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Hands-on experiments with planaria

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Meet the planarian, a fascinating flatworm with incredible biological abilities and unique and surprising ways responses to various stimuli.

Introduction

Freshwater planaria, nonparasitic invertebrate animals belonging to the phylum Platyhelminthes, the flatworms, are organisms that are well-suited to educational purposes. They are easy to maintain under laboratory conditions^[1] as they require minimal resources and can survive in a simple water-based environment. Also, they are inexpensive to propagate, reasonably macroscopic (1–10 mm in length^[2]), and easy to work with. Planaria are bilaterally symmetric animals, with a complex central nervous system and two eye spots.^[3]



An asexual specimen of *Dugesia subtentaculata* Image: Eduard Solà/<u>Wikimedia</u>, <u>CC BY-SA 3.0</u>



A single 10 mm planarian Image courtesy of the authors

Observing and studying nature plays a crucial role in the development of human thought. Hands-on teaching methods are often considered to be effective in the classroom, not only for explaining various biological processes, but also for fostering student curiosity.

Working with planaria allows students to gain a deeper understanding of various aspects of living organisms. By observing these remarkable creatures, students can explore organ systems, reproductive strategies, and the relationship between structure and function in living organisms. Planaria provide a hands-on learning experience that enables students to explore and comprehend these fundamental biological concepts.

We introduce a hands-on approach for students aged 14-19 to explore fundamental questions underlying animal responses to stimuli and their regeneration abilities.

How to culture planaria

- Cultures can be maintained in small containers (100 ml) in chlorine-free water at a pH of 7-8, at 10–20°C, in the dark.
- Once a week, defrost a small fragment of crushed calf liver and add it to the culture.
- After 1 h, remove all the liver from the culture and replace all the water.

The planaria care guide provides a comprehensive guide on how to prepare planarian food.

Activity 1: Reaction to stimuli

In this activity, students can investigate how these organisms react to stimuli of light, food, and touch. This allows students to gain insights into the sensory capabilities and behavioural adaptations of planaria and can contribute to a deeper understanding of their biology and ecological interactions.

The activity is suitable for students aged 11-19 and will take 30-45 min to complete. The planaria infosheet can be distributed before the activity or at the end before the discussion, so students learn more about these fascinating creatures.



Simplified anatomical depiction of the planarian Image courtesy of the authors

Handling tip

Planarians can be transferred from one container to another using plastic Pasteur pipettes with openings big enough to accommodate the worms without injuring them. If the opening of the Pasteur pipette is not big enough, the pipette tip can be cut with scissors.

Materials (per pair of students)

- Planaria infosheet
- Activity 1 worksheet
- 3 planaria

Getting planaria

Option 1. Freshwater planaria are one of about 1300 species that can be found in unpolluted streams and lakes, usually associated with the lower part of a rock or trunk. They can be easily collected by applying a light jet of water to the rock and transferring them to a container with the help of a plastic pipette, avoiding animal damage. After collection, they must be transported in containers filled with water (without air), at temperatures between 1 and 25°C. In the laboratory, at least half of the water should be replaced with chlorine-free water.

Option 2. Contact a biology department at a university. Some use planaria and are willing to provide animals for educational purposes.

- Aged/dechlorinated tap water
- 3 plastic pipettes (3 ml) with the tips cut off
- 3 Petri dishes
- Calf liver (1 g)
- Torch
- Toothpick

Procedure

- 1. Explain the experiments and have the students record their predictions in the results table.
- 2. Fill half of the Petri dish with dechlorinated water.
- 3. Place one planarian in the centre of the Petri dish.
- 4. Observe the planarian for 1 min in an unstimulated state and document any observations.
- 5. Touch the head of the planarian with a toothpick (touch stimulus) and record the reaction.
- 6. Repeat steps 2 to 4 with a new planarian.
- 7. Shine light on the planarian with a torch (light stimulus) and document any observations.
- 8. Repeat step 2.
- 9. Place a piece of calf liver (chemical stimulus) in the centre of the Petri dish.
- 10. Place a new planarian at the edge of the Petri dish.
- 11. Observe the planarian for 10 min and document any observations made.

Discussion

Work through the <u>worksheet</u> and discuss the following points. Sample answers can be found on the <u>answer sheet</u> in the supporting material to give educators ready-to-use content for classroom conversations.

- Why do we use a new planarian for each stimulus tested?
- Compare the predictions with the experimental data. Do they match?
- Can you explain the reactions of the planarians to different stimuli?
- What is the role of the nervous system in planarian reactions?
- Why do scientists use planaria in research on the nervous system?

Finally, challenge the students to design their own planarian stimulus experiment.

Activity 2: Planaria regeneration

Although all multicellular organisms depend on stem cells for their survival and perpetuation, planaria are capable of regenerating any missing body region^[4] in a relatively short time. This regenerative ability results from the abundance of stem cells, which are the only cells with the capacity to divide and differentiate into any type of cell.^[5] Planaria are particularly studied for their ability to regenerate quickly from any body fragment. They have abundant adult stem cells called neoblasts, comprising 20–30% of their cells, which can divide and differentiate into any cell type.

Planaria regeneration

Regeneration is a fascinating process that replaces damaged or lost structures and requires not only new tissue production but also reorganization of pre-existing tissues to ensure the correct size and proportion of the regenerated animal^[6] Body shape is preserved almost regardless of the type of injury, maintaining proper proportions even when the newly regenerated animal is significantly smaller than the original.^[7] After amputation, there is a strong contraction of the muscles to close the cut, minimizing exposure of the internal tissues and the wound area.^[8] A fragment from the previous region will continue to move, a mechanism that would allow the planarian to escape from a predator.^[7] After 30 min, a thin layer of cells is transferred to the cut zone.^[9] Then, stem cells increase their proliferation rate and, at the cut site, they generate new tissue without pigmentation, the blastema, where differentiation occurs, to replace the lost parts.^[10] Regeneration is complete when the animal has the appropriate proportions and pigmentation is homogeneous. After two weeks, the planaria can be regularly fed again. The use of a stereoscopic magnifying glass and photographic record can aid in monitoring the evolution of regeneration.

The activity is suitable for students aged 14–19 and will take two lessons to complete.

Materials (per pair of students)

- 1 planarian
- Petri dish
- Aged/dechlorinated tap water
- Slide
- Scalpel
- Plastic pipettes (3 ml) with the tips cut off
- Ethanol (96°)



Pipette, scalpel, slide, and Petri dish Image courtesy of the authors

Procedure

- 1. Stop feeding the planarian 48 h before the experiment.
- 2. Keep the planarian at 4°C for 15 min.
- 3. Sanitize the scalpel with ethanol.
- 4. Add 2 drops of dechlorinated water to the slide.
- 5. Using the scalpel, slice the animal into three fragments.
- 6. Transfer the cut fragments back into the culture container by gently squirting the fragments into the Petri dish with a pipette.
- 7. Every three days, replace the water.
- 8. Record and discuss the results.
- Optional: students can create a scientific poster to resent their findings, including the aim (research question), methods, and results.



Fragments of the sliced planarian *Image courtesy of the authors*

Discussion

Discuss the following questions. Sample answers can be found on the <u>answer sheet</u> in the supporting material.

- Why is feeding of the planarian stopped 48 h before the experiment?
- Why is it important to keep the planarian at 4°C?
- What can we learn from the regenerative abilities of planaria?
- Could we help planaria regrow faster? Design your own planarian regeneration experiment.
- What are the similarities and differences between planarian and human stem cells, and what can we learn by studying them?

Optional extension activity

Planaria also shrink under low-nutrient conditions. This can additionally be investigated as an <u>extension activity</u>, as described in the supporting material.

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 14: 298. doi: 10.1038/s41467-023-35937-y

Resources

• Read about the intriguing <u>regenerative capability</u> of flatworms.

- Read about how to teach about the nervous system: KvelloI P, GerickeI N (2021) <u>Identifying knowledge import-</u> ant to teach about the nervous system in the context of <u>secondary biology and science education-a Delphi study</u>. *PLoS ONE* 16: e0260752. doi: 10.1371/journal.pone.0260752
- Dive into the captivating world of honeybees and their sweet product through a series of engaging hands-on activities: Scheuber T (2023). To bee or not to bee: the biology of bees and the biochemistry of honey. Science in School **62**.
- Embark on a microscopic moss safari to explore the fascinating world of diverse and resilient organisms that thrive in this challenging habitat: Chandler-Grevatt A (2023) <u>Moss</u> <u>Safari: what lives in moss</u>? *Science in School* **63**.
- Gain insights into toxicology and the physiological effects of drugs through the use of Daphnia as a model organism: Faria HM, Fonseca AP (2022) <u>From drugs to climate</u> <u>change: hands-on experiments with Daphnia as a model</u> <u>organism</u>. Science in School 59.
- Read about the impacts of meat consumption and the development of lab-grown substitutes: Noble M (2023)
 <u>From Petri dish to plate: the journey of cultivated meat</u>. Science in School 63.
- Learn about the importance of animal use in research and some cutting-edge approaches to minimizing it: Schmerbeck S et al. (2021) <u>Organ-on-chip systems and</u> <u>the 3Rs</u>. Science in School **54**.

AUTHOR BIOGRAPHY

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