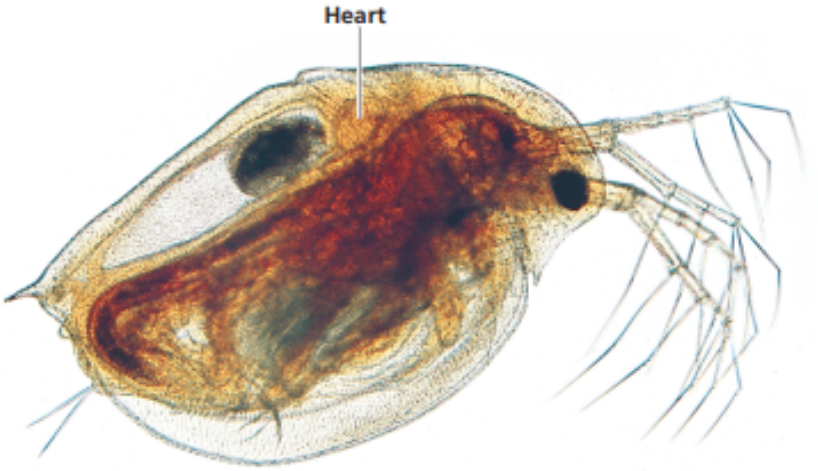
Name: _____

Partners name:_____ Due date:_____

Introduction:

Daphnia magna is a freshwater arthropod commonly found in pools and ponds throughout Canada and the northern and western parts of the U.S. Contractions of its heart are clearly visible through its transparent body. In this lab, you will measure the heart rate at room temperature and then investigate whether the heart rate is affected by exposure to different drugs. Drugs known as stimulants increase the body's metabolism. Other drugs, known as depressants slow the rate of metabolism. Heart rate is one indicator of increased or decreased metabolism. These drugs will affect the heart rate of Daphnia in the same way as humans.



Watch this video: Daphnia heartbeats and video #2

Prelab: complete in the space below [8]

1-Watch the video of measuring Daphnia heart rate twice. Initial that you have completed this step here.
2- Read this lab twice, highlighting key steps. This is a very technical lab and you need to be familiar with the procedure prior to doing the lab.
2- Circle or highlight the heart on the daphnia diagram on **page 7** of this lab. [1]
3- Reading the appendix pages and complete the summary table below for the 3 solutions you will investigate [3]

Name of drug	Predicted effect on Daphnia heart rate

4- What is the dependent variable for this lab? Include the units.[1]

5- Write a hypothesis for ONE of the solutions you will test. [1]

6- Using 2-3 of the boxes below, sketch the procedure for preparing the slide for your Daphnia [2] Include labels with your sketches.

Materials:

Daphnia
Petroleum jelly
Depression slide
Coverslip
Compound microscope
Paper towel
Distilled water
phone for timing observations
Dropper for collecting Daphnia
Nicotine
Adrenaline
Alcohol
Caffeine
Aspirin
Lactic Acid
Qtips
Styrofoam cups

PROCEDURE:

1. **Safety** –Wear goggles and gloves. Be aware that microscope light gets hot. Do not ingest any of the drugs.

2. Each person in a partnership will have their own microscope and will conduct their own research. You will help your partner when they are ready for timing and counting heartbeats.

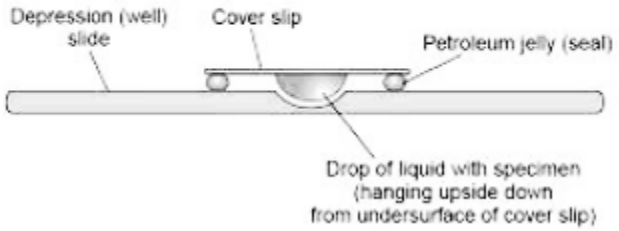
*****Never ever put your pipettes in the stock supply of anything!! Using a graduated cylinder pour 2 mL of the stock solution into a labelled medicine cup.****

3. Each person should have their own pipette and "**detox** centre" with distilled water in a medicine cup.

4. <u>Set up your glass depression slide.</u>
Living specimens do not survive long in the heat from an intense microscope illuminator bulb, usually because the specimen dries up. This problem is easily solved by preparing a vaseline chamber. Simply take a cover slip and hold it between thumb and forefinger by the edges,

using a Q tip, pick up some vaseline to and apply a small bead to the two opposite edges of the cover slip that you are not holding.

5. Using a clean medicine dropper, collect one Daphnia in fluid from the stock culture. Place the Daphnia into the centre of the well. Add your vaselined coverslip on top..



6. To collect control data, count Daphnia's heart beats for 10 seconds. Record this number under Trial 1 in your data table. Turn off the microscope light, and wait 20 seconds. Record the count for Trials 2 and 3, always turning off the light and waiting 20 seconds between each 10 second count.

7. Working in pairs, you will be assigned a team A or B.

Team A will investigate the effects of Nicotine, Adrenaline, and Alcohol on Daphnia heart rate

Team B will investigate the effects of Caffeine, Aspirin,and Lactic Acid on Daphnia heart rate.

Use a clean medicine dropper to place a drop of the desired solution on the depression slide so that it touches one edge of the coverslip. Place a tiny piece of paper towel along the opposite edge of the coverslip to draw the solution into the fluid around the Daphnia.

8. Wait one minute for the solution to take effect. Repeat step 6 for data on the Solution. Record your data in the data table.

9. Clean the slide and coverslip with soap and water. Rinse thoroughly. Prepare a slide with a fresh Daphnia by repeating step 4.

10. Repeat steps 6-8 for the remaining test solutions. Record all the data in the proper spaces on the data table.

11. Calculate the average 10 second heart rate for the control and each test solution. The formula is: (A + B + C) / 3 Record the answers in the proper spaces in column (D) of the data table.

12. Calculate the average 60 second heart rate for the control and each test solution. The formula is: D x 6 Record the answers in the proper spaces in column (E) of the data table.

13. Calculate the change in heart rate for each test solution.
The formula: is Esol- Econtrol. Record the answers in the proper spaces in column (F) of the data table.

Observations and Calculations:

1. Calculate the average 10 second heart rate for the control and each test solution. The formula is: (A + B + C) / 3 Record the answers in the proper spaces in column (D) of the data table.

2. Calculate the average 60 second heart rate for the control and each test solution. The formula is: D x 6 Record the answers in the proper spaces in column (E) of the data table.

3. Calculate the change in heart rate for each test solution.
The formula is : Esol- Econtrol. Record the answers in the proper spaces in column (F) of the data table. Note if the value is positive or negative in the table,

Data Table: 10-second Heart Rate Calculations **[7 x 1= 7]**

	10 Sec Heart Rate Trial 1 (A)	**10 Sec Heart Rate Trial 1 (B)**	**10 Sec Heart Rate Trial 1 (C)**	**Average 10 sec Heart rate (D)**	**Average 60 sec Heart rate (E)**	**Change in Heart Rate (F) Note whether the change is + or -**
Solution #1						
Control						
Solution #2						
Control						
Solution #3						
Control						

Analysis Part A [11]

1. Which solutions increased the heart rate of Daphnia? [1]

_____
_____

2. Which solutions decreased the heart rate of Daphnia? [1]

_____
_____

4. Did any of your solutions cause the heart rate of Daphnia to differ by more than 10 beats per minute? [1]

_____
_____

5. Based on your data, list which solutions contain drugs classified as stimulants? [1]

_____
_____

6. Based on your data, list which solutions contain drugs classified as depressants?[1]

_____
_____

7. Why did you need to use a fresh Daphnia for each solution? [1]

_____
_____
_____

8. Describe ONE advantage and ONE disadvantage of using Daphnia as a model organism [1 x2=2] See appendix for hints.You may need to do **additional** research, ensure that your answer is in your **own** words.

_____
_____
_____
_____

9. Find two other teams who tested the effect of the same solutions as your team. Teams will share data. Each original partner pair will select and report the results of 1 solution in the table. **Note:** each team must analyze a different solution. [3]

Team Members	**Change in heart rate for solution _____ (list name of solution)**
	Average change=

Analysis Part B: Dry Lab Analysis [7]

An experiment was performed to determine the effect of a drug, labelled X, on **human** metabolism. The rate of metabolism can be measured indirectly by monitoring changes in body temperature.

Procedure
1. Four comparable groups consisting of 50 individuals per group were used in the experiment. Each group had 25 healthy males and 25 healthy females between 40 and 50 years of age.
2. At the same time each day, all group members were given a dosage of the drug, except for group 4, which was given a placebo that did not contain the drug.
3. Each group member was monitored for changes in body temperature and in the volume of secretion from the thyroid gland.

Data

Group #	**Dosage of drug X (x 10^{-6} g / 50 kg of body mass)**	**Change in body temperature (C)**	**Perspiration**
1	1	+0.2	slight increase
2	10	+0.9	moderate increase
3	100	+1.2	large increase
4	placebo	0.0	no change

1. Identify the independent variable for this experiment. Include the units [1]

2. Identify the dependent variable for this experiment. Include the units [1]

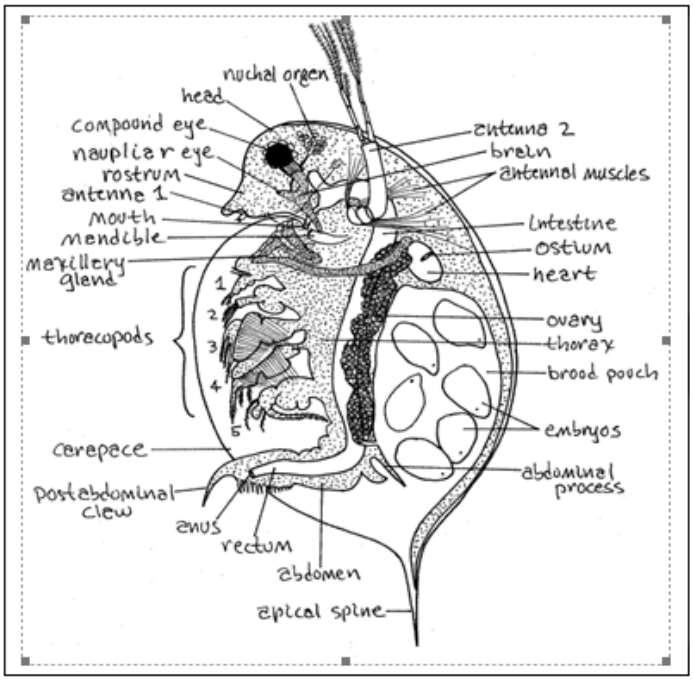
3. What is the control sample? [1]

4. What are two controlled variables for this experiment? [1 x 2=2]

5 a) Using this data, does the drug increase metabolic rate? [1]

b) Explain using **evidence** from the experimental results. [1]

DAPHNIA ANATOMY



Assessment

Safety Knowing what to do,using safety gear and equipment properly	0 1 2 3
Pre lab	0 1 2 3 4 5 6 7 8
Observation Table	0 1 2 3 4 5 6 7
Analysis	0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Communication Analysis is answered in complete sentences. Answers use appropriate terminology. Work is legible. Sentences are short and personal pronouns are avoided. Work is free of spelling and grammar errors. Work is submitted on time.	0 1 2 3
Total	/39

Appendix: Background information on the drugs in this lab and the heart

Adrenaline: Heart rate is increased by the sympathetic nervous system by the hormone adrenaline. When it is circulating in the blood, it activates cell surface receptors in the sinoatrial node - pacemaker)..This occurs during exercise or fear. The effect is to increase the rate of ejection of blood by the heart. This means that there will be more blood flow to skeletal muscle (in which exercise causes dilatation of blood vessels), so the skeletal muscle cells are supplied with more oxygen and respiratory substrates used to generate energy in respiration where it is needed. A drug that increases heart rate is called a positive chronotrope.

One of the ways adrenaline increases heart rate is through the action of what is known as a 'second messenger' in this case it is a chemical made in the cell known as cyclic adenosine monophosphate (cAMP). Transduction is the process that follows the action of a drug or hormone at a receptor. Thus, when adrenaline activates the beta-1 adrenoceptor in the sinoatrial node, this leads to an increase in cAMP in the sinoatrial node and the result is an increase in heart rate.

Caffeine: Caffeine mimics some of the effects of adrenaline in the heart. By a different mechanism not involving beta-1 adrenoceptors, caffeine also increases the amount of cAMP in the sinoatrial node. Then cAMP levels increase and this increases the electrical activity of the sinoatrial node, making it depolarize and 'beat' faster. Caffeine has additional effects on the heart. Like adrenaline, it can affect the main pumping chambers (ventricles), leading to an increase in the rate of contraction and relaxation of each heart beat. This means that, as well as beating faster, the heart's individual beats are associated with an increased volume of blood ejected into the circulation per unit time. This is called increasing cardiac output. Two or three cups of strong coffee or tea contain enough caffeine (and a similar acting compound called theobromine) to cause an increase in human heart rate of 5-20 beats/min.

Alcohol/Ethanol: Ethanol slows heart rate. Ethanol depresses the nervous system by acting as what is known as a non-selective neuro depressant. The amounts of ethanol necessary to achieve this effect in humans would also be sufficient to depress the respiratory centres of the brain, rather like the effect of an overdose of general anaesthetic, resulting in death.

Aspirin*:* Aspirin has no effect on heart rate. Despite this, aspirin has beneficial effects in the heart. By reducing the ability of platelets to adhere to damaged blood vessel walls, aspirin reduces the chance of coronary artery thrombosis, the event that precipitates a heart attack. People who take aspirin long-term for medical reasons (because they have cardiovascular disease or diabetes) may have a lower heart rate than controls, simply because they experience less coronary and peripheral thrombosis and thus have a better lubricated cardiovascular system.

Lactic Acid: Lactic acid is a byproduct produced by your body during the process of the metabolic process of glycolysis, or when your body turns glucose into energy. Lactic acid is then broken down into lactate, an action that releases hydrogen ions into the blood. Lactose has been shown to dramatically affect the rate and rhythm of the heartbeat in Daphnia. The effects include a slower heart rate and inconsistent beats.

Nicotine: Nicotine primarily acts on the cardiovascular system through stimulation of the sympathetic nervous system leading to release of norepinephrine and increases in heart rate, and blood pressure.

Notes about Daphnia anatomy

Daphnia's heart has no compartments or valves and is classified as an open circulatory system. The *Daphnia*'s heart differs from the human heart in many respects. In terms of heart rate, the *Daphnia* sinoatrial node (SA node) is actually a collection of spontaneously active nerves in a body called the cardiac ganglion. This means that it would be risky to extrapolate heart rate findings from *Daphnia* directly to humans without first validating the model.