Animal Physiology

Funk & Wagnalls (1980) defines physiology [from the French, from the Latin, from the Greek *phyisiologos* = speaker on nature] as "...the processes and mechanisms by which living animals and plants function under various conditions." In this particular laboratory, we will study the effects of temperature and certain drugs on the heart rate of the water flea *Daphnia.*

Daphnia - the Water Flea

Daphnia are crustaceans in the phylum Arthropoda and the class Branchiopoda. The class name refers to the feet of the animal (*podos*) which are feathery-like and are used in respiration. Not only are the legs used in respiration but they are also used to rake food into the mouth of the animal. *Daphnia* are freshwater branchiopods and they may form a significant portion of the zooplankton in freshwater systems. *Daphnia* have been used for years as a qualitative test for water quality. *Daphnia* found in water with low oxygen content manufacture additional hemoglobin to compensate. The more hemoglobin, the more available to pull what little oxygen present in the water out for respiration. If you see *Daphnia* which are very red or pinkish, the oxygen content of that water is poor.

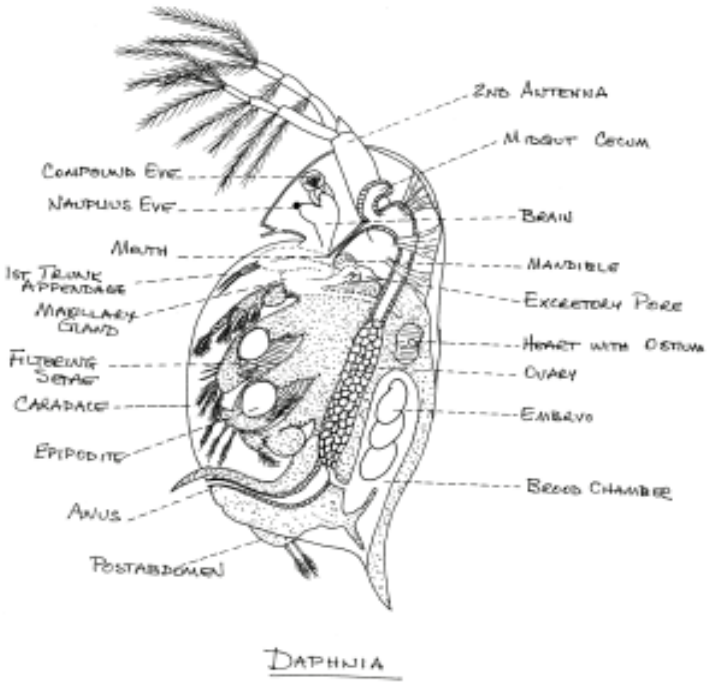

Figure 1. Daphnia.

Exercise A - Basal Heart Rate of *Daphnia*

Using a large bore pipette found in your dissection kit, pick up a *Daphnia* from the supply table. *Daphnia* is macroscopic, so you should have no trouble collecting one in your pipette. Transfer the *Daphnia* from the large bore pipette into the large end of a Pasteur pipette. Allow the animal to collect in the narrow tip of the Pasteur pipette. Use a Kimwipe© to pull some of the liquid from the tip of the Pasteur pipette so that approximately 5 mm covers the *Daphnia*. Seal the end of the Pasteur pipette with some petroleum jelly provided at the supply table.

Use the triangular file to score the Pasteur pipette 2 cm above the *Daphnia.* Break the tip of the pipette at the score. **Caution! Be sure you are wearing safety glasses when breaking the pipette tip.** Now seal the broken end of the pipette with petroleum jelly.

Place the tube with *Daphnia* in a Petri dish of water at room temperature as the original water of the culture dish of *Daphnia.* You can assume the water provided at the supply table is room temperature.

Place the Petri dish with the *Daphnia* tube under a dissection microscope. Using the diagram above, locate the heart in your living *Daphnia*. Record the number of heart beats in 15 seconds. Multiply this by 4 to get the heart beats per minute. Record the data in Table 1 provided.

Repeat the process at least 4 more times. Obtain an average heart beat rate for the four tries.

Data Collection Attempt	Heartbeats per 15 Seconds	Multiplication Factor (4)	Heartbeats per Minute
1		___ x 4	
2		___ x 4	
3		___ x 4	
4		___ x 4	
Average Heartbeats per Minute			

Data Table 1: Basal Heartbeats per Minute for Daphnia.

Exercise B. The Effect of Temperature on Basal Heartbeat of *Daphnia*

Now place the tube with *Daphnia* is a Petri dish containing water at 0°C to 5°C. Use the thermometer provided to determine the exact temperature. Give the *Daphnia* a minute to stabilize and count the heartbeats per minute as before.

Very slowly add warm water to the Petri dish until the temperature increases by 5°C. Wait a moment a record the temperature and heartbeats per minute. Continue adding warm water to increase the temperature by 5°C and recording the heartbeats per minute until you can no longer accurately record the heartbeats.

Determine Q_{10} for your *Daphnia* using the formula provided. Q_{10} has no units. It is a factor by which a rate changes, and is a useful way to express the temperature dependence of a process. In this case, we are determining the ratio of the rate of a process (heartbeat) at a given temperature to its rate at a temperature 10°C lower. You should have several temperature and heartbeat per minute determinations, therefore, you will be able to calculate several Q_{10}'s. In most living systems, Q_{10} decreases as temperature increases with a value of 2 or 3 normal at moderate temperatures.

$$Q_{10} = \frac{R_1}{R_2} \times \frac{10}{T_1 - T_2}$$

Calculate the Q_{10} for your *Daphnia* for a low temperature. _____ Q_{10} for your *Daphnia* for a moderate temperature. _____ Q_{10} for your *Daphnia* for a high temperature. _____

Attempt	Temperature (°C)	Heartbeats per Minute
0°C-5°C		
+5°C		
+5°C		
+5°C		
+5°C		
+5°C		
+5°C		
+5°C		
+5°C		
+5°C		
+5°C		
+5°C		
+5°C		

Data Table 2. Effect of Temperature on Heartbeats in Daphnia.

Carefully remove the plug of petroleum jelly from the end of the pipette tip and place your *Daphnia* in the recovery culture dish. Do not use these *Daphnia* again.

Exercise C - The Effect of Ethyl Alcohol on *Daphnia*

Obtain a depression slide from the supply table. Place a small amount of petroleum jelly in the well of the slide. Transfer a new *Daphnia* to the well of the slide using the water in the culture dish and a large bore pipette from your dissection kit.

While viewing the *Daphnia* with the dissection scope, place two drops of ethanol in the well of the slide and determine the heartbeats per minute each minute for five minutes. Remember, you are only counting the heartbeats in 15 seconds and multiplying that by 4 to obtain the heartbeats per minute. Record your data in the table provided.

Remove as much liquid from the well as possible with a Pasteur pipette. Replace with culture water from the supply table and try to collect the *Daphnia* with a large bore pipette and place in the recovery dish. Clean the depression slide with soap and water, rinse, and dry. You will need it for the next exercise.

Time	Heartbeats/ minute
0 min	
1 min	
2 min	
3 min	
4 min	
5 min	

Data Table 3. The Effect of Ethanol on Heartbeats of Daphnia.

Exercise D - The Effect of Caffeine on *Daphnia*

Place a small amount of petroleum jelly in the well of your depression slide. Obtain a new *Daphnia* from the supply table. Add two drops of caffeine solution and determine the heartbeats per minute each minute for five minutes. Again, remember you are only county the heartbeats in 15 seconds and multiplying by 4 to obtain heartbeats per minute. Record your data in the table provided.

Recover your *Daphnia* and place in the recovery dish. Wash and dry your depression slide and return to the supply table.

Time	Heartbeats/ minute
0 min	
1 min	
2 min	
3 min	
4 min	
5 min	

Data Table 4. The Effect of Caffeine on Heartbeats of Daphnia.

Produce a graph of the data of Exercise C and Exercise D.
What can you deduce from your data about ethanol and caffeine?

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Bibliography

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