Caffeine and Daphnia Heart Rate (Core Practical 1): Edexcel SNAB A Level Biology Exam Questions

20 questions · 58 marks · about 70 minutes. Answer in the spaces, then check the mark schemes at the end.

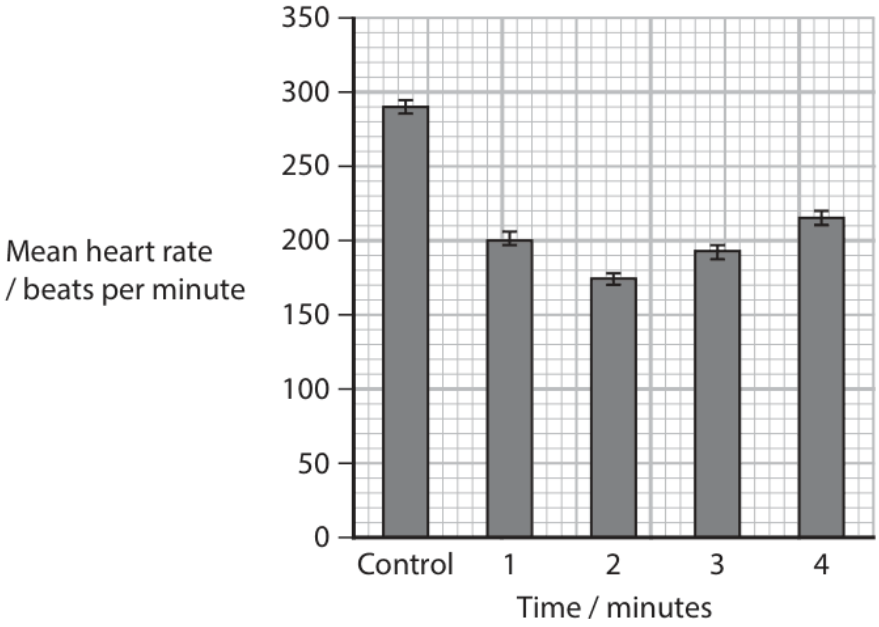
Question 1 · AS Paper 1 · June 2025 · Q7(b)(i) · 2 marks

(b) The effect of alcohol on the heart rate of *Daphnia* was investigated.

The heart rates of several *Daphnia* in a control solution were measured.

The control solution was replaced with a solution of 2.5 % alcohol. The heart rates of these *Daphnia* were measured every minute, for four minutes.

The graph shows the results of this investigation.



(i) Explain why *Daphnia* is a suitable organism to use in this investigation.

(2)

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Question 2 · information from earlier in the question

1 The photograph shows a young zebrafish that can be used to investigate the circulatory system. Zebrafish are vertebrates.



(a) The effect of caffeine on the heart rate of zebrafish was investigated.

A zebrafish was placed in water and observed using a microscope. The number of heartbeats in one minute was counted and the heart rate was recorded. This was repeated to give three measurements of heart rate.

The water was replaced with a caffeine solution and the heart rate was recorded. This was repeated to give three measurements of heart rate.

The results of the investigation are shown in the table.

Treatment	Heart rate / beats min^{-1}		
	Repeat 1	Repeat 2	Repeat 3
Water	124	120	112
Caffeine solution	184	172	156

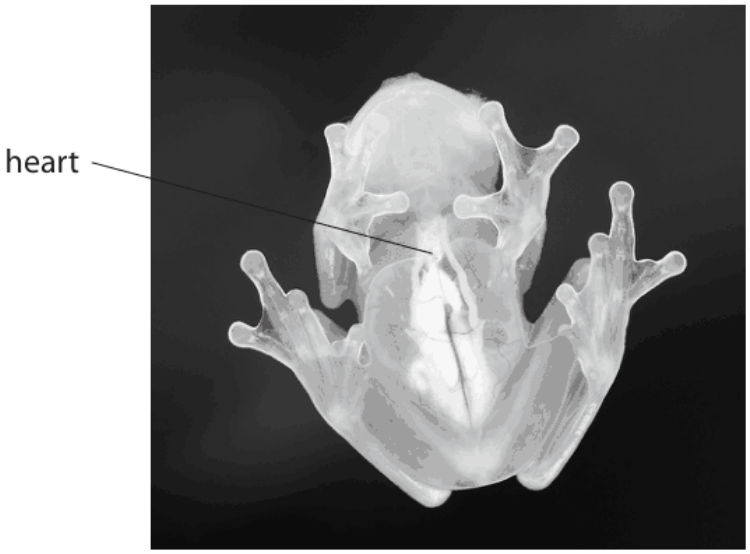
Question 2 · AS Paper 1 · June 2017 · Q1(a)(iii) · 2 marks

(iii) Explain the advantages of using young zebrafish for this investigation.

(2)

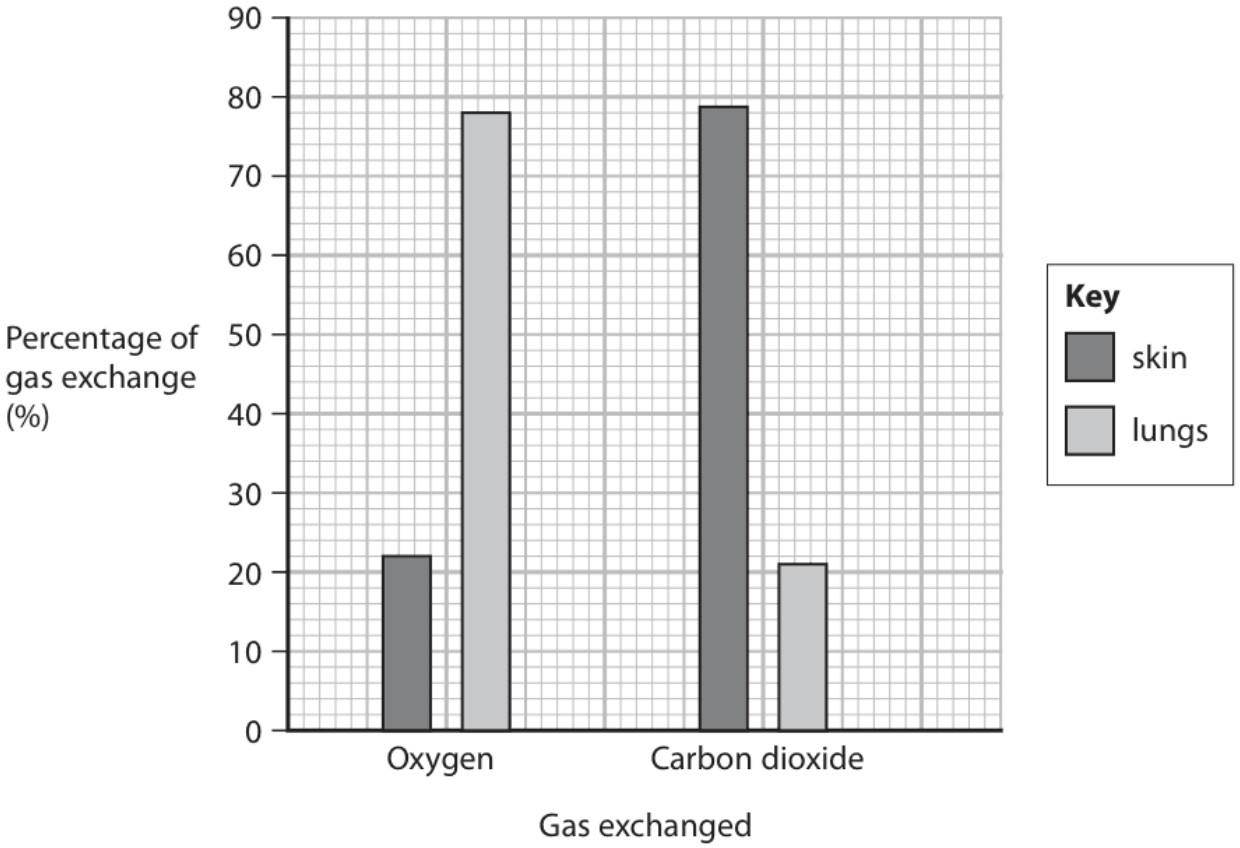
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6 The photograph shows a type of frog (*Nymphargus bejaranoi*) from South America. The bones and organs of this frog are visible through the skin on the underside of the body. The adult frogs of this species are approximately 25 mm in length.



(Source: © Rick & Nora Bowers / Alamy Stock Photo)

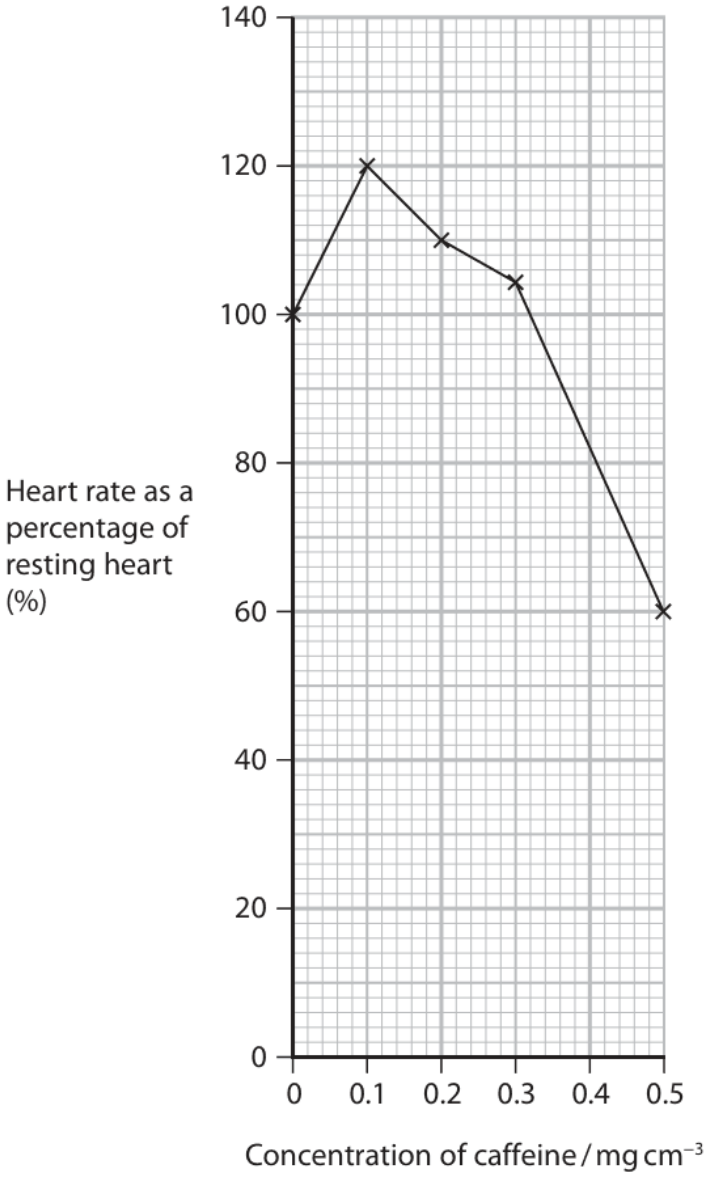
This frog can exchange gases across the surfaces of both the skin and the lungs. A scientist investigated the percentage of the gases exchanged at each surface. The graph shows the results of this investigation.



Questions, mark schemes and examiners' report extracts © Pearson Education Ltd.

Question 3 · AS Paper 1 · June 2022 · Q6(c)(i) · 2 marks

(c) The effect of caffeine concentration on the heart rate of one of these frogs was investigated.



(i) Explain the advantages of using this type of frog for this investigation.

(2)

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Question 4 · AS Paper 1 · June 2024 · Q8(c)(ii) · 2 marks

(ii) A student wished to investigate the effect of caffeine on the heart rate of some vertebrate and invertebrate organisms.

Describe two potential ethical issues which should be considered before the selection of organisms to include in this investigation.

(2)

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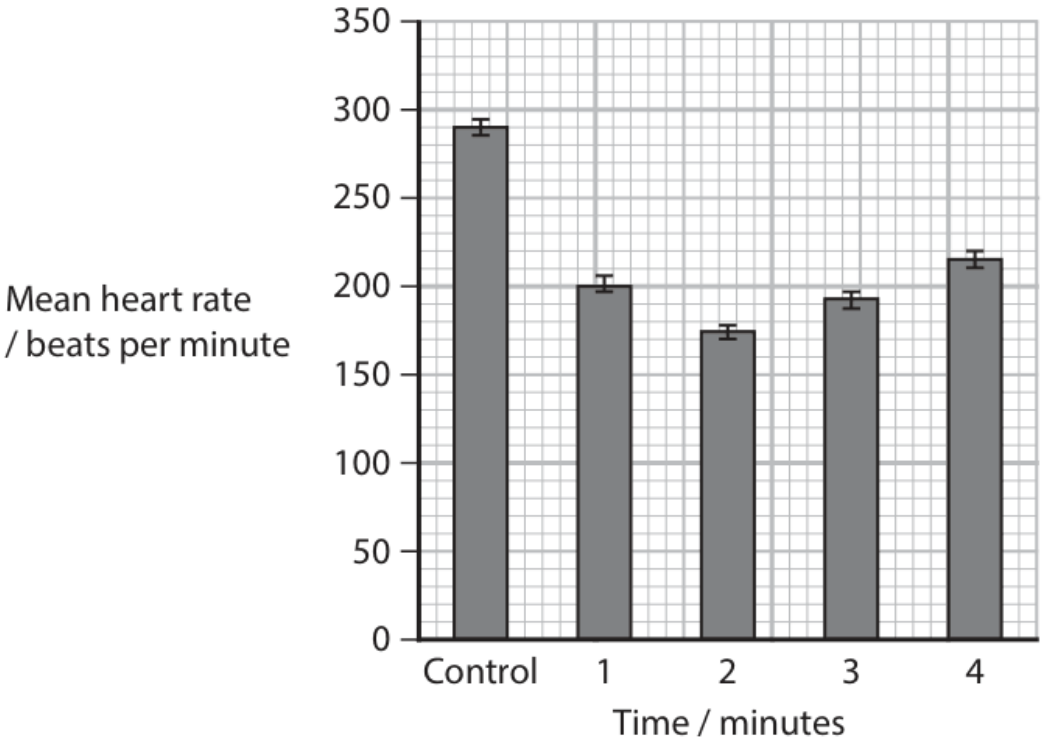
Question 5 · information from earlier in the question

(b) The effect of alcohol on the heart rate of *Daphnia* was investigated.

The heart rates of several *Daphnia* in a control solution were measured.

The control solution was replaced with a solution of 2.5 % alcohol. The heart rates of these *Daphnia* were measured every minute, for four minutes.

The graph shows the results of this investigation.



Question 5 · AS Paper 1 · June 2025 · Q7(b)(ii) · 1 mark

(ii) Give a suitable control solution.

(1)

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Question 6 · AS Paper 1 · November 2021 · Q8(a)(i) · 2 marks

8 The photograph shows a ghost shrimp that lives in fresh water.



© Nicholas Toh/Alamy Stock Photo

The effect of caffeine concentration on the heart rate of ghost shrimps was investigated.

A shrimp was placed in a caffeine solution and observed using a microscope.

The number of heartbeats in one minute was counted and the heart rate was recorded.

This was repeated for other concentrations of caffeine solution.

The investigation was repeated using two more ghost shrimps.

The table shows the results of this investigation.

Concentration of caffeine / mg cm^{-3}	Heart rate / beats min^{-1}		
	Shrimp 1	Shrimp 2	Shrimp 3
3	272	298	304
7	268	284	298
10	260	270	266
13	152	242	292

(a) (i) State and justify a suitable control for this investigation.

(2)

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Question 7 · AS Paper 1 · June 2017 · Q1(a)(i) · 1 mark

1 The photograph shows a young zebrafish that can be used to investigate the circulatory system. Zebrafish are vertebrates.



(a) The effect of caffeine on the heart rate of zebrafish was investigated.

A zebrafish was placed in water and observed using a microscope. The number of heartbeats in one minute was counted and the heart rate was recorded. This was repeated to give three measurements of heart rate.

The water was replaced with a caffeine solution and the heart rate was recorded. This was repeated to give three measurements of heart rate.

The results of the investigation are shown in the table.

Treatment	Heart rate / beats min^{-1}		
	Repeat 1	Repeat 2	Repeat 3
Water	124	120	112
Caffeine solution	184	172	156

(i) State **one** variable that should have been controlled in this investigation.

(1)

Questions, mark schemes and examiners' report extracts © Pearson Education Ltd.

8 The photograph shows a ghost shrimp that lives in fresh water.



© Nicholas Toh/Alamy Stock Photo

The effect of caffeine concentration on the heart rate of ghost shrimps was investigated.

A shrimp was placed in a caffeine solution and observed using a microscope.

The number of heartbeats in one minute was counted and the heart rate was recorded.

This was repeated for other concentrations of caffeine solution.

The investigation was repeated using two more ghost shrimps.

The table shows the results of this investigation.

Concentration of caffeine / mg cm^{-3}	Heart rate / beats min^{-1}		
	Shrimp 1	Shrimp 2	Shrimp 3
3	272	298	304
7	268	284	298
10	260	270	266
13	152	242	292

Questions, mark schemes and examiners' report extracts © Pearson Education Ltd.

Question 8 · AS Paper 1 · November 2021 · Q8(a)(ii) · 4 marks

(ii) Variables relating to the caffeine solution and the ghost shrimps should have been controlled.

State and justify **two** variables that should have been controlled in this investigation.

(4)

Variable relating to the caffeine solution .....

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Variable relating to the ghost shrimps .....

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Question 9 · information from earlier in the question

(b) In this investigation, *Daphnia* were placed into three groups of 10. Each group was placed in alcohol of a different concentration for five minutes.

After five minutes, each *Daphnia* was removed from the alcohol and its heart rate recorded every 15 seconds for one minute. A mean for each concentration was calculated.

The results are shown in the table.

Alcohol concentration / mol dm^{-3}	Mean *Daphnia* heart rate / beats per minute
0.00	221
0.17	176
0.34	97

Question 9 · A level Paper 2 · October 2020 · Q5(b)(ii) · 2 marks

(ii) Explain why the *Daphnia* were left for five minutes in the alcohol before the heart rate was recorded.

(2)

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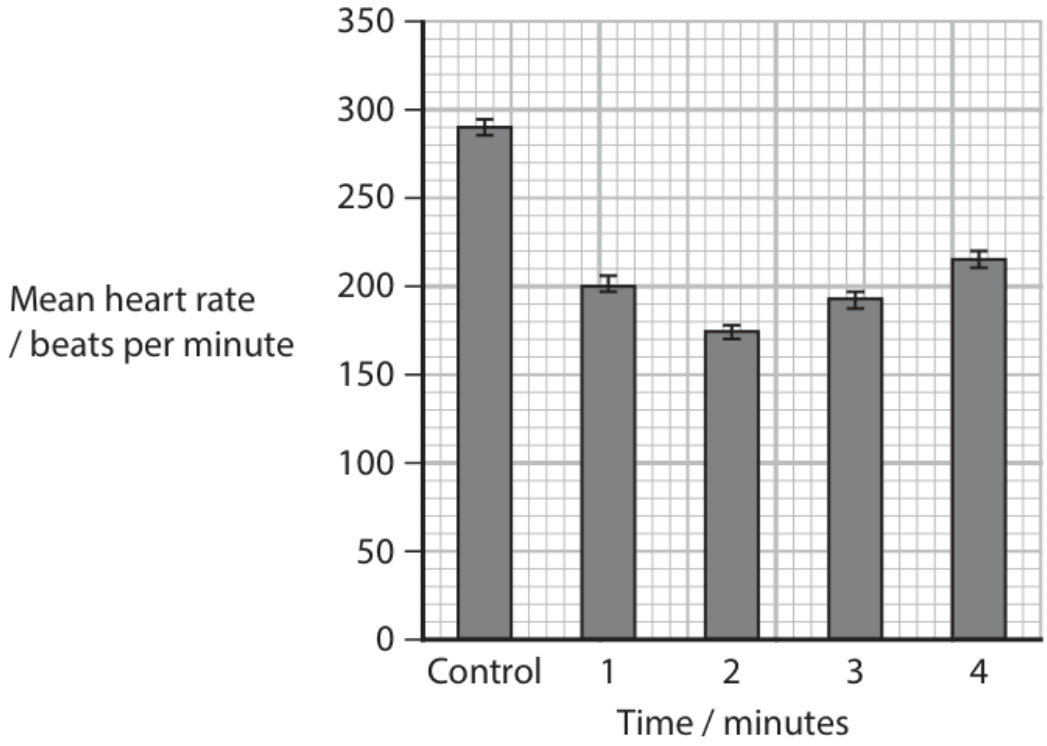
Question 10 · information from earlier in the question

(b) The effect of alcohol on the heart rate of *Daphnia* was investigated.

The heart rates of several *Daphnia* in a control solution were measured.

The control solution was replaced with a solution of 2.5 % alcohol. The heart rates of these *Daphnia* were measured every minute, for four minutes.

The graph shows the results of this investigation.



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Question 10 · AS Paper 1 · June 2025 · Q7(b)(iii) · 3 marks

(iii) Describe how **valid** data could have been collected for this investigation.

(3)

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Question 11 · information from earlier in the question

(b) Some chemicals can affect the heart rate of animals.

The effects of dopamine and caffeine on the heart rate of *Daphnia* were investigated.

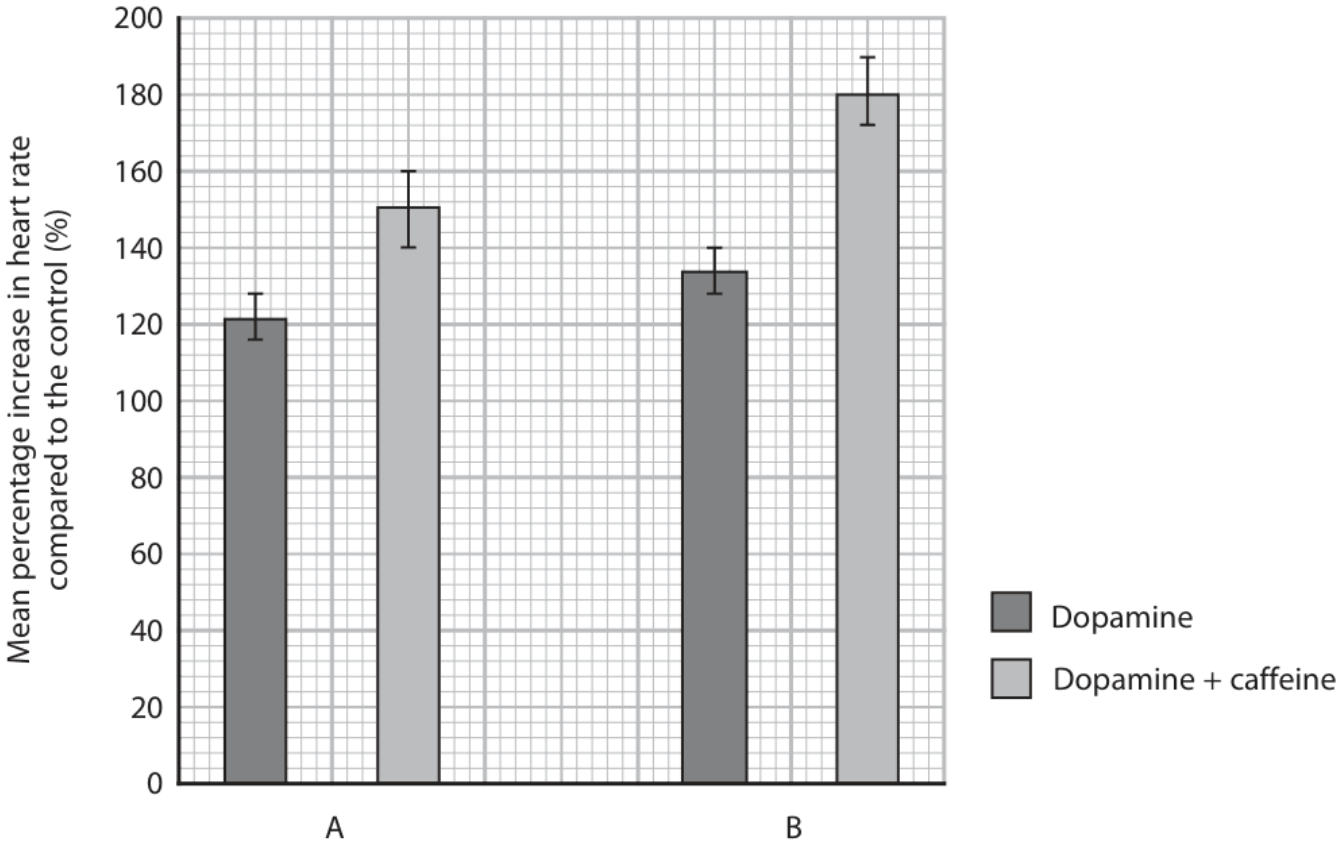
Investigation A used three different solutions:

- a control solution
- a solution containing 0.8 mg cm^{-3} dopamine
- a solution containing 80 µg cm^{-3} caffeine and 0.8 mg cm^{-3} dopamine

Investigation B used three different solutions:

- a control solution
- a solution containing 1.6 mg cm^{-3} dopamine
- a solution containing 80 µg cm^{-3} caffeine and 1.6 mg cm^{-3} dopamine

The graph shows the percentage increase in heart rate compared with the controls, for both investigations.



(Source from: https://f1000research.com/articles/7-254/v1)

Questions, mark schemes and examiners' report extracts © Pearson Education Ltd.

Question 11 · AS Paper 1 · October 2020 · Q7(b)(iii) · 5 marks

(iii) Devise a procedure that could have been used to produce the results shown in the graph.

(5)

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Question 12 · A level Paper 2 · November 2021 · Q5(b)(ii) · 4 marks

(ii) *Daphnia* can be used to investigate the effects of caffeine on heart rate.

Devise a procedure to compare the effects of decaffeinated coffee and regular coffee on the heart rate of *Daphnia*.

(4)

Questions, mark schemes and examiners' report extracts © Pearson Education Ltd.

(b) In this investigation, *Daphnia* were placed into three groups of 10. Each group was placed in alcohol of a different concentration for five minutes.

After five minutes, each *Daphnia* was removed from the alcohol and its heart rate recorded every 15 seconds for one minute. A mean for each concentration was calculated.

The results are shown in the table.

Alcohol concentration / mol dm^{-3}	Mean *Daphnia* heart rate / beats per minute
0.00	221
0.17	176
0.34	97

Questions, mark schemes and examiners' report extracts © Pearson Education Ltd.

Question 13 · A level Paper 2 · October 2020 · Q5(c) · 5 marks

(c) Devise an investigation to find the lowest concentration of alcohol that has an effect on the heart rate of *Daphnia*.

(5)

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Question 14 · information from earlier in the question

(b) Angina can lead to a raised heart rate.

A substance found in a plant, Roselle (*Hibiscus sabdariffa*), has been shown to reduce heart rate in humans.

In humans, this substance was effective at a dose of 15 mg kg^{-1} of human body mass.



(Source : © K JAYARAM/SCIENCE PHOTO LIBRARY)

Roselle (*Hibiscus sabdariffa*)

Questions, mark schemes and examiners' report extracts © Pearson Education Ltd.

Question 14 · A level Paper 2 · June 2025 · Q10(b)(ii) · 5 marks

(ii) Devise an investigation to find the mean minimum concentration of this substance that will lower the heart rate in *Daphnia*.

(5)

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(b) In this investigation, *Daphnia* were placed into three groups of 10. Each group was placed in alcohol of a different concentration for five minutes.

After five minutes, each *Daphnia* was removed from the alcohol and its heart rate recorded every 15 seconds for one minute. A mean for each concentration was calculated.

The results are shown in the table.

Alcohol concentration / mol dm^{-3}	Mean *Daphnia* heart rate / beats per minute
0.00	221
0.17	176
0.34	97

(i) Give one reason why 10 *Daphnia* were used for each alcohol concentration.

(1)

Questions, mark schemes and examiners' report extracts © Pearson Education Ltd.

Question 16 · information from earlier in the question

1 The photograph shows a young zebrafish that can be used to investigate the circulatory system. Zebrafish are vertebrates.



(a) The effect of caffeine on the heart rate of zebrafish was investigated.

A zebrafish was placed in water and observed using a microscope. The number of heartbeats in one minute was counted and the heart rate was recorded. This was repeated to give three measurements of heart rate.

The water was replaced with a caffeine solution and the heart rate was recorded. This was repeated to give three measurements of heart rate.

The results of the investigation are shown in the table.

Treatment	Heart rate / beats min^{-1}		
	Repeat 1	Repeat 2	Repeat 3
Water	124	120	112
Caffeine solution	184	172	156

Question 16 · AS Paper 1 · June 2017 · Q1(a)(ii) · 2 marks

(ii) Analyse the data to justify a conclusion for this investigation.

(2)

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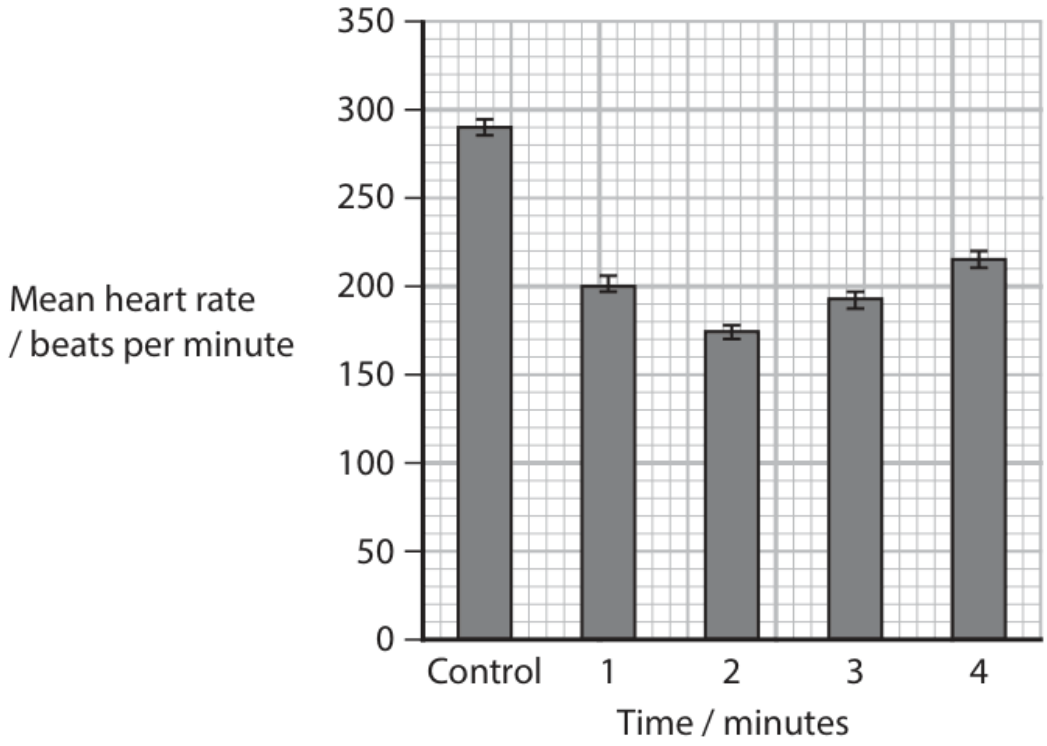
Question 17 · information from earlier in the question

(b) The effect of alcohol on the heart rate of *Daphnia* was investigated.

The heart rates of several *Daphnia* in a control solution were measured.

The control solution was replaced with a solution of 2.5 % alcohol. The heart rates of these *Daphnia* were measured every minute, for four minutes.

The graph shows the results of this investigation.



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Question 17 · AS Paper 1 · June 2025 · Q7(b)(iv) · 3 marks

(iv) Comment on the effect of alcohol on the heart rate of *Daphnia*.

(3)

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8 The photograph shows a ghost shrimp that lives in fresh water.



© Nicholas Toh/Alamy Stock Photo

The effect of caffeine concentration on the heart rate of ghost shrimps was investigated.

A shrimp was placed in a caffeine solution and observed using a microscope.

The number of heartbeats in one minute was counted and the heart rate was recorded.

This was repeated for other concentrations of caffeine solution.

The investigation was repeated using two more ghost shrimps.

The table shows the results of this investigation.

Concentration of caffeine / mg cm^{-3}	Heart rate / beats min^{-1}		
	Shrimp 1	Shrimp 2	Shrimp 3
3	272	298	304
7	268	284	298
10	260	270	266
13	152	242	292

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Question 18 · AS Paper 1 · November 2021 · Q8(b)(ii) · 4 marks

(ii) Comment on the results of this investigation.

(4)

Questions, mark schemes and examiners' report extracts © Pearson Education Ltd.

(b) Some chemicals can affect the heart rate of animals.

The effects of dopamine and caffeine on the heart rate of *Daphnia* were investigated.

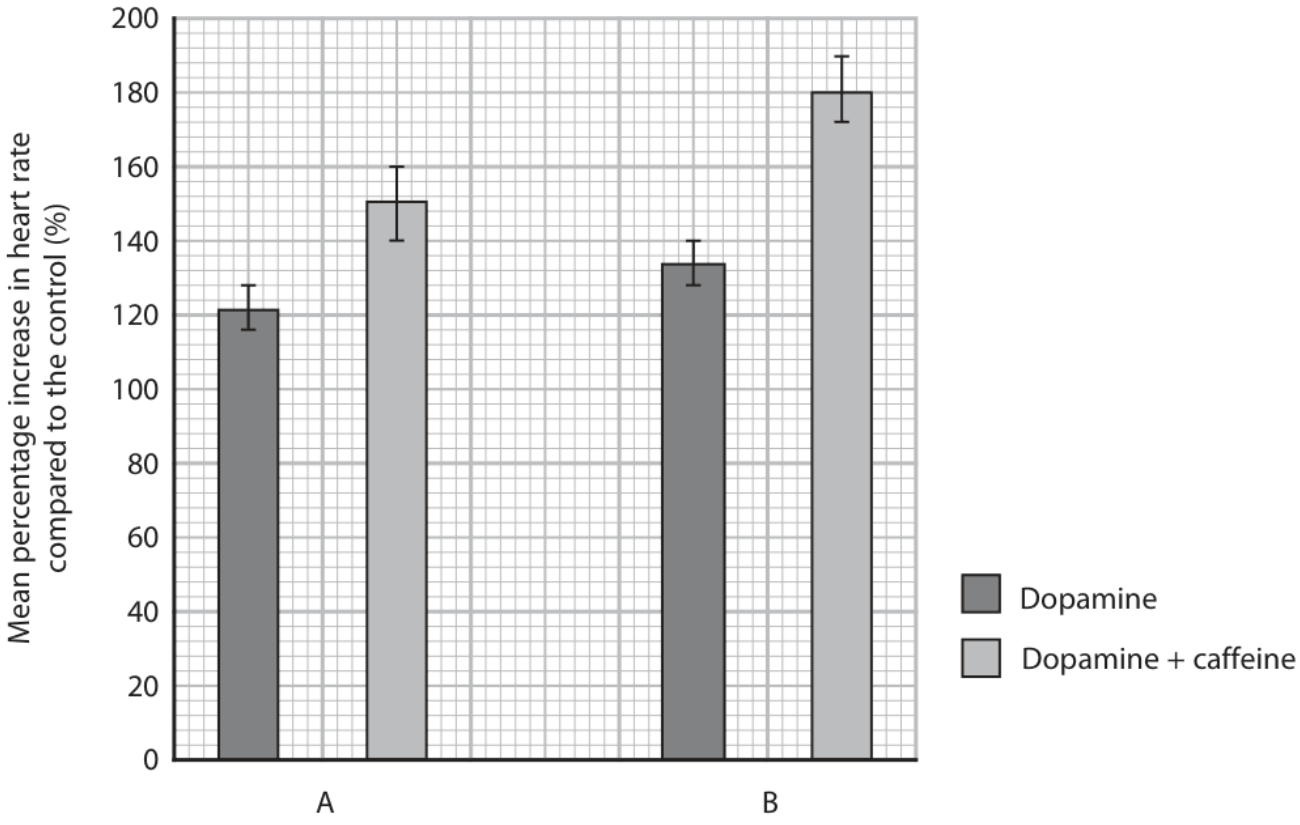
Investigation A used three different solutions:

- a control solution
- a solution containing 0.8 mg cm^{-3} dopamine
- a solution containing 80 µg cm^{-3} caffeine and 0.8 mg cm^{-3} dopamine

Investigation B used three different solutions:

- a control solution
- a solution containing 1.6 mg cm^{-3} dopamine
- a solution containing 80 µg cm^{-3} caffeine and 1.6 mg cm^{-3} dopamine

The graph shows the percentage increase in heart rate compared with the controls, for both investigations.



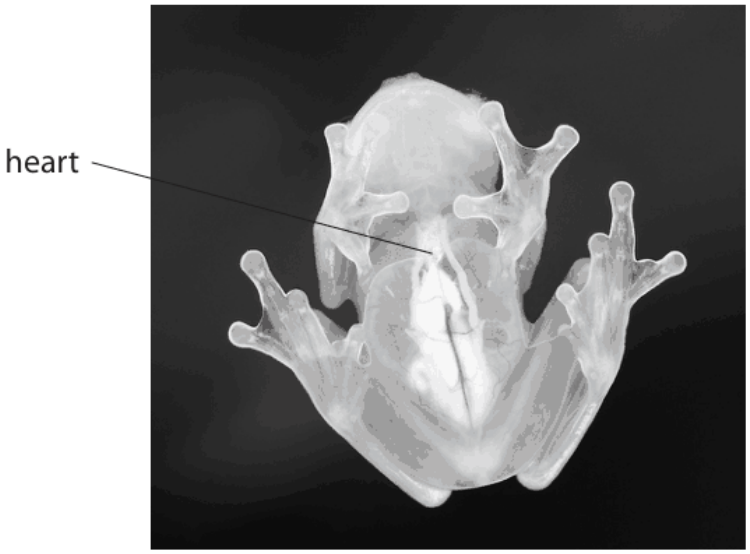
(Source from: https://f1000research.com/articles/7-254/v1)

(i) Comment on the effects of dopamine alone and dopamine with caffeine on the heart rate of *Daphnia* in this investigation.

(4)

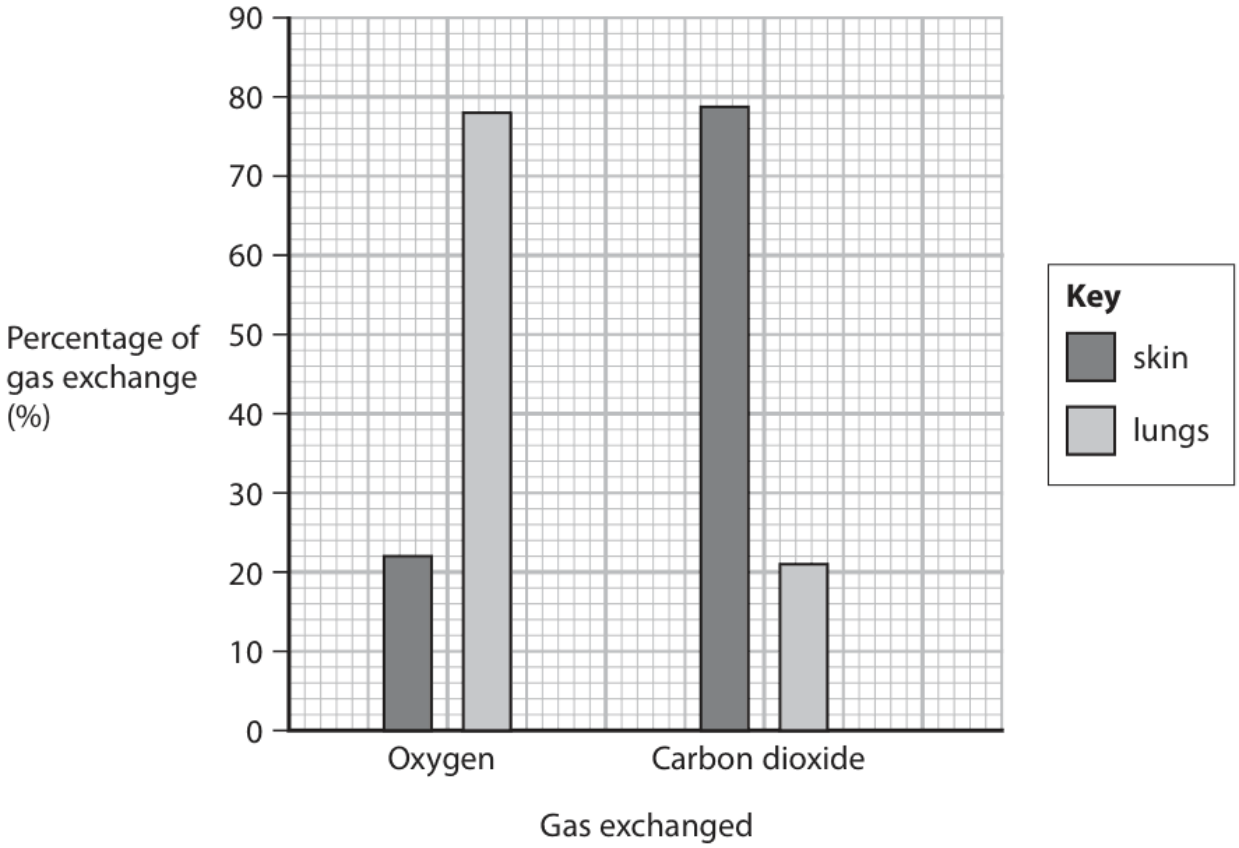
Questions, mark schemes and examiners' report extracts © Pearson Education Ltd.

6 The photograph shows a type of frog (*Nymphargus bejaranoi*) from South America. The bones and organs of this frog are visible through the skin on the underside of the body. The adult frogs of this species are approximately 25 mm in length.



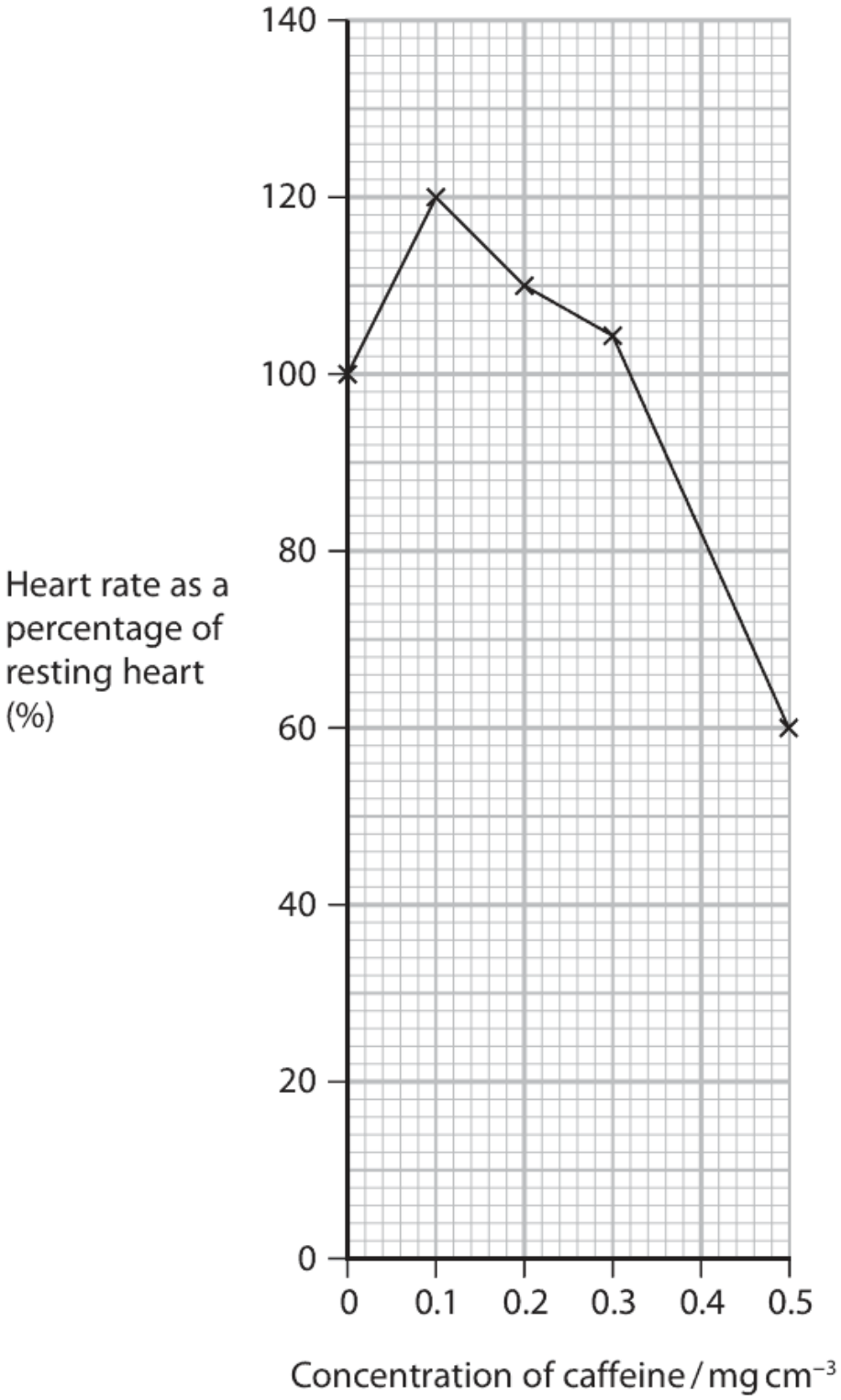
(Source: © Rick & Nora Bowers / Alamy Stock Photo)

This frog can exchange gases across the surfaces of both the skin and the lungs. A scientist investigated the percentage of the gases exchanged at each surface. The graph shows the results of this investigation.



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(c) The effect of caffeine concentration on the heart rate of one of these frogs was investigated.



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Question 20 · AS Paper 1 · June 2022 · Q6(c)(ii) · 4 marks

(ii) A student concluded that a caffeine concentration of 0.1 mg cm^{-3} would cause the largest increase in heart rate in every species of frog.

Discuss the validity of this conclusion.

(4)

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Mark schemes

Question 1 · AS Paper 1 · June 2025 · Q7(b)(i) · 2 marks

Question Number	Answer
7(b)(i)	An explanation that makes reference to the following: • relevant feature of Daphnia described (1) • appropriate explanation for feature described (1)

Additional Guidance	Mark
E.g. {transparent / translucent} (body / surface) / aquatic animal / simple nervous system / invertebrate / large surface area: volume E.g. heart is visible / monitoring of heart rate is non-invasive / alcohol can diffuse (from solution into *Daphnia*) / alcohol absorbed through skin / less likely to feel pain IGNORE cannot feel pain / easier to count heart rate	**(2)**

Question 2 · AS Paper 1 · June 2017 · Q1(a)(iii) · 2 marks

Question Number	Answer	Additional Guidance	Mark
1(a)(iii)	An explanation that makes reference to two of the following: • transparent body (1) • therefore heart is visible / procedure is non-invasive (1) **Or** • aquatic organism (1) • therefore can take in caffeine from solution (1)		**(2)**

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Question 3 · AS Paper 1 · June 2022 · Q6(c)(i) · 2 marks

Question Number	Answer	Additional Guidance	Mark
6(c)(i)	An explanation that makes reference to the following: • transparent body / translucent skin means) heart is visible / monitoring of heart rate is non-invasive (1) • (aquatic animal therefore) caffeine can diffuse (from solution into frog) through the skin (1)	ALLOW large surface area: volume for fast diffusion (of caffeine)	(2)

8BN0_01 QPEC Version

Question 4 · AS Paper 1 · June 2024 · Q8(c)(ii) · 2 marks

Question Number	Answer
8(c)(ii)	A description that makes reference to two of the following: • vertebrates {have a more developed nervous system / may feel pain} (1) • use a non-invasive procedure (1) • select organisms which are {abundant / not endangered} / select aquatic organisms so caffeine uptake can be passive (1)

Additional Guidance	Mark
ALLOW reverse argument for invertebrates nervous system ALLOW invertebrate may feel pain ALLOW organisms may {be harmed / die} ALLOW organisms cannot give consent ALLOW description of a suitable method to measure heart rate without harm to organism e.g. use transparent organisms so heart is visible ALLOW experiment on for minimal time periods ALLOW return to their habitat (after investigation)	(2)

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Question 5 · AS Paper 1 · June 2025 · Q7(b)(ii) · 1 mark

Question Number	Answer
7(b)(ii)	An answer that makes reference to one of the following: • distilled water (1) • solution used to make alcohol solution (without alcohol added) (1)

Additional Guidance	Mark
ALLOW water / pondwater / 0% alcohol solution	(1)

Question 6 · AS Paper 1 · November 2021 · Q8(a)(i) · 2 marks

Question Number	Answer
8 (a)(i)	An answer which makes reference to the following: • (pond /plain) water / solvent used for caffeine solution (1) • to compare with the caffeine solutions / to show {normal/ resting / starting} heart rate (1)

Additional Guidance	Mark
ALLOW solution with no caffeine	(2)

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Question 7 · AS Paper 1 · June 2017 · Q1(a)(i) · 1 mark

Question Number	Answer
1(a)(i)	An answer which makes reference to one of the following: • {concentration / volume / temperature / aeration} of solution / temperature (1) **Or** (zebrafish) {time to acclimatise / age / size} (1)

Additional Guidance	Mark
ALLOW surroundings ALLOW same zebrafish	**(1)**

Question 8 · AS Paper 1 · November 2021 · Q8(a)(ii) · 4 marks

Question Number	Answer	Additional Guidance	Mark
8 (a)(ii)	An answer which makes reference to the following: • {temperature / aeration} of solution / acclimatisation time (1) • correct justification relating to effect on heart rate (1) • same {sex / size / age} of ghost shrimp (1) • so caffeine would affect the ghost shrimp equally / to produce more valid results (1)	e.g. a cold temperature would decrease the heart rate ignore species ignore accuracy	**(4)**

8BN0_01 QPEC Version

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Question 9 · A level Paper 2 · October 2020 · Q5(b)(ii) · 2 marks

Question Number	Answer
5(b)(ii)	An explanation that makes reference to the following: • (because time is required) for the alcohol to be absorbed (1) • (because time is required) for acclimatisation (1)

Additional Guidance	Mark
ALLOW getting used to the new conditions / overcoming stress of being moved	(2)

Question 10 · AS Paper 1 · June 2025 · Q7(b)(iii) · 3 marks

Question Number	Answer
7(b)(iii)	A description that makes reference to three of the following: • time for acclimatisation (1) • correct reference of use of microscope to view heart rate (1) • how another (suitable stated) variable could be controlled (1) • suitable method to measure heart rate accurately (1)

Additional Guidance	Mark
e.g. same temperature, same volume of solution, same oxygen concentration, same pH, immobilisation of *Daphnia,* same age of *Daphnia* e.g. filming on phone and watching back at slow speed, dots on paper, clicker	(3)

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Question 11 · AS Paper 1 · October 2020 · Q7(b)(iii) · 5 marks

Question Number	Answer
7(b)(iii)	An answer which makes reference to the following: • Daphnia (immobilised) on (cavity) slide (1) • acclimatisation time in {control / dopamine / dopamine and caffeine} solution (1) • use of concentrations from graph (1) • suitable method for counting and recording heart rate (1) • use Daphnia of same {species / age / sex / size} (1) • repeats and calculation of {mean / SD} (1)

Additional guidance	Mark
	(5)

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Question 12 · A level Paper 2 · November 2021 · Q5(b)(ii) · 4 marks

Question Number	Answer
5(b)(ii)	An answer that makes reference to four of the following • details of the procedure (1) • record heartbeat before and after placing in regular or decaffeinated coffee (1) • controlled variable for the coffee (1) • reference to controlled variables (1) • use of a suitable statistical test to compare the results (1)

Additional guidance	Mark
e.g. use of a microscope / method of counting / allowing acclimatisation ALLOW ref to heartbeat for *Daphnia* in water and coffee e.g. concentration or volume e.g. temperature / length of time to count heartbeat / same species	**(4)**

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Question 13 · A level Paper 2 · October 2020 · Q5(c) · 5 marks

Question Number	Answer
5(c)	An answer that makes reference to the following: • alcohol concentrations between 0.00 and 0.17 mol dm^{-3} (1) • controlling one biotic variable (1) • controlling one abiotic variable (1) • determine the concentration at which the *Daphnia* show a decrease in heart rate (1) • use of microscope to count heart beats of *Daphnia* (1)

Additional Guidance	Mark
e.g. age, size, sex, species of *Daphnia,* e.g. temperature, volume of alcohol ALLOW the concentration at which the mean heart rate drops below 221 beats per minute	**(5)**

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Question 14 · A level Paper 2 · June 2025 · Q10(b)(ii) · 5 marks

Question Number	Answer
10 (b)(ii)	An answer that makes reference to five of the following: • measure mass of individual *Daphnia* (1) • range of substance given below 15mg kg^{-1} (body mass) (1) • count number of heart beats in a set time (1) • control of a relevant biotic variable (1) • control of a relevant abiotic variable (1) • use several *Daphnia* at each concentration to find the mean (1)

Additional Guidance	Mark
ALLOW measure for count, or a correct description for finding heart rate IGNORE heart rate for heart beats e.g. count the heart rate e.g. {species/age/sex} of *Daphnia* e.g. temperature ALLOW repeats to find a mean	**(5)**

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Question 15 · A level Paper 2 · October 2020 · Q5(b)(i) · 1 mark

Question Number	Answer
5(b)(i)	An answer that makes reference to one of the following: • { identify / remove } any anomalies (1) • to calculate a mean / can make valid comparisons (1)

Additional Guidance	Mark
ALLOW to gain more repeatable data ALLOW outliers for anomalies ALLOW produce / form for calculate	(1)

Question 16 · AS Paper 1 · June 2017 · Q1(a)(ii) · 2 marks

Question Number	Answer
1(a)(ii)	An answer that makes reference to the following: • caffeine increases the heart rate (of the zebrafish) (1) • the results show a calculated increase (1)

Additional Guidance	Mark
e.g. increase of at least 52 bpm / average increase of 43.8%	(2)

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Question 17 · AS Paper 1 · June 2025 · Q7(b)(iv) · 3 marks

Question Number	Answer
7(b)(iv)	An answer that makes reference to the following: • alcohol decreases heart rate compared to {resting / control / 290bpm} heart rate (1) • heart rate decreases during the first two minutes and then increases / heart rate decreases and then increases {after 2 minutes / at 3 minutes / between 3-4 minutes} (1) • all heart rates are significantly {lower / different} than the control as the {SD / error} bars don't overlap (1)

Additional Guidance	Mark
ALLOW heart rate does not return to {resting / control} heart rate ALLOW heart rate is lowest at two minutes ALLOW no significant difference between heart rate at 1 and 3 minutes {SD / error} bars overlap	(3)

Question 18 · AS Paper 1 · November 2021 · Q8(b)(ii) · 4 marks

Question Number	Answer	Additional Guidance	Mark
8 (b) (ii)	An explanation which makes reference to four of the following: • as the concentration of caffeine increases the heart rate decreases / there is a negative correlation (for 2 shrimps) (1) • heart rate for shrimp 3 increased from caffeine 10 to 13 mg cm^{-3} (1) • normal heart rate is not known (as there was no 0% caffeine concentration) (1) • largest decrease in heart rate between 10-13 mg cm^{-3} (for shrimps 1 and 2) (1) • much greater decrease in heart rate for shrimp 1 than shrimp 2 (1)	ALLOW reference to shrimp 3 showing anomalous results eg decrease of 120 compared to 56	(4)

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Question 19 · AS Paper 1 · October 2020 · Q7(b)(i) · 4 marks

Question Number	Answer
7(b)(i)	An answer which makes reference to the following: • adding caffeine (to dopamine) increases heart rate (1) • {adding / increasing the concentration of} dopamine increases heart rate (1) • larger increase when both are used together at higher concentration (1) • no overlap between error bars indicates significant difference between dopamine and dopamine with caffeine / overlap between error bars indicates no significant difference between dopamine concentrations (1)

Additional guidance	Mark
ALLOW dopamine with caffeine increases HR more than just dopamine – not to go in final MS ALLOW comparative data	(4)

Question 20 · AS Paper 1 · June 2022 · Q6(c)(ii) · 4 marks

Question Number	Answer	Additional Guidance	Mark
6 (c)(ii)	An answer that makes reference to the following: • conclusion is not valid (1) • cannot be confident that 0.1 mg cm^{-3} caused the largest increase in heart rate of this frog (1) • as no repeats were performed / result could have been anomalous (1) • investigation was on one species / investigation not representative of {this frog species / all frog species} (1)	ALLOW the largest heart rate could result from a concentration between 0-0.2 mg cm^{-3} / too few concentrations tested for this conclusion to be valid / need to test intermediate concentrations ALLOW only performed on one frog (from that species) ALLOW different species hearts' may be affected differently	**(4)**

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