



(A) Respirometers

Analysis of results from experiments involving measurement of respiration rates in germinating seeds or invertebrates using a respirometer.

A respirometer is any device that is used to measure respiration rate. There are many possible designs. Most involve these parts:

- A sealed glass or plastic container in which the organism or tissue is placed.
- An alkali, such as potassium hydroxide, to absorb carbon dioxide.
- A capillary tube containing fluid, connected to the container.

One possible design of respirometer is shown in figure 11, but it is possible to design simpler versions that require only a syringe with a capillary tube attached to it.

If the respirometer is working correctly and the organisms inside are carrying out aerobic cell respiration, the volume of air inside the respirometer will reduce and the fluid in the capillary tube will move towards the container with the organisms. This is because oxygen is used up and carbon dioxide produced by aerobic cell respiration is absorbed by the alkali.

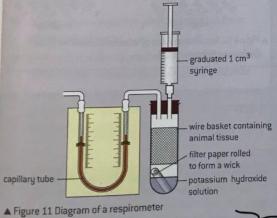
The position of the fluid should be recorded several times. If the rate of movement of the fluid is relatively even, the results are reliable. If the temperature inside the respirometer fluctuates, the results will not be reliable because an increase in air temperature causes an increase in volume. If possible the temperature inside the respirometer should be controlled using a thermostatically controlled water bath.

Respirometers can be used to perform various experiments:

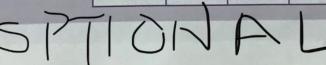
- the respiration rate of different organisms could be compared:
- the effect of temperature on respiration rate could be investigated;
- respiration rates could be compared in active and inactive organisms.

The table below shows the results of an experiment in which the effect of temperature on respiration in germinating pea seeds was investigated.

To analyse these results you should first check to see if the repeats at each temperature are close enough for you to decide that the results are reliable. You should then calculate mean results for each temperature. The next stage is to plot a graph of the mean results, with temperature on the horizontal x-axis and the rate of movement of fluid on the vertical y-axis. Range bars can be added to the graph by plotting the lowest and highest result at each temperature and joining them with a ruled line. The graph will allow you to conclude what the relationship is between the temperature and the respiration rate of the germinating peas.



Temperature (°C)	Movement of fluid in respirometer $(mm min^{-1})$		
	1st reading	2nd reading	3rd reading
5	2.0	1.5	2.0
10	2.5	2.5	3.0
15	3.5	4.0	4.0
20	5.5	5.0	6.0
25	6.5	8.0	7.5
30	11.5	11.0	9.5



2 DO DITION OF MOLECULAR BIOLOGY

Data-based questions: Oxygen consumption in tobacco hornworms

Tobacco hornworms are the larvae of *Manduca sexta*. Adults of this species are moths. Larvae emerge from the eggs laid by the adult female moths. There are a series of larval stages called instars. Each instar grows and then changes into the next one by shedding its exoskeleton and developing a new larger one. The exoskeleton includes the tracheal tubes that supply oxygen to the tissues.

The graphs below (figure 12) show measurements made using a simple respirometer of the respiration rate of 3rd, 4th and 5th instar larvae. Details of the methods are given in the paper published by the biologists who carried out the research. The reference to the research is Callier V and Nijhout H F (2011) "Control of body size by oxygen supply reveals size-dependent and size-independent mechanisms of molting and metamorphosis." PNAS;108:14664–14669. This paper is freely available on the internet at http://www.pnas.org/content/108/35/14664.full.pdf+html.

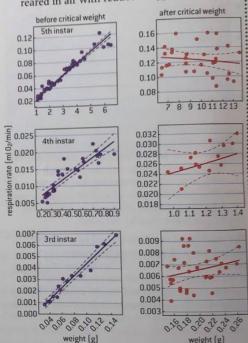
Each data point on the graphs shows the body mass and respiration rate of one larva. For each instar the results have been divided into younger larvae with low to intermediate body mass and older larvae with intermediate to high body mass. The results are plotted on separate graphs. The intermediate body mass is referred to as the critical weight.

- a) Predict, using the data in the graphs, how the respiration rate of a larva will change as it grows from moulting until it reaches the critical weight.
 - b) Explain the change in respiration rate that you have described. [2]
- 2 a) Discuss the trends in respiration rate in larvae above the critical weight.

b) Suggest reasons for the difference in the trends between the periods below and above the critical weight. [2]

The researchers reared some tobacco hornworms in air with reduced oxygen content. They found that the instar larvae moulted at a lower body mass than larvae reared in normal air with 20% oxygen.

3 Suggest a reason for earlier moulting in larvae reared in air with reduced oxygen content. [2]



▲ Figure 12 Respiration rates of tobacco hornworms [after Callier and Nijhout, 2011]



Ethics of animal use in respirometers

Assessing the ethics of scientific research: the use of invertebrates in respirometer experiments has ethical implications.

It is important for all scientists to assess the ethics of their research. There has been intense debate about the ethics of using animals in experiments. When discussing ethical issues, do

we consider the consequences such as benefits to students who are learning science? Do we consider intentions? For example, if the animals are harmed unintentionally does that change



whether the experiment was ethical or not? Are there absolute principles of right and wrong: for example, can we say that animals should never be subject to conditions that are outside what they would encounter in their natural habitat?

Before carrying out respirometer experiments involving animals these questions should be answered to help to decide whether the experiments are ethically acceptable:

- I Is it acceptable to remove animals from their natural habitat for use in an experiment and can they be safely returned to their habitat?
- 2 Will the animals suffer pain or any other harm during the experiment?

- 3 Can the risk of accidents that cause pain or suffering to the animals be minimized during the experiment? In particular, can contact with the alkali be prevented?
- 4 Is the use of animals in the experiment essential or is there an alternative method that avoids using animals?

It is particularly important to consider the ethics of animal use in respirometer experiments because the International Baccalaureate Organization has issued a directive that laboratory or field experiments and investigations need to be undertaken in an ethical way. An important aspect of this is that experiments should not be undertaken in schools that inflict pain or harm on humans or other living animals.

2.9 Photosynthesis

Understanding

- → Photosynthesis is the production of carbon compounds in cells using light energy.
- Visible light has a range of wavelengths with violet the shortest wavelength and red the longest.
- → Chlorophyll absorbs red and blue light most effectively and reflects green light more than other colours.
- → Oxygen is produced in photosynthesis from photolysis of water.
- → Energy is needed to produce carbohydrates and other carbon compounds from carbon dioxide.
- → Temperature, light intensity and carbon dioxide concentration are possible limiting factors on the rate of photosynthesis.



Applications

Changes to the Earth's atmosphere, oceans and rock deposition due to photosynthesis.



Skills

- Design of experiments to investigate limiting factors on photosynthesis.
- → Separation of photosynthetic pigments by chromatography.
- Drawing an absorption spectrum for chlorophyll and an action spectrum for photosynthesis.



Nature of science

 Experimental design: controlling relevant variables in photosynthesis experiments is essential.