DOES CAFFEINE AFFECT HEART RATE?

Purpose

- To investigate the effect of caffeine on the heart rate of *Daphnia* (water fleas).
- To develop practical skills.

Caffeine

Plants produce caffeine as an insecticide. Cocoa in South America, coffee in Africa and tea in Asia have all been used for hundreds of years to produce 'pick me up' drinks containing caffeine. These days, caffeine is also used as a flavour enhancer in a wide range of soft drinks. In addition, it has medicinal uses in painkiller preparations and is found in weight-loss drugs and as a stimulant in students' exam-time favourites like PRO PLUS™ and Red Bull™.

In humans, caffeine acts as a stimulant drug, causing increased amounts of stimulatory neurotransmitters to be released. At high levels of consumption caffeine has been linked to restlessness, insomnia and anxiety, causing raised stress and blood pressure. This can lead to heart and circulation problems.

The effect of caffeine on heart rate can be investigated using *Daphnia* (water fleas). The beating heart of a water flea can be seen through its translucent body, by placing the flea in a few drops of water in a cavity slide under the microscope. A mobile phone can be used to video the heart beat.

Investigating the effect of caffeine on heart rate

SAFETY

Wash your hands thoroughly after handling the Daphnia *or the pond water.*

1 Scientific questions and information research

- *State what you are going to investigate* – try to express this as a hypothesis to test. What do you think will be the effect of caffeine on the heart rate of water fleas? Write down your ideas and a prediction, and present relevant biological knowledge to support your suggestions.
- *Research relevant information* – to help you decide on what you are going to investigate and how you will carry out the practical work, you might need to research the background science and methods people have used to investigate similar problems. When you write up your plan remember to give full details of any information sources you use and comment on their reliability.

2 Planning and experimental design

- Design an experiment that you can use to test your hypothesis – the Developing Practical Skills Framework in the Practical Skills Support section of SNAB Online will help you plan your investigation. Note: *Daphnia* are poikilothermic (cold blooded). Turn off the microscope lamp when not viewing the fleas.

The following equipment will be available:

- Culture of *Daphnia* (water fleas)
- Cavity slides
- Dropping pipettes
- Distilled water
- Caffeine tablets
- Cotton wool
- Standard glassware (beakers, measuring cylinders, etc.)
- Stopclock
- Paper towels or filter paper
- Microscope.

Make sure your plan:

- includes the hypothesis that you are testing
- identifies the independent and dependent variables
- identifies any other variables which may affect the outcome of the experiment and, where possible, controls or allows for them
- includes a procedure which uses suitable apparatus that will give you measurements that will validly test your hypothesis, and explains why the apparatus is suitable and how the results will let you test the hypothesis
- has a control, if appropriate, and this control is fully explained
- includes replicates, and an explanation of why this is necessary
- says what measurements you will make, how they will be made and the level of precision that you can expect in your measurements
- identifies any potential sources of error (systematic or random) and how errors can be minimised
- comments on any ethical issues that arise from using invertebrates in the experiment and explains how these will be taken into account in the practical method used
- includes a risk assessment that identifies any risks and explains any safety precautions that need to be taken so as to reduce those risks.

3 Carrying out practical work safely and ethically

Either use the plan you have created after it has been checked by your teacher/lecturer or use a method supplied by your teacher/lecturer. If unexpected ethical or safety issues arise, deal with them sensibly, taking advice where needed and make a note of them. Record all measurements, including repeated ones, as soon as they are taken; with appropriate precision (i.e. a suitable number of significant figures) and units. Note any possible errors.

4 Analysis and interpretation of data

Present your data in an appropriate table and graph. For information on the features of a good table and graph see the Maths and Stats Support in the SNAB Online resources. If you have lots of repeated results, remember that you should work out mean values and present these in your report. This also lets you comment on the significance of your results. If the results that are used to calculate the means are very variable, any differences between the treatment means may not be significant. The range of values can be shown on the graph using bars on each point as a measure of the variation of the data. See Maths and Stats Support Sheet 10 – standard deviation – for details of how to work out standard error. NB: you need to make it clear what any bars on a graph are showing.

5 Conclusion and evaluation

In the discussion of your results you should use evidence from your data to identify any trends and patterns. You should quote some data that show the trend. You should then use biological knowledge to explain any patterns or trends identified. You should state a clear conclusion, summarising what you found out and comment on the validity of your conclusion. You should evaluate your experimental apparatus and methods, commenting on the accuracy and precision of your results.

Remember that the hypothesis you suggested may not be correct. In this case, the results will not show the patterns or trends that you expected. There may be a different trend or no trend at all. This is perfectly OK. You may be able to suggest an alternative explanation for your results. You may still think your hypothesis is sound, but that there are concerns about the experimental method used and that the results obtained are not very valid, i.e. they may not be testing the hypothesis appropriately. In this case, you cannot draw valid conclusions from the results and this should be explained in any write up. An experiment that does not produce the expected results is often as valuable to other researchers as a report that supports the original hypothesis. It allows other researchers to make informed decisions about the methods they will use in the future and it may allow them to suggest alternative ideas.

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Notes on the procedure

The Student Sheet that accompanies this activity guides students through planning and writing up this investigation. The Developing Practical Skills Framework can be used in conjunction with this sheet: this can be found in the Practical Skills Support section of SNAB Online. After students plan the investigation their plans can then be discussed with the group to highlight any omissions.

To support less able students, or to help with the organisation of practical work for a large group, students could be given the practical procedure at the end of these notes (see page 4). It provides a basic outline and will need to be read by students before starting the practical with decisions and modifications made as appropriate. The procedure is for a simple experiment; more able students could complete a more complex experiment using serial dilutions, with several *Daphnia* used at each concentration. Note that high concentrations of caffeine can be fatal for *Daphnia*.

The *Daphnia* hearts are fairly easily seen, but counting the number of beats can be difficult. Counting is easier if each heartbeat is recorded by tapping a pencil on a piece of paper and counting up the pencil marks after the specified time. In addition, cooling the *Daphnia* before the experiment may help slow their heart rate: heart rate is highly temperature dependent. A webcam above the eyepiece of the microscope to project an image of the slide onto a large screen may also help with counting. There are videos available on the Internet that can be used to help students distinguish between the *Daphnia* heart and gills.

A mobile phone can be used to film the heart beat and then replay the video to count the heart beats in a set period of time. For additional information, see CLEAPSS leaflet TL019.

A dissecting microscope with a light source under the stage works well for this experiment. To prevent the *Daphnia* from overheating while on the microscope turn off the microscope light between observations and use a heat sink.

Using pond water/*Daphnia* culture solution is recommended for both the control group and to dissolve the caffeine as this may give more valid results and be less stressful to the *Daphnia*. In distilled water the heart rate may rise due to lack of oxygen.

In the trial of the experiment caffeine was used at 0.1% and 0.5% w/v with no ill effect. At 1% the *Daphnia* stopped swimming after 5 minutes.

It is suggested that a 'blind' study is done. This means that the person counting the heart rate is unaware as to whether the *Daphnia* is in water with or without caffeine. It has been shown that observer expectations influence the result.

It is difficult to get clear-cut results from this experiment and significant differences between treatments may not be found. The sets of results below indicate the sorts of results that can be expected. It should be impressed upon students that it does not matter if they do not get differences between treatments. Indeed, the experiment provides a good opportunity to focus on the critical evaluation of the technique used. Published results (see the weblinks) report a slowing of heart rate by a maximum of 20% at 15–20 mM caffeine. The control mechanism is unknown.

Experiment 1

Daphnia were cooled on ice before the experiment. Beakers containing *Daphnia* in pond water were put on ice for about half an hour. This had the effect of slowing the heart rate and thus facilitating counting. The temperature of the pond water in which the *Daphnia* were swimming fell to about 5 °C.

A single *Daphnia* was placed in a beaker containing test solution for 5 minutes: either pond water, or pond water + 0.5% caffeine. After the 5 minutes, the *Daphnia* (in a few drops of test solution) was transferred to the slide for measurement of heart rate. Each individual was counted for 4 × 30 seconds. A blind counting method was used.

Overall means:

Caffeine – 173 beats per minute

Control – 172 beats per minute

There were no immediately observable ill effects of caffeine at this concentration (0.5% w/v).

Experiment 2

The experiment was conducted at room temperature. A single *Daphnia* was transferred to the slide. A paper towel was used to remove the pond water from the slide. A few drops of test solution – either pond water or pond water + 0.5% caffeine – were dripped onto the *Daphnia*. The clock was started immediately and the heart rate recorded for 15 seconds at 2, 4, 6, 8 and 10 minutes. A webcam was used to facilitate counting.

The results from the two experiments do not show that caffeine increases heart rate in *Daphnia*.

Treatment	Heart rate/beats 30 s^{-1}					Mean for individual beats min^{-1}
	Trial 1	Trial 2	Trial 3	Trial 4	Mean	
Caffeine	87	85	81	88	85	170
Caffeine	83	84	78	82	82	164
Caffeine	84	86	86	84	85	170
Caffeine	89	91	93	99	93	186
Caffeine	86	87	90	90	88	176
Control	62	70	73	74	70	140
Control	81	101	-	-	91	182
Control	88	75	74	85	81	162
Control	93	98	98	100	97	194
Control	89	91	85	94	90	180

Table 1 Results for Experiment 1.

		Number of heartbeats in 15 seconds				
Length of time in solution/minutes		2	4	6	8	10
Treatment	Caffeine	49	53	49	44	48
	Caffeine	54	54	57	51	55
	Caffeine	54	59	55	61	64
	Caffeine	59	65	64	68	62
	Mean	**54**	**58**	**56**	**56**	**57**
	Control	57	55	59	50	58
	Control	58	60	54	58	60
	Control	56	56	58	57	59
	Control	60	64	61	66	62
	Mean	**58**	**59**	**58**	**58**	**60**

Table 2 Results for Experiment 2.

Purpose

To investigate the effect of caffeine on the heart rate of *Daphnia* (water fleas).

Caffeine

Caffeine is produced by plants as an insecticide. Cocoa in South America, coffee in Africa and tea in Asia have all been used for hundreds of years to produce 'pick me ups' containing caffeine. These days caffeine is also used as a flavour enhancer in a wide range of cola and other soft drinks. In addition, it has medicinal uses in aspirin preparations and is found in weight-loss drugs and as a stimulant in students' exam-time favourites like PRO-PLUS® and Red Bull®.

In humans, caffeine acts as a stimulant drug, causing increased amounts of stimulatory neurotransmitters to be released. At high levels of consumption caffeine has been linked to restlessness, insomnia and anxiety, causing raised stress and blood pressure. This can lead to heart and circulation problems.

You need

- Culture of *Daphnia* (water fleas)
- Three cavity slides
- Three dropping pipettes
- Distilled water
- Caffeine solution
- Cotton wool
- Pipettes
- Test tubes
- Stopclock
- Paper towels or filter paper
- Microscope

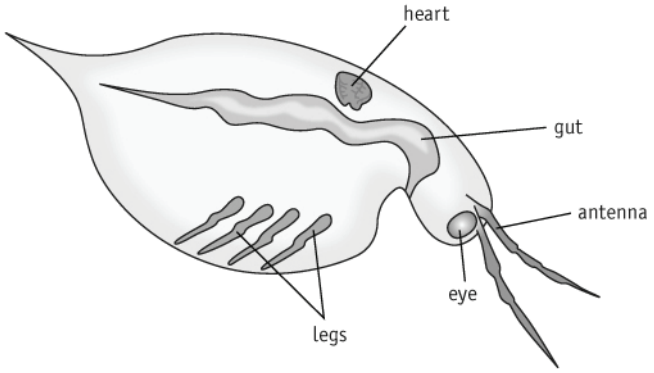

Figure 1 *Daphnia.*

Procedure

1 Place a few strands of cotton wool on a cavity slide; this will help restrict the movement of the water flea. Using a pipette, transfer one large water flea to a cavity slide. Remove the water from around the water flea using filter paper, then add one or two drops of distilled water or pond water. Use as much water as you can and do not use a cover slip. Together these precautions will help maintain sufficient oxygen supply to the flea. View the water flea under low power. Focus on its heart, which can be seen through its translucent body. The location of the heart is shown in Figure 1.

2 Use a stopwatch to record the number of heartbeats per minute. This is made easier by working in a pair, with one person counting beats while the other person tells them the time period. Tap a pencil on a piece of paper and count up the pencil marks at the end of the time period. Record the heart rate at intervals of 2 minutes over a 10 minute period. It is a good idea to do a 'blind' study to avoid bias in the results. The person counting the heartbeats should be unaware as to whether the *Daphnia* is in water or water with added caffeine.

3 Repeat the procedure using other water fleas from the culture solution and fresh, clean slides. Replace the water with caffeine solution. Repeat the procedure using several different concentrations of caffeine.

4 Record your results in a suitable format and present them in an appropriate graph.

5 Compare the treatments and try to explain the effect of each treatment on the heart rate.

6 Comment on the validity of your study. For example, would it have been better or worse to use the same *Daphnia* throughout the study?

7 If time permits, you could also look at the effect of other chemicals, for example, ethanol, on the heart rate.

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This is an activity that students may plan themselves. There is also a support sheet giving a suggested method. The list below gives the apparatus and approximate quantities needed for the suggested method. However, students may ask for things outside of this list.

SAFETY

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Requirements per student or group of students	Notes
Culture of *Daphnia*	*Daphnia* may be killed by chlorine in tap water. Any tap water used should be left to stand for 24 hours first to let the chlorine dissipate. Allow at least nine *Daphnia* per student or pair. They should be unharmed by the activity, but may need too long to recover after the caffeine to use the same one for different concentrations in the same practical. They can be bought from an aquarist or Blades Biological Supplies. To catch them use a plastic pipette with the tip cut off to make it wider.
Container for 'used' *Daphnia*	This will stop them getting mixed up with the 'fresh' ones. Dispose of *Daphnia* ethically, preferably by returning them to a freshwater pond or stream.
Six cavity slides	Students can wash and reuse the slides if needed.
Approximately 5 cm^3 caffeine solution (0.5%)	Dissolve 0.5 g caffeine in 100 cm^3 water. Instant coffee can be used. Make up the solution with culture water or distilled water. Students will need to dilute this to make weaker solutions. If time is limited it may be easier to provide made-up solutions of 0.25%. (Consult with the teacher/lecturer – they may prefer to use more concentrations, for example, 0.1%, 0.2%, 0.3%, 0.4% if time permits.)
Cotton wool	Students will only need a small amount – about as much as is in a single cotton wool ball will supply the whole class. They put a few strands on the slide to stop the *Daphnia* moving.
Three dropping pipettes	
5–10 cm^3 distilled water or water from the *Daphnia* culture	For adding to the *Daphnia* during the experiment.
Test tubes/small beakers	For students to collect or mix their caffeine solutions in. They will need more if they are doing more concentrations.
Small measuring cylinder or 5 cm^3 syringe	For making dilutions of caffeine solution.
Approximately 100 cm^3 distilled or pond water	For making dilutions of caffeine solution. Amount required will depend on the dilutions made.
Stopclock	
Paper towels or filter paper	
Microscope	For use at low power. Some centres find that it is useful, if available, to use a microscope-mounted video camera to show students the heart.
Mobile phone with video function	