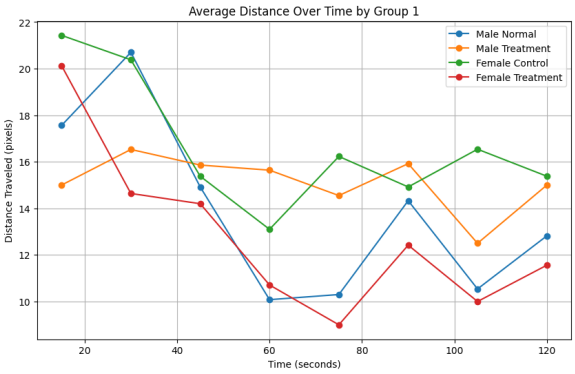


Figure 5: Method 1: Average distance of flies in Group 1

### By Method 2:

After analyzing the 4 videos(Group 1, Group 2, and Group 3 - Video 1 and Video 2): The male treatment group shows more stability compared to the male control group. The male treatment group stabilizes at certain positions, resulting in minimal fluctuations in the distance traveled. In contrast, the male control group exhibits more variability in movement, leading to fluctuations in

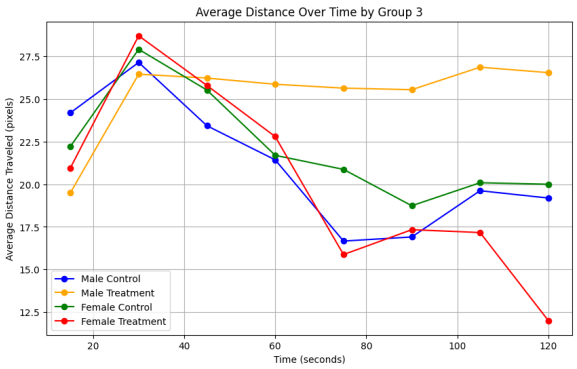


Figure 6: Method 1: Average distance of flies in Group 3

the average distance.
While the difference traveled by the female control and treatment group isn't showing enough difference. We even tried to detect the positive and negative movement separately. In this part, we haven't observed such a difference in trends as compared to the total distance traveled.

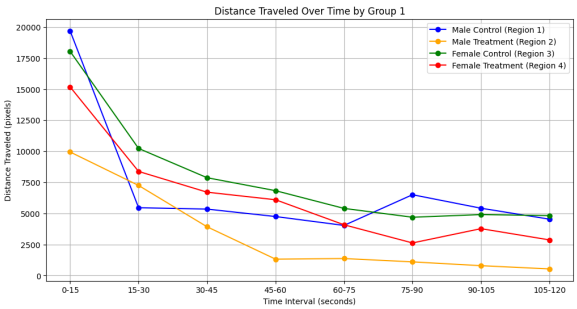


Figure 7: Method 2: Distance traveled by Flies in Group 1

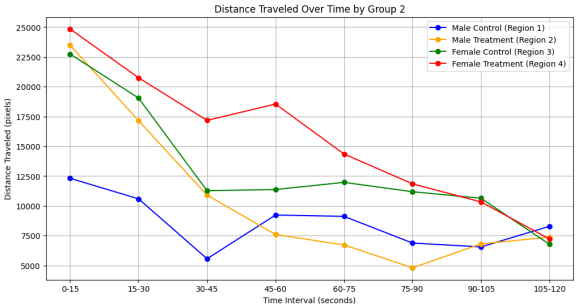


Figure 8: Method 2: Distance traveled by Flies in Group 2

## Discussion

Male control was moving too fast and reaching the top earlier than male treatment. While the female control and treatment groups did not show such a significant difference in time taken to reach the top of the column. After performing the Welch T-Test, we found the effect of larval crowding in males but failed to observe a significant change in the female control and treatment group.

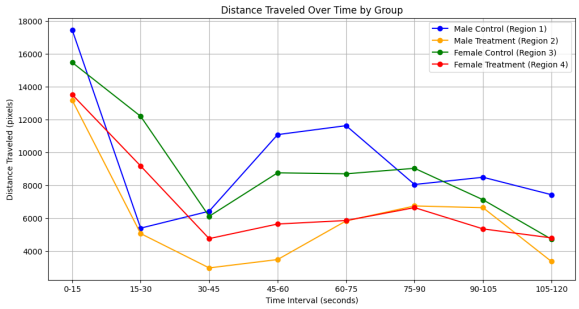


Figure 9: Method 2: Distance traveled by Flies in Group 3 (Trial 1)

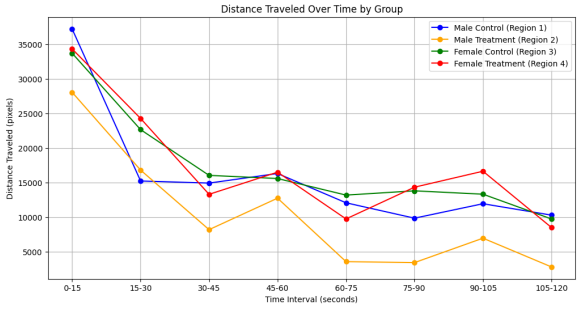


Figure 10: Method 2: Distance traveled by Flies in Group 3 (Trial 2)

### Limitations of the assay

1. Grouping of flies may introduce social interactions that affect climbing behavior. So we can't explicitly predict its behavior when observed as an individual.
2. After repeating the experiment on the same flies, they may show different behavior. 3.It is very hard if we are having limited data and trying to generalize the behavior to every fly.
4. It is hard to make conclusion regarding it's behavior in free space behavior as we operating it in a confined space.

## Conclusion

We can draw any conclusion of its behavior in open space, but in the confined space, it shows a significant difference in the male treatment group. So we can put our hypothesis that there is an effect on the climbing behavior of males because of larval crowding.
**Note:** Conclusion is drawn taking the results the hypothesis of **control = treatment** and observation.
We concluded as in the case of the male 2/3 trials of method 1 and also in method 2, that control and treatment are not the same, while in the female 2/3 trials of method 1 and also in method 2, control and treatment are same.
**T-Test Results are on the next page.**

Table 3: Method 2: Welch's t-test Results (Rejection column for the hypothesis of control is same as treatment)

| Group | T-Value | Critical T-Value | Reject |
|---|---|---|---|
| Male | 1.6997 | 1.6698 | Yes |
| Female | 0.1776 | 1.6704 | No |

Table 4: Method 1: Welch's t-test Results (Rejection column for the hypothesis of control is same as treatment)

| Group | T-Value | Critical T-Value | Reject |
|---|---|---|---|
| Group 1 Male | −0.8635 | 1.8464 | No |
| Group 1 Female | 2.4001 | 1.7674 | Yes |
| Group 2 Male | 7.1683 | 1.7633 | Yes |
| Group 2 Female | −1.2788 | 1.8204 | No |
| Group 3 Male | −2.7425 | 1.7817 | Yes |
| Group 3 Female | 0.9160 | 1.7961 | No |

### *(III) Mating Behavior Assay*

#### Methodology

Virgin Drosophila from crowded and normal populations are separated into male and female and kept separately until they reach sexual maturity. A single male and female are introduced in a vial, kept undisturbed, and observed for their mating behavior. A total of 18 such vials (9 from each population) are observed for roughly an hour. The duration of mating latency and copulation is noted for each vial.

Mating Latency = mating initiation time - observation start time

Copulation Duration = mating end time - mating start time

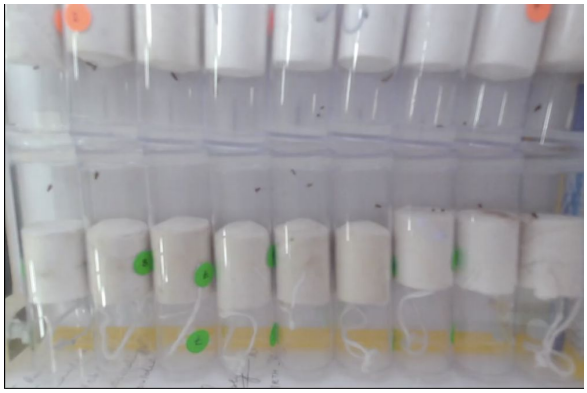

Figure 11: Mating Behavior Assay

#### Analysis

Question to be Answered

Is there a significant difference between the duration of mating latency and copulation of the control and treatment (crowded) groups?

We use Welch's unpaired T-test to calculate if the difference between the control and treatment groups is significant. We use a significance value of 0.05 for the hypothesis testing.

**Null Hypothesis:** There is no significant difference between the two populations.

**Alternate Hypothesis:** There is a significant difference between the two populations.

This would mean that crowding in the larval stage has an effect on mating behavior in Drosophila.

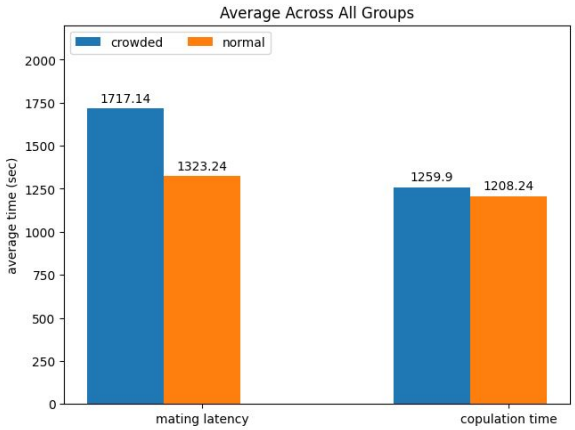


Figure 12: Mating Latency and Copulation Time average across all groups

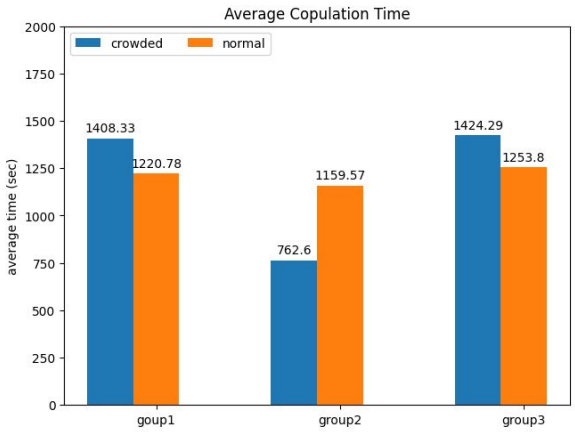


Figure 13: Average Copulation time

#### Discussion and Conclusion

Using the Welch's T-test, we cannot reject the null hypothesis and hence the differences observed between the control and treatment populations are not statistically significant. This may be due to errors in the experiment. No trend can be observed between the control and treatment populations when data for each group is analyzed individually. This may be because of the small sample size. Also, copulation was not observed in some of the vials, reducing the sample size further. However, the average mating latency and copulation duration for all groups together is longer for the crowded populations than for the normal populations. The longer mating latency

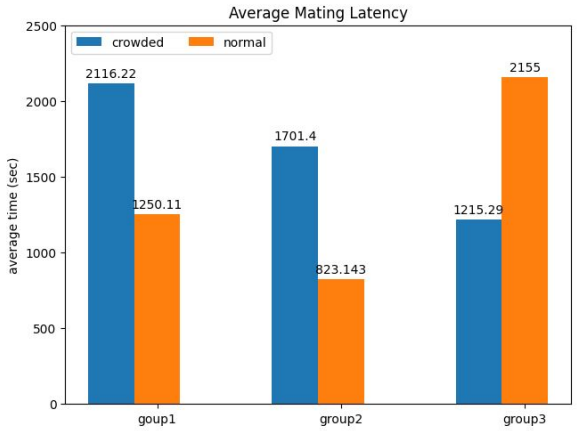


Figure 14: Average Mating Latency

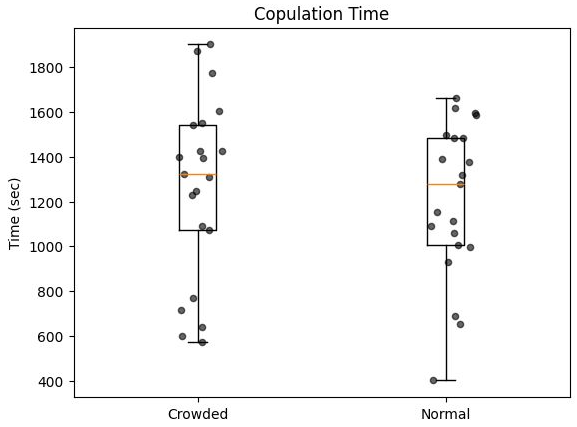


Figure 15: Copulation Time

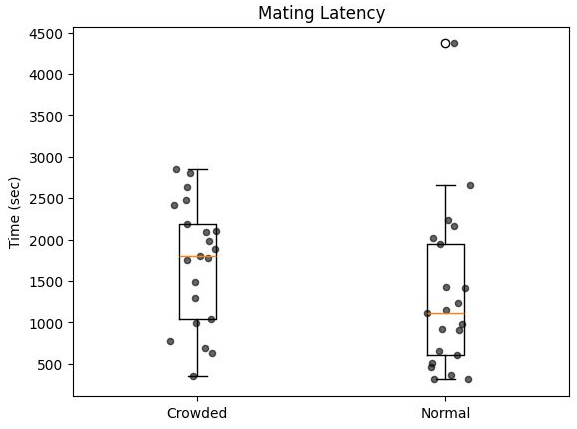


Figure 16: Mating Latency

could be due to the lower mating success in crowded populations. Lower mating success is shown to increase courting duration in males (reference from discussion of a research paper - Narasimhan, Kapila, Meena, and Prasad. "Consequences of Adaptation to Larval Crowding on Sexual and Fecundity Selection in Drosophila melanogaster." Journal of Evolutionary Biology, vol. 36, no. 4, 2023, https://doi.org/10.1111/jeb.14168).

The difference in the copulation duration between the two populations is relatively modest, so it suggests that flies raised under crowded (and potentially stressful or malnourished) conditions might engage in slightly prolonged copulation.

| Type | T-Statistic | P-Value | Significant? |
|---|---|---|---|
| Group 1: Mating Latency | 3.5047 | 0.0029 | Yes |
| Group 1: Copulation Time | 1.3897 | 0.1836 | No |
| Group 2: Mating Latency | 1.9693 | 0.0772 | No |
| Group 2: Copulation Time | -1.9446 | 0.0805 | No |
| Group 3: Mating Latency | -1.4654 | 0.1735 | No |
| Group 3: Copulation Time | 0.7369 | 0.4781 | No |
| All Groups: Mating Latency | 1.4582 | 0.1526 | No |
| All Groups: Copulation Time | 0.4419 | 0.6609 | No |

Table 5: Welch's T-test results for Mating Latency and Copulation Time

## Sources of Error

1. Copulation in some pairs was interrupted and hence only the longest uninterrupted copulation time was considered( especially in Group 2 readings).
2. The other vials may have gotten disturbed when other vials were being placed under the camera for observation.
3. For the very first few vials, the space given to the flies was lesser compared to the subsequent vials in the experiment conducted by group 1.
4. The flies may have been slightly injured while they were being transferred to the vials.

## References

1. Consequences of Adaptation to Larval Crowding on Sexual and Fecundity Selection in *Drosophila melanogaster*. Journal of Evolutionary Biology, vol. 36, no. 4, 2023, https://doi.org/10.1111/jeb.14168
2. LLMs are used for programming and analysis purposes.
3. https://graphpad.com
4. https://trikinetics.com
5. https://www.criticalvaluecalculator.com