

**Submission ID #:** 67108

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**Project page Link:** [https://review.jove.com/files\\_upload.php?src=20467988](https://review.jove.com/files_upload.php?src=20467988)

**Title:** Climbing Behavior of *Drosophila melanogaster* Larvae in Response to Hypoxia

**Q: Filming location:** Will the filming need to take place in multiple locations? **No.**

**Current Protocol Length**

Number of Steps: 06

Number of Shots: 14

# Protocol

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## 1. Protocol

- 1.1. To begin, store the portioned *Drosophila* fly food vials in a designated refrigerator until the start of the experiment **[1]**. Maintain experimental *Drosophila melanogaster* in a controlled environment under standard temperature, humidity, and light–dark conditions **[2]**.
  - 1.1.1. WIDE: Talent placing labeled vials with fly food into the refrigerator.
  - 1.1.2. Shot of the experimental flies in a controlled environment
- 1.2. Transfer the *Drosophila melanogaster* flies into vials containing food **[1]** and incubate them for a minimum of 7 days and a maximum of 9 days **[2]**.
  - 1.2.1. Talent transferring the flies from one vial to the food vial.
  - 1.2.2. Talent placing fly containing food vials into the incubator and closing the door.
- 1.3. For hypoxia induction, place the flies along with their food vials into the hypoxia chamber **[1]**. Place LED (*L-E-D*) flashlights inside the chamber to provide illumination for the incubator and visualize the escape behavior of the flies **[2-TXT]**.
  - 1.3.1. Talent transferring food vials with flies from the incubator into the hypoxia chamber.
  - 1.3.2. Talent arranging multiple LED flashlights inside the hypoxia chamber and switching them on. **TXT: LED lights regulate the circadian rhythm of the flies**
- 1.4. Then pre-set the hypoxia chamber to a temperature of 24.5 degrees Celsius and a carbon dioxide concentration between 0 and 0.1 percent **[1]**. Set the oxygen concentration in the chamber to 1 percent **[2]**. ~~Once the chamber reaches 1 percent oxygen, open the outer chamber door~~ **[3]**.
  - 1.4.1. Talent adjusting the hypoxia chamber temperature to 24.5 degrees Celsius, and setting the carbon dioxide concentration to 0–0.1 percent.
  - 1.4.2. Talent setting the oxygen concentration to 1%.
  - 1.4.3. ~~Talent opening the outer chamber door.~~ **Videographer's NOTE:** Shot 1.4.3. was skipped at author's request

- 1.5. Start a timer immediately upon opening the chamber door to mark the start of the experiment at 0 minutes [1]. While keeping the transparent door closed to maintain hypoxic conditions [2], count the number of larvae climbing out of the fly food and along the walls of the vial [3].
  - 1.5.1. Talent pressing the start button on a digital timer right after opening the chamber door.
  - 1.5.2. Shot of the closed transparent door.
  - 1.5.3. Talent counting larvae on the vial walls.
- 1.6. Use the timer to perform a second count of the larvae at the 10-minute mark [1-TXT].  
~~Without activating the hypoxia chamber, allow it to sit under normal atmospheric conditions with 21 percent oxygen concentration [2].~~
  - 1.6.1. Talent looking at the timer reading 10 minutes and recounting larvae climbing the vial walls. **TXT: Perform larval counts at 0 min and 10 min; Let the hypoxia chamber remain at 1% O<sub>2</sub>** **Videographer's NOTE:** Shot 1.6.0. "Timer Reading at 10 minutes." was added at author's request
  - 1.6.2. ~~Talent leaving the chamber untouched with flies inside and the oxygen setting screen displaying 21 percent oxygen. **NOTE:** Not filmed, VO moved as on-screen text~~

1. Drosophila  
Pronunciation link: <https://www.merriam-webster.com/dictionary/Drosophila>  
IPA: /drə'sa:fɪlə/  
Phonetic Spelling: druh·sah·fuh·luh
2. melanogaster  
Pronunciation link: <https://www.merriam-webster.com/dictionary/melanogaster>  
IPA: /'melə'nɑ:gæstər/  
Phonetic Spelling: meh·luh·nah·gas·ter
3. hypoxia  
Pronunciation link: <https://www.merriam-webster.com/dictionary/hypoxia>  
IPA: /haɪ'pə:ksiə/  
Phonetic Spelling: hy·paak·see·uh
4. incubator  
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubator>  
IPA: /'ɪŋkjə,beɪtər/  
Phonetic Spelling: ing·kyuh·bay·ter
5. circadian  
Pronunciation link: <https://www.merriam-webster.com/dictionary/circadian>

IPA: /sər'keidiən/

Phonetic Spelling: ser·kay·dee·uhn

6. larvae

Pronunciation link: <https://www.merriam-webster.com/dictionary/larvae>

IPA: /'la:rvi/ , /'la:rveɪ/

Phonetic Spelling: laar·vee; laar·vay

7. Celsius

Pronunciation link: <https://www.merriam-webster.com/dictionary/Celsius>

IPA: /'selsiəs/

Phonetic Spelling: sel·see·uhs

8. hypoxic

Pronunciation link: <https://www.merriam-webster.com/dictionary/hypoxic>

IPA: /haɪ'pə:ksɪk/

Phonetic Spelling: hy·paak·sik