Reducing yeast concentration in diet of Drosophila melanogaster negatively impacts female fertility

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Abstract
Developmental timing and female fertility are dependent on a wide variety of factors. Before female flies can allocate resources to reproduction, they must account for the risk-benefit ratio. It is known that changing protein levels in fly food, among other manipulations, affects female fertility and developmental timing. However, the influence due to only manipulating yeast levels is not fully characterized. We evaluated the impact of decreased yeast concentration on the fertility and developmental timing in *Drosophila melanogaster*. We also tested if there was a difference in the effect of yeast concentration on the fertility and developmental timing of differently aged flies. We found that the yeast concentration played a significant role in decreasing female fertility and increasing developmental timing regardless of age, though age had a negative effect on its own.

Introduction
The strength and survival of animals is dependent on their diet. Thus, altering the diet of an animal will lead to visible as well as cellular differences in animals (Grangeteau et al. 2018). There are multiple ways to test the effect of food on the health of an animal. One such measure is through female fertility and developmental timing. Fertility refers to the ability for the female to produce offspring. Developmental timing refers to the timeframe in which the offspring reach certain measurable changes during their development. In order to test for these parameters, it is helpful to choose an animal with a short lifespan and early maturity. One such model organism is *Drosophila melanogaster*. *Drosophila* is a common model organism and one that is relatively easy to take care of in the lab. *Drosophila* is one of the most used model organisms, not only because of the low cost, short lifespan, and well-documented genetics, but also because most of the genes present in *Drosophila* are homologous to those in humans (Tolwinski 2017). There are multiple possibilities that could be explored when experimenting on the effect of food on *Drosophila*, but for this experiment, we only chose to test the effect of decreased yeast concentrations on fertility and developmental timing. We chose to test yeast because it is the protein source for the flies. Proteins are known to be important for development, and previous studies have shown that decreasing the yeast concentration emulates overcrowding and has similar effects on the flies during larval development (Klepsatel et al. 2018). Overcrowding is when there are too many flies present and so the availability of nutrients is depleted. Larval crowding has been shown to affect multiple aspects of fly development, including decreased body size, an extended life span, lower fecundity, etc. (Klepsatel et al. 2018). Thus, decreasing yeast concentration will likely lead to smaller flies and other physical changes. Food and access to adequate nutrients are also important aspects for mating. Mating is not necessary for survival but is necessary for reproduction, therefore it is an action that can be put off in times when survival is not guaranteed or when nutrients are low. Mating has been shown to take a toll on female fly health, and so the female flies need high nutritional food in order for the benefits of mating to outweigh the risks (Gorter et al. 2016). This indicates that a decrease in the food quality will lead to a decrease in the amount of female mating. Decreased mating will directly influence the measured fertility of the female. Protein rich environments have been shown to lead to higher levels of mating which is associated with the levels of offspring produced (Gorter et al. 2016). In addition, the percentage of protein present in the environment affects the development of larva (Gorter et al. 2016; Grangeteau et al. 2018). Therefore, there are previous studies that support testing the effect of yeast concentration on female fertility and developmental timing (Good and Tatar 2001). For our experiment, we measured female fertility by the number of eggs laid over a certain timeframe and we measured developmental timing by counting the number of flies that eclose over a series of days. Eclosion is the process when an adult fly emerges from the pupal casing. This is a physical change that is easily seen. More information on how fertility and developmental timing were measured will be provided later in the paper. Since there are common recipes to make food for *Drosophila* kept in the lab, we decided that we would keep the control group with the usual concentration of 4% yeast. In order to change the yeast concentration enough to have an effect, we halved the concentration of yeast in the experimental group down to 2% yeast. Since the concentration of the control is already relatively low, we hoped that a change of 2% yeast would be large enough, especially since we did not want to remove all yeast from the food source. As mentioned before, previous literature shows a preexisting connection between yeast concentration and the health and fertility of *Drosophila*, however further characterization is always necessary. Therefore, we hypothesize that if the yeast concentration in the *Drosophila* food source is halved, then there will be a negative impact on the fertility and development of the flies. To test this hypothesis, we manipulated both the yeast concentration as well as the time frame in which the flies were mated, five-day and twenty-day old female virgins, our "young" and "old" flies respectively. We tested both young and old flies to see if the effect of yeast stayed the same throughout the lifespan or if the effect was confounded in older flies.

Methods
Production of Fly Food
To make food with lower concentrations of yeast, the food had to be made from scratch as follows: 1% agar, 7% dextrose, 1500 mL water, and 5% cornmeal were combined and heated until boiling, stirring occasionally. Once heated the mixture was divided into equal parts. In separate beakers, the control and experimental food was created by adding 2% yeast and 4% yeast, respectively, then filled with water to desired volume and boiled again. The mixtures were cooled and 1.6% tegosept was added into each. 5 mL of food was aliquoted into separate vials for the control and experimental food groups. The food for both the in-lab group and at-home experiments were made at the same time in lab weeks prior to starting the experiment and stored in the fridge until used.
Fly maintenance and handling.
Fly stocks of genotype Canton-special (CS) were passed to fresh commercial food about twice a week to prevent microbial contamination and maintain freshness. When passing flies, the old vial was tapped on a foam pad so that flies fell to the bottom of the vial. The old vial was uncapped, and the new vial quickly aligned on top not allowing any flies to escape. The connected vials were then inverted, and the flies were then tapped down into the new culture vial. The new culture vial was then labeled with the date, genotype, food condition, student initials, and number of times the culture had been passed. Flies in the in-person lab were stored in an incubator at 25°C and 60% humidity to shorten the generation time. Flies reared in the at-home lab were kept at room temperature, which fluctuated throughout the experiment. To account for these fluctuations, the temperature on the home thermostat of the storage room for the fires was recorded and averaged over a period of two weeks.
Fly anesthetization in-person & at-home
To best sort, move, and identify the sex of flies over longer durations of time the flies were first anesthetized. Due to the variability of the availability of resources, the methods of anesthetization for the in-person and at-home labs differed from each other but remained consistent within each location. The in-person lab used carbon dioxide tanks to anesthetize flies. The at-home lab immobilized flies by lowering their body temperature via refrigeration. When handling and moving anesthetized flies, small paint brushes were used, taking special note to handle all flies delicately as not to injure, squish, or unnecessarily jostle them.
Developmental timing vial set up
To test developmental timing, two males and two females were placed together in vials of either experimental or control food groups. These flies were left to mate and lay eggs for roughly two days (~48 hours). The same parent pairs were removed from the vials to be used to start the egg lay pates. The vials were checked every few days for visual changes and dryness. If the food in any of the vials looked dry or began lifting from the sides of the vials 2-3 drops of water were added to moisten the food. Roughly 8 days after the start of the developmental timing test, vials were checked every 24 hours for eclosed flies. Each day after eclosed flies were observed, counts were taken of the number of eclosed female and male flies in each vial. This was continued until all flies had eclosed and then the vial was discarded.
Egg Lay Plate set up to measure fertility
To measure fertility, egg lay plates were assembled to enable egg counting

data to be collected. Grape agar plates were kept at 4°C until ~5-10 min before the preparation of the egg lay plates, and any condensation was wiped away before use. Granulated yeast (~¼ to ½ tsp) was mixed in a weighing boat with a few drops of water and spread in the center of the plate to attract females to lay eggs. Parents from developmental timing vials were anesthetized and transferred onto the edge of the corresponding plate, avoiding the yeast paste. The egg lay plate was then topped with a mating chamber, secured with tape, and incubated for 24 hours at 25 °C for in-person lab. The at-home lab mating cages were kept in a box at room temperature. After a day (~24 hours) the parents were discarded in the fly morgue. Data collection of the egg lay plates consisted of counting the visible eggs on the plate. This was done under a light microscope for the in-lab groups and with a camera and flashlight setup for the at-home experiment. While counting embryos the egg lay plates were marked on the reverse side with a line to signify where counting began. Eggs were counted until returning to this marked line. Each plate was counted at least twice to retrieve accurate counts and the average of each count was used for statistical analysis.

Statistical analysis of fertility and developmental timing

To determine significance of the data, two programs were used. Initially, the data analysis add-on in Excel was implemented to perform a two-sample F-Test for variances, the corresponding two-sample t-test assuming equal or unequal variances, and Two-Factor ANOVAs with replication for data sets with equal n in each condition. For days to eclosion, Jamovi was used to generate an ANOVA with unequal sample sizes and an estimated marginal means graph.

COVID-19 safety precautions

To maintain safe working environments, in-person lab and at-home lab safety precautions were taken to protect the health and well-being of students and other various individuals in labs. Materials used for this experiment were discarded in biohazard bags, in-person lab, or separate trash bags, at-home lab. While in lab and handling flies and materials, students refrained from touching their faces and frequent and proper hand washing procedures were followed. In-person students wore face masks and remained socially distanced as to maintain COVID-19 safety practices.

Results

Lower yeast concentration in food decreases female fertility

In order to assess whether the concentration of yeast in the food affected female fertility, we first needed to define fertility. For this study, fertility was defined as the number of eggs laid in a 24-hour period. To measure the number of eggs, grape juice agar plates with a small circle of yeast paste in the center were assembled, then two young male and two virgin female flies were added, along with a mating cage. We predicted that the young virgin female flies (5 days old) in the 4% yeast condition would be the most fertile based on previous literature. Initially, we counted eggs from the 2% yeast experimental and 4% yeast control food types using young virgin females. The average number of eggs laid by young females on 4% yeast was 23.65 eggs (n = 10), while the average for 2% yeast was significantly lower at 5.1 eggs (n = 10), $t(11) = 3.76$, $p = 0.003$ (Figure 1). It was also important to examine fertility in aged virgin female flies (20 days old). We used the same apparatus and counted the eggs 24 hours after set-up. The average number of eggs in the 4% yeast condition (n = 10) was 13.25 eggs, while the 2% yeast (n = 10) was similar to the young condition with 5.8 eggs (Figure 1). These results indicate significantly lower fertility in the aged females on 2% yeast food, $t(18) = 2.77$, $p = 0.01$. Because the averages for eggs laid in the 4% yeast condition by young and aged females was qualitatively different by about 10 eggs, we did a two-factor ANOVA with replication to examine whether age and food condition are jointly influencing fertility (Table 1). We found that the ANOVA supported the previous result that there were significantly less eggs laid in the 2% yeast condition ($F(1, 36) = 21.38$, $p < 0.0001$). However, any effects due to age were not significant ($F(1, 36) = 2.98$, $p = 0.09$), and there was not a strong significant interaction between age and food type ($F(1, 36) = 3.90$, $p = 0.06$). Thus, these results suggest a relationship between the concentration of yeast in diet and female fertility.

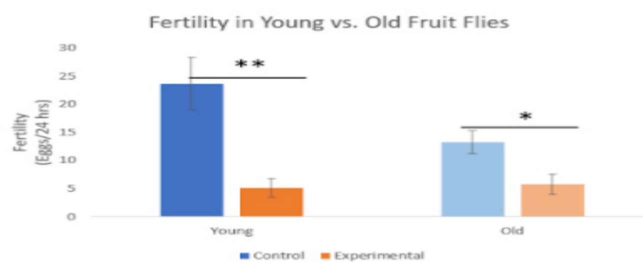

Figure 1. Fertility of young and old females decreases significantly in the 2% yeast condition (orange) compared to the 4% yeast control (blue). Fertility was measured by the number of eggs laid over 24 hours by virgin females of two ages, 5 day and 20 day. N = 10 for each diet and age. (**p < 0.01; *p < 0.05)

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample (Age)	235.225	1	235.225	2.976	0.0931	4.113
Columns (Food)	1690	1	1690	21.380	0.0000471	4.113
Interaction	308.025	1	308.025	3.897	0.0561	4.113
Within	2845.65	36	79.046			
Total	5078.9	39				

Table 1. Type of food significantly decreases fertility of young and old female flies. A two-factor ANOVA with replication was run to examine whether age and food have any interaction. The 2% yeast condition resulted in significantly lower fertility ($p < 0.0001$), while fertility based on age was not significantly impacted ($p = 0.09$). There is a slight interaction between age and food type, but this was not significant ($p = 0.056$).

Developmental timing differs with decreased yeast concentration

After determining that reducing the concentration of yeast in female diet affects fertility, we investigated whether there were any developmental timing differences caused by the different diets. To begin, we placed two young male and two young virgin female flies together in a developmental timing (DT) vial for 48 hours, after which they were removed. Once flies began to eclose in the DT vials, we recorded the number of flies that eclosed every 24 hours and the number of days to eclosion. For young female flies in 4% yeast, the average time to eclosion for their progeny was 10.67 days (n = 10), while the 2% yeast progeny was 11.4 days (n = 7) (Figure 2A). This was repeated in 20-day old females with similar results of the 4% yeast condition averaging 10.73 days (n = 10) and the 2% yeast averaging 11.71 days (n = 3) (Figure 2B). Qualitatively examining this data, the progeny from females on 2% yeast food had delayed developmental timing by about one day. This was determined to be true for the aged condition by a two-sample t-test assuming equal variances that indicated a significant increase in time to eclosion in the 2% yeast condition ($t(11) = -5.37$, $p < 0.001$). For the young female condition, we implemented a t-test assuming unequal variances due to significance in an F-test two-sample for variance ($F(9, 6) = 0.23$, $p = 0.02$). The t-test revealed that there was no significant difference in time to eclosion between diets for young females ($t(8) = -2.17$, $p = 0.06$), despite the qualitative difference. We again tested for any interaction between age and diet that might affect time to eclosion using an ANOVA. For this test, it was necessary to use a different program, Jamovi, because we had an unequal n for each condition. In the DT experiments, there were multiple vials in the 2% food condition that had no flies eclose and thus needed to be excluded from this specific data set. The ANOVA revealed similar results to the individual t-tests, in that the lower concentration of yeast significantly increased the time to eclosion of flies, ($F(1, 26) = 17.40$, $p < 0.001$), as seen by the higher orange line in Figure 3. However, there were no significant differences as a result of age ($F(1, 26) = 0.75$, $p = 0.393$) or due to the interaction between the two variables ($F(1, 26) = 0.32$, $p = 0.578$).

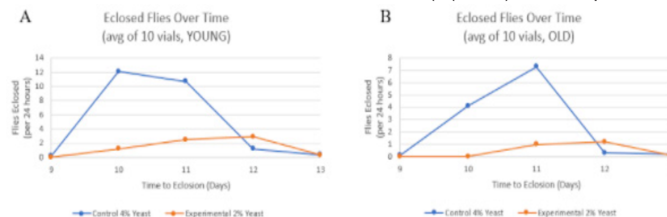

Figure 2. Flies on the 2% yeast food took longer to develop than those on 4% yeast food. The number of newly eclosed flies in each vial were counted every 24 hours until flies stopped eclosing. **A**) Flies eclosing from the young, 4% yeast condition (blue) averaged 10.7 days to eclosion while the young, 2% yeast condition (orange) took longer to eclose, averaging 11.4 days, but this was not significant (p = 0.06) Young 4% N =10, Young 2% N = 7. **B**) In the old, 4% yeast condition (blue), flies eclosed after 10.73 days, while the old, 2% yeast condition (orange) they averaged 11.7 days to eclosion, significantly longer (p < 0.001) Old 4% N= 10, Old 2% N = 3.

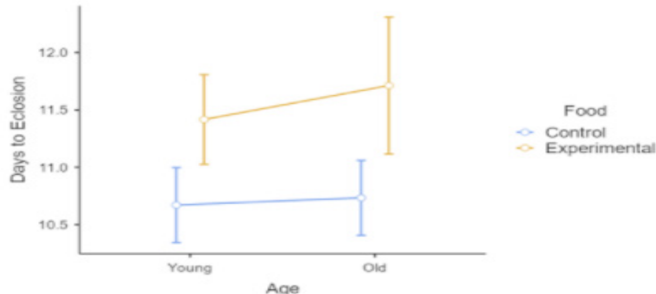

Figure 3. Flies took significantly longer to eclose on the 2% yeast food than the 4% yeast. A two-factor ANOVA was run in Jamovi due to different N in conditions (Young 4% N =10, Young 2% N = 7, Old 4% N= 10, Old 2% N = 3) and an estimated marginal means was generated. The experimental 2% yeast condition is in orange while the control 4% yeast is in blue. The food significantly influenced the time to eclosion (p < 0.001), but age did not have a significant effect (p = 0.393) and there was no significant interaction between food and age (p = 0.578).

Reduced fertility results in a smaller total number of eclosed flies

The previous experiment not only provided information about differential developmental timing, but also gave the total number of flies eclosed in each condition. This data can also be used as a qualitative measure of fertility, as it can be assumed that when more eggs are laid, more flies will eclose. Therefore, we expected to see the most flies eclosed in the vials where young females on 4% yeast laid eggs, and the least in the aged females on 2% yeast vials. In the young condition, the average flies eclosed across the ten DT vials was 25.7 flies in the 4% yeast and 6.9 flies in the 2% yeast condition. The average number of flies eclosed in the aged females were 12 flies for 4% yeast and 2.3 flies for 2% yeast (Figure 4). We next ran two t-tests to evaluate whether lower yeast concentration significantly decreased the total number of eclosed flies, and we found this to be supported. In both the young and aged females, there was a significant decrease in the total number of eclosed flies (t(18) = 4.46, p < 0.001 and t(18) = 3.98, p < 0.001 respectively). We next asked the question of whether the age of the female flies had any impact on the total number of eclosed flies. Through a two-factor ANOVA with replication (Table 2), we determined that both age and yeast concentration significantly decreased the number of eclosed flies across the DT vials (F(1, 36) = 14.11, p < 0.001 and F(1, 36) = 34.24, p < 0.00001 respectively). While both variables resulted in significant changes to the number of flies, there was no significant interaction between the two (F(1, 36) = 3.49, p = 0.07). Thus, these data suggest that both age and yeast concentration have an independent effect on female fertility.

Figure 4. Female flies on 4% yeast food produced greater number of flies than the 2% yeast flies. Two male and two virgin female flies mated for 48 hours, then the number of eclosed flies were counted each day. Significantly less flies eclosed in the 2% yeast condition in both the young and old conditions (***p < 0.001) N = 10 for each age and food condition.

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Sample (Age)	837.225	1	837.225	14.116	0.000608	4.113
Columns (Food)	2030.625	1	2030.625	34.238	1.10E-06	4.113
Interaction	207.025	1	207.025	3.491	0.0699	4.113
Within	2135.1	36	59.308			
Total	5209.975	39				

Table 2. Total number of eclosed flies was decreased significantly by old age and 2% yeast food type. A two-factor ANOVA with replication was generated with the number of eclosed flies data (N =10 for all conditions). This test showed significant reduction in total flies across the young and old variable (p < 0.001), as well as across the 4% yeast and 2% yeast food conditions (p < 0.00001). The interaction between variables was not significant (p = 0.07).

Results from at-home lab experiments

No correlation between decreased yeast concentration and female fertility

The same experiments described above were performed outside of the laboratory. Figure 5 shows the measured fertility of young and intermediate aged flies cultured outside of the lab. Five-day old flies on 2% food conditions averaged 0.6 eggs while flies on the control 4% yeast conditions averaged 1.33 eggs. An F-test performed to account for variances (F(2) = 4.0, p = 0.20) prompted the use of a t-test assuming equal variances that showed no significant difference between food conditions in young flies (t(4) = -0.89, p = 0.422). Intermediate flies cultured on 2% yeast food conditions averaged production of 8.5 eggs. The control 4% yeast conditions of intermediate 12-day old flies produced an average of 17 eggs. To calculate variances, an F-test was employed (F(1) = 0.25, p = 0.295), from which a t-test assuming equal variances indicated no significant difference of the number of eggs laid on either food conditions (t(2) = -2.53, p = 0.127). There was a qualitative difference between the young and intermediate female fertility on different food conditions, so a two-factor ANOVA was performed on the data to test significance (Table 3). The results of this analysis suggested there was no significant difference in the number of eggs laid by females on the experimental food (F(1,8) = 0.562, p = 0.46). There was also no significant difference due to age (F(1, 8) = 5.06, p = 0.054) or due to an interaction between age and food (F(1, 8) = 0.90, p = 0.370).

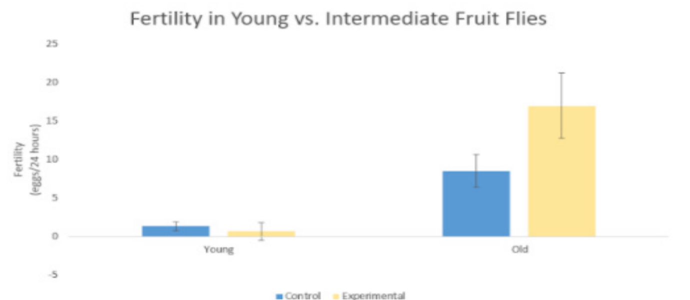

Figure 5. Fertility of young, aged 5 days, and intermediate aged flies, 12 days, measured by the number of eggs laid over a 24-hour period. Fertility of intermediate females shows an increase in fertility in the 2% yeast concentration (yellow) compared to the 4% yeast control (blue). An overall increase in fertility of intermediate females compared to young flies exists. However, due to error these differences are statistically insignificant (p=0.42, p=0.13) No observed differences yeast conditions in young flies.

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Sample (Age)	168.75	1	168.75	5.0625	0.05456	5.3176
Columns (Food)	18.75	1	18.75	0.5625	0.47473	5.3176
Interaction	30.08333	1	30.08333	0.9025	0.36992	5.3176
Within	266.6667	8	33.33333			
Total	484.25	11				

Table 3. Food type and age showed no statistical difference in fertility of young nor intermediate flies. A two factor ANOVA with replication revealed no significant change in fertility due to age (p = 0.055), food type (p = 0.475), or interaction between age and food (p = 0.370).

No differential developmental timing of altered yeast concentration
To further analyze the data for the developmental timing, the timing of when most flies eclosed needed to be addressed. Figure 6A and 6B displays the number of eclosed flies from parents cultured on control 4% yeast and experimental 2% yeast, as well as the time to eclosion. Young flies on both food conditions averaged 15.21 days to eclosion while intermediate aged flies averaged 11.67 days to eclosion. There was no significant difference in times to eclosion between either food conditions as all p-values were above the standard 5% threshold ($p > 0.05$) of significant difference.

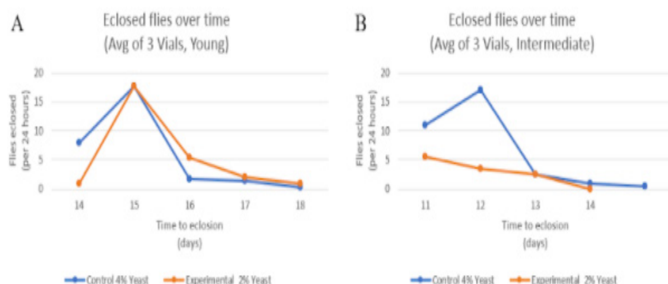

Figure 6. Mapped is the number of newly eclosed flies counted in each vial every 24 hours until flies stopped eclosing. A) Developmental timing of young five-day old flies. Flies on both food conditions had similar times to eclosion. The number of eclosed flies each day was not significantly different between the food conditions. B) Intermediately aged flies (12 day old) on 4% yeast conditions had a higher average of overall flies eclose. There is no significant difference in the number of flies eclosed between food conditions.

Female fertility by total number of eclosed flies at home
In extending the results shown in figure 6, the total number of eclosed flies was also used as a measure of fertility (Figure 7). The average number of eclosed young flies was 29 in controlled 4% yeast food condition. The average eclosed young flies on experimental 2% yeast was 27. As for the intermediately aged flies, the average number of eclosed flies was 16.6 under the controlled 4% food condition; while the average of eclosed flies for the experimental 2% yeast was 13. Any possible interaction of these results had no statistical difference on account of error and variance. Neither age ($F(1, 8) = 4.17$ $p = 0.075$) nor food type ($F(1, 8) = 0.09$, $p = 0.77$) were statistically different, and there was no significant interaction between age and food as well ($F(1, 8) = 0.005$, $p = 0.94$).

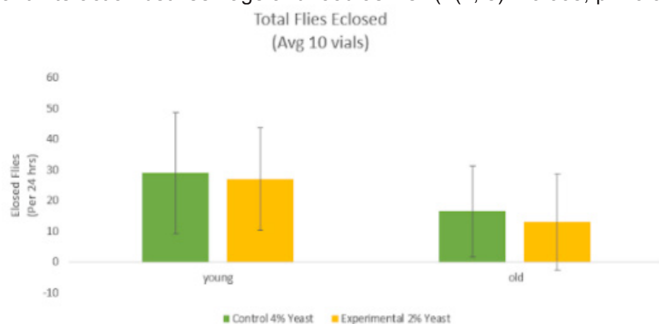

Figure 7. Comparison of the total number of eclosed flies of young and intermediate aged flies. Values of eclosed flies comparing young and intermediate flies implies a decrease in fertility with aging. However, the large error indicates there is not real significant difference. No significant difference between the control and experimental yeast condictiones within the young nor the intermediate groups.

Discussion
Based on our results, it is evident that the concentration of yeast in a female fly's diet directly influences the measured fertility. We found that with lower concentrations of yeast, young female flies laid fewer eggs and produced less progeny than those in the higher control concentration. The same result was observed in the old female flies. This indicates that flies that were placed on the 2% yeast food had reduced fertility compared to those on the control 4% yeast food. The type of food also negatively impacted the developmental timing of progeny, as eggs laid on 2% yeast took close to one day longer than the 4% yeast condition on average to eclose. Our results are supported by numerous previous studies that found decreasing the protein source in fly food also decreases female fertility, among other effects that we did results. Analysis of the at-home lab suggested there was no significant change in fertility or developmental timing due to reduction of yeast concentration in food conditions. Additionally, these results suggest that fertility increased as the flies aged when looking at female fertility but had reverse results when looking at the total eclosed flies in each food condition. The at-home lab did not have access to an incubator, resulting in all experiments being conducted at a fluctuating room temperature. The at-home experiments were subjected to large open spaces for longer periods of time. The lower temperatures alone gave reason to understand the inconsistencies of the at-home replication of these experiments. Additionally, the at-home lab also had slightly varied procedures and was subject to trial and error when conducting experimental methods. Lastly, the at-home lab had a much smaller sample size and fewer replications than that of the in-person lab. A comparison of in-person and at-home lab results would suggest conflicting analysis of both the female fertility and developmental timing. Notably, an understating of reviewed literature would also support that these are highly unsupported conclusions. Furthermore, with consideration of the multitude of external factors such as lower temperature, humidity and smaller sample size, it was determined best to refrain from statistically comparing the results from the two different lab environments.
There were multiple limitations of this study that might have impacted our results. To begin, the food we made had the tendency to dry out after larvae were present in the vials and moving through the tube, even with the addition of water. However, the food dried out evenly across the experimental and control conditions, and between in-person and at-home labs. So, while the dryness of food was not ideal, it equally influenced the results from each condition and, therefore, is not the most important factor to consider. Another surprising effect that we did not anticipate was that many of the 2% yeast developmental timing vials did not show any pupae and had no eclosed flies. We initially had ten DT vials in each condition and the 4% yeast DT vials maintained an n = 10, but over the course of experimentation, the 2% vials in the young condition were reduced to n = 7, while the vials in the old condition were reduced even more to only three vials eclosing flies (n = 3). There could be multiple explanations for why the lower yeast concentration reduced mating. One possibility is that it could be more difficult for the developing eggs to survive with lower protein levels. It is also important to note that there may have been slight differences between how the 2% and 4% yeast DT vials were handled, as the conditions were not equally distributed, instead each author in-person recorded data for all the flies in one food condition. So, despite similar timing of data collection and similar techniques, there might have been some differences that impacted the 2% yeast flies. Another more likely explanation is that flies are less likely to mate on food with lower nutrition (Gorter et al. 2016). This could be supported by the fact that the same flies that mated in a DT vial that yielded no new flies were recorded to lay eggs on an egg lay plate that contained an unknown, but higher concentration of yeast paste. Unfortunately, we did not investigate whether the eggs laid on plates were fertilized, so it is difficult to predict whether the females were not fertile, or if they were not willing to mate on 2% yeast food to increase their survival. In terms of how this impacted our results, the smaller n in the old female condition could influence the significance of our results or whether age and food type interact to change time to eclosion.
In the future, it would be interesting to explore whether the reduced fertility observed in low protein conditions is seen in multiple generations of flies. To begin the experiment described in this paper, we collected virgin female flies from mass produced commercial food, not our specific 2% and 4% yeast food. So, the female flies used in the fertility experiments developed on nutrient rich food rather than the nutrient poor 2% yeast. In following experiments, we would begin with a culture of flies on the control 4% and experimental 2% yeast foods conditions we made to investigate whether having lower levels of protein during development negatively impacts the adult fertility in a comparable way to the results of this study. It is possible that there could be an additive effect on the fertility of female flies and successive generations would lay fewer and fewer eggs and have fewer total flies eclose. On the other hand, the flies might have some adaptive mechanism that enables them to mate and survive on the low yeast food just as well as they do on the control food. Another study of interest would be to examine whether there is a relationship between measured fertility and lifespan of female flies on low-protein food. Some previous literature has noted that flies reared on low-yeast food show longer lifespans (Tatar 2007), while others have shown a more

complex relationship between yeast level, lifespan, and sex-specific responses (Duxbury and Chapman 2020). It is reasonable, based on results from Gorter et al. (2016), that the flies on low-yeast food reduce the energy intensive process of reproduction and mating to extend their lifespans. However, it would be interesting to see the difference of fly lifespans on 2% and 4% yeast and whether increased measured fertility is associated with decreased lifespan of female flies in one or both conditions. These results, and any future experiments, could be applicable to the importance of diet both before and during pregnancy for many organisms, including humans. If female flies who did not consume an adequate amount of protein are less fertile, then it may be reasonable to hypothesize humans who do not get the proper nutrition will have more difficulty in becoming pregnant. This interaction has been observed in human females, but the ideal amount of protein to consume remains unknown, although it is suggested that plant-based proteins are more likely to increase fertility than animal-based proteins (Silvestris et al. 2019). Overall, diet is extremely important to the survival and propagation of all living organisms, so a thorough understanding of nutrition is necessary to optimize survival.

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