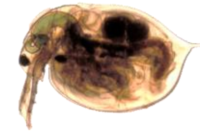
ISSF Lesson: Water Strand

Daphnia magna and Water Monitoring



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Logistics

This lesson is intended for high-school students studying either biology or environmental science. This lesson is developed in conjunction with the 14th Annual International Student Science Fair to be held this July at IMSA.

The structure of the lesson has students move through a series of stations that feature environmental pollutants. As such, a science laboratory is necessary. Students will use a bioassay to monitor water quality by subjecting *Daphnia magna* to various pollutants and measuring the heart rate response. This data will be used to evaluate toxicity of water samples.

Materials

per station:

Daphnia magna in culture liquid (about 30 per station)
1 – Labeled container for treated *Daphnia,* with spring water
1 – Plastic cup for used water sample collection
200 ml – water sample (see Advanced Preparation)
Chart paper

per partner team at each station:

Daphnia magna in culture liquid
2 – Depression (or well) slides
1 – 6-well microplate
1 – Microscope – low power, transmission (or dissecting)
2 – Safety gloves (one pair for each student)
1 – Scissors
1 – Stopwatch
2 – Disposable transfer pipettes
1 – Vis-à-vis marker (for labeling microplate)

per student:

1 – Copy of Student Pages

Time: One 90-minute class period (two 45-minute sessions)

Objectives/Standards

Students will:

- Investigate the effects of different environmental toxins on a metabolic process. **HS-LS1-3, SEP3**
- Collect and analyze heart rate data for *Daphnia magna* after exposure to different water-borne pollutants. **SEP4**
- Make and defend a claim based on evidence. **SEP7**
- Discuss implications of human exposure to water pollutants. **SEP6**

References to Next Generation Science Standards adapted from NGSS Lead States. 2013. Next Generation Science Standards: For States, By States. Washington, DC: The National Academies Press

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Introduction

Daphnia magna, commonly referred to as "water flea", are small, freshwater crustaceans commonly used in water toxicity studies. These aquatic animals are highly sensitive to chemical changes in their environment, making them model organisms for use in water quality testing. *Daphnia* are an important part of the aquatic ecosystem as a food source for many fish and as a filter-feeder ingesting algae, bacteria and detritus.

The anatomy of *Daphnia* also makes them an ideal bioindicator. Due to the presence of a transparent carapace, or outer skeleton, the internal organs are visible, including the beating heart. Thus, heart rate measurements can be conducted by viewing live *Daphnia* through a low-power transmission microscope.

Advanced Preparation:

- ❑ Prepare approximately 200 ml of 1% concentrated solution for each station prior to this laboratory experiment. These will represent water sample from the various wells. Use the following protocols:

Station	Solution Protocol
1. Simulated Wastewater	Dissolve in 1 L of tap water: • peptone, 160 mg; • meat extract, 110 mg; • urea, 30 mg; • anhydrous dipotassium hydrogen phosphate, (KH2PO4), 28 mg; • sodium chloride, (NaCl), 7 mg; • calcium chloride dihydrate, (CaCl2.2H2O), 4 mg; • magnesium sulfate heptahydrate, (MgSO4.7H2O), 2 mg Dilute to 1% solution: 2 ml wastewater to 198 ml spring water
2. Ammonium Sulfate	Dissolve 2 grams ammonium sulfate in 200 ml spring water

NOTES

3. Acetaminophen	Dissolve 2 grams powdered acetaminophen in 200 ml spring water
4. Aspirin	Dissolve 2 grams acetylsalicylic acid in 200 ml spring water
5. Caffeine	Dissolve 1 gram caffeine powder in 200 ml spring water
6. Motor oil	Dilute to 1% solution: 2 ml motor oil to 198 ml spring water

- ❑ Label one plastic cup "Treated *Daphnia*" for each station and fill with at least 50 ml of spring water for use in storing *Daphnia magna* that have been soaked in testing solution.

- ❑ Culture enough *Daphnia magna* to provide a minimum of one animal per student pair at each station. Information on how to culture Daphnia magna can be found here: http://ei.cornell.edu/toxicology/bioassays/daphnia/culture.html

  Daphnia can be purchased through Carolina Biological Supply– https://www.carolina.com/daphnia/daphnia-magna-living/142330.pr

- ❑ Provide quantities of materials at each station based upon the number of partner teams for the class. See the materials section for a breakdown of materials per station and per partner team.

- ❑ Print copies of student pages for each student. However, print the last page (Potential Contaminants) separately to hand out later in the investigation.

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Health and Safety: need SDS for chemicals used in this investigation

Students should use safety gloves for this investigation. The use of protective eyewear is encouraged. Students will be exposed to low-level concentrations of chemicals. However, follow all safety protocols as shown in the SDS for each chemical. Avoid exposure to skin and do not swallow any of the samples provided.

Activity

Students will work in partner teams for this activity.

Begin the activity by passing out the student pages. Ask one student to read aloud the *Background Information* and the *Problem* for this investigation.

Student teams may independently view the *Daphnia magna* video or project the video from https://www.youtube.com/watch?v=2g-04Uk0ut0. Encourage students to make careful observations and to identify the location of the beating heart.

Students will then be tasked with determining a method for calculating the heart rate of *Daphnia* in beats per minute. Allow student teams several minutes to collaborate on this procedure and then ask teams to share their ideas. Since *Daphnia* heart rates are in the order of approximately 300 bpm, counting the heart rate for one minute will undoubtedly lead to errors. Student methods should focus on accurately counting beats for a fraction of a minute (10 seconds or 15 seconds) and then using multiplication to calculate bpm. Students should divide the work of timing and counting. If available, tally counter clickers may be utilized to keep track of *Daphnia* heartbeats. Additional suggestions include using a permanent marker to tap out an s-shaped pattern representing each heartbeat or counting to five or ten and recording a tally to track the number of fives and/or tens.

Once teams have decided upon a procedure to calculate heart rate, equally divide student teams at each station. Discuss appropriate safety measures before beginning the investigation.

Students will follow the procedure in their student pages to extract one *Daphnia magna* from culture solution and measure three different heart rate trials prior to treatment exposure. Once control measurements have been recorded, students may place *Daphnia* in a 10 ml water sample from

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their current station. Observe *Daphnia* in the solution for five minutes and then take three heart rate measurements.

Allow student teams to visit as many stations as they can in the allotted time. Reserve approximately 30 minutes at the end of the class session for students to compile data, write and share a recommendation and discuss the class findings.

To facilitate compiling class data, use chart paper or a white board and write the station headings along the top of the paper/board. Students can record their average control heart rate and average treatment heart rate for each visited station under the correct station heading. This data can be used by the class to write a recommendation on which wells have water that is “safe” to drink.

Safe	Unsafe

Once teams have written their recommendation, ask teams to share which wells they deem safe and which wells they deem unsafe. **Teams must justify their decisions using data and observations!** Use a t-chart for each station to record the safe/unsafe responses from each team.

Finally, pass out the last student page (Potential Contaminants) and ask students to decide which station corresponds to which contaminant.

Debrief Activity:
The discussion questions provided on the student pages can be used to facilitate small-group discourse between two or more partner teams. Following small-group discussion, the following questions may be used to facilitate a whole-class discussion:

- ***Many pharmaceutical pollutants are found in low-level or trace amounts in our water supply. Should we be concerned?***
- ***What implications might changes in heart rate for* Daphnia magna *under certain environmental pollutants have for humans?***
- ***What additional questions or concerns do you have about the water quality in the wells?***
- ***What steps can be taken to mitigate the adverse impacts of pollution on drinking water?***

Extensions

- If desired, provide students with multi-factor drinking water test strips and/or pH paper. Ask students to decide if this additional

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information would change their minds about any of the water samples.

- Test the effect on *Daphnia* heart rate after combining two or more of the water samples.
- Prepare solutions with two different concentrations and observe the effect on *Daphnia* heart rate.

Resources

Header Image:
Are We Underestimating Species Extinction Risk? (2005). *PLoS Biology,3*(7). doi:10.1371/journal.pbio.0030253

Corotto, F., Ceballos, D., Lee, A., & Vinson, L. (march 2010). Making the Most of the Daphnia Heart Rate Lab: Optimizing the Use of Ethanol, Nicotine & Caffeine. *The American Biology Teacher,73*, 3rd ser., 176-179. doi:10.1525/abt.2010.72.3.9

Goal 6: United Nations Sustainable Development Knowledge Platform. (n.d.). Retrieved February 12, 2018, from https://sustainabledevelopment.un.org/sdg6

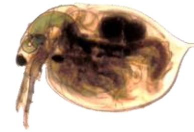
Investigating factors affecting the heart rate of Daphnia. (n.d.). Retrieved February 05, 2018, from http://www.nuffieldfoundation.org/practical-biology/investigating-factors-affecting-heart-rate-daphnia

Pau, C., Serra, T., Colomer, J., Casamitjana, X., Sala, L., & Kampf, R. (2013). Filtering capacity of Daphnia magna on sludge particles in treated wastewater. *Water Research,47*(1), 181-186. doi:10.1016/j.watres.2012.09.047

Science Buddies Staff. (2017, July 28). *Using Daphnia to Monitor Water Toxicity.* Retrieved February 5, 2018 from https://www.sciencebuddies.org/ science-fair-projects/project-ideas/EnvSci_p043/environmental-science/using-daphnia-to-monitor-water-toxicity

Siciliano A, Gesuele R, Guida M (2015) How *Daphnia* (Cladocera) Assays may be used as Bioindicators of Health Effects?. J Biodivers Endanger Species S1:005. doi:10.4172/2332-2543.S1-005

T., Smith, D., & S. (2017, May 17). Your Tap Water Likely Contains at Least 8 Drugs. Retrieved February 05, 2018, from https://www.institutefornaturalhealing.com/2017/05/tap-water-likely-contains-least-8-drugs/



Background Information:

Goal 6 of the United Nations' *Sustainable Development Goals* aims to ensure the availability and sustainable management of water and sanitation for all. According to the United Nations, millions of people die across the globe each year due to water-borne diseases. By 2030, the U.N. has targeted improvements in water quality through the reduction of pollutants, elimination of unauthorized dumping into water sources, reduction of untreated wastewater, and increase in recycling efforts to reuse water safely.

Pollutants in the form of industrial and agricultural effluents are currently discharged both directly and indirectly into available water sources. Emerging pollutants such as pharmaceuticals and personal care products, heavy metals, and endocrine disrupting chemicals are also entering the water supply. These pollutants are likely to have adverse effects on both the aquatic environments of these water bodies and humans (Siciliano, 2015). Thus, it is desired to monitor the impact of these contaminants, even at low concentration levels.

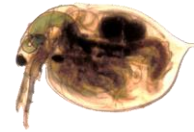
Daphnia magna is a small crustacean that can be found in freshwater environments. *Daphnia* are often used in bioassays as a sensor organism due to their high sensitivity to toxic substances and their relative importance in the aquatic food chain. *Daphnia* are characterized by a transparent carapace (outer skeleton) which allows the small, oval-shaped heart of *Daphnia* to be viewed under transmitted light using a low power microscope. Changes in *Daphnia* heart rate after exposure to environmental toxins can be measured through direct observation of the beating heart. While a change in the heart rate of *Daphnia* may not directly correlate to a similar change in the heart rate of humans, this bioindicator may be a useful tool in monitoring water quality.

Problem:

Six wells in a remote village that are used for drinking water may have been exposed to various contaminants. As the resident scientists in the village, your team will conduct toxicity testing on samples obtained from each of the six wells. Available to your team is a culture of *Daphnia magna* that you can use to evaluate the relative safety of the water. Unfortunately, you do not have access to more sophisticated testing equipment and the remoteness of the village prevents other sources of drinking water from being brought quickly into the village.

Which wells will your team deem "safe" for the villagers to access drinking water?

You will conduct tests, collect data and use this data to support your recommendation.



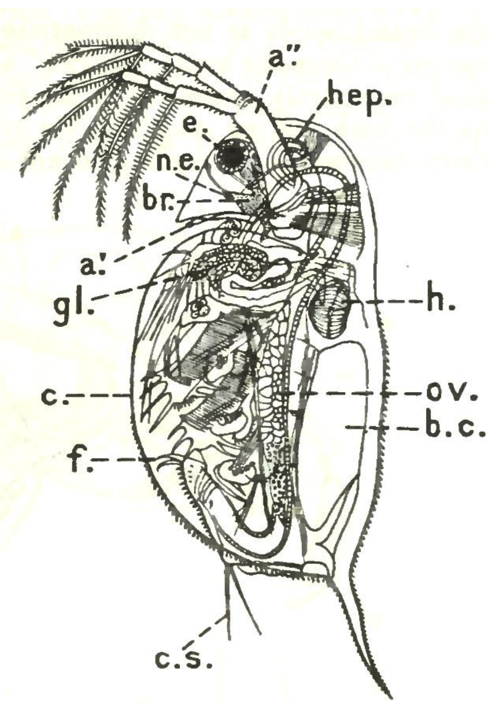
Materials at each testing station:

• *Daphnia magna* in culture water • Depression slide	• Plastic cup for treated *Daphnia* contains spring water
• Gloves	• Scissors
• Six-well microplate	• Stopwatch
• Microscope	• Vis-à-vis Marker
• Pipettes	• Water sample from a "well"

Procedure:

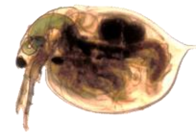
1. You and a partner will obtain a six-well microplate. Using a Vis-à-vis marker, label each well of your microplate with the numbers 1 through 6. These numbers correspond to the six stations that you will visit to test water quality using *Daphnia magna*.

2. View the video of *Daphnia magna* at https://www.youtube.com/watch?v=2g-04Uk0ut0. Mute the sound if desired. On the diagram below, identify the heart and record observations of *Daphnia magna* as you watch the video.



Daphnia, female. *a'*, antennule; *a"*, antenna; *b.c*, brood-chamber; *br*, brain; *c*, margin of carapace; *c.s*, caudal setae; *e*, compound eyes coalesced into one; *f*, furca; *gl*, maxillary gland; *h*, heart; *hep*, hepatic diverticulum of gut; *n.e*, nauplius eye; *ov*, ovary. (After Claus and Grobben.)

Observations from video



3. Determine a method to measure the heart rate (beats-per-minute, bpm) of a single *Daphnia magna* while viewing under a 10X dissecting microscope. Record your method below.

4. You and your partner will be assigned an initial testing station to begin testing water samples. Be sure to wear safety gloves prior to handling any water sample.

5. Using a disposable transfer pipette, squeeze the bulb of the pipette and place into the *Daphnia* culture jar. Slowly release the bulb to draw one *Daphnia magna* into the pipette. If the *Daphnia* is too large to fit through the pipette tip, use a pair of scissors and cut away the tip of the pipette at a 45-degree angle.

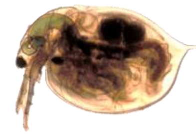
6. Gently expel excess culture water from the pipette back into the culture jar without also expelling the *Daphnia magna* that has been captured.

7. Place the *Daphnia magna* onto a depression slide and place the slide under the microscope to find the heart. If the *Daphnia* is still able to swim out of the field of view, remove more of the culture water. Use your devised method to measure the heart rate and record in the data table under the CONTROL column for this station. Repeat the heart rate measurement twice more for three total trials. Dispose of the pipette.

8. Using a clean disposable transfer pipette, carefully fill the well corresponding to your current testing station with approximately 10 ml of water sample.

9. Using the same pipette, transfer the *Daphnia* from the depression slide into the water sample. Let the *Daphnia* soak in the sample for 3 minutes. Observe the Daphnia in the sample and record your observations in the data table.

10. After three minutes has elapsed, extract the *Daphnia magna* from the water sample. Place onto a clean depression slide and measure the heart rate using your devised method for three trials. Record these values in the data table under the “WATER SAMPLE” column.



11. After taking measurements, place the *Daphnia* into the cup labeled "Treated *Daphnia*" which contains spring water. Do not return any treated *Daphnia* back to the culture jar. Dispose of the pipette.

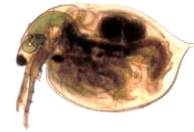
12. Pour your water sample into a collection cup at the station. Do not return the water sample back to the original water sample container.

13. As time permits, rotate through the other testing stations, repeating the entire experiment. Record your observations and heart rate measurements of *Daphnia* in the data table.

14. Calculate an average heart rate (bpm) for each station for untreated and treated *Daphnia*.

Data Table

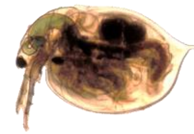
STATION SAMPLE OBSERVATIONS	CONTROL Heart Rate (bpm)	WATER SAMPLE Heart Rate (bpm)
Station 1:	**Trial 1:** **Trial 2:** **Trial 3:** **Average:**	**Trial 1:** **Trial 2:** **Trial 3:** **Average:**
Station 2:	**Trial 1:** **Trial 2:** **Trial 3:** **Average:**	**Trial 1:** **Trial 2:** **Trial 3:** **Average:**
Station 3:	**Trial 1:** **Trial 2:** **Trial 3:** **Average:**	**Trial 1:** **Trial 2:** **Trial 3:** **Average:**
Station 4:	**Trial 1:** **Trial 2:** **Trial 3:** **Average:**	**Trial 1:** **Trial 2:** **Trial 3:** **Average:**



Station 5:	**Trial 1:** **Trial 2:** **Trial 3:** **Average:**	**Trial 1:** **Trial 2:** **Trial 3:** **Average:**
Station 6:	**Trial 1:** **Trial 2:** **Trial 3:** **Average:**	**Trial 1:** **Trial 2:** **Trial 3:** **Average:**

15. Add your average heart rate data (CONTROL and WATER SAMPLE) to the class data set for each station according to your teacher's instructions. Use the table below to organize the class data.

Station 1:
Station 2:
Station 3:
Station 4:
Station 5:
Station 6:



16. Write a recommendation to your village council on which wells are safe sources of drinking water for the citizens of your village. Use your data and observations to justify your recommendation. Be prepared to share and defend your recommendation.

Discuss the following questions with another partner team:

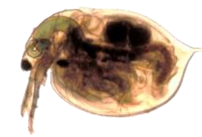
1. Why was it necessary to establish a control heartbeat for each Daphnia tested? What might account for any variability in the heart rates prior to treatment?

2. Comment on the variability of the data for each station and infer what the variability might mean in terms of the validity of the experimental procedure.

3. How are the recommendations for which wells are safe to drink from each team similar? How are they different?

4. Your teacher will now share information about the various water samples from each well. Match the water sample with its associated well.

5. Would you change your recommendation based upon this new information? Why or why not?



Potential Contaminants – match to each well

Urban runoff Storm water runoff carries pollutants such as oil, dirt, chemicals, and lawn fertilizers often directly to streams and rivers because of paved roadways, parking lots and sidewalks.	**Acetaminophen** Acetaminophen, a common anti-inflammatory drug, has been found in low-levels in drinking water sources. This drug enters through wastewater treatment plant effluent.
Untreated Wastewater Untreated wastewater can percolate down into groundwater supplies, contaminating drinking water through excess sewage, septic tanks, leaking sewer lines and land application of sludge.	**Ammonium Sulfate** This chemical is found in many agricultural fertilizers and pesticides and can enter water sources through agricultural runoff.
Caffeine Found in coffee, tea, chocolate, and some medications, caffeine's presence in water sources is a sign of human pollution.	**Aspirin** This over-the-counter pain medication can enter into water supplies through human waste and disposal of unused medication into sewage systems.