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From drugs to climate change: hands-on experiments with *Daphnia* as a model organism

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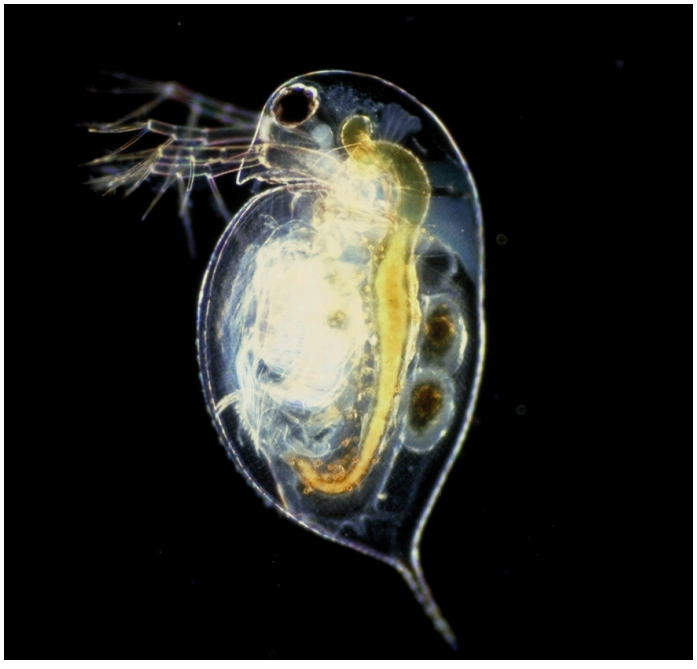
How do social drugs affect metabolism? How is toxicity measured? How does climate change affect water ecosystems? Promote active learning by investigating these questions with *Daphnia*.

Introduction

Daphnia, a freshwater microcrustacean keystone species, is a model organism with many practical advantages: simplicity of culture under laboratory conditions, small body size (2–5 mm), short life cycle, easy handling, high fecundity, parthenogenetic reproduction, and low maintenance costs.[1,2] Due to their translucent chitinous exoskeleton, it is possible to observe *Daphnia*'s internal organs under a microscope or binocular magnifying glasses.[3] Since they are very sensitive animals, and because they have fundamental biological responses very similar to those of humans, they are used as models to test the effects of various social drugs, such as coffee, tobacco, and alcohol, and are used to evaluate water quality.[2] Freshwater ecosystems, which are among the most threatened in the world, provide drinking and irrigation water, food, climate regulation, erosion prevention, and recreation for human society. Climate change is increasing the salt concentration of freshwater, with drastic effects on the health and survival of freshwater organisms.

The hands-on activities outlined in this article are suitable for students aged 14–19 and are a valuable way to evaluate the effects of social drugs on the cardiovascular system and the influence of salinization on *Daphnia* survival.

By investigating these effects, students learn to record and manage experimental data to draw their own conclusions. From the conclusions, students can consider the implications for human health, and understand the effects of climate change on freshwater ecosystems.



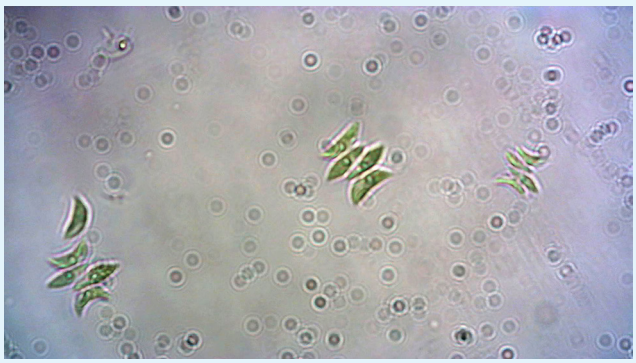
Daphnia Pulex
Image: Paul Hebert/Reference [4], CC BY 2.5

How to culture *Daphnia*

Cultures can be maintained in containers with a maximum of 30 *Daphnia* per litre in chlorine-free water with a pH of 7–8, at 18–25°C, over a controlled photoperiod of 16 h and fed 2–3 times per week with microalgae or yeast (5 drops/litre). Low levels of dissolved oxygen are lethal.

Yeast suspension

To prepare a yeast (unicellular fungus) suspension, add chlorine-free water to a 1 litre container and stir in 2 g of baker's yeast. Store the suspension in a refrigerator (4°C) and always agitate before use to resuspend the yeast. Add 5 drops/litre of this food to *Daphnia* cultures 2–3 times per week.



Scenedesmus
Image courtesy of the authors

How to culture Microalgae

Microalgae are microscopic photosynthetic organisms. They play a central role in life on Earth, as they form the basis of the food web in many aquatic ecosystems. *Scenedesmus* is a nonmotile green microalgae, typical of freshwater phytoplankton, that is easy to culture and maintain in the laboratory and is routinely used to feed *Daphnia*.

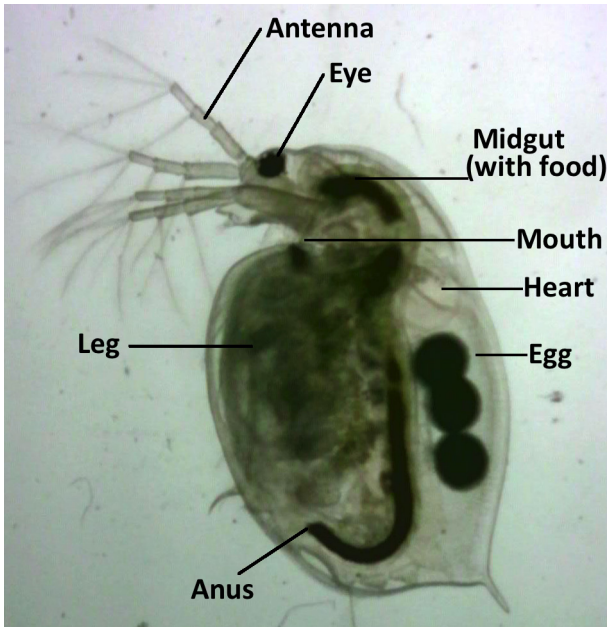
Cultures of Scenedesmus require the use of chlorine-free water with a pH of 7–8, at a minimum temperature of 16°C (18 to 25°C), and with a minimum photoperiod of 12 h provided by an LED (or fluorescent) aquarium light. The microalgae culture should be kept in an aerated container (bioreactor) to provide the CO_2 required for photosynthesis and prevent sedimentation of microalgae in the bottom of the vessel, which greatly limits their reproduction. Every 15 days, the culture should be supplemented with mineral salts from 3–5 drops/litre of commercial liquid plant fertilizer.

The algae bioreactor
Image courtesy of the authors

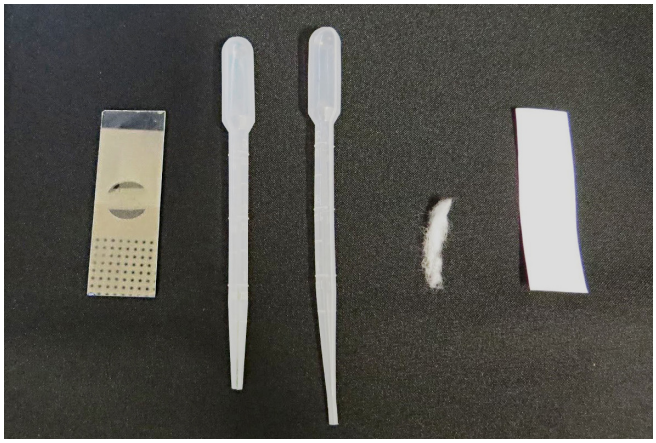
Activity 1: The rise and fall of a heartbeat

Social-drug consumption causes changes to the central nervous system, resulting in physiological and/or behavioural changes. Stimulant drugs (caffeine, nicotine) are characterized by increasing body metabolism, whereas depressor drugs (alcohol) decrease it. The heart rate can be monitored as an optimal indicator of these changes.

Observing how drugs affect a living organism in real time allows students to establish a relationship with the possible effects of drugs in their body. The transparent body of *Daphnia* allows measurement of different physiological parameters using noninvasive optical methods. A bioassay is a procedure that uses living organisms to determine the effects of a chemical. The activity requires around 2 h to complete but can be done in 1 h if the solutions are prepared beforehand.



Female *Daphnia magna* from a laboratory culture of a clone originating in lake Oued Mellah (Morocco)
Image courtesy of the authors



Materials: concave slide, plastic pipettes (with and without the tip cut off), cotton wool, and filter paper
Image courtesy of the authors

Materials

- Aged/dechlorinated tap water
- Jar containing living, young *Daphnia* (aged less than 48 h)
- Stereo- or optical microscope
- Concave slide
- Plastic pipettes (3 ml) with the tips cut off
- Cotton wool
- Filter paper
- Beakers
- Alcohol (6%, 12%, and 20%)
- Coffee 30% (70 ml distilled water + 30 ml instant coffee)
- Tobacco

Preparation of tobacco solution

1. Weigh 2 g of tobacco, add distilled water to bring it to a volume of 100 ml, and leave to macerate (12 h), stirring at least 4 times.
2. Filter through filter paper. The tobacco solution lasts for 2–3 days.

Procedure

On the provided worksheet (see sample table), students predict the effects of the social drugs on the heart rate of *Daphnia*. Before starting experimental work, watch this video to learn how to find the *Daphnia* heart and how to count heartbeats.

1. Add 2 drops of water from the *Daphnia* culture medium (enough to immerse the animal) on the concave slide.
2. Use a pipette (with the tip cut off) to carefully place a *Daphnia* on the concave slide.
3. Place some cotton threads in the depression of the slide (just enough to immobilise the *Daphnia*).
4. Using a stereo- or an optical microscope, determine the basal heart rate of each *Daphnia* (control). Make three measures of 30 s each.
5. Remove the concave slide from the microscope and add one drop of the social-drug solution (experiment).
6. After a minute, repeat step 4 and determine the heart rate. Make three measures of 30 s each.
7. Repeat the procedure with the other social-drug samples using a new *Daphnia* each time.
8. Record and discuss the results.
9. Construct and present a scientific poster.

Notes

Caution: if excess cotton is used, it may be difficult to observe the *Daphnia* heart.

The optical microscope and solutions (alcohol, tobacco, coffee)
Image courtesy of the authors

	Prediction (according to key below)	Control heart rate (bpm)	Experimental heart rate (bpm)
Tobacco			
Alcohol (6%)			
Alcohol (12%)			
Alcohol (20%)			
Coffee			

Key:

+++	big increase in heart rate
++	increase in heart rate
+	small increase in heart rate
0	no change in heart rate
–	small decrease in heart rate
––	decrease in heart rate
–––	big decrease in heart rate
x	heart stops

Control counts below 200 beats per minute (bpm) or above 300 bpm should be repeated.

Safety notes

Care must be taken to ensure that experimental solutions do not come into contact with students' skin, eyes, or mouth.

At the end of the activity, students must wash their hands.

Discussion

The transparent bodies of *Daphnia* allows visual observation of internal organs and measurement of physiological characteristics, such as heart rate. They can be used as a model system for toxicology because they are highly sensitive to environmental disturbances and exhibit rapid physiological responses when exposed to water contamination.[5]

The use of live animals, as part of authentic research experiences, has been shown to increase understanding of scientific concepts. After the experiment, ask students the following questions to promote their understanding of the influence of social drugs on the nervous system using *Daphnia* as a model organism:

- What is the purpose of having some *Daphnia* in water only?
- Why is the average of three heartbeat counts used?
- Why is a new *Daphnia* used for each experimental solution (social drug) tested?
- What is the advantage/importance of using clones?
- Compare predictions with experimental data.

Discuss how *Daphnia* is a suitable biological model for studying multiple stressors.

Activity 2: Climate change: how does salt exposure affect *Daphnia*?

Salinization of freshwater ecosystems is an increasing problem due to human activities such as irrigation, resource extraction, accelerated weathering of rocks by acid rain, use of agricultural fertilizers and road salts, mining, and urban construction. This is further exacerbated by climate change due to decreased rainfall, increased evaporation, and an increased need for irrigation.[6,7] The use of bioassay investigations can develop students' understanding of the effects of salinization on *Daphnia* survival, thus promoting a conceptual connection between the survival of simple organisms and the effects on freshwater ecosystems. These organisms need to maintain an osmotic balance between the ion concentration within their cells and their body fluids, and this is strongly influenced by the salinity of the surrounding water. In this activity, students determine the toxicity of NaCl for *Daphnia* by calculating the LC_{50} (lethal concentration, 50%) value, which is defined as the toxicant concentration that kills 50% of the exposed organisms. This experiment should take around 20 min.[1]

Materials

- Aged/dechlorinated tap water
- Jar containing young *Daphnia* (aged less than 48 h)
- NaCl solutions (0.5%, 1%, 1.5%, and 2%)
- Graduated cylinder (10 ml)
- 5 small beakers or cups for mixing solutions
- 5 test tubes, 10 ml minimum (or exposure chambers and test tube racks or small cups)
- plastic pipettes (3 ml) with the tips cut off

Test tubes, NaCl solutions, graduated cylinder, and pipettes
Image courtesy of the authors

Procedure

1. Prepare solutions with different salt concentrations (0.5%, 1%, 1.5%, and 2% NaCl).
2. Label five test tubes and add aged water or one of the salt solutions (10 ml).
3. Use a pipette (with the end cut off) to carefully place 10 *Daphnia* in each test tube.
4. After 5 min, 10 min, and 20 min, observe the behaviour of the living *Daphnia* and count the number of *Daphnia* that have died (are immobilized).
5. Record the results in a table.
6. Graph data on *Daphnia* mortality (percentage of *Daphnia* that died after 20 min) versus concentration of salt solution.
7. Determine the LC_{50} value of salt for *Daphnia*.
8. Construct and present a scientific poster.

Note

In the control, the mortality cannot be higher than 10%.

Note

Do not use detergent to clean the glass material. Detergent residues may be toxic to *Daphnia*.

Discussion

After the experiment, discuss the results to promote an understanding of the influence of climate change on the behaviour and survival of *Daphnia* and other aquatic organisms.[8,9] Questions could include the following:

- What is the purpose of having some *Daphnia* in water only?
- Calculate the LC_{50} of sodium chloride for *Daphnia*.
- What do you think the long-term effect of salinization would be on *Daphnia* populations?
- In what ways can climate change affect freshwater ecosystems?
- Can you think of any implications of this research for human populations?

This activity could also be the basis for a discussion on the ethics of using live animals in research. The use of very simple organisms like *Daphnia* is fairly uncontroversial, but where would students draw the line? More information on why the use of animals in research can be important and how we can minimise it can be found in this article. «

NaCl (%)	Number of *Daphnia* that died after			% mortality (after 20 min)	Observations of *Daphnia* behaviour
	5 min	10 min	20 min		
0					
0.5					
1					
1.5					
2					

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Resources

- An informative website detailing the effect of climate change on freshwater ecosystems: http://www.climate-and-freshwater.info/climate_change/
- Try some engaging experiments with bioluminescence that integrate biology, chemistry, and engineering: Wegner C, Hammann M, Zehne C (2021) Bioluminescence: combining biology, chemistry, and bionics. *Science in School* **53**.
- Explore antibiotic resistance and drug development with these fun microbiology activities: Deumal Fernandez M, Lladonosa Soler M, Godinho T (2021) Microbiology: Discovering antibacterial agents. *Science in School* **55**.
- Learn about the importance of animal use in research and some cutting-edge approaches to minimizing it: Schmerbeck S et al. (2021) Organ-on-chip systems and the 3Rs. *Science in School* **54**.

Acknowledgements

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AUTHOR BIOGRAPHY

Hugo Miguel Faria has been a science teacher for 32 years. His main research interest is in the use of model organisms (*Daphnia*, *Planaria*, *Lymnaea*) in K-12 teaching, concept mapping, and computer adaptive assessment. He is a collaborator at Coastal and Marine Environmental Toxicology (CIIMAR-Universidade do Porto).

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