J. Eur. Opt. Society-Rapid Publ. 2024, **20**, 24

https://doi.org/10.1051/jeos/2024022
Available online at: https://jeos.edpsciences.org

**Journal of the European Optical Society-Rapid Publications**

EOSAM 2023
Guest editors: Patricia Segonds, Guy Millot and Bertrand Kibler

RESEARCH ARTICLE

OPEN ACCESS

# Design of an optical system equipped with blue LEDs for the irradiation of *Drosophila melanogaster* cultures

Mónica López-Bautista[1], Jorge Enrique Mejía-Sánchez[1,*], Francisco Javier Ornelas-Rodríguez[2], and Viviana Matilde Mesa-Cornejo[1]

[1] Universidad de Guadalajara, Centro Universitario de Los Lagos, Lagos de Moreno, Jalisco C.P. 47460, México
[2] Instituto Politécnico Nacional, Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada, Unidad Querétaro, Querétaro C.P. 76090, México

Received 31 January 2024 / Accepted 22 April 2024

**Abstract.** *Drosophila melanogaster*, better known as the fruit fly, has become a widely used model organism that has allowed us to understand many biological behaviors, from sleep to neurological diseases, behavioral patterns, reproduction, and the circadian cycle, which coordinates biological rhythms in a 24-hour daily cycle through its main *Zeitgerber*, light, especially blue light. Therefore, the aim of this work was to build an optical setup with a hexagonal design that allowed a large number of *D. melanogaster* cultures to be irradiated homogeneously with blue light simultaneously. This array can cover an illuminance range from 0 to approximately 600 lux by applying a current variation from 0 to approximately 1 A. It also has a real-time timer to turn the lights on and off, programmed in a 12:12 LD cycle for 24 h. The optical setup with its unique design can become a very useful tool for developing experiments and understanding paradigms related to blue light at genetic, behavioral and neuronal levels, among others that are still unanswered.

**Keywords:** *Drosophila melanogaster*, Optical setup, Circadian cycle, Blue light.

## 1 Introduction

Light has been fundamental to the evolution of all organisms on Earth and has a profound effect on behavior, physiology and metabolism, which can have a significant impact on the health of mammals, including humans [1]. Light is perceived by a specialized light-sensitive tissue at the back of the eye called retina, which function is to detect light and form images. The retina transmits visual information from the optic nerve to the brain, which can affect both our physical and mental state [2]. Light not only allows us to illuminate the environment for vision, but is also responsible for regulating processes that occur independently of image formation, such as the circadian cycle, which is present in most organisms from cyanobacteria to humans [3, 4]. These rhythms synchronize biological functions such as sleep/wake cycles and hormone levels with the external environment through signals known as *zeitgebers*, with light being the most important for "photoentrainment" of the rhythm's activity over a period of about 24 h [5].

It should be noted that the disruption of circadian rhythms by light is due to the desynchronization of the internal biological clock with external environmental conditions and depends on the duration, wavelength and intensity of light [6]. This is common due to living and working conditions, 24 h a day, 7 days a week [7]. This is because we live in a globalized and widely digitalized society, which brings profound and irreversible changes in our social, domestic and professional environment, leading to an increasing use of LED technology, applied in various technological devices, lighting, medical treatments, etc. Although it gives us as humans independence in daylight, we are enhancing exposed to light in the blue spectrum as we spend more time indoors. This is because LED light emits a higher intensity in the blue wavelength range, often peaking at 460 nm [8]. Long-term exposure to blue LED light leads to changes in the circadian cycles of humans, ranging from disruption of the cycle to deregulation of their coupled functions, including sleep and mood [9].

*Drosophila melanogaster* is an excellent model to study how light affects various processes such as hatching, courtship, locomotion, reproduction, etc. It is also often used to study light-influenced circadian rhythms [10]. In humans, prolonged exposure to blue light has been shown to cause sleep disturbances by suppressing the melatonin production. Additionally, it can cause visual fatigue and elicit emotional brain responses such as depression and anxiety [11].

* Corresponding author: enrique.mejia@academicos.udg.mx

It also shortens the lifespan of *D. melanogaster*, accelerates aging and leads to sleep disturbances, etc. [12].

Although there are some devices for evaluating the effect of blue light in flies, they have several disadvantages such as a limited number of cultures and thus a smaller sample size, as well as a limited range of application. Therefore, the aim of this work was to carry out the optical design of a system for homogeneous irradiation of *D. melanogaster* cultures with blue LED light. This system allows for the observation of various areas of interest, including strain strain development, longevity, reproduction, courtship to stress response gene expression, brain neurodegeneration, retinal degeneration, antioxidant compound assessment, etc., over an extended period of time and several generations simultaneously. This setup is cost-effective and easy to manipulate. It is programmed in a cycle of 12 light hours and 12 dark hours (12:12 LD) to simulate the alternation of day and night.

## 2 Materials and methods

### 2.1 Light source

High-power blue 1W LED with aluminum heat sinks (LED-P1B25-120/41, SiLed®) were used. The dimensions were 7.0 mm × 4.5 mm × 5 mm for the encapsulated LED and 18.6 mm × 19.8 mm × 15 mm with the aluminum heat sink. The technical sheet indicates that each LED has a minimum luminous flux of 25 and maximum of 45 lm and a threshold voltage of 3.0–3.8 V, with a viewing angle of 120° and a forward current of 350 mA.

The emission spectrum was measured with the Flame Miniature Spectrometer (FLAME-S-XR1, Ocean Insight Inc., Florida, USA). The LED emission spectrum had a wavelength central at 457 nm with a spectral width of $\Delta\lambda$ 45 nm at 25 ± 1 °C and providing an energy per photon of $4.352 \times 10^{-19}$ J.

### 2.2 Circuit assembly

The HanMatek HM305 variable power supply (HanMatek, China) was used to realize the overall connection of the circuit with a current output of 0–5 A (≤0.2% ± 3 digits) and an output voltage of 0–30 V (≤0.1% ± 1 digit). The LEDs used for the assembly were connected in parallel with a CORDON SPT-1 300 V (20 AWG) cable (Indiana, Ware & Cable, USA) which was connected with the SOL60-100 solder (Electrónica Steren S.A. de C.V., Mexico), with a diameter of 1 mm, a composition of 60/40 tin/lead and a melting point of 183 °C, using the WLC 100 soldering station and a conical ST7 soldering tip (Weller, USA).

### 2.3 Temperature control

The temperature and humidity are controlled by the laboratory's air conditioning system, then to verify the thermal gradients within the arrangement, two thermohygrometers were used, the USB-502-LCD thermohygrometer (Logicbus, USA) with a measurement range of 0%–100% humidity and a temperature of −35 to +80 °C and an accuracy of ±0.5 °C (±1.0 °F) and ±3.0% relative humidity and a DeltaTrak model 13309 thermohygrometer (DeltaTrak® Inc., USA) with NIST certification. The first one was used to measure variations throughout the day (24 h) allowing for data storage and export.

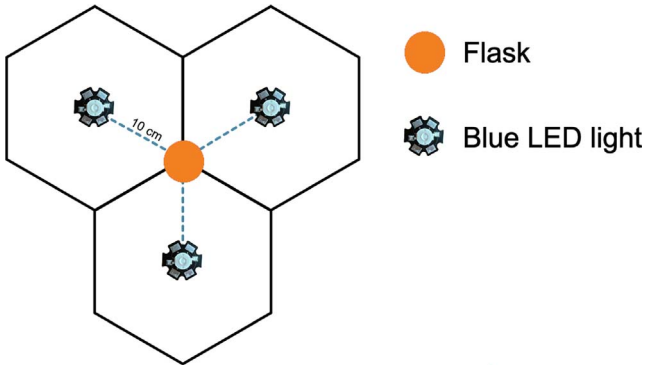


**Fig. 1.** Location plane of the LED and the flask (at each vertex of the hexagon) separated 10 cm from each other.

On the other hand, the second one was used to record the temperature and humidity while the experiment was running.

### 2.4 Real-time lighting control system

The lights were controlled by an Arduino Uno microcontroller (Arduino, Italy) of 16 MHz in which a real-time lighting control system was implemented to control the on/off operation of the array equipped with blue LED lights operating at a clock rate. The control system was designed to switch the LED power supply in a 12:12 LD cycle. The voltage sources that supply power to the LED assemblies were independent and operate on 120 volts AC. Since the controller of the Arduino Uno board does not have a real-time clock, it was necessary to install the ARD-374 board, which is a Real Time Clock (RTC). This RTC card allowed for programming of the real-time schedule and accurate tracking of the date and time. This type of card contains a backup battery, such as an SR225, which allowed the clock to continue functioning even when the main power source was disconnected.

### 2.5 Illuminance

The illuminance was measured with the luxmeter (GM-1010, Shenzhen Jumaoyuan Science and Technology Co., Ltd., Shenzhen, China) with an accuracy of ±3% rdg ± 0.5% f.s. (<10,000 lux) and ±4% rdg ± 10 dgts. (>10,000 lux), at a surface distance of 10 cm and a sensor height of 5 cm. Five replicates of measurements were taken at each point where a *Drosophila* culture could be positioned.

### 2.6 Fly strains and maintenance

The wild strain of *D. melanogaster* (Canton, CS flies) was acquired from the *Drosophila* Stock Center Mexico by the Universidad Nacional Autónoma de México (UNAM). *D. melanogaster* parents were reared at 25 ± 1 °C under

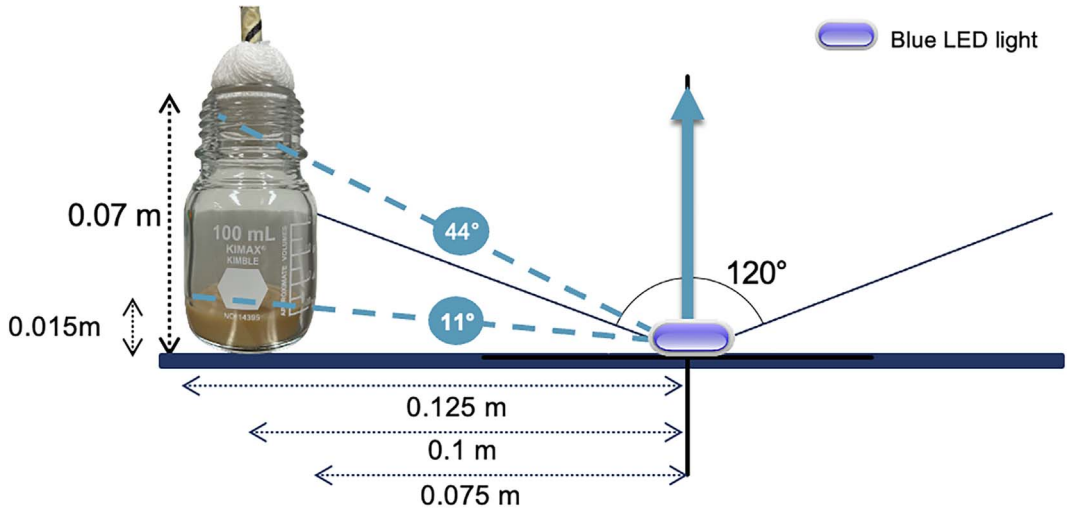


**Fig. 2.** Distribution of light in relation to the angle of irradiation towards the flask, considering the culture medium, the flies and the cap.

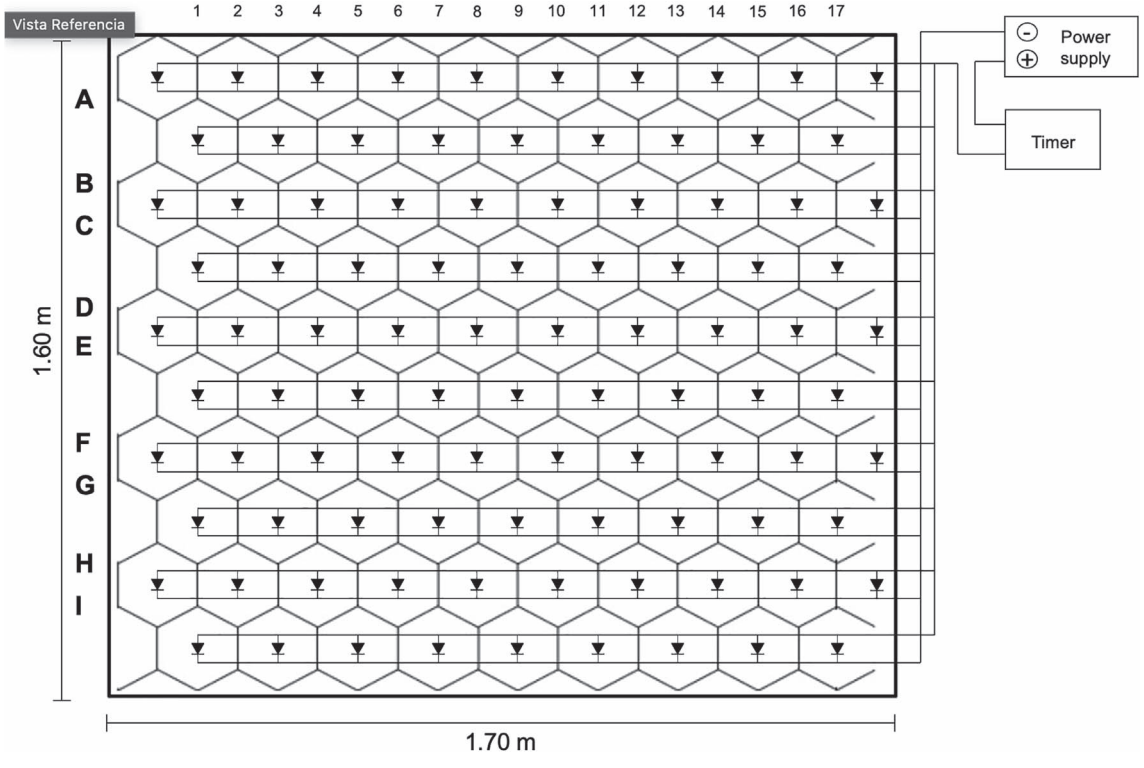


**Fig. 3.** Electrical diagram of the circuit used to connect 95 blue light LEDs in parallel to a variable power source and consecutively to the timer.

conventional light. Virgin adult *Drosophila* were separated by sex to avoid copulation and anesthetized with ether. Subsequently, 3 pairs of flies (3 males – 3 females) were paired in a 100 mL borosilicate flask previously filled sterilely with 20 mL of culture medium enriched with yeast. This was carried out in five replicates; flies were mated for 8 days at 25 ± 1 °C with a humidity of over 40% and an illuminance of ~100 lux in a LD 12:12 cycle. Then, the parents were sorted out to obtain the offspring, which were kept under the same conditions and separated by sex in different flasks.

## 2.7 Data analysis

The illuminance data were analyzed with a Shapiro–Wilk test and a QQ-plot using OriginPro statistical software.

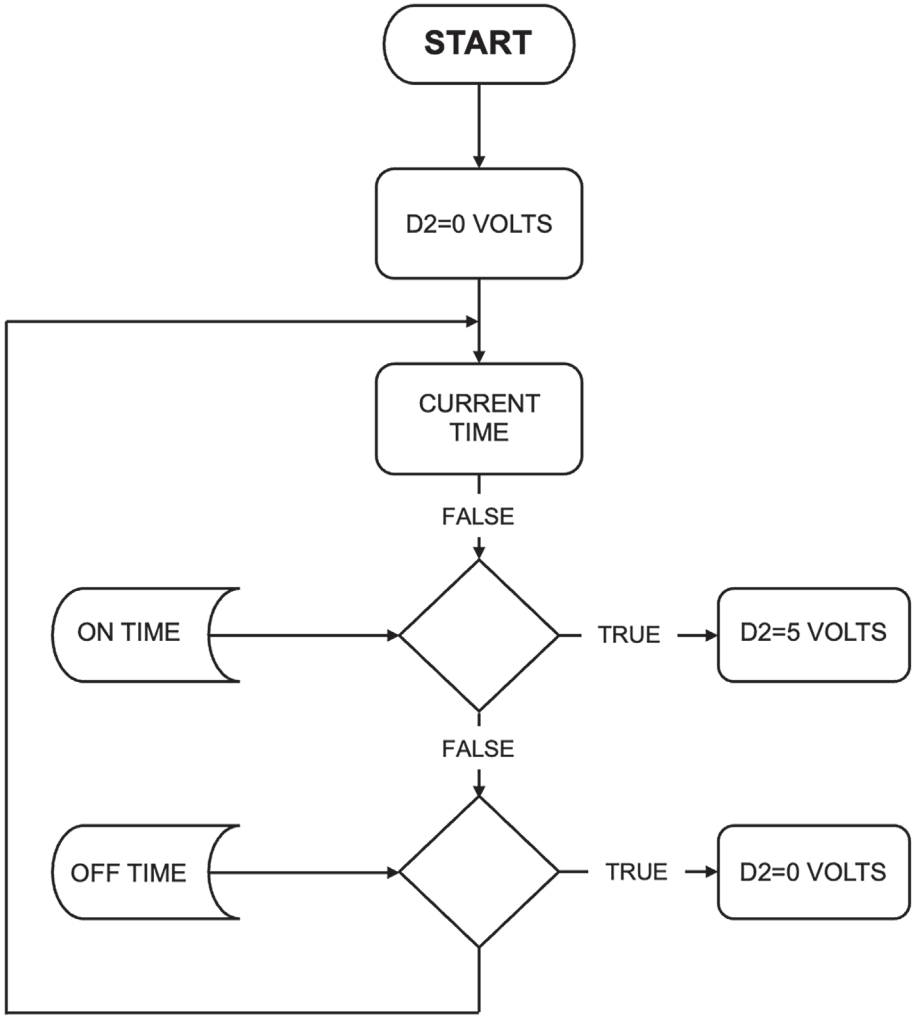


**Fig. 4.** Flow diagram of the timer program. The timer programmed with ON/OFF change at 7:00 am and 7:00 pm respectively.

The biological assay data were subjected to a two-way analysis of variance (ANOVA) using GraphPrism 8 statistical software.

## 3 Design and construction of optical assembly

To observe the effects of light on organisms, various devices with light sources, usually of the LED type, have been proposed for the irradiation of *D. melanogaster* [13, 14], one of the drawbacks was to ensure that each organism receives the same amount of light regardless of the number of cultures to be prepared, so a design was initially developed to solve this problem by simple assembly and handling.

A design was developed featuring a hexagonal arrangement within a defined area, with a flask located at each vertex and a blue LED positioned in the center. In this way, each flask receives the illuminance of 3 LEDs around it, at a distance of 10 cm (Fig. 1).

According to the vertical plane of the arrangement, the irradiation angle was 33°, with angles below 11° corresponding to the culture medium and angles above 44° referring to the cotton plug and unused space. Furthermore, the dimensions of the flask, the culture medium, and their distances from the LED position are shown (Fig. 2).

In order to assemble an optical setup suitable in size for the development and evaluation of *D. melanogaster* cultures, this design was placed in a specific area inside the laboratory that would allow the layout of the plan according to the previously proposed design. Therefore, the final dimensions of the arrangement were of 1.6 m × 1.7 m × 0.2 m, which allowed a circuit of 95 LEDs connected in parallel, that were soldered in 10 rows of 10 and 9 LEDs as shown in Figure 3.

Figure 4 shows the operation of the software that controlled the lighting, initially, a 0-volt value was sent to the digital output D2 to ensure that the control started with the light off. Next, the real-time clock (RTC) card was read to obtain the current time (hour and minutes) and this was compared with the programmed time switching on the lighting (7 am). When the real time and programmed switch-on time match, a 5-volt signal was sent through digital output 2, which activated the relay and closed the 3.8 DC-volt circuit to power the lights.

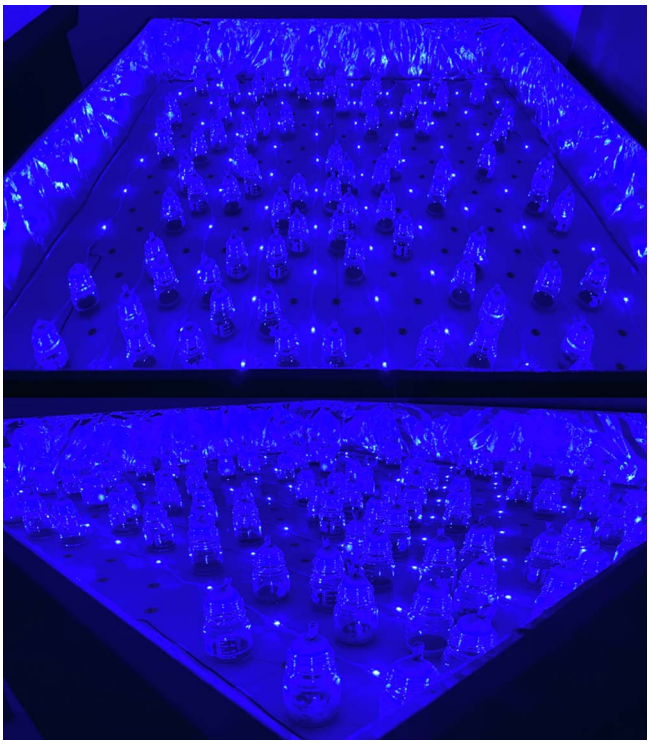

**Fig. 5.** Prototype of the optical assembly, tested and used for cultures of the development of *Drosophila* flies.

Once the power supply was switched on, it remained active while the software continuously compared the real-time with the programmed switch-off time. When the real-time matched, the scheduled turn-off time, a 0-volt signal was sent through digital output 2, deactivating the relay switch and turning off the 3.8 DC-volt circuit. This cycle was repeated until the real-time matched the programmed switch-on time again.

Additionally, the optical array was protected with walls and ceiling covered internally with aluminum foil to maintain the reflection emitted by the LEDs. This arrangement allowed the manipulation and development of up to 153 *Drosophila* cultures in 100 mL flasks in an area of 2.72 $m^2$ (Fig. 5), which it is suggested can be organized randomly in future experiments. The low-cost blue LEDs were equipped with a useful aluminum heat sink or PCBs (Printed Circuit Boards), which facilitates heat dissipation from the LED to the environment, and helps to maintain the temperature of the LED within safe levels.

## 4 Characterization of optical system

A characteristic group of insects has been used in chronobiological experiments, from the honeybee (*Apis mellifera*) to the monarch butterfly (*Danaus plexippus*), due to their ease to manipulate and well-defined circadian cycles *D. melanogaster* is the model organism par excellence to understand these processes. Furthermore, the molecular basis of circadian rhythms is fully characterized in *D. melanogaster*, which, coupled with its fully sequenced genome, represents a significant advantage over other models [15, 16]. When exposing these insects to a stimulus such as light, the source must be quantified, either in the form of measurements such as illuminance or correlated color temperature to directly elucidate their behavior [17].

The illuminance of the light source, the blue LED, was measured in the optical assembly at each point where a culture can be positioned at 190 ± 4.8 mA. Each flask was illuminated by 3 LEDs according to the proposed hexagonal design. Therefore, the illuminance was indicated by three measurements with the luxmeter, each taken in the normal direction of the light source. The uncertainty in measurements was estimated according to the Guide for the Uncertainty in Measurements (GUM), the relative uncertainty contribution from the instrument (calibration error resolution) was considered as 3%, as reported by the manufacturer for the measurement range and scales used, which is consistent with previous accuracy reports for this metrological class of luxmeters [18]. Additionally, a 1.25% for the reading repeatability. This relative combined standard estimated uncertainty is represented in figure with a coverage factor $k = 2$, ensuring a 95.4% confidence. The data obtained were grouped into 9 classes as shown in Figure 6A. These values were arranged in columns from 1 to 17 and in rows from A to I, where each circle represents a place for a possible culture in the array. Each shade of blue represents the range of illuminance according to an interval of 1.515 lux. In this way, a minimum value of 86.28 ± 2.8 lux (light blue circle at A2) was recorded, while the maximum value was 99.92 ± 3.24 lux (dark blue circle at B12). Overall, the arrangement had an average value of 92.14 ± 2.99 lux. These values correspond to the behavior of a normal distribution, which was verified with the Shapiro–Wilk with $p > 0.05$ (Figs. 6B and 6C).

This optical arrangement was able to achieve illuminance levels ranging from 0 to 601.12 ± 19.53 lux at a current variation of 0–1.05 A with an uncertainty ± (2% +10 digits), (Fig. 7). It should be noted that different illuminance levels can be programmed locally via the variable power supply by adjusting the current into the system. The desired illuminance value to test will depend on the objective of the project and the specific design parameters under observation. This flexibility enables a wide range of possibilities, allowing for the analysis of the blue light under various conditions.

On the other hand, the temperature remained stable within the range required by the biological model thanks to the laboratory's air conditioning system. Figure 8A shows that over a period of 24 h, during which the temperature was measured every 10 s with an injected current of 190 ± 4.8 mA, there was only a change of 0.5 °C. Furthermore, to evaluate the change in the temperature caused by illuminance, the current was modified in increments of 0.05 A every 60 min, starting at 0.05 A and ending at 1.05 A with an uncertainty ± (2% +10 digits), observing a rise 24.5 ± 0.5 °C to 25 ± 0.5 °C at 0.25 ± 0.006 A shown in Figure 8B, after this value, no further temperature changes were detected.

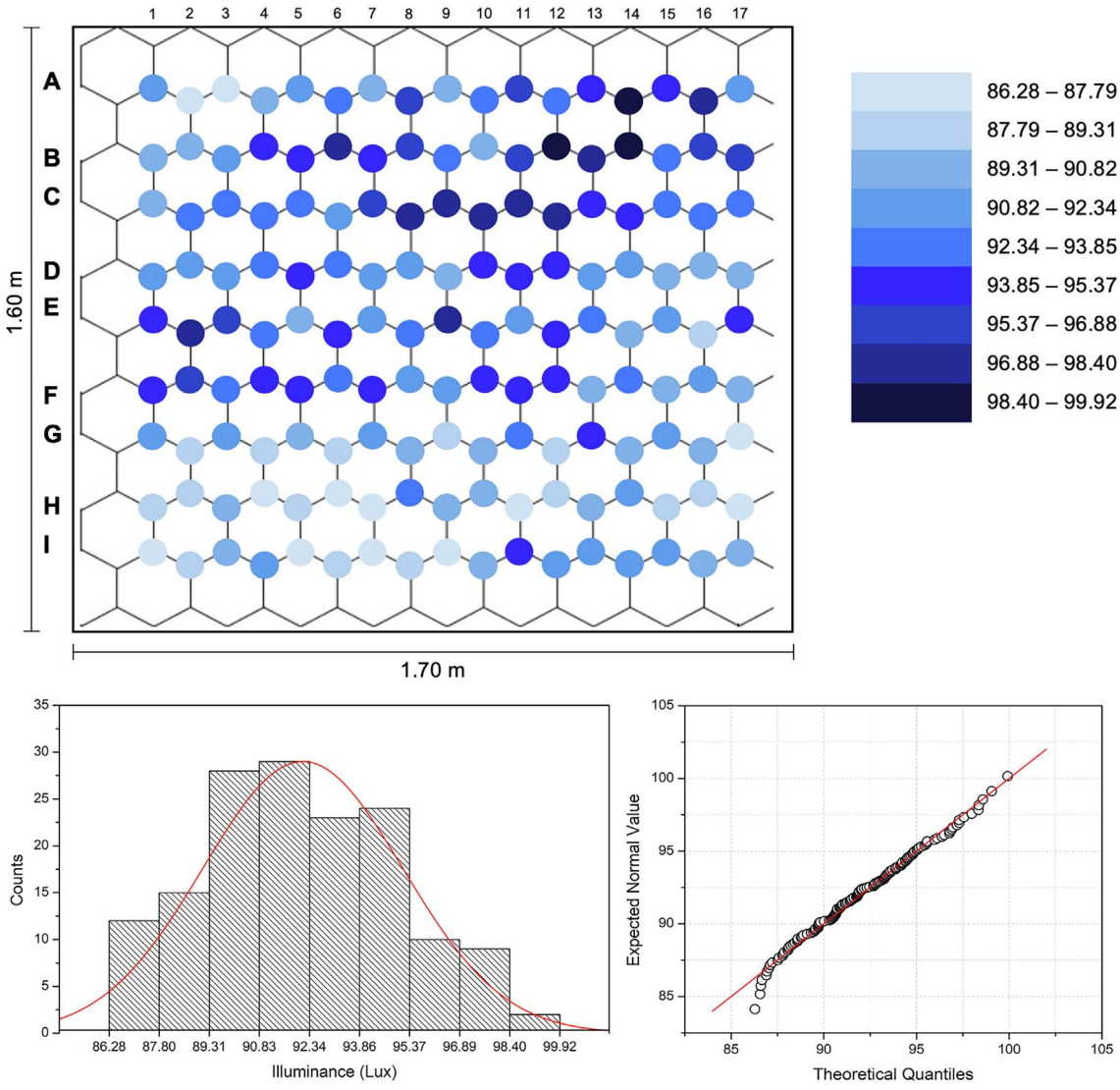


**Fig. 6.** Representation of the illuminance (uncertainty of 3.25%) in the LED assembly area (A) distribution of the illuminance at the 153 points where each culture flask can be positioned. (B) Histogram of the data grouped into 9 classes. The Shapiro–Wilk test gives a value of $p = 0.17754$ C. QQ-plot normality with those expected.

## 5 Biological assay

To verify the non-toxicity of the proposed optical design, an illuminance of ~100 lux was used, which did not register a toxic effect on the development of *Drosophila* [19] and could be compared with its development under conditions of total darkness (DD). To set the array to ~100 lux, the current in the variable source was manually changed to 205 ± 5.1 mA. Five culture repetitions with three pairs of flies each were exposed to blue light for 8 days, then the parents were discarded. The culture was maintained under the same illuminance and temperature conditions (25 ± 1 °C) during all stages of metamorphosis until adult flies were obtained and the number of males and females was recorded.

Figure 9 shows the SD of the population averages obtained during the LD/DD periods by sex. For males: 34.2 ± 8.9 and 33 ± 4.94; while for females: 38.4 ± 5.17 and 37.2 ± 4.60, respectively.

It was observed that the number of flies obtained during the LD 12:12 period with the proposed optical design compared to the dark period (DD) did not present a significant difference according to the two-way ANOVA with $p = 0.669$ for LD conditions and $p = 0.147$ by sex.

## 6 Discussion

The use of *D. melanogaster* as a model organism has been of fundamental importance for the understanding of various biological processes, particularly the circadian cycle that underlies the human being. This cycle is closely linked to temperature and, in general, to light, which serves as the primary driving stimulus due to the presence of rhodopsins

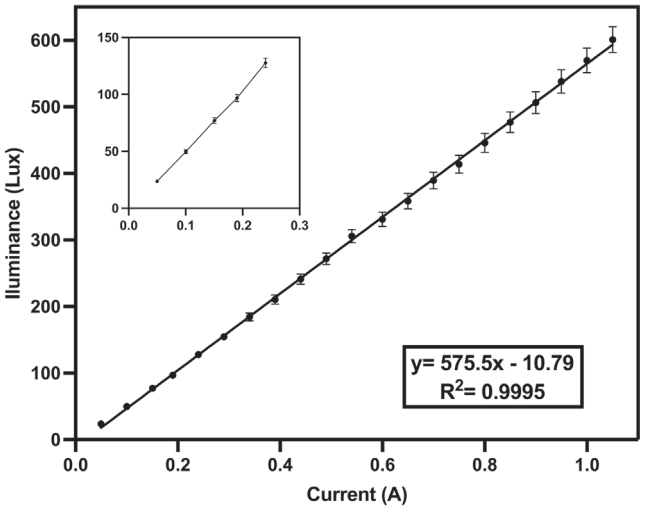


**Fig. 7.** Measurement of illuminance with respect to the variation of current injected (± (2% +10 digits)), into the circuit.

in the visual organs (ocelli and compound eyes) and the Hofbauer–Buchner (HB) eyelets [20]. Since blue LED light is a relatively new light source, its long-term effects on humans are unknow. Therefore, the design of a novel optical setup was proposed as a hexagonal network over a defined area, allowing for the simultaneous and straightforward evaluation of up to 153 *Drosophila* cultures to understand the effect of blue light on flies. Generally, plastic and/or glass vials are used for maintenance of *D. melanogaster* [21, 22] due to their easy handling and low cost. However, limitations arise in the number of offspring compared the proposed configuration, which allowed the monitoring of a large number of cultures, ensuring more precise results in complex biological processes susceptible to being affected by light, such as reproduction, oviposition, sex determination, etc. These processes have not yet been fully understood, unlike studies that were limited by the number of replicates [23, 24]. Additionally, the transgenerational effect of blue light could cause genetic changes that can be passed on to the next generation [25].

The completion of the construction of this arrangement required covering the walls with aluminum foil, which, in addition to being an economical and easily accessible material, also offers a light reflectance of 70%–75% in the blue light range [26]. This reflectance is necessary to ensure the quasi-homogeneous distribution of light in the spaces that are on the sides of the arrangement. While various reflective materials such as mirrors, silver, chrome, and polymers may be highly efficient, they often come with disadvantages such as high cost and difficult handling when applied in the arrangement in question. On the other hand, materials that disperse light, such as softboxes and diffuser paper, aim to soften light but may lead to a loss of light intensity.

Conversely, according to the proposed design and upon completing the construction of the optical setup, the illuminance was found to be quasi-homogeneous, reaching an average value of 92.14 ± 2.99 lux at 190 ± 4.8 mA. The variability in the illuminance can be mitigated by randomly placing the cultures at the specified positions. Moreover, this type of study is often conducted using an incubator equipped with LED lighting [27]. However, a drawback of this approach is the lack guarantee that all cultures receive the same amount of light and the number of replicates may be limited depending on the experiment setup and workspace constraints. One of the disadvantages of using this type of equipment to study the effect of blue light is the low practicality of variating the illuminance, which often requires changing in the type of light installation. However, the optical setup presented has the advantage that the illuminance range, following a linear trend from 0 to 601.12 ± 19.53 lux, can be modified in a direct and simply by adjusting the injected current from 0 to 1.05 A, respectively. It is important to note that in various studies where light has been used as a stimulus, organisms are often exposed to abnormal light intensity or wavelength outside their normal ecological conditions. Similarly, the incubators normally used for the maintenance of *D. melanogaster* are programmed to around 2000 lux for common laboratory experiments, with treatment depending on the exposure time [13]. Although some devices have been developed to assess exposure to LED light in *Drosophila*, they are often limited by space, cost and design, and also focus on studying a single issue, such as retinal degeneration caused by blue light at high illumination [14]. This limitation can result in obtaining an insufficient number of samples for subsequently analysis at the molecular, immunological, physiological and other levels.

In addition to the above, it is known that the developmental cycle of the fruit fly at 25 ± 1 °C is about 10 days, so temperature fluctuations can either slow down and/or accelerate this process [28]. Furthermore, temperature serves as a driving stimulus for circadian cycles, and since *D. melanogaster* is an ectothermic insect (body temperature varies with ambient temperature) [29], it is important to maintain this parameter as constant as possible. However, studies with *Drosophila* exposed to light are usually carried out with a variation of temperature up to 25 ± 1 °C [24]. In this way, the temperature in the system was set to 25 °C with a small deviation of 0.5 °C to 190 ± 4.8 mA. In fact, with the change in current from 0.05 to 0.5 A, the temperature varied from 24.5 ± 0.5 °C to 25 ± 0.5 °C. This is due to the fact that the setup is located in a dark room with a stable temperature year-round, allowing the device can operate under the same conditions of temperature, humidity and, especially, luminance, as the light-dark cycle can be easily programmed.

It should be noted that in different modified strains of *D. melanogaster*, illuminance levels of up to 300 lux were observed without altering the response in terms of reproduction and longevity [30]. For this experiment, the population was obtained from flies maintained in darkness and exposed to blue light at ~100 lux to validate that the proposed design was suitable for evaluating the effect of light on flies. It was expected that there would be no significant differences in both the 12:12 LD and darkness cycle and by sex. The results confirmed that the proposed design is valid and easily reproducible setup. Furthermore, it was reaffirmed that there are no toxic effects on the development of *D. melanogaster* in terms of reproduction at ~100 lux [19].

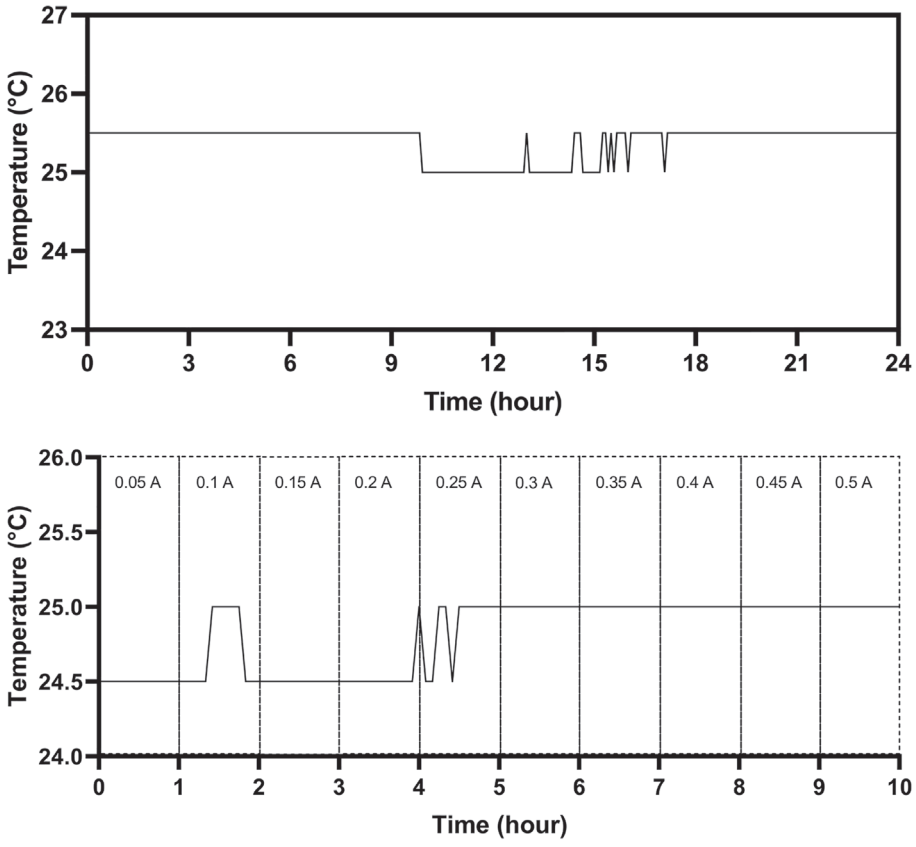


**Fig. 8.** (A) Temperature measurement throughout 24 h a day at constant current of 190 ± 4.8 mA. (B) Temperature measurement during current variation from 0.05 to 0.5 A (uncertainty of ±0.5 °C).

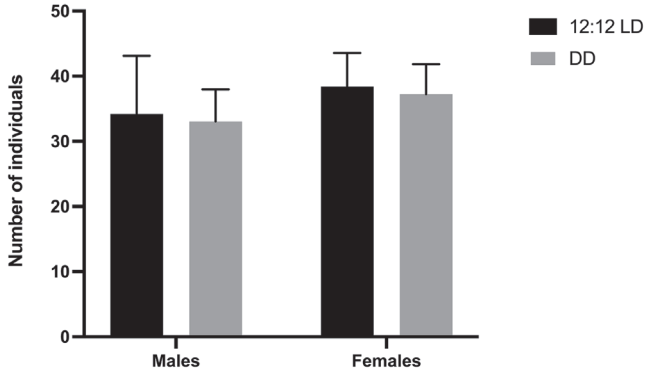


**Fig. 9.** Effect of blue light on the development of *Drosophila melanogaster* flies. Number of individuals obtained separated into females and males in LD conditions 12:12 h and DD: 24 h – dark conditions at 100 lux.

## 7 Conclusion

Fruit flies are widely used in laboratory settings to understand various chronobiological processes, where light is employed to observe patterns consistent with the circadian cycle. In particular, exposure to short-wavelength blue light has been linked to proliferation of various problems human body function, making it an increasingly studied area of interest. Therefore, in this work, a novel optical setup is presented for the development of *D. melanogaster* flies, that allowed the generation of a large number of transgenerational populations with the ability to modulate illumination intensity depending on the experimental design and offered the scientific community the possibility to apply it quickly, easily and inexpensively.

Finally, the optical assembly equipped with LED light represents a tool for studying the effect of blue light on *D. melanogaster* cultures across a wide range of illumination levels. With temperature control facilitated by a real-time system, the light can be switched on and off according to programmed settings. This optical assembly demonstrates quasi-homogeneity in the distribution of illuminance across the work area. Its application extends to various fields of science, engineering and medicine. With its carefully designed alignment, it allows for optimal results as a considerable number of fly cultures can be developed simultaneously, resulting in a high population, according to the conditions required at the experimental level.

### Funding

Author Mónica López Bautista to acknowledge the scholarship provided by Consejo Nacional de Humanidades, Ciencia y Tecnología (CONAHCyT, México) for PhD studies 857105.

## Conflicts of interest

The authors declare that they have no competing interests to report.

## Data availability statement

The data associated with this study is available upon request. Please contact the corresponding author to request access to the data.

## Author contribution statement

All authors made significant contributions to this work.

## References


1 Duffy J.F., Czeisler C.A. (2009) Effect of light on human circadian physiology, *Sleep Med Clin.* **4**, 2, 165–177. https://doi.org/10.1016/j.jsmc.2009.01.004.

2 Palczewski K. (2012) Chemistry and biology of vision, *J. Biol. Chem.* **287**, 3, 1612–1619. https://doi.org/10.1074/jbc.R111.301150.

3 LeGates T.A., Fernandez D.C., Hattar S. (2014) Light as a central modulator of circadian rhythms, sleep and affect, *Nat. Rev. Neurosci.* **15**, 7, 443–454. https://doi.org/10.1038/nrn3743.

4 Hastings M., Reddy A., Maywood E. (2003) A clockwork web: circadian timing in brain and periphery, in health and disease, *Nat. Rev. Neurosci.* **4**, 649–661. https://doi.org/10.1038/nrn1177.

5 Grabe S., Mahammadov E., Olmo M.D., Herzel H. (2022) Synergies of multiple zeitgebers tune entrainment, *Front. Netw. Physiol.* **1**, 803011. https://doi.org/10.3389/fnetp.2021.803011.

6 Golombek D.A., Rosenstein R.E. (2010) Physiology of circadian entrainment, *Physiol Rev.* **90**, 3, 1063–1102. https://doi.org/10.1152/physrev.00009.2009.

7 Klerman E.B., Brager A., Carskadon M.A., Depner C.M., Foster R., Goel N., Harrington M., Holloway P.M., Knauert M.P., LeBourgeois M.K., Lipton J., Merrow M., Montagnese S., Ning M., Ray D., Scheer F.A.J.L., Shea S.A., Skene D.J., Spies C., Staels B., St-Onge M.P., Tiedt S., Zee P.C., Burgess H.J. (2022) Keeping an eye on circadian time in clinical research and medicine, *Clin Transl Med.* **12**, 12, e1131. https://doi.org/10.1002/ctm2.1131.

8 Behar-Cohen F., Martinsons C., Viénot F., Zissis G., Barlier-Salsi A., Cesarini J.P., Enouf O., Garcia M., Picaud S., Attia D. (2011) Light-emitting diodes (LED) for domestic lighting: any risks for the eye? *Prog. Retin. Eye Res.* **30**, 4, 239–257. https://doi.org/10.1016/j.preteyeres.2011.04.002.

9 Hanifin J.P., Lockley S.W., Cecil K., West K., Jablonski M., Warfield B., James M., Ayers M., Byrne B., Gerner E., Pineda C., Rollag M., Brainard G.C. (2018) Randomized trial of polychromatic blue-enriched light for circadian phase shifting, melatonin suppression, and alerting responses, *Physiol. Behav.* **198**, 57–66. https://doi.org/10.1016/j.physbeh.2018.10.004.

10 Menegazzi P., Yoshii T., Helfrich-Förster C. (2012) Laboratory versus nature: the two sides of the *Drosophila* circadian clock, *J. Biol. Rhythms.* **27**, 6, 433–442. https://doi.org/10.1177/0748730412463181.

11 Wahl S., Engelhardt M., Schaupp P., Lappe C., Ivanov I.V. (2019) The inner clock-Blue light sets the human rhythm, *J. Biophotonics.* **12**, 12, e201900102. https://doi.org/10.1002/jbio.201900102.

12 Nash T.R., Chow E.S., Law A.D., Fu S.D., Fuszara E., Bilska A., Bebas P., Kretzschmar D., Giebultowicz J.M. (2019) Daily blue-light exposure shortens lifespan and causes brain neurodegeneration in *Drosophila*, *NPJ Aging Mech. Dis.* **5**, 8. https://doi.org/10.1038/s41514-019-0038-6.

13 Ramakrishnan P., Joshi A., Tulasi M., Yadav P. (2023) Monochromatic visible lights modulate the timing of pre-adult developmental traits in *Drosophila melanogaster*, *Photochem. Photobiol. Sci.* **22**, 4, 867–881. https://doi.org/10.1007/s43630-022-00358-1.

14 Chen X., Leon-Salas W.D., Zigon T., Ready D.F., Weake V.M. (2017) A programmable optical stimulator for the Drosophila eye, *HardwareX* **2**, 13–33. https://doi.org/10.1016/j.ohx.2017.07.001.

15 Beer K., Helfrich-Förster C. (2020) Model and non-model insects in chronobiology, *Front. Behav. Neurosci.* **14**, 601676. https://doi.org/10.3389/fnbeh.2020.601676.

16 Huang R.C. (2017) The discoveries of molecular mechanisms for the circadian rhythm: The 2017 Nobel prize in physiology or medicine, *Biomed. J.* **41**, 1, 5–8. https://doi.org/10.1016/j.bj.2018.02.003.

17 Spitschan M., Stefani O., Blattner P., Gronfier C., Lockley S.W., Lucas R.J. (2019) How to report light exposure in human chronobiology and sleep research experiment, *Clocks Sleep.* **1**, 3, 280–289. https://doi.org/10.3390/clockssleep1030024.

18 Rosas E., Estrada-Hernández A. (2016) Effect of photometric detector spectral response quality on white LED spectral mismatch correction factors, *Appl. Opt.* **55**, 5267–5272. https://doi.org/10.1364/AO.55.005267.

19 Shen J., Yang P., Luo X., Li H., Xu Y., Shan J., Yang Z., Liang B. (2021) Green light extends *Drosophila* longevity, *Exp. Gerontol.* **147**, 111268. https://doi.org/10.1016/j.exger.2021.111268.

20 Helfrich-Förster C. (2020) Light input pathways to the circadian clock of insects with an emphasis on the fruit fly *Drosophila melanogaster*, *J. Comp. Physiol. A* **206**, 259–272. https://doi.org/10.1007/s00359-019-01379-5.

21 Shibuya K., Onodera S., Hori M. (2018) Toxic wavelength of blue light changes as insects grow, *PLoS One* **13**, 6, e0199266. https://doi.org/10.1371/journal.pone.0199266.

22 Schlichting M., Menegazzi P., Rosbash M., Helfrich-Förster C. (2019) A distinct visual pathway mediates high-intensity light adaptation of the circadian clock in *Drosophila*, *J. Neurosci.* **39**, 9, 1621–1630. https://doi.org/10.1523/JNEUROSCI.1497-18.2018.

23 Liu Z., Zhao Z. (2014) Effects of light interruption on sleep and viability of *Drosophila melanogaster*, *PLoS One* **9**, 8, e105678. https://doi.org/10.1371/journal.pone.0105678.

24 Hall H., Ma J., Shekhar S., Leon-Salas W.D., Weake V.M. (2018) Blue light induces a neuroprotective gene expression program in *Drosophila* photoreceptors, *BMC Neurosci.* **19**, 1, 43. https://doi.org/10.1186/s12868-018-0443-y.

25 Vandegehuchte M.B., Janssen C.R. (2011) Epigenetics and its implications for ecotoxicology, *Ecotoxicology* **20**, 3, 607–624. https://doi.org/10.1007/s10646-011-0634-0.

26 Echazú R.D., Cadena C.A., Saravia Mathon L.R. (2000) Estudio de materiales reflectivos para concentradores solares, *Avances en Energías Renovables y Medio Ambiente* **4**, 11–16.

27 Cho E., Oh J.H., Lee E., Do Y.R., Kim E.Y. (2016) Cycles of circadian illuminance are sufficient to entrain and maintain circadian locomotor rhythms in *Drosophila*, *Sci. Rep.* **6**, 37784. https://doi.org/10.1038/srep37784.
28 Fernández-Moreno M.A., Farr C.L., Kaguni L.S., Garesse R. (2007) *Drosophila melanogaster* as a model system to study mitochondrial biology, *Methods Mol. Biol.* **372**, 33–49. https://doi.org/10.1007/978-1-59745-365-3_3.
29 Goda T., Hamada F.N. (2019) *Drosophila* temperature preference rhythms: An innovative model to understand body temperature rhythm, *Int. J. Mol. Sci.* **20**, 8, 1988. https://doi.org/10.3390/ijms20081988.
30 Johnson J.C., Munneke A.S., Richardson H.M., Gendron C.M., Pletcher S.D. (2023) Light modulates *Drosophila* lifespan via perceptual systems independent of circadian rhythms, *Aging (Albany NY)* **15**, 2, 396–420. https://doi.org/10.18632/aging.204472.