ISSN: 3068-9538

Research Article

Journal of Environmental Sciences and Plant Research

Measurement of Lifespan and Impact of Inhaled Pollution on Health Outcomes in Drosophila Melanogaster

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ABSTRACT
The effects of environmental changes on an organism's lifespan have been extremely successful in elucidating features of aging that are conserved across taxa and in generating innovative approaches to prolong lifespan and prevent age-related diseases in mammals. Because of its short lifetime, ease of care, and simple genetics, Drosophila melanogaster is a desirable model organism for researching aging mechanisms. However, demographic measurements of mortality and aging are extremely sensitive to even small changes in experimental and ambient conditions, and it is necessary to maintain stringent laboratory procedures throughout the course of aging studies. Environmental factors like temperature and food have an impact on the longevity of Drosophila; mature males usually live much shorter lives as the temperature rises. Similarly, mature female flies on a less concentrated diet (5% SY) usually survive much longer than those on a more concentrated diet (15% SY). All living things are at risk from air pollution, a major worldwide issue. The purpose of the study was to ascertain whether face masks may improve health outcomes in D. melanogaster by significantly reducing inhaled particulate matter with a diameter of smaller than 2.5 microns (PM 2.5). It was predicted that Drosophila's lifespan and fertility would decline if it were exposed to high PM 2.5 levels. The exhauster was used to expose flies to pollutants in a controlled environment. PM 2.5 values were recorded using an air pollution monitor. The doctor's mask and N 95 mask reduced inhaled PM 2.5 by 69.04% and 89.20%, respectively, according to the study's findings. The longevity of Drosophila was reduced by 78.90% when exposed to pollution, while it was reduced by 51.79% when wearing a N 95 mask. Compared to the N 95mask (1.6), the doctor's mask (0.5), or no mask (0.28), the control group had much more mean offspring. Although they can effectively filter out particulate pollution, face masks are not the best solution to our current environmental crisis. First and foremost, we must continue to be dedicated to cleaning up our planet.

Keywords: Longevity, Drosophila Melanogaster, Mortality, Particulate Pollution, Temperature.

Received: March 16, 2026;
Accepted: March 23, 2026;
Published: March 30, 2026

Introduction

Because of its short lifetime, ease of care, and simple genetics, Drosophila melanogaster is a desirable model organism for researching aging mechanisms. However, environmental factors including temperature, pollution, and food have an impact on Drosophila lifespan; adult males usually live much shorter lives as temperature and pollution exposure rise. In almost every creature studied, aging causes a continuous physiological decline that lowers physical performance and raises the risk of illness. In order to understand the aspects of aging that are conserved across taxa and to inspire new strategies for extending lifespan and preventing age-associated disease in mammals, experiments that aim to quantify the extent to which genetic or environmental

Citation: Tasneem Amina and Venkataramana GV (2026) Measurement of lifespan and Impact of Inhaled Pollution on Health Outcomes in Drosophila Melanogaster. J Envir Sci Plant Res 2: 1-9.

manipulations will impact lifespan in simple model organisms have proven remarkably successful physical performance and a higher chance of illness.

Nowadays, environmental pollution is a serious worldwide problem that negatively impacts both food security and human health. Every day, both humans and animals are subjected to crippling levels of contamination. According to the World Health Organization (WHO), 99% of people breathe air that contains contaminants above recommended levels. Air pollution alone causes millions of premature deaths worldwide each year, primarily from lung cancer, chronic obstructive pulmonary disease (COPD), stroke, heart failure, and respiratory infections [1,2]. In addition to biodiversity and climate change, the United Nations (UN) has set a pollution-free earth goal as one of its three pillars for 2022–2025. The effective utilization of conventional in vivo testing is painfully limited by a number of reasons, including high operating costs and ethical concerns with the use of higher vertebrates [3,4]. Because D. melanogaster has homologs for 75% of the genes linked to human disorders, making it easier to study various abnormalities, simpler and more dynamic model animals like D. melanogaster may be preferred for toxicity studies. In an effort to provide a thorough review of the dangers associated with exposure to environmental contaminants, the study attempts to convey the image of studies about environmental pollutants that used D. melanogaster.

Nowadays, 90% of people worldwide breathe contaminated air, which causes 7 million deaths annually [5]. One of the main causes of air pollution is the burning of fossil fuels, which is linked to climate change. Particulate matter and gases are the two primary forms of air pollution. Fine inhalable particles that is typically smaller than 2.5 micrometers (PM 2.5) and particles smaller than 10 micrometers (PM 10) are included in particulate matter (PM) [6]. The majority of particles in the atmosphere are created by complicated chemical reactions between pollutants released by factories, power plants, and cars, such as sulfur dioxide and nitrogen oxides. Research indicates that harmful health consequences including heart attacks and asthma are significantly influenced by tiny particles like PM 2.5 [1,7].

Many people in nations like China and India try to shield themselves from air pollution by using face masks. Today's market offers a wide variety of masks, but little is known about their effectiveness [5,8]. In the current study, we assessed two widely used masks. The first is a basic surgical mask that is available at any doctor's office and has a single layer of cloth filter that is fastened to the face with elastic straps. It is generally believed to provide very little protection from the PM 2.5 particles in the air, even though it is frequently worn by people in nations with high levels of pollution. The second mask under evaluation, the N 95mask, employs several layers of filter material, such as carbon and microfiber fabric. Because it uses valves to maintain a good seal and make it easier for the wearer to exhale, it is regarded as a respirator. The purpose of this mask is to shield wearers from dangerous PM 2.5 particles [4,9].

We asked the following question after conducting a comprehensive analysis of the scientific literature: Do face masks considerably reduce the amount of particulate matter inhaled, and does this result in better, quantifiable health outcomes? For a number of reasons, we decided to investigate these issues using a practical animal model like D. melanogaster. According to Roeder and Linford et al., Drosophila has a short, straightforward reproductive cycle that lasts between eight and fourteen days, at least one lung equivalent, and an airway system that is strikingly comparable to mammals in terms of its physiology and response to pathogens [10]. Furthermore, humans and flies share 75% of the genes that cause disease. In this study, we used a sensor-based model to calculate the amount of particulate matter recorded by the pollution meter both with and without the insertion of each face mask. Using exhaust from a 2 HP Kirloskar diesel engine, we designed a pollution chamber to mimic a polluted environment. We then used an air quality monitor to test the ambient air quality within the chamber. Groups of D. melanogaster kept in vials with and without air pollution masks were then exposed. Inter-component metabolic interference and metabolic saturation may be responsible for the observed interactions and changes in their kind or degree in relation to mixture component concentrations.

Materials and Methods

Preparation of Experimental Food

Storing experimental foods, yeast paste, and grape agar plates at 4 °C and using them within 1-2 months as long as mold and dryness have not set in. Standard environmental conditions for both the larval and adult stage involve maintenance of flies in an incubator at 25 °C with a 12:12 hr light dark cycle and 60% relative humidity. For larval growth, we use a modified Caltech Medium, abbreviated as CT, diet for adult Drosophila (SY) that consists of sugar (sucrose) and yeast (lyophilized whole brewer's yeast) in a 2% agar base, that has been boiled, supplemented with antibiotics and anti-fungal agents, and distributed (10 ml per vial [11,12]). Food should be allowed to solidify and evaporate for 12-24 hr prior to storage. The yeast paste consists of combine 5-6 ml of water with 3 g of active dry yeast and mix well.

Collection of Synchronized Eggs

Media used, i.e. yeast, grape agar plates, CT and 10% SY food, should be at room temperature. Spread a 2-3 cm diameter layer of yeast paste on a grape agar plate and set aside. Place a large egg collection cage on a CO2 pad with the mesh-side down to anesthetize the flies [13]. Using a funnel, transfer 150-200 pairs of flies into the egg collection cage, Place the grape agar plate to cover the open end of the cage and secure with an end cap, lay the cage on its side until flies wake up. Aliquot 32 µl of eggs into CT bottles using a wide-bore pipette tip, eggs in the pipette tip should be compact, with little to no liquid aspirated. Place seeded CT bottles back in the incubator throughout fly development.

Collection of Age-matched Adult Flies

Adults usually close on day 10, discard the flies that emerged on the first day, and put the bottles back in the incubator overnight. The day-old adult flies are then transferred into 10% SY feeding bottles 16–22 hours later. Put the flies back in the incubator, give them two days to gain sexual maturity and mate, and note the day they are transferred to 10% SY bottles as their first day of adulthood.

Maintaining the Longevity Experiment

The vials containing fresh food should be at room temperature for each transfer. During the experimental period, transfer flies onto new vials containing fresh food every 2 days (young females), or 3 times a week (males or females >3 weeks of age). This step will ensure that the feeding environment for young females is not disrupted by the presence of larvae. This transfer should be completed without anaesthesia, which can induce acute mortality, particularly in older flies [5,14].

Data Analysis

The survivorship curve displays the probability that an individual survives to a given age and is typically calculated using a Kaplan-Meier approach, the formula can be simplified such that age-specific survivorship at age x (Sx) is determined by dividing the number of individuals alive at the start of a census time at age x (Nx) by the total number of flies in the experiment (N0); Sx=Nx/N0. 9.2) data [15-17]. Mortality measures are independent from one age to another, and the shape of the mortality curve is useful for inference about the dynamics of aging [18].

Construction Design of a Pollution Chamber/ Environment

The chamber was made by using plywood sheets box with a metal dryer vent assembly to introduce pollutants, plexiglass cube, window to introduce flies into the chamber, atmos real time air quality monitor inside to measure PM in the chamber and vials fitted with masks as shown in Figure 4, and 5. The Wi-Fi enabled electrochemical sensor devices (Atmos) for monitoring PM2.5 were installed within the chamber and pollutant levels were recorded for a 24-hour period. Real time data was retrieved from the company's cloud storage interface (Figure.5). Dependent variable = PM 2.5 reading, independent variable = Mask type, to determine how effective the pollution masks are at filtering out PM 2.5 pollutants. Pollution was generated using the exhaust from a 2 HP kirloskar diesel engine, measured PM 2.5 using an air quality monitor in real time. To determine the amount of pollution given off by the motor, connected the exhaust to one end of the 4” diameter dryer vent pipe and placed the air quality monitor about 6” from the open end. We ran the motor for 30 seconds and recorded the PM 2.5 level from the meter. Then we turned off the motor, waited 30 seconds, and recorded the PM 2.5 level again. We conducted three trials and used a leaf blower to introduce fresh air through the pipe after each trial. To test the effectiveness of the masks, we repeated the same process with each mask secured over the pipe so that the exhaust had to flow through the mask to get to the pollution meter.

Longevity and Fertility: Drosophila were placed into 4 experimental groups: no pollution, pollution and no mask, pollution and vials covered with doctor’s mask, pollution and vials covered with N 95mask, after 6 hours of pollution exposure, flies in groups 2, 3, and 4 were transferred to an incubator with unexposed flies, and their lifespan was monitored, to assess fertility, 3 male and 3 female flies were placed into each vial and pupae were counted on day 5. b) Dependent variable = Longevity, Independent variable = Mask type, wild type Drosophila melanogaster was acquired from laboratory biological company Bangalore and maintained at room temperature under normal lighting conditions, twelve vials of culture media were prepared and labelled, three for each of four experimental conditions: 1) No exposure to pollution; 2) Pollution only, no mask; 3) Pollution + doctor’s mask; 4) Pollution + N95mask. The control vials were covered with regular plugs, and the pollution, no mask vials were covered with cling wrap that had holes pierced in a size that allowed pollutants through but blocked flies. The doctor’s mask and N 95mask were cut into circular pieces that were used to cover the doctor’s mask and N 95mask vials respectively.

The vials for experimental conditions 2, 3, and 4 were placed in pollution chamber. Exhaust of the 2 HP kirloskar diesel engine was fed into the dryer vent. We recorded PM 2.5 levels every 30 minutes for a total of 6 hours, after the exposure, all 12 vials were covered with regular vial plugs and placed in an incubator with the unexposed vials. The adult flies were transferred to new vials every 3-4 days to avoid including their offspring in the longevity count. During each transfer we recorded the number of dead flies in the old vial and living flies in the new vial, and the percentage of flies remaining alive in each of the experimental groups was calculated. Data were tabulated and graphed on Kaplan-Meier type of survival curves. c) Dependent variable = Fertility, Independent variable = Mask type vials of culture media were prepared and labelled, three for each of the four experimental groups as described above. Drosophila from Biological laboratory Bangalore was obtained where gender is matched to eye colour. Flies were carefully anaesthetized using Flynap and separated by sex. Exposure to pollution was conducted as described above, for a total of 6 hours. Mean pupae and standard error were recorded for each group on day 5. Pollution levels of PM 2.5 for our longevity and fertility experiments.

Results

Schematic of a Drosophila lifespan assay, effects of different temperature and diet on adult lifespan flies were maintained through adulthood and flies were exposed to either a 15% SY or 5% SY diet and day 10 of development of synchronized eggs, containing aliquot are shown Figure.1, 2 (A&B) and 3. Simplified scheme of protocol was the collected adult flies males and females were separately setup to on longevity experiment and transferred flies into new food for every two days to analyse survivorship as presented in Figure.1. The synchronization part of the protocol can be used for various assays that require age-matched adult flies. Adult males usually live shorter, with both populations achieving a mean and median longevity of >50 days on a 10% SY food at 25 °C. The survivorship remains high in the early part of the experiment and then declines exponentially. Figure 2 shows the effect of temperature (18 °C, 25 °C and 29 °C) with adult control male flies group increases temperature decreases the survivorship the maximum death was observed when exposure to 29 °C followed by 25 °C and 18 °C (Figure.2 A). The adult control female group flies were exposed to 5% sugar yeast and 15% sugar yeast mixture shows maximum survivorship was observed at 5% SY than compared with 15% SY female group at 80 days exposure (Figure.2 B). Drosophila lifespan is affected by environmental conditions, such as temperature and diet, adult males typically live markedly shorter as temperature is increased likewise, the effect of diet on lifespan is presented, adult female flies on a less concentrated diet (5% SY) typically live significantly longer than those on a more concentrated diet (15% SY).

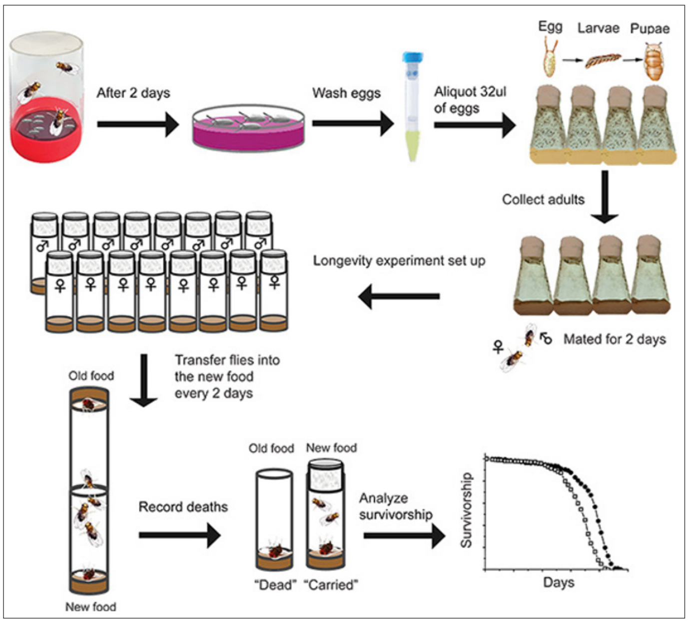

Figure 1: Schematic of a Drosophila lifespan assay aliquot 32 ul of eggs, longevity and analyse survivorship.

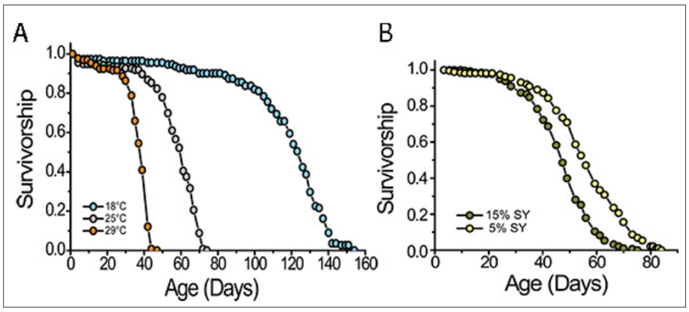

Figure 2 (A): Shows the effect of different temperature (18, 25 and 29 °C) on adult lifespan of control (Canton S) male flies were maintained through adulthood and diet. **(Figure. B):** adult control female flies were exposed to either a 15% SY and 5% SY on adult lifespan

The density of cohorts during development can influence adult lifespan and alter developmental timing, adult fly yield is poor and the food surface is susceptible to drying when the number of eggs is too low. On the other end of the spectrum, larval development is retarded in over-crowded bottles, and the yield of adult flies is reduced (Figure.3). The survivorship curve of the cohort as a whole can be influenced significantly by anomalous vial effects. Irregular survival data for individual vials may have several causes, such as poor food quality or bacterial/fungal accumulation and infection. Figure 3 shows day 10 of development (at 25 °C) of synchronized eggs aliquot in CT food bottles. Volume of the embryo-containing aliquot is as shown beneath each bottle.

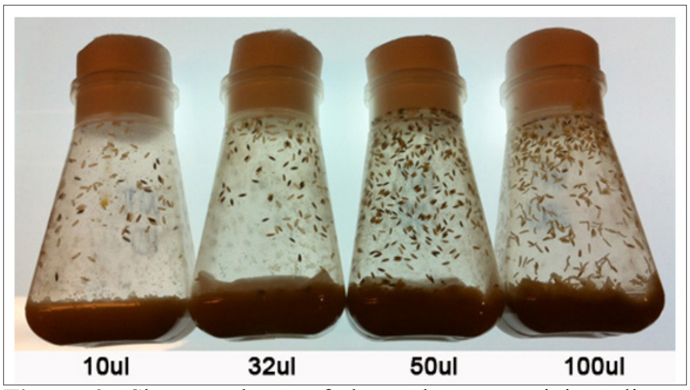

Figure 3: Shows volume of the embryo containing aliquot is as shown beneath each bottle of day 10 synchronized eggs development, the aliquoted in CT food bottles maintained at 25 °C.

Efficacy of Face Masks

The masks did diminish the amount of transmitted pollution to varying degrees as shown in Table 1, an N95 respirator is a respiratory protective device designed to achieve a very close facial fit and very efficient filtration of airborne particles. Note that the edges of the respirator are designed to form a seal around the nose and mouth. Surgical N95 Respirators are commonly used in healthcare settings and are a subset of N95 Filtering face piece respirators (FFRs), often referred to as N 95s. At 30 seconds, no mask, the doctor's mask and N 95mask allowed transmission of 70.15%, 21.43% and 6.41% of PM 2.5 pollutants, and at 90 seconds, only 39.88%, 4.55% and 1.83% of pollutants, respectively, were being transmitted (Table 1).

Table 1: Efficacy of mask filtration did diminish the amount of transmitted pollution to varying degrees, an N 95 respirator was a respiratory protective device designed to achieve a very close facial fit and very efficient filtration of airborne particles at different intervals.

Mask type	PM 2.5 Measured in chamber in µg/m3		
	At 30 seconds	At 60 seconds	At 90 Seconds
No Mask			
Trial 1	71.01	42.12	50.42
Trial 2	59.12	39.11	31.12
Trial 3	80.32	48.21	38.12
Mean (s.e)	70.15±3.2	43.15±4.12	39.88±3.1
Doctors Mask			
Trial 1	21.65	6.12	5.11
Trial 2	20.12	5.72	4.32
Trial 3	22.52	6.26	4.23
Mean (s.e)	21.43 ±3.7	6.03±0.5	4.55±0.5
95 Mask			
Trial 1	9.25	2.45	2.06
Trial 2	6.12	2.61	2.11
Trial 3	3.04	2.23	1.32
Mean (s.e)	6.41± 2.4	2.43± 0.9	1.83± 0.9

Table 2 shows a snapshot (days 5-10) of our raw data where we calculated the percentage of surviving flies, under each experimental condition. At each time point studied, the control group did the best in terms of survival (day -5, 67.5%, day -6, 66.4%, day- 7, 67.2 %, day- 8, 61.8%, day- 9, 62.11% and day -10, 60.1% respectively) and the vial with pollution + no mask did the worst (day-8 to day-10 an average of survival was 5/31, 15%). Among the three groups exposed to polluted air, the N 95mask group did best (day-8 to day10 an average of survival was 15/36, 40 %). Using this data, survival curves were plotted using a Kaplan meir model, then calculated the time taken for 50% of each experimental group to die (Figure.7), showing that the Drosophila exposed to no pollution lived the longest, while exposed Drosophila, without any mask, died the earliest. Importantly, the group that was exposed to pollution but shielded by the N 95mask did better than the pollution group without a mask but not as well as the controls. This demonstrated that the N 95mask was able to protect the Drosophila from the hazards of pollution to some extent but posed more health risks than being in clean air.

Table 2: Shows a snapshot (days 5-10) of data calculated the percentage of surviving flies under each experimental condition over time (G-M-1,2,3 no pollution (Control),G+M-1,2,3 pollution + no mask, G+DM+1,2,3 pollution with doctors mask and G+N 95M+ 1,2,3 Pollution with N 95 mask)

Experimental condition	Flies Remaining over time																	
	Day 5-10/25			Day 6-10/26			Day 7-10/27			Day8-10/28			Day 9-10/29			Day 10-10/30		
	Dead	Alive	% Surviving	Dead	Alive	% Surviving	Dead	Alive	% Surviving	Dead	Alive	% Surviving	Dead	Alive	% Surviving	Dead	Alive	% Surviving
G-M-1	0	5	23/34 67.5%	0	5	22/34 66.4%	0	6	23/34 67.2%	0	5	21/34 61.8%	0	5	21/34 62.11%	1	5	21/34 60.1%
G-M-2	0	10		0	10		0	11		0	10		0	10		0	10	
G-M-3	1	5		0	6		0	5		2	3		1	2		0	3	
G+M-1	0	5	11/31 32.1%	1	4	9/31 29%	0	4	8/31 27.1%	4	0	5/31 15%	0	0	5/31 16.2 %	0	0	5/31 14.1%
G+M-2	0	3		0	3		0	3		0	3		0	3		0	3	
G+M-3	0	2		0	2		0	2		1	2		0	2		0	2	
G+DM+1	0	6	13/36 34.1%	0	6	11/36 31%	0	6	10/36 29.1%	0	6	8/36 24.1%	1	5	7/36 19%	1	5	6/36 15%
G+DM+2	0	4		1	3		1	2		0	2		1	1		1	1	
G+DM+3	0	3		0	3		0	3		2	1		0	1		0	1	
G+N 95M+1	0	13	18/35 48%	2	11	17/36 42.6%	0	11	16/36 41.6%	2	9	15/36 40%	0	9	15/36 39%	0	9	14/36 39%
G+N 95M+2	0	4		0	4		0	4		0	4		0	4		0	4	
G+N 95M+3	0	1		0	1		0	1		0	1		0	1		0	1	

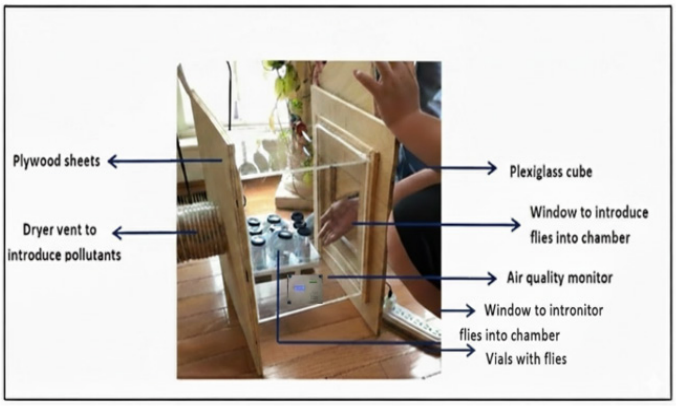

Figure 4: Shows experimental setup of D. melanogaster within population chamber, the chamber was made by using plywood sheets box with a metal dryer vent assembly to introduce pollutents, plexiglass cube, window to introduce flies into the chamber, and atmos realtime air quality monitor inside to measure PM in the chamber and vials fitted with masks are marked.

Longevity and Fertility: Drosophila were placed into 4 experimental groups: 1) No pollution; 2) Pollution and no mask; 3) Pollution and vials covered with doctor's mask; 4) Pollution and vials covered with N 95mask. After 6 hours of pollution exposure, flies in groups 2, 3, and 4 were transferred to an incubator with unexposed flies, and their lifespan was monitored. To assess fertility, 3 male and 3 female flies were placed into each vial and pupae were counted on day 5. Fertility was measured by counting pupae 6 days after exposure to the polluted environment, means and standard errors were calculated for each experimental group and data is shown in Figure. 8. On day 5 the control group had significantly more offspring (4 pupae) than any of the pollution exposed groups. Among those exposed to pollution, the Drosophila covered by the N 95mask (1.7 pupae) did better than the doctor's mask (0.5 pupae), while the no mask group did the worst (0.2 pupae). Breathing polluted air significantly decreased the reproductive potential of D. melanogaster however, the N 95 mask appeared to offer some protection from this adverse health effect.

Figure 5: Shows the Wi-Fi enabled electrochemical sensor devices (Atmos) for monitoring PM2.5 were installed within the chamber and pollutant levels were recorded for a 24-hour period. Real time data was retrieved from the company's cloud storage interface [19,20].

Figure 6: (a) Medical masks are loose-fitting and disposable, protect droplets and sprays, also called surgical masks, they filter out large particles in the air. (b) N95 masks are made in several layers, the middle filtering layers are made of polypropylene fibres with an embedded electrostatic charge, filtering efficiency (FE) is achieved by both the mechanical structure of the polypropylene filter layer and the electrostatic charge. The electrostatic charge can augment the mechanical filtering efficiency by as much as 10 to 20 times.

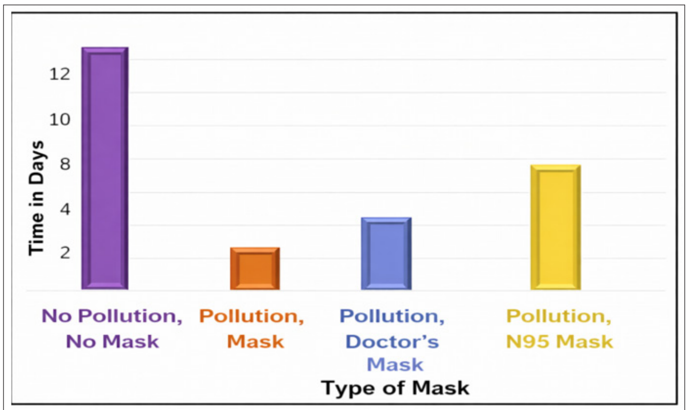

Figure 7: Shows the survival curves were plotted using a Kaplan meir model, then calculated the time taken for 50% of each experimental group that the Drosophila exposed to no pollution, without any mask, pollution with Doctors mask and pollution with N 95 mask.

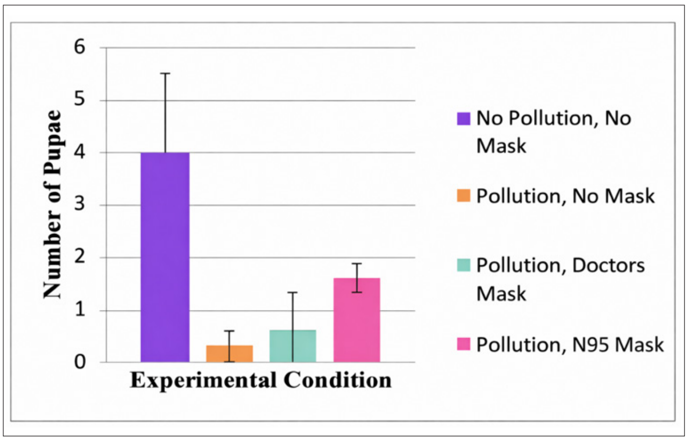

Figure 8: Shows the assess fertility of male and female flies were placed into each vial and pupae were counted on day 5 , was measured by counting pupae for 6 days after exposure to the polluted environment.

Discussion

This procedure outlines a technique for generating repeatable adult longevity assessments in Drosophila that may be modified to evaluate pharmacological and environmental interventions. Carefully regulating the larval growth environment, reducing adult stress, and minimizing bias between experimental groups and controls are all essential components of the technique. Additionally, we demonstrate how the entire lifetime experiment can help with data collection for every measurement and survivorship curve plotting. This experiment management tool could be readily modified for use in other organisms, with different types of population chambers, or for additional measures of survival, such as stress resistance and drug toxicity, even though it is currently most appropriate for studies of fly lifespan using vials [3,4]. One must carefully regulate the generation of parental stocks before beginning any longevity assessment. Both the health of parental strains and genetic diversity are significant factors. Due of these reasons, early research that indicated potential longevity regulators have generated a great deal of public discussion [21]. It is advisable to investigate the impact of environmental interventions on multiple strains (e.g., pharmacological treatment, nutrition, temperature, etc.). Lastly, healthy young adults should be selected for egg production because stress and parental age can affect the lifetime of the F1 generation [1,22-24].

Preventing overcrowding and maintaining a controlled environment with stringent regulation of light-dark intervals, humidity, and temperature are important parts of controlling the larval/pupal environment. Both the timing of development and the physical characteristics of the resulting individuals will be impacted by these factors. Stress-inducible proteins (such as heat shock protein expression) that are known to affect adult longevity may be activated by unfavourable larval conditions, such as high larvae density [5,25].

In this work, we have identified potential sources of stress in the feeding environment, such as physical food anomalies (such as bubbles, froth, fractures, pathogens, etc.) that can physically imprison the animals. Many of these challenges can be solved by regularly substituting fresh food for stale food at least three times a week. Additionally, lifespan can be influenced by temperature, humidity (personal observations), lighting, and the presence of conspecifics (social environment [1,26-28]). To prevent bias in the results, it is crucial to control these factors within the experiment. Even in an apparently controlled environment, the exact location of the vials in an incubator plays a role, even though the number of flies within a vial decreases over time. We have discovered that using anaesthesia to modify the number of flies can increase mortality in an age-dependent manner [1,16]. Proper statistical inference requires a randomized physical distribution of experimental groups with controls, which can reduce bias related to vial placement. The use of within-experiment controls is crucial since small changes might be seen between studies even under tightly controlled settings. Indeed, conclusions drawn from lifespan studies in yeast, worms, flies, and mice that have been linked to genetic or environmental artifacts are the subject of numerous noteworthy disputes [29]. In this methodology, we provide a series of steps that have been refined over many years of utilizing lab vials to measure longevity in Drosophila. By using optimal experimental design, streamlining fly handling and data collecting, and standardizing data analysis, Life increases throughput and encourages best practices. We will also go over the several potential hazards that might arise while designing, gathering, and interpreting lifespan data, and we offer solutions to prevent these risks.

The evaluation of statistical significance is the last factor to take into account. The possible biological importance of such a difference must also be taken into account, even though high cohort sample sizes offer great power to distinguish minute variations across treatments. Although the overall effect of the intervention on health status may be negligible, variations as small as 1-2% are frequently highly statistically significant in moderately sized longevity research. Therefore, while assessing the experiment's overall results, both statistical and biological importance must be taken into account markers of age-related decreases in behavioural or physiological health markers, such as gastrointestinal wall integrity and climbing ability, might supplement inferences about the aging process from survival trials [13,28]. An attractive option for researching aging mechanisms is the Drosophila model organism. Robust demographic analysis combined with meticulous experimental methodology can shed light on how pharmaceutical, environmental, and genetic factors affect the aging process.

According to the World Health Organization, air pollution is the biggest threat to world health in 2019. Reducing pollution and figuring out the best ways to protect ourselves from it are two sides of this complicated issue that need to be addressed [1]. In this experiment, we successfully designed a pollution chamber, measured the effectiveness of two masks (the doctor's mask and N 95mask), and investigated whether the masks may lessen some of the detrimental impacts of pollution on Drosophila lifespan and fertility. Part of our theories came true. We demonstrated that D. melanogaster's lifespan was considerably shortened by particulate pollution, and that the N 95 mask outperformed the doctor's mask in mitigating this effect. On the other hand, the doctor's mask was comparable to the N 95 mask in terms of its ability to filter out contaminants.

Our investigation supported earlier findings that D. melanogaster can be utilized as a useful model organism to comprehend the health consequences of pollution on adult flies as well as larvae [1,31,32]. Despite lacking lungs, flies' respiratory systems are sufficiently comparable to those of mammals to make them valuable model species for inhalation toxicity. We subjected flies to six hours of pollution, which is equivalent to around a year's worth of exposure for humans. The majority of PM 2.5 levels fell between 150 and 550 μg/m3. The typical indoor PM 2.5 levels in cities like Bangalore vary from 68 to 468 μg/m3 [3-5]. PM 2.5 was the major pollutant for 66.8% of the extremely polluted days in China. According to Wang et al., and, flies in our experiment were subjected to conditions that were worse but somewhat similar to those seen in the most polluted cities in China and India [2,33].

Similar to human studies where PM 2.5 caused a detectable reduction in lifetime due to cardiovascular and pulmonary disorders, we discovered that polluted air dramatically reduced Drosophila lifespan by 80% [34]. The longevity was reduced by 53% when the 95mask was used. In terms of fertility, we found that Drosophila exposed to pollution without a mask produced 92% fewer pupae than controls. It's unclear, though, if this significant decline was also brought on by a shorter lifetime [2]. While this sort of surgical mask has been proven to filter 60–90% of PM 2.5 particles in prior research, masks like the N 95mask do better [9]. In our investigation, the doctor's mask prevented nearly 90% of PM 2.5 pollution, which looked shockingly high. The doctor's mask could not considerably lessen the harmful health impacts of the contaminated Drosophila environment in our investigation, despite its excellent filtering effectiveness [2,33]. We did not examine the possibility that the exhaust fumes employed in our investigation contained significant noxious gas pollution in addition to PM 2.5 particles that the masks were unable to filter [35-37].

Conclusion

The Drosophila model organism is a desirable option for researching lifespan and aging mechanisms, robust demographic analysis and precise experimental methods can shed light on how environmental and pharmacological factors affect the aging process. Although it was not comparable to the lifespan and fertility of Drosophila living in clean air, the N 95mask consistently performed better in reducing the harmful effects of particle pollution on D. melanogaster's lifespan and fertility. Face masks are only a small part of the solution to our environmental emergency; we need to be equally committed to cleaning up our planet in the first place and keep researching more effective ways to protect our health from environmental pollution.

Disclosures

No conflicts of interest declared.

Acknowledgments

The authors thank the Department of Science and Technology (DST) and the Vision Group of Science and Technology (VGST) Karnataka for funding this study. We also grateful to the Department of Studies in Environmental Studies at the University of Mysore and the Department of Zoology at Al-

Ameen Arts, Science, and Commerce College on Hosur Road in Bangalore for their assistance and laboratory facilities.

Authors' Contributions

Author A' created the protocol, carried out the statistical analysis, planned the study, and authored the initial draft of the publication. "Author B" oversaw the study's analyses, literature searches, and edits. The final text was reviewed and approved by all authors.

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