IB Biology Investigations

Volume 1 (Standard Level)

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(For use with the IB Diploma programme)

(Fourth edition)

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Author profile

Paul Billiet B.Sc. (Biology), M.Sc. (Ecological Systems), P.G.C.E., C. Biol.

Paul has devoted his career to the teaching of IB Science and Biology in particular. He is Head of Science at *Ecole Active Bilingue Jeannine Manuel* (EABJM) in Paris and he has taught Biology for over 38 years, including 32 years teaching IB Biology.

During his involvement with the IB, Paul has been examiner for various components of IB Biology and Environmental Systems as well as authoring several exam papers. He served as Deputy Chief Examiner for Biology from 1997 to 2002.

Paul has been involved in curriculum reviews for biology since 1986. He has taken part in the development of internal assessment in both Biology and Sports Exercise and Health Science. As Principal Moderator for Biology, Paul was part of the committee that developed the current Internal Assessment (IA) model.

Author's acknowledgements

Firstly I would like to thank my School's Administration for its support throughout and my students who, over the years, have trialled these Investigations.

Colleagues, too numerous to cite all by name, at EABJM and around the world, have provided me with useful tips and guidance over the years. Whether in formal workshops or over a coffee, their combined experience was an enormous asset. Special thanks for pointing me in the right direction go to Andrew Allott, Shirley Burchill, Alan Cadogan, Catherine Casalis, Alan Damon, Alison Davis, Guy Décarie, Sandra Deighton, Paul Fairbrother, Karen Frey, Robert Gaurenne, Lonnie Guralnick, Lionel Guerin, Jazcqueline Gout, Deborah James, John Knopp, David Mindorff and Adrian Thompson.

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I would also like to thank to my editor David Greig, who encouraged me to write for IBID Press and has reminded me, diplomatically, ever since of the 'deadlines'.

Finally, a very special thanks to my wife, Evelyne, who has patiently put up with the impact of those 'deadlines' during the writing of successive editions of 'those books' over the years. Without her support it would never have happened.

Paul Billiet *June 2014*

Editor profile

David Greig (B.Sc., Dip. T(Sec)., Dip. Ed., Grad. Cert. Sc. Ed.)

David has been a teacher of senior Biology for 40 years or so in South Australian schools. For the last 15 years or so he has worked as a consultant on a wide variety of writing, editing and publishing projects, producing Science materials for use in Australian Schools, and also internationally, for use with the International Baccalaureate Programmes.

Editor's acknowledgements

David wishes to acknowledge the author Paul's very high level of expertise, enthusiasm and the excellent work done by him over the years on the successive editions of these Biology Practical Investigations. It has not always been 'a walk in the park' on a warm and sunny autumn afternoon in Paris!

In addition David wishes to acknowledge the invaluable support, friendship and encouragement offered by technical guru Colin Flashman, publisher Rory McAuliffe, proof reader Jenna Crierie, my wife Mary and other friends and family. A project like this does not happen by accident!

Foreword

This collection of Investigations/Activities is part of a series which has been written specifically to support the teaching of the practical component of current International Baccalaureate Science courses (IBO). Volumes have been written for Biology, Chemistry and Physics at both Standard (SL) and Higher level (HL). These Investigations and Activities have been written by an experienced IB teacher with very close reference to the syllabus, which is current at the time of writing. It is vital that staff consult syllabus and assessment guidelines which are current at the time they use this material because these syllabus documents are subject to review and modification.

These copy Masters and accompanying Teaching Notes may be used by anyone in the purchasing school but may not be passed on to another school. The Investigations and Activities in this volume will fit comfortably into a Practical Scheme of Work as required (see below), but they are not intended to be exclusive or exhaustive. Syllabus Correlation Tables are provided showing the relevant Skills and Applications for the various Topics and sub-Topics and suggested focus for each Investigation.

This collection of Investigations does not in any way form a proscribed Practical Scheme of Work (PSOW). They are meant primarily to give teachers ideas of the sort of experimental work that is suitable for a PSOW and to assist teachers in designing their own PSOW. In some of the activities the method of analysis is quite clearly laid out but where possible the students must be encouraged to design and draw up their own data tables, decide the best way to analyse the data of a particular experiment and make their own evaluation of an experiment. These are all necessary skills that the students need to acquire if they are to be successful in their assessed Individual Investigation. It is hoped that this set of Investigations will help the students achieve this requirement. Suggestions are made where planned Investigations may be developed from the Investigations in this collection.

For the sake of consistency, the Options have been numbered as follows: Option A will be Chapter 12, Option B will be Chapter 13, Option C will be Chapter 14 and Option D will be Chapter 15.

The author has trialled all of these Activities and he has suggested various safety precautions but will not accept any responsibility whatsoever for any accident that may arise during the conduct of these Investigations and Activities. The publisher (IBID Press) will be pleased to receive any suggestions and comments from staff or students using this material. The ideas for the Practicals appearing in these volumes arise of a variety of sources and multicultural exchanges.

Within this Volume, we hope you will find many new ideas. Many have been redesigned from ideas generated from conversations or observations. We are indebted to those scientists and teachers who designed and developed the original experiments, from which many of these Practicals have been developed, and on behalf of the teachers and students that use them we warmly acknowledge these pioneers. We also acknowledge and thank those that generated ideas and suggestions that helped us.

Please refer to www.ibid.com.au for current information about our complementary full colour IB Biology Textbook and other publications.

P. Billiet, (Author)

Paris 2014

Health and Safety Symbols

Laboratories can be hazardous places. Often scientists, Science teachers and students handle equipment and materials which can be dangerous to their health and safety. Throughout these Volumes of Investigations you will see a number of symbols and warnings which will represent particular hazards. For each of these we will briefly describe the hazard and indicate what precautions you should take to avoid damage and/or what responses are appropriate. In all cases, of course, you should seek advice and assistance from the teacher or laboratory technician.

A biohazard is any organism or body fluid which could possibly cause illness or disease in your body. This particularly includes micro-organisms.

A flammable substance is one which will readily burn in air. It may be a solid, liquid or gas. If you are using such a substance it is vital that there are no sparks or naked flames which could ignite it. It is vital that you know what to do in the event of fire. This may include the use of fire extinguishers and evacuation procedures.

A radioactive substance is one which emits particles or 'radiation'. This radiation is known to cause damage to cells and may also be cancer causing. If you are using radioactive substances it is vital that you wear protective clothing, use metal tongs and listen carefully to instructions given by your teacher or laboratory technician.

Sharp instruments are often used in Science and particularly in Biology, to cut sections through plant or animal tissue. These instruments, which include scalpels and razor blades are very sharp and obviously will also cut through your tissues. When using these instruments it is essential that you always cut away from your body and preferably onto a cutting board. It is also important to be very careful when carrying these instruments and also ensure they are placed on the workbench in a safe place.

When certain chemicals are mixed together they can become explosive. An explosion is caused by rapid expansion of gas in a confined space and can be very dangerous. Sometimes it is important to ensure that the space is not confined and sometimes it is important to conduct these reactions behind a protective screen.

It is often necessary to protect your hands from heat, chemicals or other hazards and gloves will be made available for these situations. The type of glove needed will depend on the particular hazard and your teacher will provide further advice. In some cases you will be advised to dispose of the gloves after use and in other cases to wash and dry them carefully.

Your eyes are the most vulnerable and easily damaged external part of your body. This is why they must be protected if you are using solids and liquids which could get into them. Whenever you are heating things or using corrosive liquids, and in other cases as instructed by a teacher, you should wear safety goggles. You should also do this if possible even if you wear spectacles to correct your vision. In the event that something gets in your eye you should immediately make use of the eyewash facility in the laboratory as instructed and then notify your teacher.

Some chemicals, which are used in a laboratory, are *corrosive*. This means that they can react with and 'eat away' materials like the bench, your books, clothing and skin. It is essential that you handle these materials, which are usually liquids, with care. Always tip from the container with the label uppermost, never add water to concentrated acid and never have your face anywhere near the container. It is usually advisable to wear both safety goggles and gloves. If protective aprons are available you should also wear one.

As a general rule, 12 or 24 volt *electrical* appliances are unlikely to cause serious injury. However, 'mains' voltage (110V or 240V or higher) can cause serious injury or death. The appliances you use should be regularly tested and certified safe. If you notice sparks or smell insulation burning, turn the power off immediately and notify staff. Be particularly careful not to allow water to get into any appliance as it may cause a short circuit.

ELECTRICITY

Some chemicals are *poisonous* and should not be inhaled or ingested. It will be necessary to use a fume cupboard when using poisonous gases or volatile liquids. They could make you very ill and you may require medical assistance. It is vital that you listen to instructions, follow them carefully and notify your teacher immediately if there is accidental exposure to poisonous or toxic substances.

POISON

Lasers are very intense beams of light. They are capable of causing burns to the skin and permanent damage to the eyes. It is essential that these are only ever used under the supervision of a teacher and in a situation where people can not see the beam directly or when it is reflected from a shiny surface. Sunglasses or welding masks do not provide sufficient protection and special 'laser glasses' must be used where there is a risk.

LASER

UV light is harmful to skin and especially eyes. Do not expose these areas directly to a UV light source. If it is not avoidable, sunscreen can be applied to the skin and special goggles should be worn.

UV

There are other *dangers* or hazards as well, for example carrying heavy or hot objects. This may also include chemicals which are not poisonous but which may smell unpleasant or irritate the skin. Whenever you see this icon more information will be provided in the adjacent text about the specific danger.

DANGER

In Science and particularly in Biology, there are situations when ethics and ethical issues need to be considered in experimental work. This is particularly the case when human volunteers are being used, not just for experimental work but also when they are being surveyed to collect personal information. In these cases a consent form should be used to explain the nature of their involvement and to get their approval. Ethics will also be an issue whenever animals are used in experimentation or when they are collected in the field. They should not be exposed to conditions that are outside their natural range of tolerance and wild animals must be released back where they were sampled with the minimum of disturbance.

ETHICS

The environment and environmental issues become important when hazardous substances are used or produced during an experiment. Their disposal must result in minimal impact on the environment. In field work the protocol that is used must reflect practices that minimise the impact of the investigation on the site.

ENVIRONMENT

IMPORTANT NOTE

Although every care has been taken in preparing and trialling these investigations, absolutely no responsibility or liability whatsoever can be accepted for any damage or accident which may occur for whatever reason during the conduct of any of these activities. The Safety Warnings and Icons are advisory only and are not intended to be exhaustive or exclusive. It is a strict condition of sale that safety in the laboratory is the responsibility of the staff and students doing the laboratory work and not the author, editor or publisher of this work.

Scientific Investigations

Each time you design an investigation check that you have done the following.

Design

- Have I consulted the literature and written a short concise introduction establishing the theoretical background?
- Have I correctly cited my sources?
- Have I stated my aim or objective (research question)? It should contain reference to the independent and dependent variables.
- Does my investigation have a clear purpose?
- Have I identified the origin of the material used and/or the species used by their scientific names?
- If it is valid, have I written my hypothesis (a justified prediction)?
- Have I clearly identified the variables?
- Which variable will I change (the independent or manipulated variable)?
- Which variable will I measure/observe (dependent or measured variable)?
- Which other variables do I need to control (which ones will affect the experiment)?
- How significant are each of these control variables?
- Which variables cannot be controlled but need to be monitored?

Method

- What equipment and materials will I need?
- Can this be best described using a labelled diagram or photograph of the experimental setup?
- What safety factors should I bear in mind?
- How exactly will I measure the dependent variable?
- What must I do to control all the other variables identified in the controlled variables?
- If I cannot control these variables, then how will I monitor them or do I need a control experiment? If so, have I described it? (Note: controlled variables and a control experiment are not the same thing).
- How can I make my experiment a fair test?
- What range am I setting for the independent variable?
- How often will I measure the independent variable, or at what intervals?
- How many trials do I need to be sure of my results?
- What safety factors should I bear in mind?

Results/Data

- How should I present the data (e.g. tables, annotated drawings, map)?
- How precise must I be with my data?
- What was the precision of the instruments I used?
- Where are the errors in my measurements and how big are they likely to be?
- Have I set up a data table for my raw results? Try visualising this first and sketching it out roughly by hand.
- Has the table got a clear title ('The results' is insufficient)?
- Are the headers of my table columns clear?
  e.g. the name of the variable, the units, the uncertainties (e.g. Time / s ± 0.1s).
- Have I explained my uncertainties?
- Is the independent variable first in the table (top line or left hand column)?
- Are all my data to the same degree of precision (same number of decimal places)?
- Have I centred my data in each column?
- Are my data to the same precision as my uncertainties?
- Did I see anything else happen during the investigation that needs to be described?
- During the experiment, did I make any significant qualitative observations?(Photographs could help here)

Processing and Analysis of Data

- Do I calculate a change, a proportion, a percentage, an average of the repeats, and a standard deviation of the repeats?
- Are my calculations easy to follow? For those done in a spreadsheet, a screen shot can be included.
- Are my processed results to the same precision as my raw data?
- Do I present these data as a table, graphically or both?
- If graphically, what sort of graph is best? Line graph, scatter plot, bar chart histogram, pie chart…?
- Try visualising by hand what you want to produce as a graph BEFORE you go to a program like MSExcel.
- Am I respecting the conventions for graphs?
- Do I need to analyse the graph to obtain a result?
- Do I look for intercepts (e.g. where a line cuts an axis)?
- Do I calculate a gradient to establish a rate?
- Do I establish an optimum? Do my graphs have clear titles? What, precisely, do they show and what are the error bars representing?
- Have I presented the results such that the uncertainties are clear? For example have I used error bars or drawn a trend line if one can be drawn?
- Do I need to analyse the data statistically to obtain a result (e.g. t-test, χ^2 test, correlation)? If so, which is the correct one to use?

Discussion of results

- What do the results show? Are there any trends?
- What can I interpret from the results?
- Can I explain the results in a systematic way and come to a conclusion?
- Are the results consistent with what I expected?
- Do they fit in with the theoretical background (the literature values)?
- Have I correctly cited my sources?
- How consistent are the results?
- Is there a lot of scatter beside the trend line?
- Are the standard deviations particularly large?
- Have any results been justified as outliers and excluded?
- Can I explain any unexpected results?
- What are the sources of error: in my method, in the manipulation, in the analysis?
- How significant were each of the weakness that I have identified?
- What improvements could be made?
- Do I need to suggest a new hypothesis to account for the results?
- How could I take the investigation further?
- Have I added footnotes where there are direct quotes or citations?
- Have I included a bibliography of the references that were consulted?

Style

- Keep it impersonal (e.g. "The tubes were left for 10 minutes to incubate" instead of "I left the tubes for 10 minutes to incubate").
- Use subheadings to organise your report (Discussion, Conclusion, Evaluation etc).

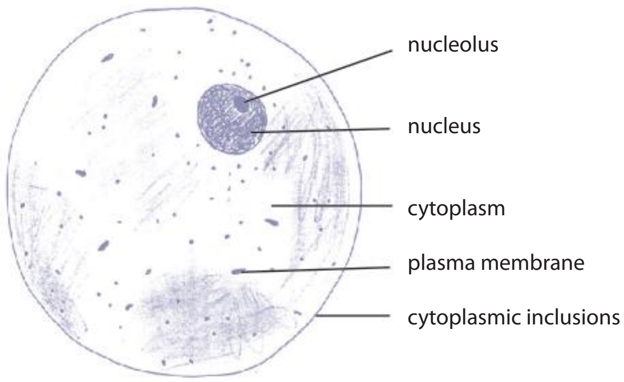
Table of Contents

Syllabus reference: Sub-Topic 1.1 Skills

Cells were first recognised as fundamental structures in biology by Schleiden and Schwann in 1839 when they formulated the Cell Theory. Studying cells requires the use of the microscope.

1A.1 Animal Cells

Animal cells compared to plant cells viewed under light microscope are characterised by what they do not possess. They usually do not possess an easily defined extracellular matrix, plastids and a sap vacuole. They tend to be smaller than plant cells and their structure is usually determined by the cytoskeleton which is not visible without special stains.



An animal cell as seen with a light microscope

Preparation of a smear of liver cells (hepatocytes)

Materials

microscope	forceps	filter paper
slides	dropping pipette	methylene blue stain
coverslips	mounted needle	liver tissue
micrometer eyepiece	cotton bud	

Liver cells are gland cells. There are involved in a large number of functions including storing glucose as glycogen, storing vitamins and detoxifying chemicals such as alcohol. These cells divide relatively slowly sometimes at intervals of over a year.

Method

1. Gently scrape the piece of freshly cut liver with a cotton bud.
2. Smear the cells removed onto the middle of a microscope slide. Add one drop of methylene blue and two drops of water, then place the coverslip using the mounted needle or forceps.

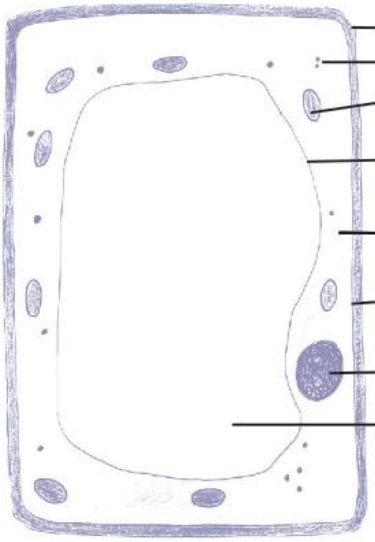
Warning: Sharp instruments, handle with care

3. Observe the preparations at low power, then medium power. Scan over the slide to get a general idea of the appearance of the liver cells. Select one that appears typical, centre it and move to high power. Draw one of the liver cells from the stained preparation and label the structures that are visible. Note: Sometimes liver cells are binuclear. How does this contradict the Cell Theory?
4. Find the average size of the cells seen in your slide preparation, in micrometres (μm) by measuring a sample of the cells. Add these data on your cell drawing, showing all the data you collected clearly. Add a scale bar next to your drawing indicating the largest diameter of the cell that you have drawn.

1A.2 Plant Cells

Plant cells characteristically are surrounded by an extracellular matrix of carbohydrate, in particular cellulose. This cell wall supports the plant cells and determines their shape. In general plant cells are bigger than animal cells mainly due to this cell wall.

Internally plant cells have two characteristic organelles that can be observed under the light microscope; plastids and the sap vacuole. Plastids are complex organelles that include green chloroplasts. The sap vacuole, a fluid filled sack, is usually not visible without staining but some store pigments.



A plant cell as seen with the light microscope

Structure	Function	Structure	Function
Cell wall	a cellulose framework.	Chloroplast	a plastid pigmented green.
Sap vacuole	an inflated sac containing a solution which may be pigmented.	Starch grain	a food reserve inclusion coloured blue-black in the presence of iodine solution. Starch grains are laid down in amyloplasts.

Materials for the following Parts A, B, C and D activities

microscope
micrometer eye piece
stop watch
slides
coverslips
small beaker
forceps

lancet
scalpel
dropping pipette
filter paper
marker pen
distilled water bottle

Canadian pond weed (*Elodea*) or another suitable transparent leaf
20% sucrose solution
iodine solution
red onion
banana

Part A: Canadian pond weed (*Elodea*)

Method

1. Using forceps, pick a healthy green leaf of Elodea from a terminal bud and mount it in a drop of water.
2. Observe the leaf under medium and high power. The chloroplasts should be visible as small green spheres. Draw and label a few of these cells.
3. Look for cyclosis (cytoplasmic streaming). The chloroplasts will move slowly around the cell. The best area to start looking is around the midrib (midline) of the leaf. The chloroplasts that seem to disappear are moving out of the field of focus. Use the fine-focus control to follow them and to get a three-dimensional impression of the cell.
4. Use the micrometer eyepiece to measure the size of a sample of chloroplasts and using a stopwatch estimate how fast they travel. Do the chloroplasts travel at different speeds under different conditions?

Elodea sp.

Part B: Red onion (*Allium*) epithelium tissue

Method

1. Using a scalpel, cut a square, about $1cm^2$, from an onion leaf scale. Then peel off the outer red layer cells (epithelium) using forceps.

Warning: Sharp instruments, handle with care

SHARP

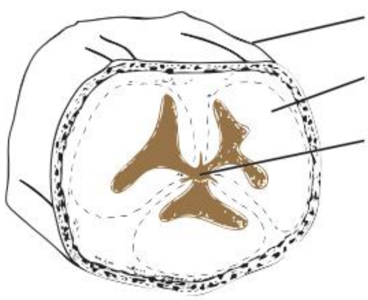
2. Mount the tissue in a few drops of distilled water. Observe the cells under medium and high power.
3. Set up another slide, this time using a few drops of 20% sucrose solution. The sucrose will make the vacuole shrink. As the vacuole shrinks, the cytoplasm will also shrink pulling the cell surface membrane away from the cell wall. In the tissue mounted in distilled water, the cells swell up with water and the cell surface membrane is pushed onto the cell wall so it is no longer visible. Mounting the cells in sucrose allows this structure to be observed.
4. Draw and measure a few of these cells, as seen in distilled water and in the sucrose solution. Label your drawing.
5. Compare the cells from inner and outer scales of the onion. Are the cells on the outer scales bigger, or are there just more of them?

Part C: Banana (*Musa*) pulp

Some plant tissues, such as ripe fruit, are soft enough to spread the cells out into a single layer. This technique can also be used on more solid tissues that have been softened by soaking them in warm acid. This hydrolyses the cell wall material.

Method

1. Using a scalpel, cut a slice of banana.



Warning: Sharp instruments, handle with care

2. Take a very small sample of pulp (the size of a pinhead) and mount it in a drop of iodine solution. Allow the fragment of banana to soak in the iodine solution for two minutes. Then place a coverslip over it and crush it lightly.
3. Observe the preparation under the microscope. Focus the image and adjust the diaphragm carefully so that the outline of the cells can be clearly seen. Draw a cell at high power and label it. Measure the cell and add a scale bar to the drawing.
4. The iodine solution will stain the starch grains blue-black. Measure the longest dimension of a sample of starch grains. Compare this to the overall size of these cells.
5. Compare bananas of different degrees of ripeness. As a banana ripens it tastes sweeter. What happens to the starch grains?

Part D: Making a squash from tomato (*Solanum*) fruit pulp cells

Method

1. Cut out a small piece of tomato pulp using a lancet. Mount the tissue in a few drops of water in the middle of a slide and cover with a cover slip.

Warning: Sharp instrument, handle with care

2. Place the slide in the middle of a piece of filter paper. Wrap the filter paper over the slide and press down firmly on the centre of the slide using your thumb.
3. Unwrap the slide and observe the cells and their contents under medium and high power. You should be able to see the coloured chromoplasts that give this tissue its red colour.
4. Using the materials available try to prepare the tissue to see if you can determine more of the cellular structure.
5. Compare normal tomatoes with cherry tomatoes. Does the cell size or shape reflect the overall shape of the fruit?
6. Compare an unripe green tomato with a ripe red one. The green colour is caused by chloroplasts and the red colour by chromoplasts. These plastids can develop from one another as do the starch grains in amyloplasts. Plastids can also be seen in the epidermal cells of red and green capsicum peppers (*Capsicum annuum*). These can be observed by pealing off the epidermal layer like the red onion cells.

1A.3 Drawing cells and tissues

Drawing is still a very important skill in Biology. Drawings help to record data from specimens. Drawings can highlight the important features of a specimen. Photographs can be very useful for recording data but they are not very selective - they show more detail of a specimen than you might want.

A drawing is the result of a long period of observation at different depths of focus and at different magnifications.

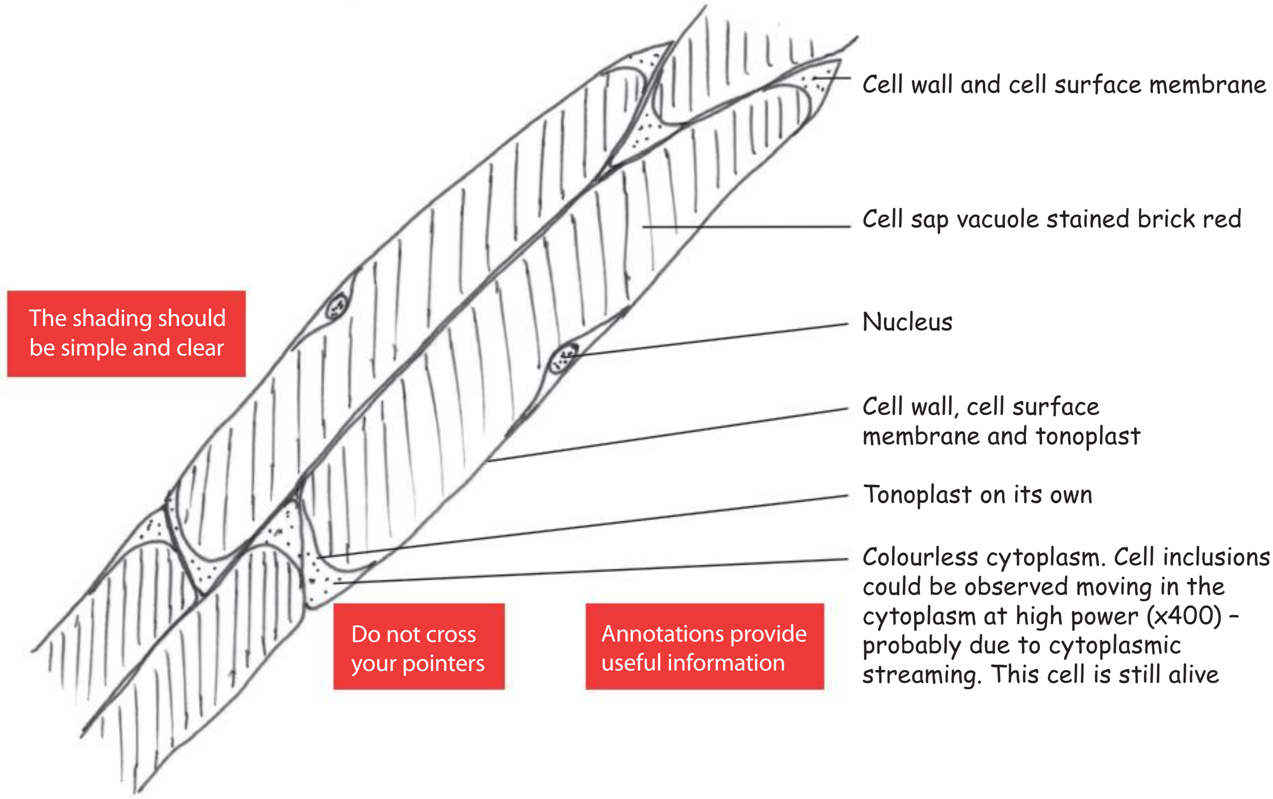
Photographs can be taken to support the drawings. It is possible to combine many photographs taken at different points of focus by stacking in programs such as Photoshop. This increases their depth of field.

Some guidelines for drawing from specimens in Biology

- Move the specimen around, do not just concentrate on one part. Observe the general appearance first.
- Identify the most significant features (only include detail that is necessary in your drawing).
- Determine which part or parts you are going to draw.
- Use a sharp HB (medium grade) pencil.
- Use white, unlined paper for drawing.
- Make a large, clear drawing, it should occupy at least half a page.
- Keep looking back at your specimen whilst you are drawing. When drawing from a microscope it is useful to look down the eye piece with one eye and at the drawing paper with the other - it takes practice but it is possible.
- Whilst you are observing, increase the magnification to observe more details and reduce the magnification to get a more general view. Use the focusing controls on the microscope to observe at different depths of the specimen.
- A drawing is incomplete without a full title and a scale or magnification. Annotations are particularly important, they permit you to put your observations where they will have the most impact.

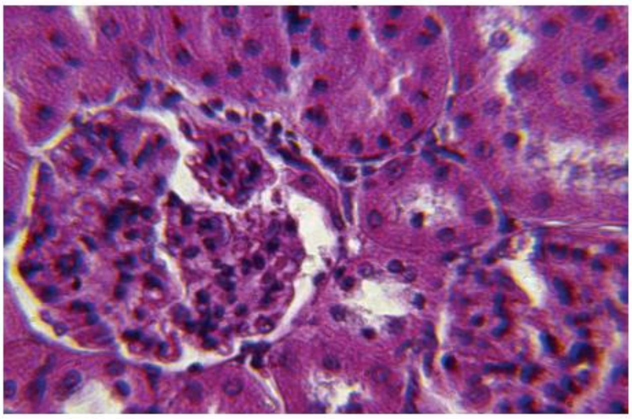
Example from observations on onion cells

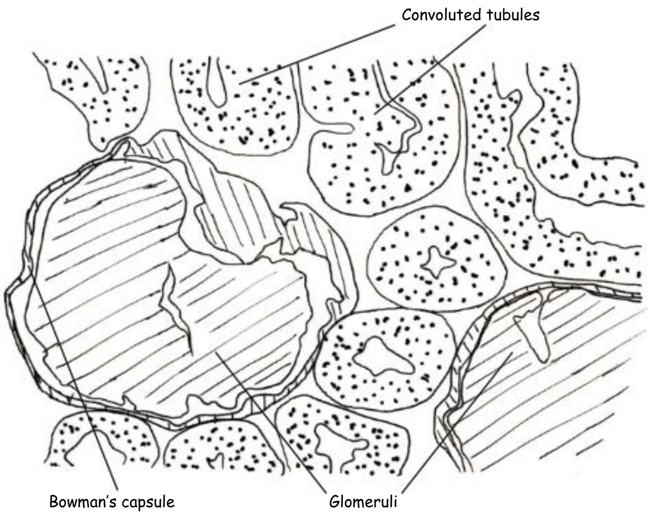
Epithelial cells from an onion bulb (*Allium cepa*) stained with neutral red at pH 7.6 maintained at 20°C.
Viewed from ×100 to ×400



Example 2 Drawing a plan view

Identify the tissues, select your area and draw without including details of the cells.





1A.4 Using a Micrometer Eyepiece

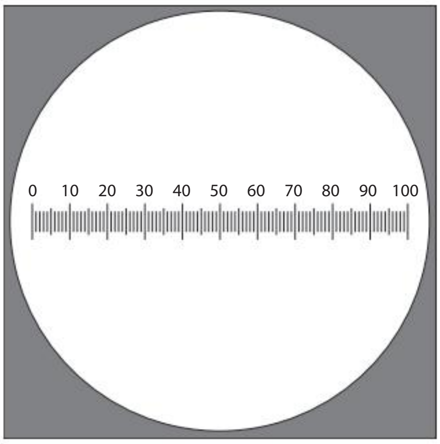
Measuring objects under the microscope requires a microscopic ruler. When using the light microscope, the most frequently used units are micrometres (µm). (1mm = 1000µm)

Materials

microscope lamp   eyepiece micrometer   micrometer slide   lens cleaning tissue

Method

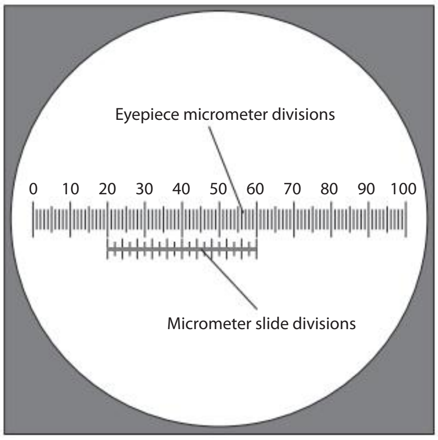
1. Replace the usual eyepiece lens by the micrometer eyepiece lens. Switch on the microscope lamp and look down the eyepiece. You should see a scale of 100 divisions with the numbers in the correct orientation, as shown opposite.



2. Calibrating the eyepiece micrometer: The scale in the eyepiece will depend upon the objective lens being used. You must calibrate the eyepiece for each different lens that you use.
3. The micrometer slide usually consists of 1 millimetre subdivided into microscopic divisions. (e.g. 1mm divided into 100 divisions).

   Find out how many divisions on your micrometer slide equal 1000µm, 100µm and 10µm.

4. Place the micrometer slide on the microscope and focus on it using the low power objective.
5. Line up the divisions on the slide with those in the eyepiece (see opposite).



   How many divisions on the eyepiece = the divisions on the slide?

6. Recall how many micrometres there are to each division on the slide.
7. Now calculate the number of micrometres that each division in the eyepiece represents when you are using the low power objective.

Example for the low power objective

X eyepiece divisions = Y slide divisions

If one slide division = 10µm

Then one eyepiece division on low power = $\left[\dfrac{\textbf{Slide divisions}}{\textbf{Eyepiece divisions}}\right] \mathbf{x10\mu m} = \left[\dfrac{\mathbf{Y}}{\mathbf{X}}\right] \mathbf{x10\mu m}$

For example

If 10 eyepiece divisions = 25 slide divisions

Then one eyepiece division on low power = $\left[\dfrac{25}{10}\right] \times 10\,\mu m$

Therefore, on low power of this microscope, each division in the eyepiece will measure 25µm.

Repeat this for the other Objective lenses and keep a record of your results. If you use the same microscope in the future you will not need to calibrate it again.

Now the eyepiece micrometer can be used like a simple ruler.

You might use the micrometer to measure the size of an animal cell, the size of a plant cell, the size of a nucleus or the speed of moving objects under the microscope.

Syllabus reference: Sub-Topic 1.4 Skill

In this experiment osmosis will be measured in a piece of tissue made of millions of cells. The water potential of the tissue can be determined by the change in size of the tissue or the change in mass of the tissue or the change in density of the liquid surrounding it.

Sucrose is a disaccharide the easily dissolves in water lowering its water potential. However, it is a molecule that is too large to pass across the cell membranes of the plant cells.

These methods can be used to compare the water potentials of plant tissues that can be cut into regular shapes.

Materials

6 specimen tubes with caps

marker pen

potato chip cutter

scalpel

fine forceps

ruler

electronic balance

paper towel

6 labelled beakers:

one containing 50cm^3 of distilled water (i.e. 0 mol dm^{-3} sucrose) and the other five containing 50cm^3 of:

- 0.2 mol dm^{-3}
- 0.4 mol dm^{-3}
- 0.6mol dm^{-3}
- 0.8 mol dm^{-3} and
- 1.0 mol dm^{-3} sucrose

12 clean, dry test tubes in a rack

methylene blue powder

spatula

Pasteur pipettes

10cm^3 syringe

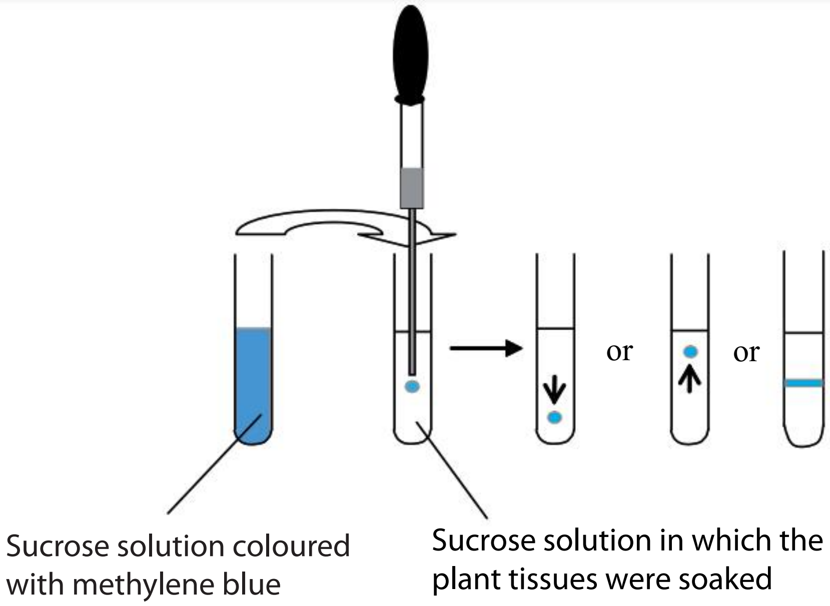
plant tissues: potato, sweet potato, carrot, swede, cassava, manioc, apple or turnip

Method

1. Using the potato chip cutter, cut 30 chips from the plant tissue. Cut the chips lengthwise in four then trim each chip, as precisely as possible, to 50mm lengths using the scalpel. Weigh each chip and record their masses.

Warning: Sharp instrument, handle with care

2. Place five chips into each of six specimen tubes, one tube for each of the different sucrose solutions. Pour in enough of each respective solution to cover the tissues. Put a cap on each one of the tubes. Make your initial observations on the tissues bathing in the liquid and leave for 24 hours.
3. Prepare a set of six large test tubes, adding 25cm^3 of each of the solutions mixed with the smallest amount of methylene blue powder.
4. Leave the tubes overnight in a refrigerator, drain off the liquid from each of the tubes into a second set of clean test tubes. Set these tubes aside. Measure the length of each chip of tissue. Measure the mass of the chips. Make your observations and record them in an organised way.
5. Analyse your data by processing them. The initial masses of the chips should be similar but they will not be identical. Therefore, you should calculate the percentage change in mass in order to compare them.
6. Plot the percentage change in mass of the tissue against the sucrose concentration. The isotonic sucrose solution, which has the same water potential as the tissue, can be determined where the line of best fit cuts the x-axis (where there is no change in the tissue). From this and the calibration curve on the next page, you can estimate the water potential of the plant tissues.
7. Do the same thing with the percentage change in length and compare the results you get for both methods.
8. Take your series of test tubes of sucrose solutions coloured with the smallest amount of methylene blue and the series of solutions drained from the tubes containing the plant tissue.
9. Using a clean, dry Pasteur pipette and a steady hand, carefully transfer one drop from each of the methylene blue coloured solutions to its equivalent tube in the series of solutions which soaked the tissues.



10. Either the blue drop sinks, the solution has become less dense. The tissue has lost water.

    Or it rises; the solution has become denser. The tissue has absorbed water.

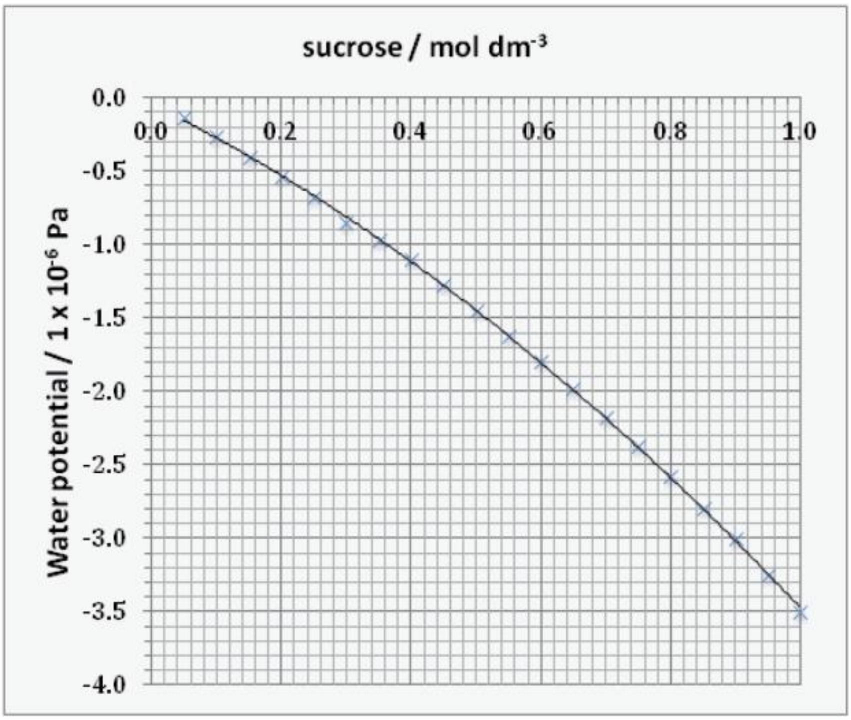
    Or it spreads out at the same level; the density has not changed.

Discussion

Discuss your results, make your conclusions and evaluate the three methods of determining the water potential of these tissues.

Additional information

The water potential of different sucrose solutions can be calculated. It is measured in units of pressure (Pascal). The conversion graph below shows this relationship.



Syllabus reference: Sub-Topic 1.4 Aim 6

Red cabbage leaf epidermal cells are pigmented purple with anthocyanins. The pigments are held in their sap vacuoles. If the membranes of the cell are damaged these pigments will leak from the cell.

What will be the effect of heat or ethanol on the membranes of red cabbage tissue?

Materials

data logger

colorimeter sensor

10 cuvettes and caps

thermometer or temperature probe

electronic water bath

marker pen

dropping pipette

10 test tubes in a rack

test tube holder

10cm^3 syringe

distilled water

series of solutions of ethanol:

(15%, 30%, 45%, 60%, 75%, 80%, 90%, 100%)

washed red cabbage disks

Method

The effect of temperature

1. Using a cork borer discs have been cut from the red cabbage and washed for several hours. Select 30 that are of uniform thickness. Place three discs in each test tube and add 10cm^3 of distilled water solution to each tube.
2. Put the tubes of water in the water bath and set the temperature to 40°C. Monitor the temperature of the water using a thermometer or a temperature probe linked to the computer with the data logging program installed. When the temperature reaches 40°C remove a tube and label it. Continue raising the temperature and remove a tube at 5°C intervals.
3. Withdraw liquid from a test tube and fill a colorimeter cuvette until it is 1 cm from the top (about ¾ full). Wash the pipette, take a sample from the next tube and fill a fresh cuvette. When labelling the cuvettes mark one side only near the top. Handle the cuvettes near the top too.

The effect of ethanol

1. Select another 30 discs that are of uniform thickness. Place three discs in each test tube and add 10cm^3 of ethanol solution to each tube. This includes one with pure distilled water which is 0% ethanol.
2. Leave the discs to soak in the ethanol solutions for 20 min.
3. Withdraw liquid from a test tube and fill a colorimeter cuvette until it is 1 cm from the top (about ⅔ full). Wash the pipette, take a sample from the next tube and fill a fresh cuvette. When labelling the cuvettes mark one side only near the top. Handle the cuvettes near the top too.

Calibrating the sensor

1. Plug the colorimeter probe into the first channel on the data logger.
2. The data logger should detect the colorimeter sensor, set the data collection parameters, and display the current sensor reading. If you have a choice between transmission and absorption select absorption.
3. To calibrate the colorimeter set up a blank cuvette containing distilled water. Put a cap on it and place it in the colorimeter.
4. Select the appropriate light source. Remember red cabbage is purple in neutral conditions. This is the light that is transmitted. You want to choose a wavelength of light that is absorbed by the liquid. Try a yellow or green wavelength. Calibrate the colorimeter for the selected wavelength.

Collecting data

1. Put a cap on the cuvette with the first sample. Set the cuvette in the colorimeter, take care not to touch the sides where the light will pass.
2. When everything is ready begin data collection using an event entry mode.
3. Remove the cuvette and replace it with the next. Collect the data for every 5°C from 40 to 80°C or every ethanol solution from 0 to 100%. The data will appear in the spreadsheet of the data set. Click on 'Stop' to end the data collection. DO NOT LEAVE THE LAST CUVETTE IN THE COLORIMETER
4. Copy the data set into your laboratory logbook or record it on your USB key. The data can then be processed directly in data logging program or it can be cut and pasted into a spreadsheet like MS Excel.
5. Present your data, both quantitative and qualitative, in an appropriate way, process the data to determine the effect of temperature on the membranes of this tissue. Discuss and evaluate your results.

Trouble shooting

As usual when evaluating investigations it is not sufficient to simply quote equipment malfunction.

However, if you get readings that appear strange or impossible try the following:

- Check the connections.
- Reboot the data logger.
- Check the liquid that you have sampled. Is it homogeneous and transparent?
- Check the cuvette that you are using is not dirty or wet on the outside.
- Check the cuvette is in the holder the right way round
- Check the cuvette holder in the colorimeter. Is it clean and dry?
- Try a different wavelength of light.

Note: You should try to keep your results between 0.050 – 0.550 absorbance. Outside this range the calibration curve is not linear. If your results are outside this range try a different wavelength (light source) or the liquid may need diluting by a known factor.

Syllabus reference: Sub-Topic 1.6 Skills

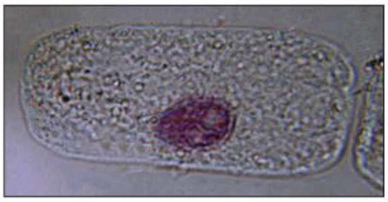
The organisation of a plant is essentially on a vertical axis (stem and roots) with branches. The plant develops by extending from the tips. Development requires cell division, then cell growth (elongation), followed by cell specialisation (differentiation). In plants, cell division occurs in zones called **meristems**. There is a meristem at the tip of the root.

Root tips are fixed to stop and preserve the cell activities. They are then sectioned longitudinally (sliced lengthwise) and stained. Each section, therefore, shows the state of the cells at one point in time.

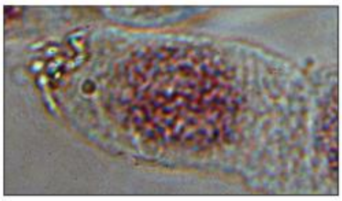
If the length of the cell cycle of the tissue is known, then by identifying and counting the stages of the cell cycle, it should be possible to make an estimate of the time the cells spend in each stage. This assumes that the cells are all randomly going through the different stages of the cell cycle.

Method

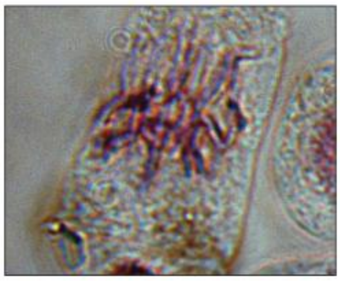
1. Prepare a slide of a root tip (or select a prepared slide) and place it on the microscope. Focus on low power then medium power, finally high power.
2. Observe the cells just behind the root tip and look for the different stages in the cell cycle. The photomicrographs of garlic root tip cells below will be helpful.



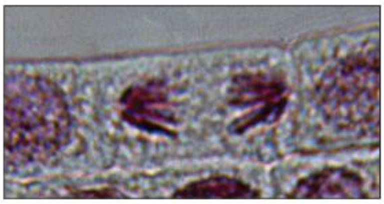
Interphase



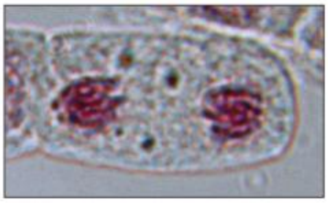
Prophase



Metaphase



Anaphase



Telophase

(All photos are copyright property of the author Paul Billiet.)

3. Systematically, observe at least 100 cells and record how many are in each stage of the cell cycle.
4. Present your results on the class bulletin board.
5. Search online for information on the duration of the cell cycle of the plant tissue you are observing. Estimate the time spent by the cells in each stage of the cell cycle.
6. Pool the data from the class and present your data in a suitable manner.
7. Estimate the mitotic index of this tissue = $\frac{\text{number of cells in mitosis}}{\text{total number of cells}}$

Syllabus reference: Sub-Topic 2.1 Skill

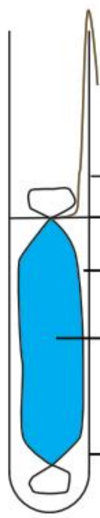
Dialysis allows the selective diffusion of molecules or ions of different sizes across a selectively permeable membrane that acts as a molecular sieve.

Materials

A solution containing a mixture of starch, glucose and sodium chloride
small beaker
15 cm of dialysing tubing
glass rod
cotton thread
large test tube
electronic water bath
dropping pipette
pasteur pipette
3 test tubes in a rack
test tube holder
stop clock or watch
wash bottle of distilled water
iodine solution
silver nitrate solution
Fehlings or Benedicts solution
safety glasses

Method

1. Wet the piece of dialysing tubing and open it using a round-ended glass rod. Tie a secure knot in one end of the tubing. Inject the mixture of starch, glucose and sodium chloride into the open end of the dialysing tubing using the dropping pipette.



2. When the tubing is nearly full, tie a thread of cotton tightly around the open end. Wash the outside of the tubing thoroughly, under the tap, and then wash it again using distilled water.
3. Suspend the dialysing tubing in the large test tube and fill it with distilled water up to the level of the knot (as shown opposite). Immediately start your stop clock and, using the Pasteur pipette, take three small samples from the distilled water in the large test tube. Pipette the samples into the three test tubes.
4. Test the samples for the presence of:

- STARCH by adding a few drops of iodine solution to a sample in a test tube. Positive result = blue/black colour.
- GLUCOSE by adding an equal volume of Fehlings or Benedicts solution to the sample in a test tube. Heat this mixture gently in a water bath at 80°C for a few minutes. Positive result = green, yellow or red, depending upon the amount of sugar present.
- CHLORIDES by adding a few drops of silver nitrate solution ($AgNO_3$) to the sample in a test tube. Positive result = a white precipitate.

Warnings: Very hot water can scald, use a test tube holder. Fehling's solution is caustic. Wear safety glasses. Iodine solution is an irritant and silver nitrate is toxic and an irritant. Wash off spills with water.

5. Leave the mixture for five minutes, take a second set of three samples and place them in clean test tubes. Repeat the test at 5-minute intervals for 25 minutes. Record your results in the form of a table. At the end of 25 minutes add several dropper-fulls of iodine solution to the large test tube and observe what happens. Record your results for this last part of the experiment and record your observations and conclusions on the whole experiment. What are the sources of error that you may have encountered?

Additional information

Particle	Diameter/nm
Chloride ion	0.362
Iodide ion	0.452
Glucose molecule	70.0
Starch molecule	17500.0

Syllabus reference: Sub-Topic 2.2 Application

It is well known that sweat is used to cool the body. The heat of the body excites the water molecules and their evaporation helps the body to cool down. How effective is water at cooling a body?

Materials

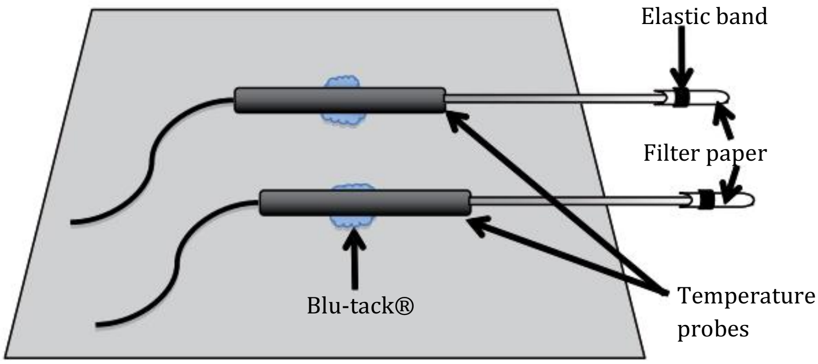
data logger
2 temperature probes
2 elastic bands
Blu-tack® or adhesive tape

filter paper
scissors
ruler

2cm of solvents in test tubes:
water
ethanol
propanone

Method

1. Wrap an elastic band around each temperature probe. Cut several pieces of filter paper into to squares 2cm × 2cm.
2. Roll a piece of paper around the tip of each of the temperature probes so they fit tightly and hold them in place using the elastic bands.
3. Plug them into two channels of a data logger and set to record the temperature every 0.5s for 3 min.



4. Stick a piece of Blu-tack® on the bench where a probe will be attached. Dip one of the probes into the liquid being tested and leave the other one dry. Stick the two temperature probes to the bench, as shown above. Start recording the temperature.

Warning: Ethanol and propanone are volatile and inflammable. Use in a well ventilated area. Do not expose to a naked flame.

5. What variables should be controlled during the course of the experiment?
6. When the run is complete, save the data and remove the filter paper. Clean and dry the probe that was dipped in liquid and repeat the experiment using a different liquid.

Some points for consideration

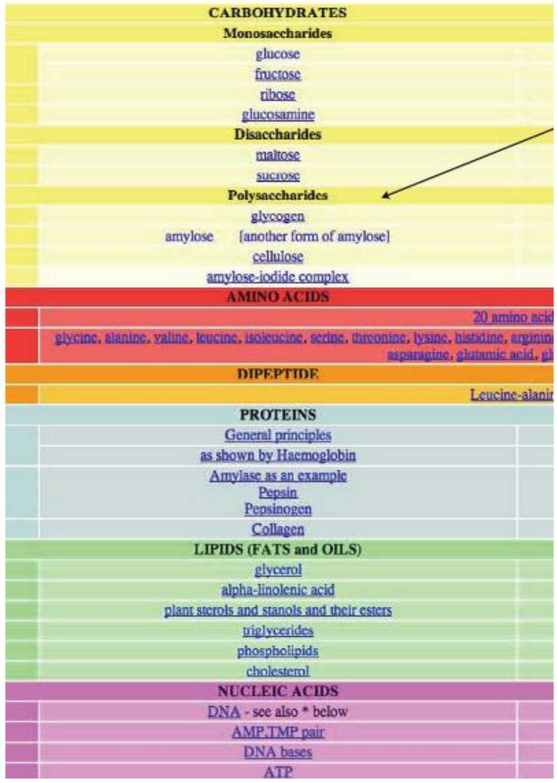
- How much did the temperature drop by for each liquid?
- Compare the size of each of these molecules. The bigger the molecule, the more energy it takes to make it evaporate.
- Compare the polarity of these molecules. Can they easily form hydrogen bonds? The stronger the hydrogen bonds between the molecules, the more energy it takes to make them evaporate.
- What are the advantages of animals and plants using water as coolant?
- How have animals and plants adapted to hot arid conditions where water is limiting?

Syllabus reference: Sub-Topic 2.3 Skills

Carbohydrates are one of the four fundamental groups of molecules that living systems use. The basic building block, the monosaccharides, can be used to build large macromolecules, the polysaccharides.

The structures of these molecules can be visualised on the Biotopics site using:

<http://www.biotopics.co.uk/JmolApplet/jcontentstable.html>



Select the following molecules on viewed in Jmol

Under Carbohydrates / Polysaccharides

- Cellulose
- Starches: amylose and amylopectin
- Glycogen

Note that these are only parts of the complete molecules. For example,a complete molecule of cellulose may contain 2 000 to 14 000 glucose units.

Observations

Compare these molecules: their size and shape.

Some points for consideration

Compare their functions in living organisms

Branched molecules are more compact than non-branched molecules.

Large molecules are less soluble than small molecules.

Plants store starches in plastids (amyloplasts and chloroplasts). These large insoluble molecules are built up from simple sugars and broken down by hydrolysis to simple sugars when they are needed.

Unlike plants, animals store glycogen, not starches. These are stored in relatively small amounts and their glycogen stores are not long term.

Compare the structures of glycogen and the starches from the point of view of energy storage

Why are animals inclined to store fats rather than polysaccharides?

Simple sugars would be more rapidly available for use than polysaccharides.

What is the advantage of storing carbohydrates as polysaccharides?

Cellulose is used in the cell walls of plants.

How is the structure of cellulose appropriate to its function in plant cell walls?

Syllabus reference: Sub-Topic 2.5 Skill

Hydrogen peroxide (H_2O_2) is a toxic substance produced by the metabolism of cells. To stop this substance building up, cells break it down into water and oxygen.

$H_2O_2 \longrightarrow H_2O + ½O_2\uparrow$

This reaction is catalysed by a group of enzymes called peroxidases. One well known peroxidase is catalase. The reaction is exothermic which means it releases heat energy.

As the reaction proceeds, the substrate will be used up, so the reaction will slow down. Therefore, where possible, when measuring the reaction rates, we take the initial reaction rates where the enzyme is performing at its maximum rate under the conditions it is exposed to.

Catalase is found in many tissues. In this case a suspension of yeast cells is used.

Method A: Using flotation to measure enzyme activity

A product of the reaction is oxygen gas. When the enzyme extract is soaked into a piece of filter paper and dropped into the hydrogen peroxide it will sink and then float back to the surface.

Materials

large test tubes in a stand	$100cm^3$ measuring cylinder	filter paper
yeast suspensions (1%)	$10cm^3$ syringes	safety glasses
10 volume H_2O_2	forceps	paper towel
$50cm^3$ beaker	hole punch	stop watch

1. Make up 100 cm^3 of a 1 Vol solution of H_2O_2 in a 100 cm^3 beaker.

Warning: Hydrogen peroxide is corrosive, avoid contact with the skin and the eyes and wear safety glasses. Wash off any spills with plenty of water.

2. Cut discs of filter paper using a hole punch. Holding a disk using forceps, dip it into the yeast suspension and drain off the excess liquid.
3. Put the filter paper into the beaker of 1 Vol H_2O_2. Observe what happens. Repeat it a few times with fresh discs soaked in yeast suspension.

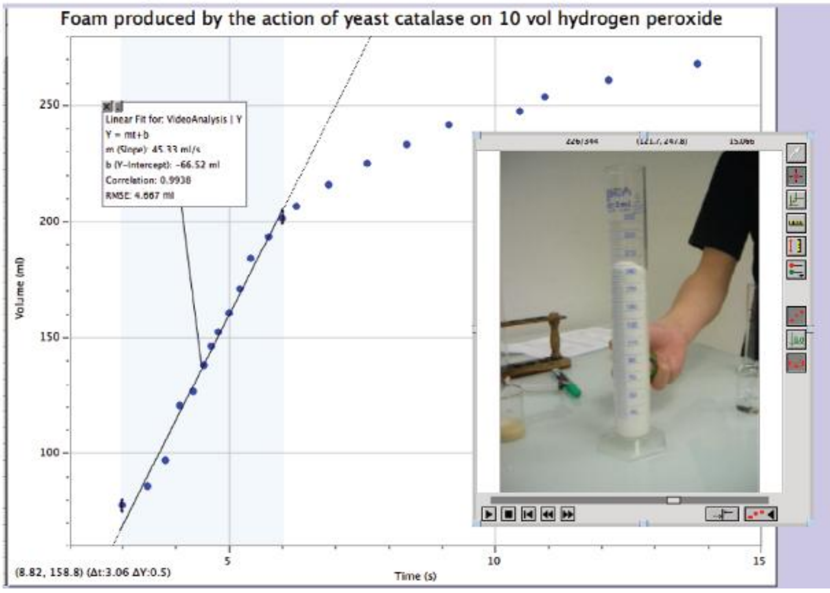
Method B: Using foam production to measure the enzyme activity

As seen in method A the production of the oxygen gas can be measured. A foam is produced when the reaction mixture is mixed with a detergent.

Materials

Yeast suspension 8%	$10cm^3$ syringes	Detergent in dropper bottle
20 volume and 10 volume H_2O_2	Safety glasses	Video camera
$250cm^3$ measuring cylinders	Paper towel	Computer with video capture

1. Add $10cm^3$ of hydrogen peroxide and two drops of detergent to a large measuring cylinder. Mix a little then add $10cm^3$ of 8% yeast suspension.
2. Record your observations.
3. The reaction can be filmed using a video camera and processed in a video capture program. The graduations on the side of the cylinder can be used a scale to calibrate the video analysis.



Method C: Using the heat released as an indicator of the reaction

The reaction is an exothermic reaction that releases heat. The rise in temperature of the reaction medium can be used as an indicator of the rate of reaction.

Materials

thermometer	10 volume H_2O_2	safety glasses
large test tubes in a stand	$10cm^3$ syringes	paper towel
yeast suspensions (8%)		

1. Measure $20cm^3$ 10 Vol H_2O_2 into a large test tube. Place the thermometer into the H_2O_2 and let it come to the temperature of the liquid.
2. Add $10cm^3$ of 8% yeast suspension and stir it in and observe what happens.

Method D: Using data logging and a temperature probe

Materials

Data logger
Temperature probe
Large test tube in a stand
Marker pen

Yeast suspension (8%)
20 volume H_2O_2
10cm^3 syringe or graduated pipette with pump

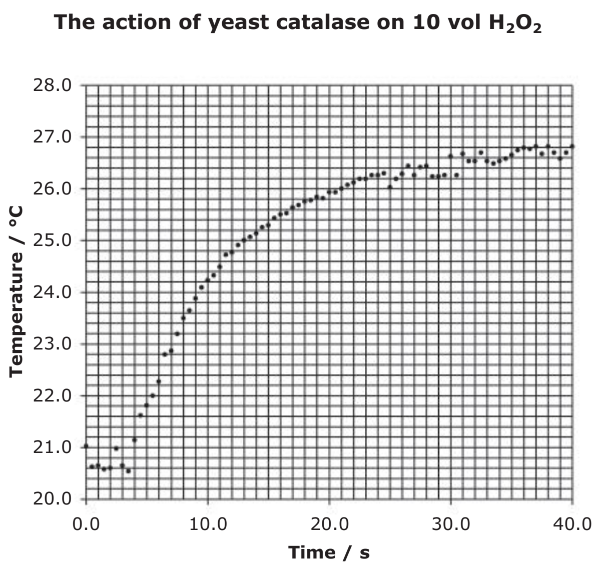
Measuring cylinder
Safety glasses
Paper towel

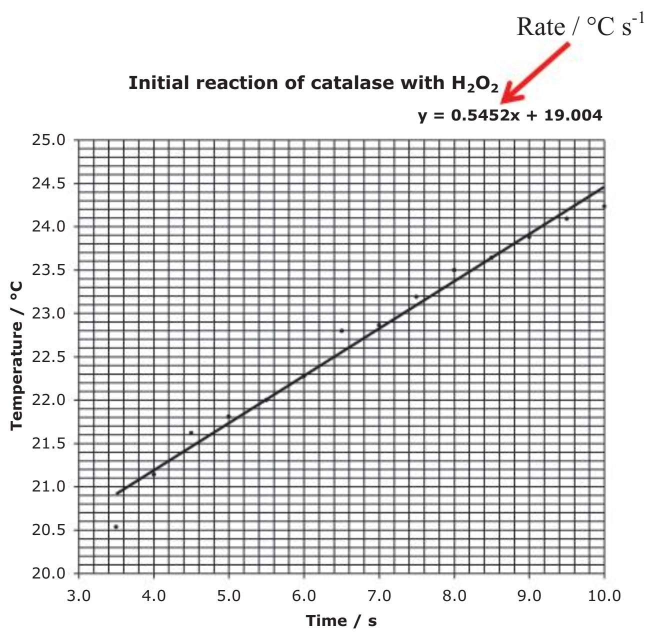
Method

1. Plug the temperature probe into the first channel of the data logger. The data logger should automatically recognise the temperature probe and it should not need calibrating.
2. Measure 20cm^3 H_2O_2 into a large test tube. Slide the temperature probe into to the H_2O_2 and let it come to the temperature of the liquid (let the probe equilibrate).

Recording and Analysing the data

1. Select a time-based mode in the data collection menu. The experimental length should be about 3 minutes for a trial run and the frequency of measurement should be 60 per minute.
2. Collect 10cm^3 of yeast suspension in a syringe. Stir the yeast suspension before taking the sample.
3. Begin data collection. A live graph should appear on the screen. Wait 15 seconds and add 10cm^3 yeast suspension and stir it in.
4. To save the data; transfer it to a USB key or send it home as an e-mail attachment.
5. Wash the probe and wipe it dry.
6. Analyse your data using a linear fit trend line of the initial reaction. The equation of this line will give the initial reaction rate. An example is given below.





Method E: Using data logging and a pressure sensor

A product of the reaction is oxygen gas. As this builds up in a closed vessel (e.g. a flask) the pressure can be used as an indicator of the rate of the reaction.

Materials

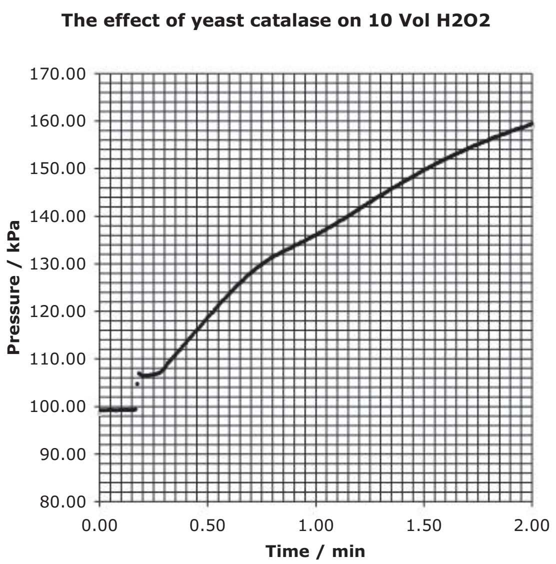
data logger,
pressure sensor syringe and connectors
$50cm^3$ flask in a clamp and stand
marker pen
yeast suspension (1%)
10 Vol H_2O_2
$5cm^3$ syringe or graduated pipette with pump
measuring cylinder
safety glasses
paper towel

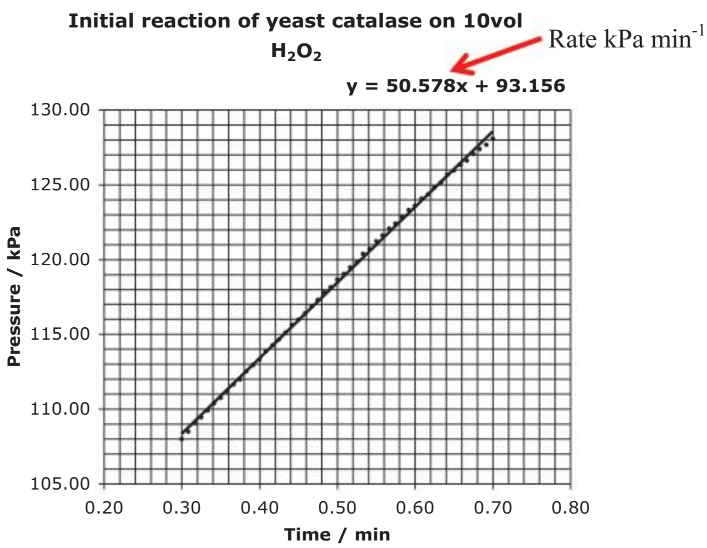
Method

1. Plug in the sensor to the first channel of the data logger. The data logger should automatically recognise the pressure sensor.
2. Measure $5cm^3$ yeast suspension into the small flask. Stir the yeast suspension before taking the sample.
3. Connect the pressure sensor to the rubber bung that fits in the flask, using a piece of plastic tubing.

Recording and Analysing the data

1. Select a time-based mode in the data collection menu. The experimental length should be about 3 minutes for a trial run and the frequency of measurement should be 60 per minute.
2. Collect $5cm^3$ of H_2O_2 in the syringe. Make sure the valve is turned off on the connector and screw the syringe into the connector on the rubber bung and attach the bung to the flask.
3. Begin the data collection. A live graph will appear on screen. Let it run for 10s, open the valve and inject the H_2O_2 into the flask, then close the valve. If the reaction is too vigorous dilute the hydrogen peroxide to 5 Vol and repeat.
4. Disconnect the syringe and wash it out.
5. Analyse your data using a linear fit trend line of the initial reaction. The equation of this line will give the initial reaction rate. An example is given below.





ENZYME ASSIGNMENT

Design investigations to determine the effects of temperature, pH and substrate concentration on the activity of catalase.

You will find that some of the protocols proposed are appropriate for a factor but not others.

Design

1. Establishing the theoretical background. This process will require some research on the properties of your enzyme and its source.
2. State a focussed research question.
3. Write a hypothesis (a justified prediction).
4. Clearly identify the variables (the 'independent' or manipulated variable, 'dependent' or measured variable and the important 'control' variables).
5. State the significance of each of the control variables and which variables cannot be controlled but need to be monitored.

Method

1. What equipment and materials will you need? This may be best described using a labelled diagram of the experimental setup.
2. Consider any safety factors.
3. Explain exactly how the independent variable will be changed (range and intervals) and how the dependent variable will be measured and how frequently.
4. Explain how you intend to control all the other variables (how will you make your experiment a fair test) or monitor them if they cannot be controlled. Perhaps a control experiment will be needed.

   (Note: 'Controlled variables' and a 'control experiment' are not the same thing).
5. Using a water bath to control temperature is best but it may be possible for the enzyme to operate at room temperature. Nevertheless, the room temperature should be monitored to see if it varies significantly during the experiment.
6. To control pH you should consider using buffers. Buffers resist changes in pH, acids such as hydrochloric acid may be diluted to appropriate pH values but they will not remain stable once the reaction starts. Solutions such as lemon juice and vinegar are inappropriate when pH needs to be controlled.

Syllabus reference: Sub-Topic 2.5 & 2.8 Applications

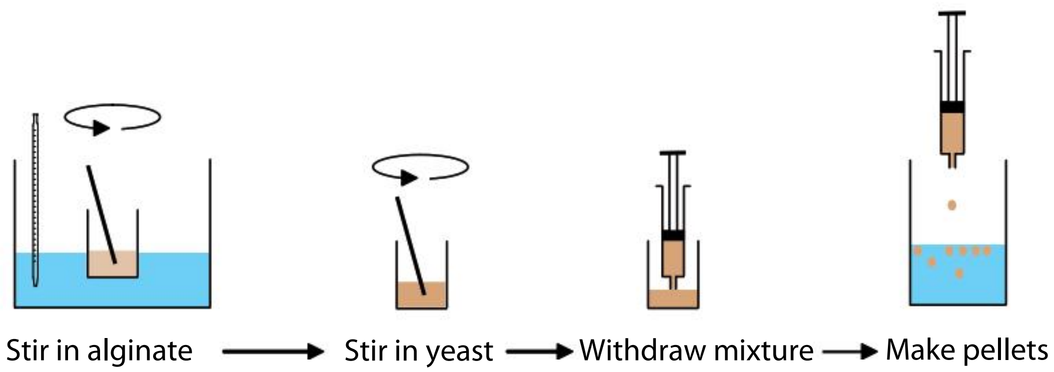
Sodium alginate is a gel that forms solid pellets when it is dripped into a solution of calcium chloride. Enzymes, or cells containing enzymes, which control reactions can be mixed in with the alginate gel so that they are immobilised. Thus, when the reaction is completed they can be recuperated and used again. In the biotechnology industries, where the strains of microbes or the types of enzymes may be very expensive, producing reusable immobilised material is an important technique.

Materials

balance and spatula
2g sodium alginate powder
100cm^3 distilled water in 250cm^3 beaker
water bath at 50°C
glass rod
2g baker's yeast
50cm^3 calcium chloride solution in 100cm^3 beaker
filter funnel and filter paper
2 conical flasks 250cm^3
sterile water wash bottle
50cm^3 10% sucrose solution
Clinistix glucose testing strips
scissors
stop watch
10cm^3 syringe

Method

1. To prepare sodium alginate pellets, heat 100cm^3 distilled water in a beaker to 50°C in a water bath. Slowly add 2g of sodium alginate and then stir for 5 to 10 min.
2. Cool the beaker of alginate to 35°C and add 2g of yeast. Stir in well to mix.
3. To make the pellets, withdraw the alginate-yeast mixture into a syringe and drip it slowly into a beaker of calcium chloride solution. Stir the solution whilst the pellets are being made. Leave the pellets in the calcium chloride for 5min, then filter into a conical flask and recuperate the calcium chloride. Wash the pellets with sterile water.



Hydrolysis of sucrose

4. Measure out 50cm^3 of sucrose solution into a flask. Add 30 alginate-yeast pellets. Mix and test for glucose using a *Clinistix* tester. Repeat the test every 10min for 30min.

Fermentation of sucrose

5. Place 10 alginate-yeast pellets into the sucrose solution. They should sink. As the yeast cells begin to ferment the sucrose, carbon dioxide gas will be produced. The pellets will float to the surface of the solution. Observe and record the time taken for each pellet to rise to the surface.

Design

6. On the basis of this experiment, plan an investigation using immobilised yeast cells, bacteria, algae or pure enzymes.

Syllabus reference: Sub-Topic 2.8 Skill

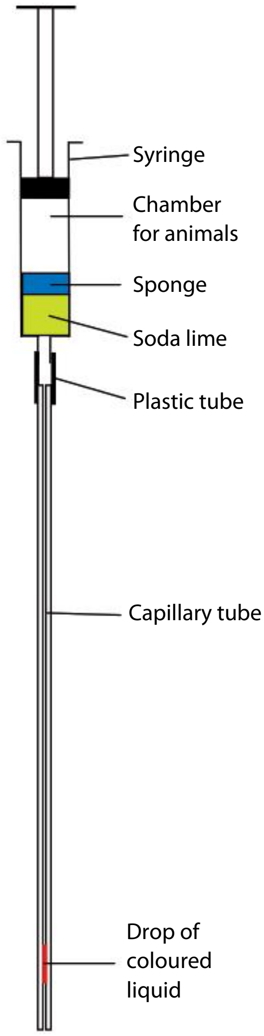
Soda lime is a mixture of calcium oxide granules and sodium hydroxide. It will absorb carbon dioxide. It can be used in a simple apparatus to calculate the respiration rate of a small organism, such as a meal worm, blow fly larvae, a germinating seed or even a piece of living tissue.

Mealworms (*Tenebrio molitor*) are insects, they are the larvae of a beetle.

Materials

2 plastic syringes ($10cm^3$)
2 pieces of plastic tubing 2cm long
2 pieces of 1mm bore capillary tubing 30cm long
Ink or methylene blue solution
Marker pen
Ruler
Stop watch
Electronic balance
3 to 5g meal worms
Soda lime
Clamp and stand

Method



The complete apparatus is set up as shown opposite:

Set up the apparatus as follows:

1. Remove the plunger from one of the syringes. Use a spatula to add the soda lime into the syringe then slide the sponge in also to cover the soda lime.
2. Measure the exact mass of the animals. Place them in the syringe. Push the plunger of this syringe up to the $10cm^3$ mark. Leave the apparatus for 2 minutes. The syringe can be supported in a burette stand.

Warning: Soda lime is caustic, avoid contact with the skin. Return it to the correct container for disposal after the experiment.

3. Set up a control using the materials provided.
4. Add a drop of coloured liquid (e.g. methylene blue or ink) to the end of each capillary tube and attach them to the syringes using the short piece of plastic tubing.
5. Mark the position of the coloured liquid and start the stop clock.
6. Using the ruler, measure the distance travelled by the drop of coloured liquid at one-minute intervals for 10 minutes, in both pieces of apparatus, in the experimental apparatus and the control. Record these results in a table.

Meal worm larvae and pupa

Meal worm pupa

Meal worm adult

Analysis

1. Calculate the average rate of respiration of these animals over the 10 minute period in: mm min^{-1}
2. What is the gas that the animals are absorbing?
3. What is the gas they are producing?
4. The diameter of the capillary tube is 1mm. Therefore, the volume of gas breathed in by these animals per minute can be calculated from the formula:

   Volume = $\pi r^2 h$

   Where: $\pi = 3.142$ r = 0.5mm (the radius of the capillary tube)

   h = the average distance travelled by the drop of coloured liquid in mm min^{-1}
5. Calculate the volume of gas absorbed per minute by your animals: $mm^3\ min^{-1}$
6. Now calculate the volume of gas inspired by the meal worms as: $mm^3\ g^{-1}\ min^{-1}$
7. Discuss and criticise this method for calculating the rate of respiration of an organism.

Ethics: Return the animals to their container

ETHICS

Some points for consideration

- If you were using a similar apparatus to measure the respiration rate of a green plant, what modifications would you need to make to the apparatus?
- What might happen if the rate of metabolism of the organism varies during the experiment?
- What might happen if the organisms start to respire anaerobically?
- Do different stages in the life cycle of an insect respire in the same way?

Research

- What is the Respiratory Quotient (RQ)?
- Could you use the above apparatus to measure an RQ value?
- How would you modify the apparatus so that the RQ values can be calculated?
- Would the energy substrates that the organism metabolises influence the results of the RQ values?

Additional information (equations)

Complete oxidation of sugar:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$

Complete oxidation of a fat:

$C_{18}H_{34}O_2 + 25.5O_2 \rightarrow 18CO_2 + 17H_2O$

Anaerobic respiration of a sugar to lactic acid:

$C_6H_{12}O_6 \rightarrow 2CH_3CH(OH)COOH$

Syllabus reference: Sub-Topic 2.9 Skill

Leaf pigments are organic compounds that absorb particular wavelengths of light. Many of them are involved in photosynthesis.

Materials

Extraction:	**Separation**:
mortar and pestle + spatula of fine sand Scissors	Pasteur pipette, 20cm^3 of solvent
funnel + filter paper	large test tube + bung
test tube + test tube rack	10cm^3 pipette + bulb
10cm^3 of pure ethanol	Whatman's paper no.1 (chromatography paper)
green leaf material	scissors, pin, hair dryer, ruler

Method

Extraction with alcohol

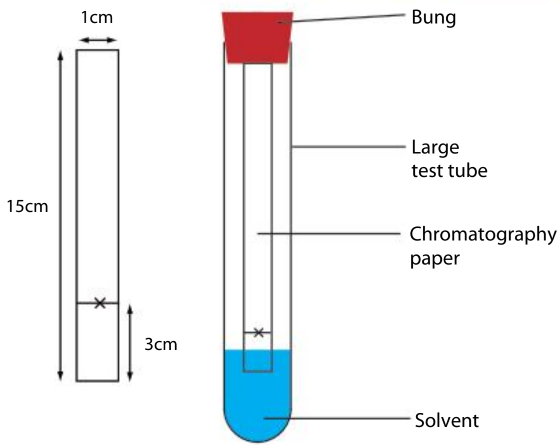
Warning: The ethanol is volatile and inflammable.
Use in a well ventilated area. Avoid contact with the eyes.
Wear gloves and eye protection.

1. Put one spatula of sand in the mortar and add 5cm^3 of 90% ethanol.
2. Chop a few leaves into small pieces and place them in the mortar. Grind the leaves, the pigment solution obtained should be very dark green.
3. Filter the contents of the mortar into a test tube.

Separation by paper chromatography

Warning: The solvent is volatile and inflammable.
Use in a well ventilated area. Avoid contact with the eyes.
Wear gloves and eye protection.

4. Cut a strip of chromatography paper about 15cm long (the length of the large test tube) and 1cm wide. Hold the paper by the edges. Draw a pencil line 3cm from the bottom of the paper. In the middle of this line mark a cross. *Refer to the diagram opposite.*
5. Using the pipette, put 3cm^3 of solvent into the large test tube. Make sure that the solvent does not go on the sides of the cylinder. Put in the bung to allow the air inside the cylinder to become saturated with the solvent.
6. Using the Pasteur pipette put a tiny spot of the pigment solution onto the marked cross. Dry this spot with the hair dryer. Repeat this eight times to obtain a small concentrated spot.



7. Remove the bung from the test tube. Fold the chromatography paper over and stick a pin into the bung through the paper. Suspend the paper in the test tube, the solvent should be below the line marked 3cm from the bottom of the paper. Make sure that the chromatography paper does not touch the sides of the cylinder. *See diagram above.*
8. Observe what happens for 1-2 minutes, then leave this aside in the dark for 1 hour.

Analysis of the chromatogram

As soon as the chromatogram is removed from the measuring cylinder, draw a line at the highest point the solvent has risen. This is the solvent front.

Mark the highest and widest points seen for each pigment that has been separated out by drawing a small cross at these points.

Calculate the Rf values and compare them to the values given in the table below.

$$R_f = \frac{\text{Distance moved by the pigment}}{\text{Distance moved by the solvent}}$$

Pigment	R_f value	Colour
Chlorophyll b	0.45	Green
Chlorophyll a	0.65	Green-blue
Xanthophyll	0.71	Yellow
Carotene	0.95	Orange

Discuss and criticise this investigation.

Some points for consideration

- Why did you grind the leaves to extract the pigment? Are you certain to extract all the pigments in this way? Explain your answer.
- How many pigments did your green leaf contain?
- Why do the different pigments separate out along the chromatogram?
- Can you be certain to have observed all the different pigments of chlorophyll?
- Is it best to identify the pigments by colour or by R_f values? Justify your answer.

Research

- Explain why the leaf is green despite the orange and yellow pigments that it contains.
- Explain why a plant has so many different pigments.

To investigate further

- Leaf pigments in leaves during Autumn
- Leaf pigments in leaves at different heights on a tree or bush
- Leaf pigments in leaves kept in the sun and in the shade
- Leaf pigments in plants subjected to mineral deficiencies (e.g. Iron and Magnesium)
- Compare the separation of plant pigments using thin layer chromatography (TLC) and paper chromatography. For TLC, photosynthetic pigments can be separated using the solvent 2 parts of hexane : 1 part ethyl ethanoate.

Warning: Hexane is inflammable work in a well ventilated area.

- Other leaf pigments; some leaves have red pigments. These are usually anthocyanins or betalains. They do not move in the solvents that transport photosynthetic pigments. For these pigments use 4 parts butan-1-ol : 1 part glacial ethanoic acid : 5 parts water. Betalains are soluble in water and very insoluble in methanol and give low R_f values. Anthocyanins are soluble in methanol but not water and give higher R_f values.

Warning: Butan-1-ol is toxic and inflammable. Ethanoic acid is an irritant. Use safety glasses and work in a well ventilated area.

Syllabus reference: Sub-Topic 2.9 Skill

The spectroscope, due to a prism or a diffraction grating, separates out the white light into its constituent colours. The spectrum is seen when you look into the spectroscope. By putting a specimen tube containing a pigmented solution in between the light entering the apparatus and the prism, a different spectrum is obtained.

Materials

Extraction of pigments

mortar and pestle + spatula of fine sand

funnel + filter paper

test tube + test tube rack

$10cm^3$ of 90% ethanol,

spinach or parsley

Analysis of extract

spectroscope

specimen tubes

pigment extract

Method

Extraction

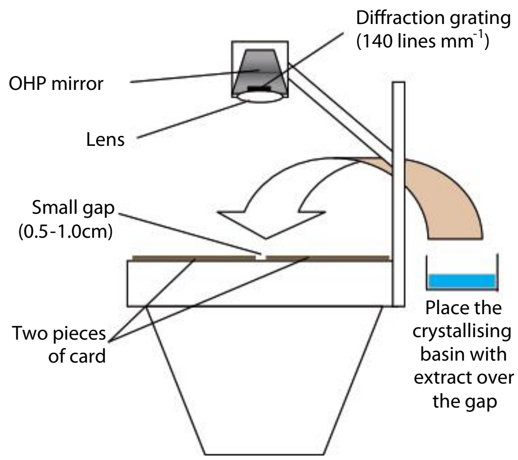
1. Put one spatula of sand in the mortar and add $5cm^3$ of 90% ethanol.
2. Tear a few leaves into small pieces and place them in the mortar. Grind the leaves, the pigment solution obtained should be very dark green.
3. Filter the contents of the mortar into a test tube.

Analysis

1. Fill a small specimen tube with the chlorophyll pigment solution and slot the tube into the spectroscope.
2. Hold the spectroscope towards a source of light.
3. Prepare the control that may be placed at the same time into the spectroscope.
4. Draw the two spectra that you see when you look into the eyepiece.
5. Comment on the significance of your observations.

Using the overhead projector as a spectroscope

Set up the overhead projector as shown in the diagram.



Using a spectrometer

If a spectrometer is available it can be used like a colourimeter than scans the whole width of the visible spectrum. This will give a graphical read out of the absorption spectrum of the leaf extract.

Fluorescence

Under bright light the chlorophyll extract gives out red light. In the absence of electron acceptors, the electrons are exited out of their orbitals by the light. They fall back to their lowest energy levels giving out light energy. The effect is particularly noticeable under 'black' light (UV light source).

Warning: UV light is harmful to skin and especially eyes.
Do not expose these areas directly to a UV light source.

Some points for consideration

From your spectroscope results, deduce what properties the leaf pigments have.

To investigate further

- Leaf pigments in leaves during Autumn.
- Leaf pigments in leaves at different heights on a tree or bush.
- Leaf pigments in leaves kept in the sun and in the shade.
- Leaf pigments in plants subjected to mineral deficiencies (e.g. Iron and Magnesium).
- Analysing the absorption spectra from the different pigments separated by chromatography.

New growth of pine needles clearly pigmented differently from that of the previous year

Syllabus reference: Sub-Topic 2.9 Skill

The equation shows that the amount of oxygen given off equals the amount of carbon dioxide taken up by the plant.

$$nCO_2 + nH_2O \underset{chlorophyll}{\overset{light}{\rightleftharpoons}} (CH_2O)n + nO_2$$

It is therefore possible to estimate the rate of photosynthesis by measuring the rate of oxygen production or the rate of carbon dioxide consumed by the plant. Several methods are outlined on the following pages.

Oxygen does not dissolve easily in water, so many water plants release oxygen as a gas. Carbon dioxide dissolves very easily in water, so a change in the level of CO_2 will affect the pH of the water surrounding the water plant.

Method A: Determining oxygen production in a water plant directly

Materials

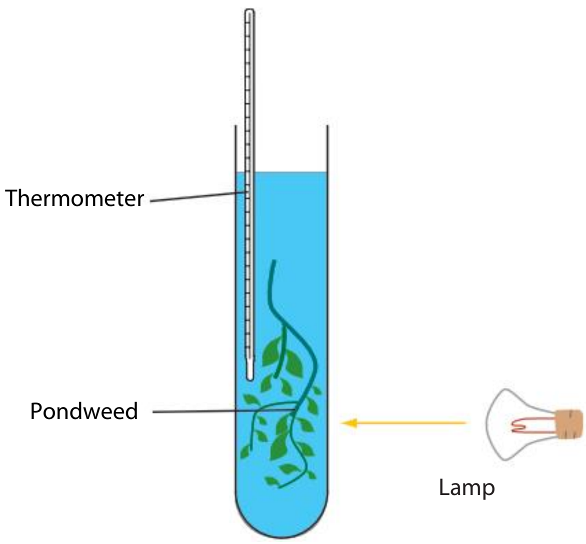
large test tube	lamp with 60 Watt bulb	stop watch
test tube rack	ruler	water enriched with carbon dioxide
glass rod	razor blade	1 branch of a water plant

Method

1. Take a branch of pondweed that is healthy and green. Using the razor blade cut the branch 5cm from the terminal bud.

Warning: Sharp instrument, handle with care

2. Half fill the test tube with a solution of hydrogencarbonate.
3. Using a glass rod, push in the branch, terminal bud end first, so that the cut end is 1cm below the surface of the water (see diagram below).
4. Illuminate the tubes with the lamp and leave for five minutes. Ensure that the plant is bubbling steadily after this time. If it is not, change the plant and start again.
5. Count the bubbles over a regular period of time. Repeat the measurement. Leave the plant to adapt for 2min between each change of conditions.



Method B: Determining oxygen production by the displacement of a liquid

Materials

- 10cm^3 syringe,
- 2cm tubing attached to the syringe,
- 30cm capillary tubing (1mm bore)
- 250cm^3 beaker
- glass rod
- lamp
- stopwatch
- razor blade
- marker pen
- clamp and stand
- 20cm^3 of 0.1 mol dm^{-3} hydrogencarbonate solution,
- distilled water
- 2 branches of pond weed

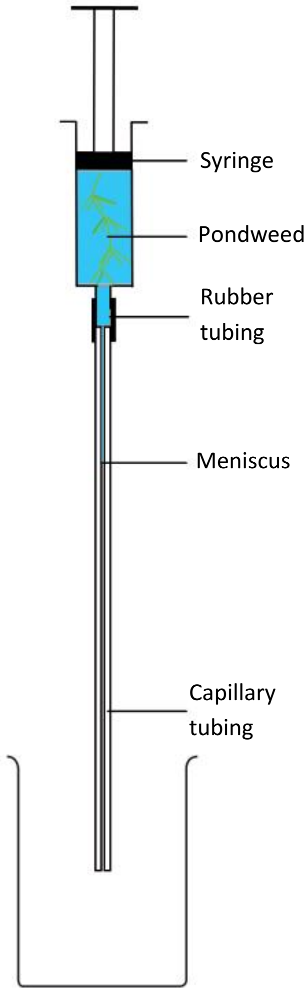
Method

1. Using a razor blade cut two branches of pondweed 5cm from the buds. Using the glass rod, push the branches, bud end first, into the syringe, with the cut ends, towards the piston of the syringe.

Warning: Sharp instrument, handle with care.

SHARP

2. Clamp the syringe to the support stand, about 40cm above the bench. Place a small beaker on the bench just below the syringe to catch drips
3. Cover the end of the syringe with your finger. Remove the piston and fill the syringe with the hydrogencarbonate solution. Replace the piston and push it up to the 10cm^3 graduation. Any unwanted liquid is collected in the beaker.
4. Attach the capillary tube to the end of the syringe using the 2cm of tubing. Make sure that there are no leaks. Gently push in the piston until a meniscus appears just under the bottom of the tubing. Mark the level of the meniscus with a marker pen.
5. Position the lamp 20cm from the apparatus and make observations on the level of the meniscus.



Method C: Determining oxygen production using a pressure sensor

A product of the reaction is oxygen gas. As this builds up in a closed vessel (e.g. a large test tube) the pressure can be used as an indicator of the rate of the reaction.

Materials

data logger	pond weed	glass rod
pressure sensor and connectors	50cm^3 $NaHCO_3$ 0.1 mol dm^{-3}	thermometer
large test tube	bench lamp (60W)	2m tape measure
clamp and stand		

Method

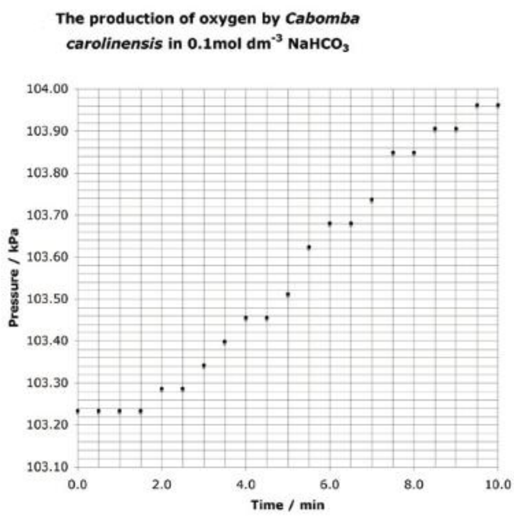
1. Plug in the pressure sensor to the first channel of the data logger. The data logger should automatically recognise the pressure sensor.
2. Using a razor blade cut a branch of pondweed 10cm from the bud. Using the glass rod, push the branch into the syringe. Fill the test tube with hydrogencarbonate solution.

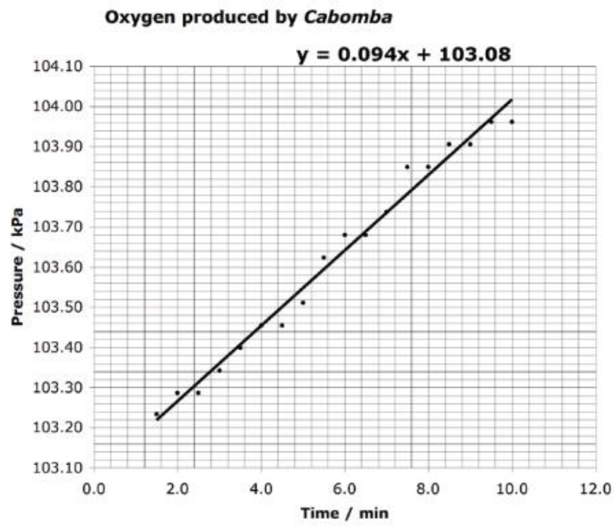
Warning: Sharp instrument, handle with care

3. Connect the pressure sensor to the rubber bung that fits in the test tube using a piece of plastic tubing. Mop up any spilt liquid that the bung displaces.
4. Put a bench lamp 20cm away facing the test tube. Leave the plant 2min to adapt. Verify the temperature of the liquid in the test tube after each run. You may need to consider controlling the temperature.

Recording and Analysing the data

1. Select a time-based mode in the data collection menu. The experimental length should be about 10 minutes for a trial run and the frequency of measurement should be 60 per minute. Begin the data collection and a live graph will appear on screen.
2. Analyse your data using a linear fit trend line of the reaction where the line becomes regular. The equation of this line will give the reaction rate. An example is given below.





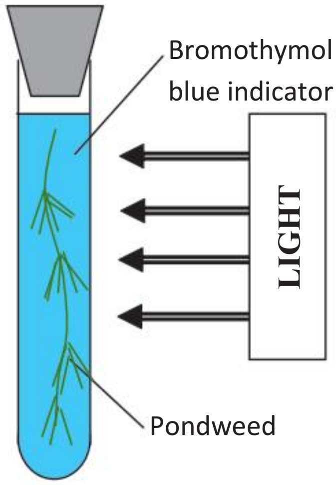
Method D: Determining the consumption of carbon dioxide by a water plant using a pH indicator

Bromothymol blue is blue when there is no CO_2 (above pH 7.6), green when there is a little CO_2 (between pH 6 and 7.6) and yellow when there is a lot of CO_2 (below pH 6).

Method

Warning: Sharp instrument, handle with care.

1. Take a branch of pondweed that is healthy and green. Using the razor blade, cut the branch 5cm from the terminal bud.
2. Fill the test tube with bromothymol blue indicator to 3cm from the top. Blow into the indicator using the drinking straw until it is green. Using a glass rod, push in the pondweed so that it is 1cm below the surface of the water (see diagram opposite).
3. Position the lamp at 20cm to illuminate the apparatus and observe at intervals over 30 min.



Elodea canadensis flowering

Method E: Determining carbon dioxide absorption using a data logger and pH probe

Calibrating the pH probe

Materials

data logger
pH probe
3 small beakers
buffers pH 4 and pH 7
distilled water wash bottle
pond weed

Method

1. Plug in the probe to the first channel of the data logger. The data logger should automatically recognise the pH probe and it will need to be calibrated.
2. Prepare a beaker with pH 4 buffer and another with pH 7 buffer. Carefully remove the container of electrolyte from the end of the probe and store it in a safe place. Wash the probe with the wash bottle.
3. Follow the steps to calibrate the pH probe with both the pH 4 and the pH 7 buffers, washing the probe with distilled water between the different buffers and at the end of the calibration.
4. Replace the probe in the electrolyte until you need it for measurements.

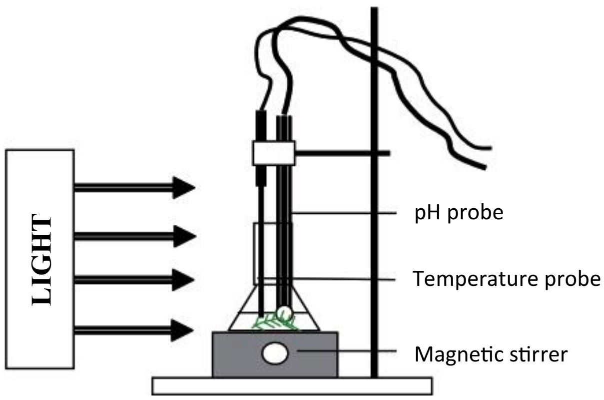
Using the pH probe to measure photosynthesis

Materials

Data logger
pH probe
Temperature probe
Conical flask 50cm^3
Magnetic stirrer
Magnetic flea
Stand and clamp
100cm^3 beaker
Marker pen
Stop watch
Bench lamp 60W
Tape measure 2m
pond weed

Method

1. Prepare a flask with a magnetic stirrer. Add 50cm^3 of tap water and a healthy piece of pondweed (approximately 10cm long).
2. Remove the probe from the electrolyte and clamp it in a stand so that it dips into the liquid in the flask. Add the temperature probe to the second channel of the data logger and clamp it so that it also dips in the liquid.
3. Select a time-based mode of data collection. The experimental length should be about 10 minutes for a trial run and the frequency of measurement should be 60 per minute.



4. Place a bench lamp 20cm from the flask. Begin recording the pH. The temperature of the liquid should be monitored at the same time to ensure that the lamp is not heating it up.
5. When the run is complete, remove the probe and wash it off. Clean out the flask, recuperate and wash off the magnetic flea. Store your latest data run.
6. Analyse the data by using a spread sheet program to draw a linear fit on the reaction rate.

Assignment

Use your experience gained from the methods given below, to design an investigation into the effect of an environmental factor on the photosynthesis of a water plant.

You may use other materials that you know are available in the school laboratories.

Your method should build upon these simple protocols. You will find that some of the following protocols are appropriate for a limiting factor but not others.

Design

- Establish the theoretical background. This will require some research on your species of plant.
- State a focussed research question.
- Write a hypothesis (a justified prediction).
- Clearly identify the variables (the independent or manipulated variable, dependent or measured variable and the important control variables).
- State the significance of each of the control variables and which variables cannot be controlled but need to be monitored.

Method

- What equipment and materials will you need? This may be best described using a labelled diagram of the experimental setup.
- Consider any safety factors.
- Explain exactly how the independent variable will be changed (range and intervals) and how the dependent variable will be measured and how frequently.
- Explain how you intend to control all the other variables (how will you make your experiment a fair test) or monitor them if they cannot be controlled. Perhaps a control experiment will be needed.

(Note: Controlled variables and a control experiment are not the same thing)

Notes

Using a water bath to control temperature is best but it may be possible for the plant to operate at room temperature. Nevertheless, the room temperature should be monitored to see if it varies significantly, especially if a bench lamp is close to the apparatus.

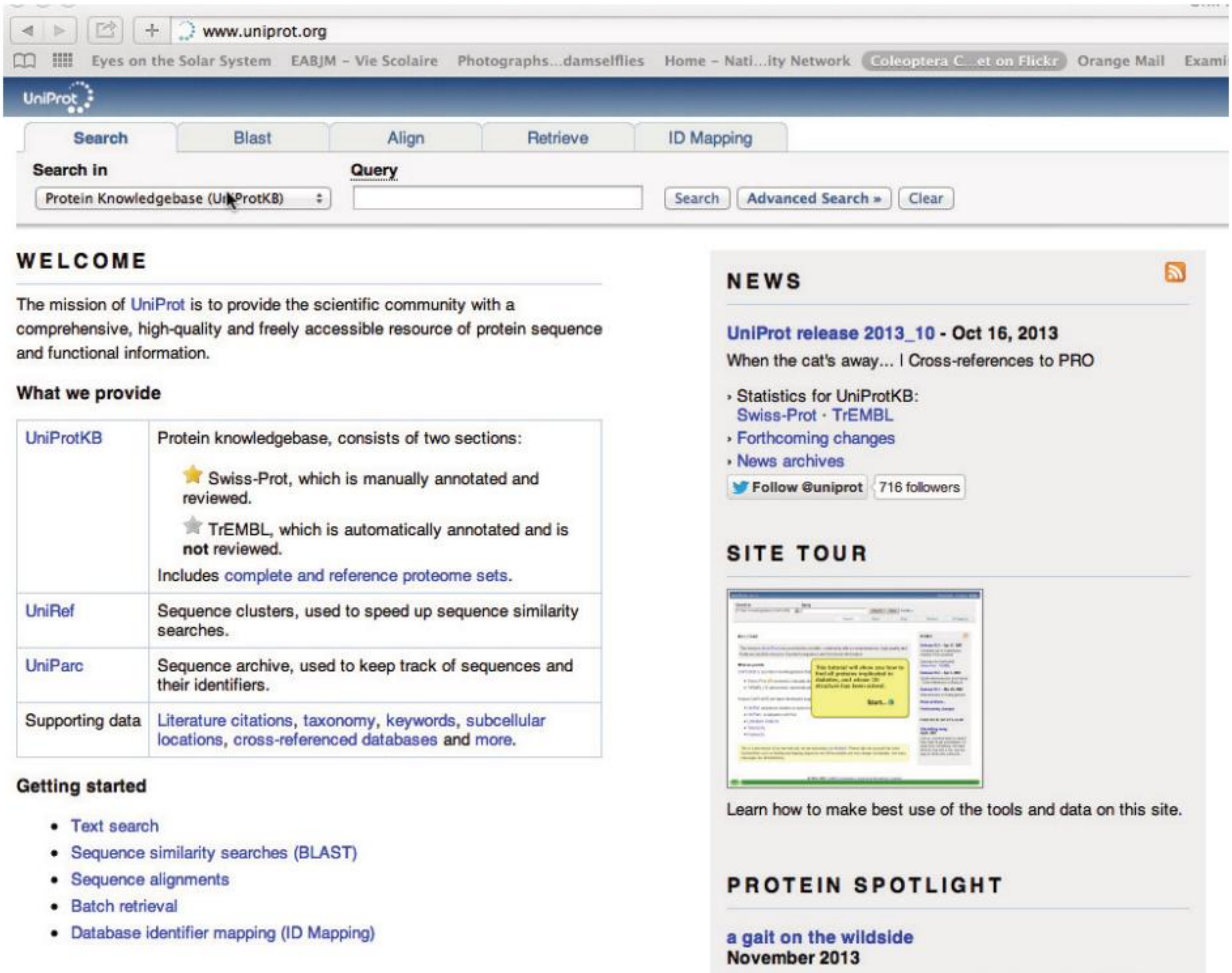
To control pH you should consider using buffers. Buffers resist changes in pH, acids such as hydrochloric acid may be diluted to appropriate pH values but they will not remain stable once the reaction starts. Solutions such as lemon juice and vinegar are inappropriate when pH needs to be controlled.

Syllabus reference: Sub-Topic 3.1 Skill

Thanks to automated gene sequencing there are large databases that permit researchers to find out about the structure of different proteins. Here we shall investigate the structure of a well known protein; beta hemoglobin.

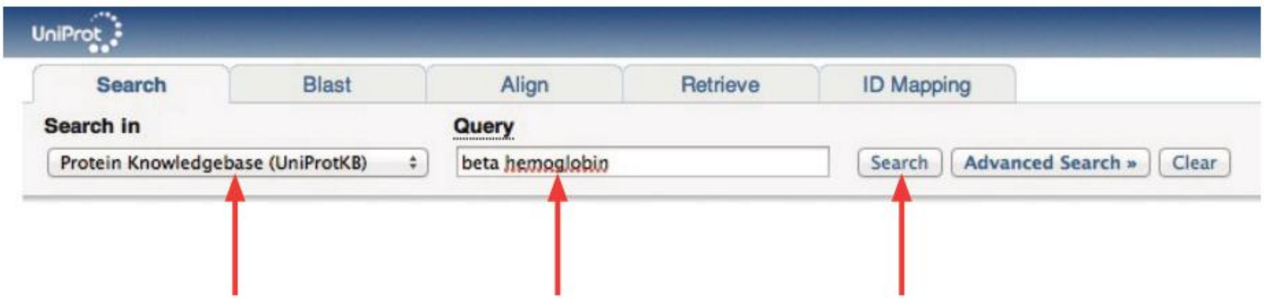
Open UniProt database: <http:/www.uniprot.org>

Select Protein Knowledgebase (UniProtKB)



Type in **Query**: (start with beta hemoglobin)

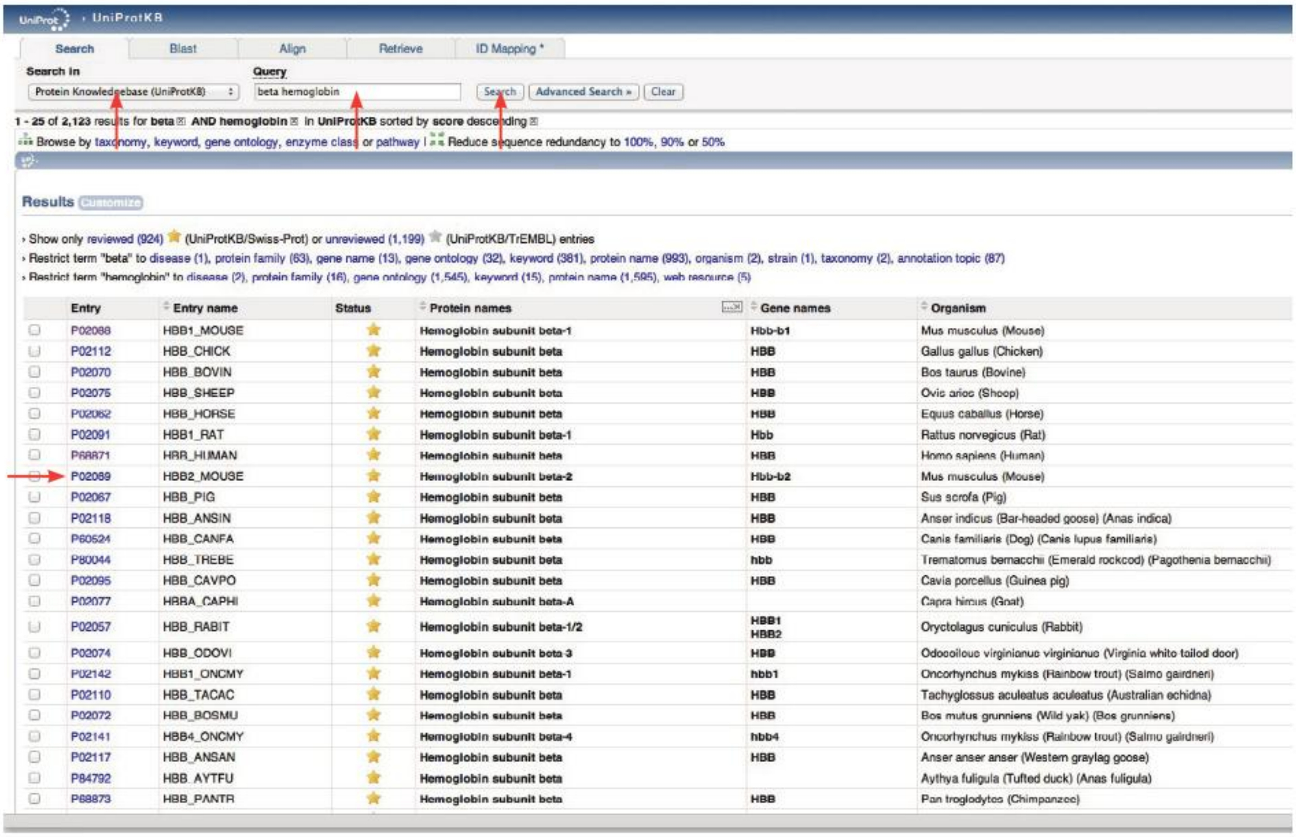
Then click on **Search**



A list of species for which the sequence of amino acids is known will appear.

To see a sequence click on the **Entry**.

Try the house mouse (*Mus musculus*)



UniProt › UniProtKB

Search | Blast | Align | Retrieve | ID Mapping *

Search in: Protein Knowledgebase (UniProtKB) — Query: beta hemoglobin — Search | Advanced Search » | Clear

1 - 25 of 2,123 results for beta AND hemoglobin in UniProtKB sorted by score descending

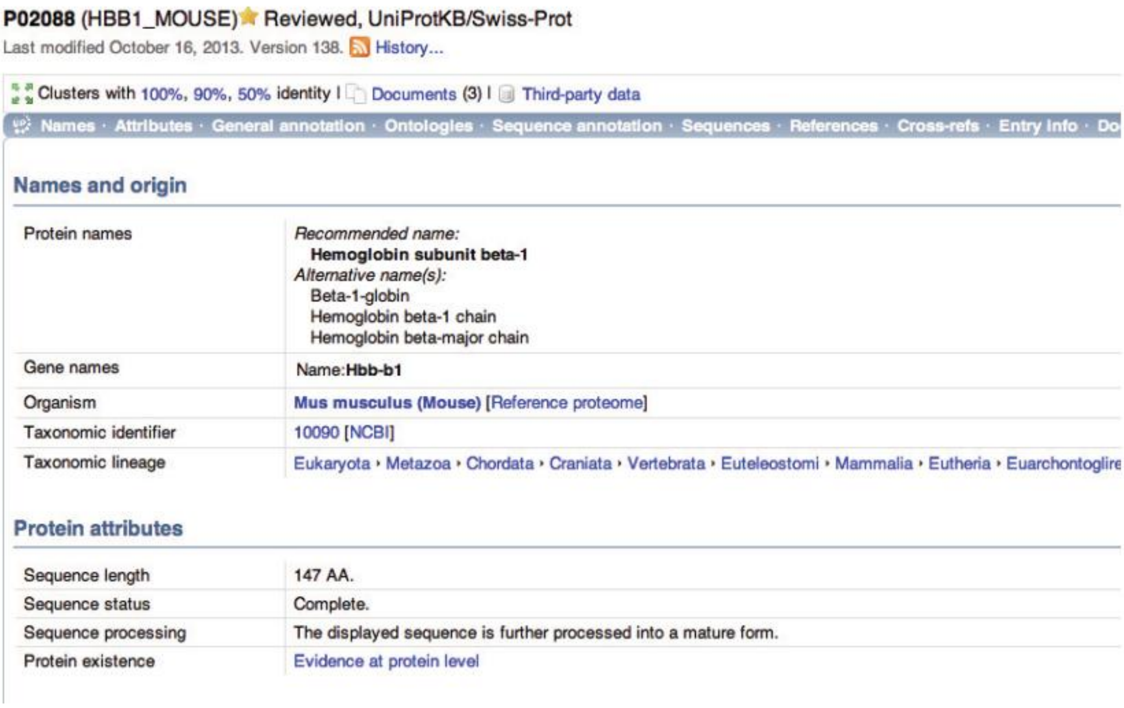
Browse by taxonomy, keyword, gene ontology, enzyme class or pathway | Reduce sequence redundancy to 100%, 90% or 50%

Results Customize

› Show only reviewed (924) (UniProtKB/Swiss-Prot) or unreviewed (1,199) (UniProtKB/TrEMBL) entries
› Restrict term "beta" to disease (1), protein family (63), gene name (13), gene ontology (32), keyword (381), protein name (993), organism (2), strain (1), taxonomy (2), annotation topic (87)
› Restrict term "hemoglobin" to disease (2), protein family (16), gene ontology (1,545), keyword (15), protein name (1,595), web resource (5)

Entry	Entry name	Status	Protein names	Gene names	Organism
P02088	HBB1_MOUSE	★	Hemoglobin subunit beta-1	Hbb-b1	Mus musculus (Mouse)
P02112	HBB_CHICK	★	Hemoglobin subunit beta	HBB	Gallus gallus (Chicken)
P02070	HBB_BOVIN	★	Hemoglobin subunit beta	HBB	Bos taurus (Bovine)
P02075	HBB_SHEEP	★	Hemoglobin subunit beta	HBB	Ovis aries (Sheep)
P02062	HBB_HORSE	★	Hemoglobin subunit beta	HBB	Equus caballus (Horse)
P02091	HBB1_RAT	★	Hemoglobin subunit beta-1	Hbb	Rattus norvegicus (Rat)
P68871	HBB_HUMAN	★	Hemoglobin subunit beta	HBB	Homo sapiens (Human)
P02089	HBB2_MOUSE	★	Hemoglobin subunit beta-2	Hbb-b2	Mus musculus (Mouse)
P02067	HBB_PIG	★	Hemoglobin subunit beta	HBB	Sus scrofa (Pig)
P02118	HBB_ANSIN	★	Hemoglobin subunit beta	HBB	Anser indicus (Bar-headed goose) (Anas indica)
P60524	HBB_CANFA	★	Hemoglobin subunit beta	HBB	Canis familiaris (Dog) (Canis lupus familiaris)
P80044	HBB_TREBE	★	Hemoglobin subunit beta	hbb	Trematomus bernacchii (Emerald rockcod) (Pagothenia bernacchii)
P02095	HBB_CAVPO	★	Hemoglobin subunit beta	HBB	Cavia porcellus (Guinea pig)
P02077	HBBA_CAPHI	★	Hemoglobin subunit beta-A		Capra hircus (Goat)
P02057	HBB_RABIT	★	Hemoglobin subunit beta-1/2	HBB1 HBB2	Oryctolagus cuniculus (Rabbit)
P02074	HBB_ODOVI	★	Hemoglobin subunit beta-3	HBB	Odocoileus virginianus virginianus (Virginia white-tailed deer)
P02142	HBB1_ONCMY	★	Hemoglobin subunit beta-1	hbb1	Oncorhynchus mykiss (Rainbow trout) (Salmo gairdneri)
P02110	HBB_TACAC	★	Hemoglobin subunit beta	HBB	Tachyglossus aculeatus aculeatus (Australian echidna)
P02072	HBB_BOSMU	★	Hemoglobin subunit beta	HBB	Bos mutus grunniens (Wild yak) (Bos grunniens)
P02141	HBB4_ONCMY	★	Hemoglobin subunit beta-4	hbb4	Oncorhynchus mykiss (Rainbow trout) (Salmo gairdneri)
P02117	HBB_ANSAN	★	Hemoglobin subunit beta	HBB	Anser anser anser (Western graylag goose)
P84792	HBB_AYTFU	★	Hemoglobin subunit beta		Aythya fuligula (Tufted duck) (Anas fuligula)
P68873	HBB_PANTR	★	Hemoglobin subunit beta	HBB	Pan troglodytes (Chimpanzee)

This will show the page for mouse beta hemoglobin.



P02088 (HBB1_MOUSE) ★ Reviewed, UniProtKB/Swiss-Prot

Last modified October 16, 2013. Version 138. History...

Clusters with 100%, 90%, 50% identity | Documents (3) | Third-party data

Names · Attributes · General annotation · Ontologies · Sequence annotation · Sequences · References · Cross-refs · Entry info · Do

Names and origin

Protein names	*Recommended name:* **Hemoglobin subunit beta-1** *Alternative name(s):* Beta-1-globin; Hemoglobin beta-1 chain; Hemoglobin beta-major chain
Gene names	Name: **Hbb-b1**
Organism	**Mus musculus (Mouse)** [Reference proteome]
Taxonomic identifier	10090 [NCBI]
Taxonomic lineage	Eukaryota › Metazoa › Chordata › Craniata › Vertebrata › Euteleostomi › Mammalia › Eutheria › Euarchontoglire

Protein attributes

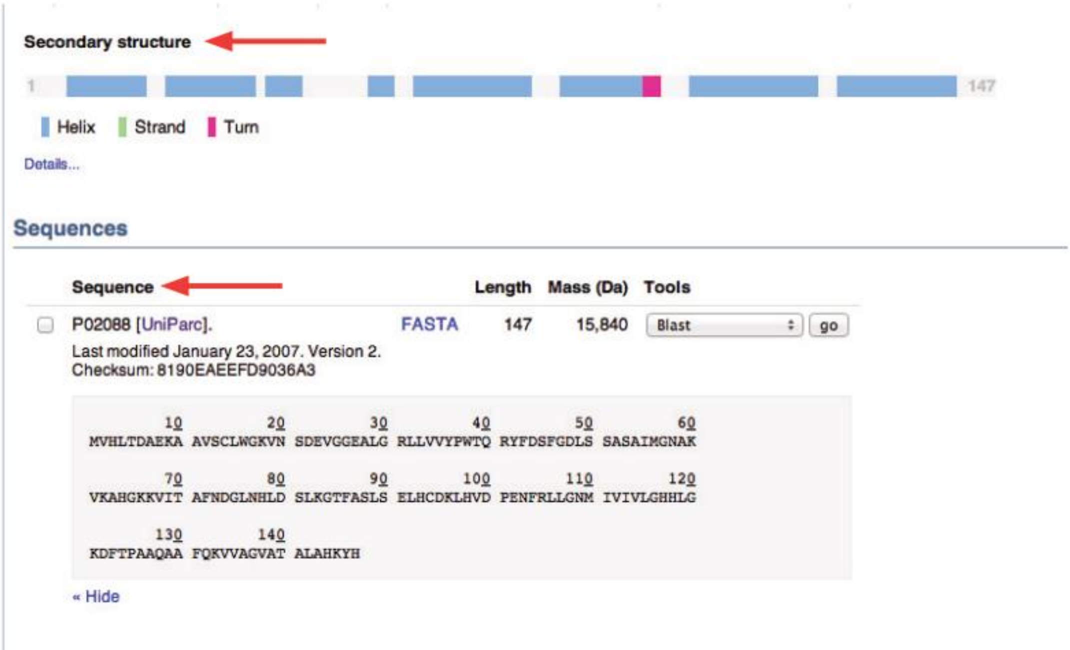
Sequence length	147 AA.
Sequence status	Complete.
Sequence processing	The displayed sequence is further processed into a mature form.
Protein existence	Evidence at protein level

Scroll down to **Sequence**.

The amino acid sequence is given using the single letter code.

This is the primary structure of the protein. It is presented here in FASTA format (pronounced fast-a).

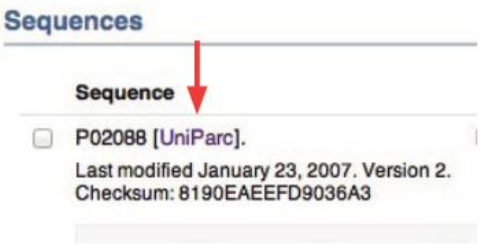
Details of the secondary structure of the protein are given above '**Sequences'**.

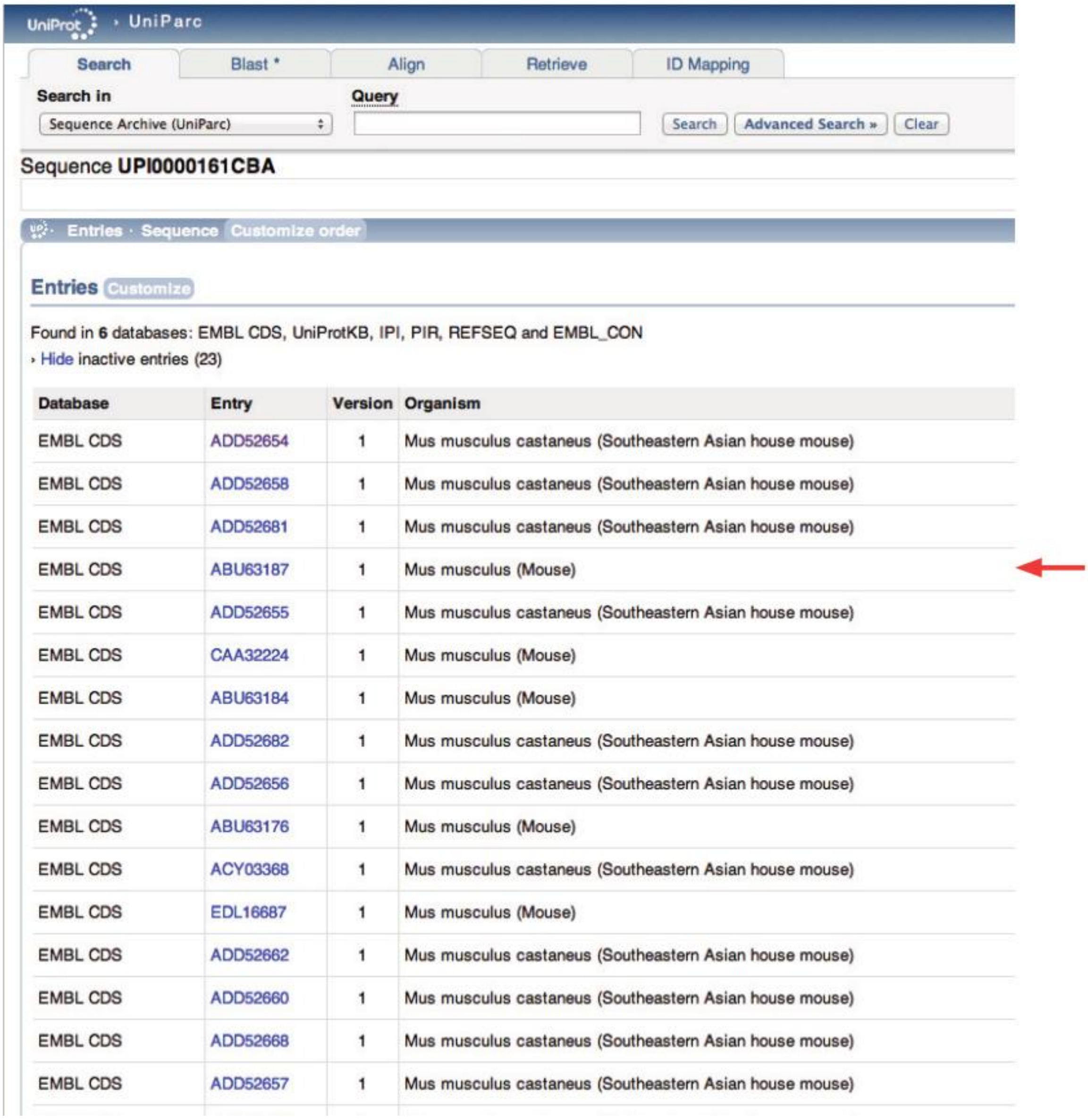


The Nucleotide Sequence

To get to the nucleotide sequence for this protein click on the 'UniParc' entry next to the amino acid sequence.

This takes you to the list of all the mouse beta haemoglobin molecules in the database.




UniProt › UniParc

Search | Blast * | Align | Retrieve | ID Mapping

Search in: Sequence Archive (UniParc) — Query — Search | Advanced Search » | Clear

Sequence UPI0000161CBA

Entries · Sequence Customize order

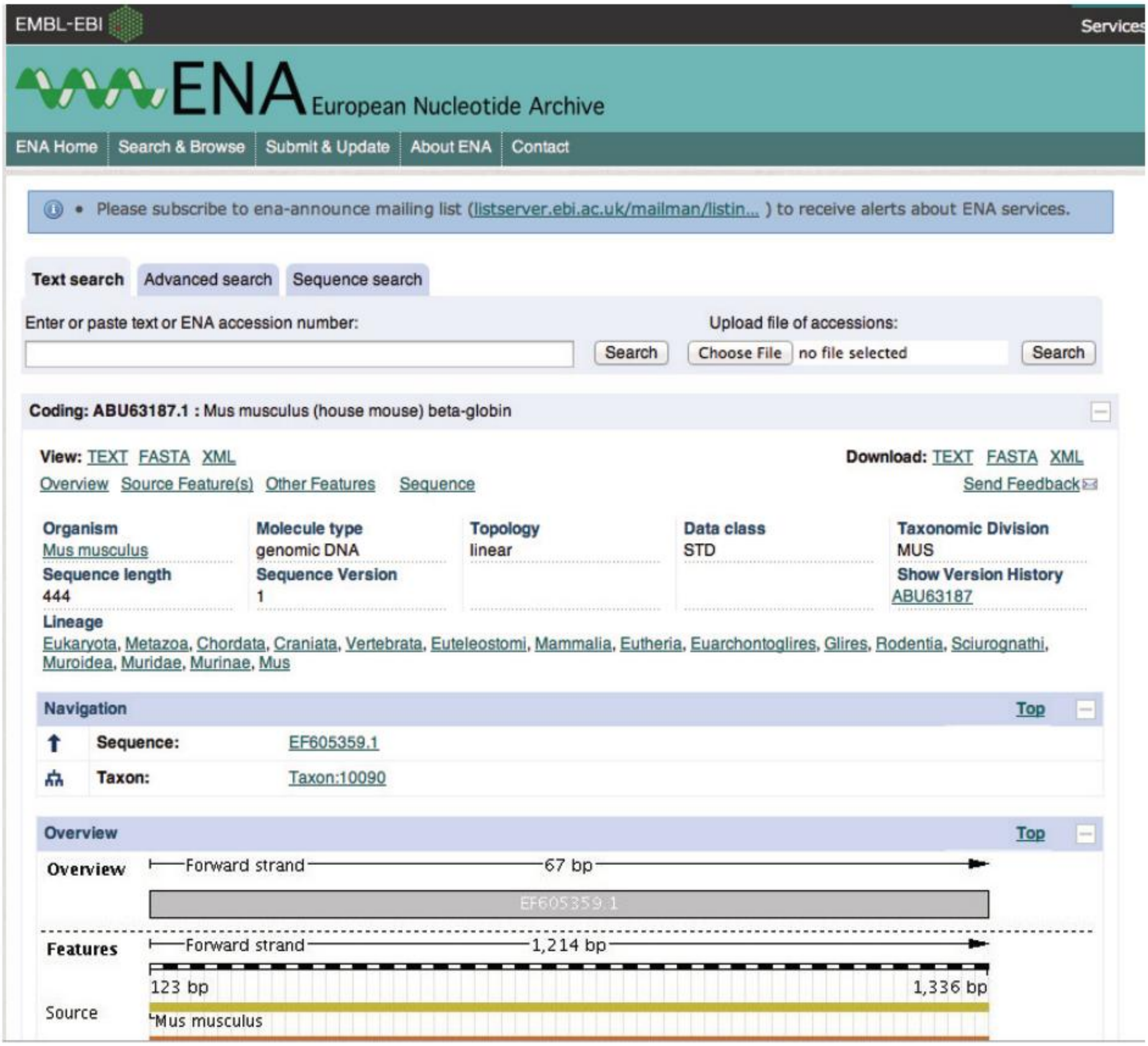
Entries Customize

Found in 6 databases: EMBL CDS, UniProtKB, IPI, PIR, REFSEQ and EMBL_CON

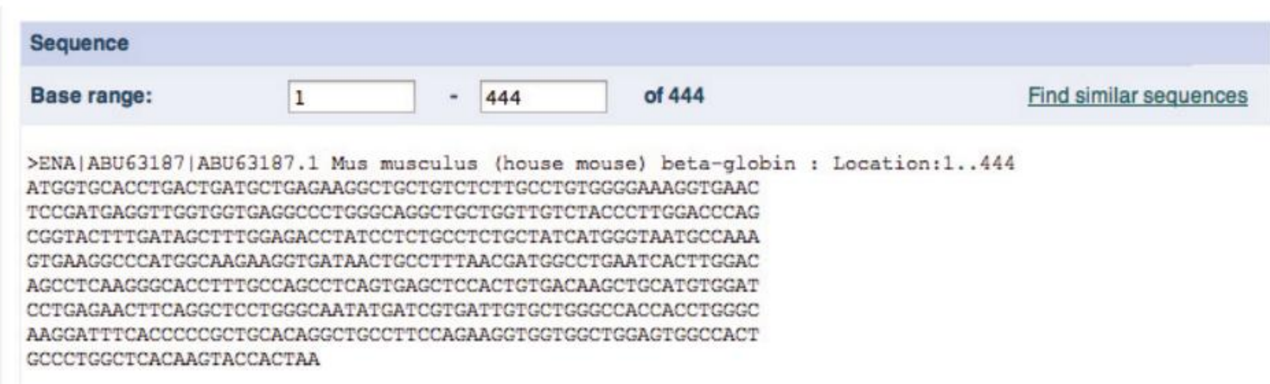
› Hide inactive entries (23)

Database	Entry	Version	Organism
EMBL CDS	ADD52654	1	Mus musculus castaneus (Southeastern Asian house mouse)
EMBL CDS	ADD52658	1	Mus musculus castaneus (Southeastern Asian house mouse)
EMBL CDS	ADD52681	1	Mus musculus castaneus (Southeastern Asian house mouse)
EMBL CDS	ABU63187	1	Mus musculus (Mouse)
EMBL CDS	ADD52655	1	Mus musculus castaneus (Southeastern Asian house mouse)
EMBL CDS	CAA32224	1	Mus musculus (Mouse)
EMBL CDS	ABU63184	1	Mus musculus (Mouse)
EMBL CDS	ADD52682	1	Mus musculus castaneus (Southeastern Asian house mouse)
EMBL CDS	ADD52656	1	Mus musculus castaneus (Southeastern Asian house mouse)
EMBL CDS	ABU63176	1	Mus musculus (Mouse)
EMBL CDS	ACY03368	1	Mus musculus castaneus (Southeastern Asian house mouse)
EMBL CDS	EDL16687	1	Mus musculus (Mouse)
EMBL CDS	ADD52662	1	Mus musculus castaneus (Southeastern Asian house mouse)
EMBL CDS	ADD52660	1	Mus musculus castaneus (Southeastern Asian house mouse)
EMBL CDS	ADD52668	1	Mus musculus castaneus (Southeastern Asian house mouse)
EMBL CDS	ADD52657	1	Mus musculus castaneus (Southeastern Asian house mouse)

Select one for *Mus musculus* the common house mouse.



This will take you to another database; the European Nucleotide Archive (ENA).



Scroll down to the nucleotide sequence.

Exercise

Try the same thing for beta hemoglobin in another species of animal.

Syllabus reference: Sub-Topic 3.5 Skill

Many species of flowering plants can easily regenerate their tissues if they are damaged. Gardeners take advantage of this to clone plants by taking cuttings. Stimulating plants to grow from cuttings can be carried out in liquid media or solid media.

Materials

cutting tool

alcohol

cuttings from a plant e.g. *Tradescantia, Impatiens*, geranium, bamboo, African violet, begonia

General Method

1. Make sure that the plant to be cloned is well watered; it should not be wilting. Cuttings taken early in the day will be more successful as the plant will be fully turgid.
2. Clean the knife with alcohol and select healthy tissue from the plant to be cloned.

Warning: Sharp instruments, handle with care

3. Take the cuttings from the upper parts of the plant showing recent growth and remove any flower heads or flower buds. Lateral branches are better than the terminal branch.
4. Keep the cuttings cool and moist before they are used. Store the cuttings in damp paper in a fridge if they are not to be used immediately.
5. Remove the leaves from the lower third of the cuttings.

Method A: Using a liquid medium

Materials

wide-necked conical flasks 50cm^3

glass tubing 20cm

plastic tubing

aquarium pump

non-absorbant cotton wool

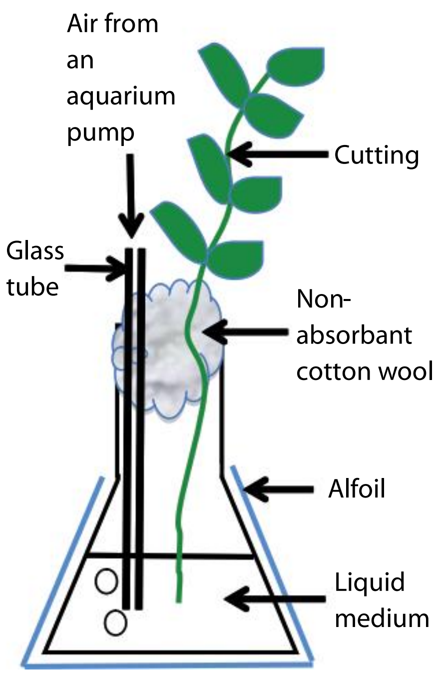
liquid media

distilled water

aluminium foil

Method

1. Place the cutting in the flask so that the cut end is immersed in the culture medium.
2. Insert the glass tube in to the medium and hold the cutting and tube with non-absorbent cotton wool.
3. Attach the glass tube to an aquarium pump using glass tubing. Adjust the height of tube in the flask, it should be under the surface of the liquid but not touching the bottom. Adjust the aquarium pump so there is a steady flow of air to aerate the liquid.
4. Cover the flask in aluminium foil. This stops light entering the flask so algae will not grow in it.
5. Leave the plants in a cool, humid environment out of direct sunlight.



Method B: Using a solid medium

Materials

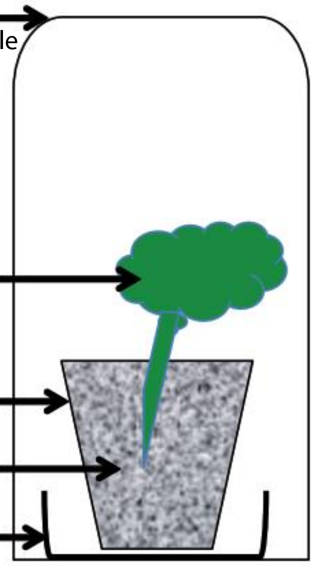
small pots
tray

cut off plastic bottle

medium: 40% medium grade vermiculite & 60% perlite or 50% vermiculite & 50% sand

Method

1. Make a hole in the cutting medium big enough to put the stem of the cutting in. Gently push the cutting into the hole and firm the medium around it so it is supported.
2. Water the pot until the liquid starts to flow out into the draining tray. The medium is then at field capacity.
3. Place the plastic bottle over the pot and the cutting. Leave the plant in a cool place out of direct sunlight.



To investigate further

- Does a particular species of plant root better in solid or liquid media?
- Hormone rooting powder or gel can be bought in garden centres these contain synthetic hormones such as indole acetic acid (IAA) or 1-naphthylactetic acid (NAA). Do they have the same effect on different species of plants? The cutting will need to be treated before they are planted.

Warning: The rooting powders or gels are biologically active compounds. Use gloves. Do not agitate the powder form. Use in a well ventilated area.

- Honey is said to stimulate rooting in plants. How could you test this?
- Do certain mineral nutrients affect rooting and root growth?

  Standard plant mineral nutrient solutions can be prepared (e.g. Sachs solution or Knop's solution) and equivalent solutions lacking one of the minerals can also be prepared.

- Mycorrhizal fungi are said to improve the rooting of their host species. Soil supplements containing mycorrhizal fungi can be obtained from organic garden suppliers.

Warning: The mycorrhiza may cause allergies. Use gloves. Do not agitate the powder form. Use in a well ventilated area.

Syllabus reference: Sub-Topic 4.1 Skill

Sealed microbial ecosystems can be set up and studied in a laboratory where simple experiments can be carried out under controlled conditions.

Such a sealed microbial ecosystem can be used to study energy flow and nutrient recycling in ecosystems and may be referred to as a mesocosm.

Materials

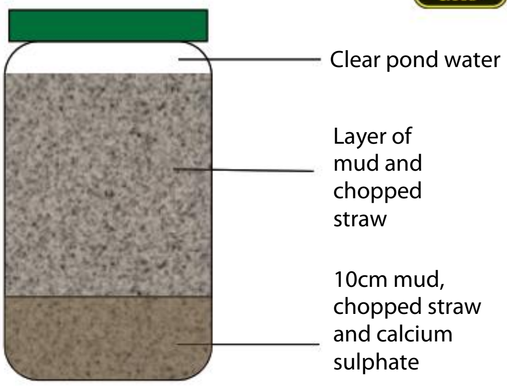
straw	mud taken from a pond or a ditch	glass rod
scissors	calcium sulphate	spatula
2 tall glass jars with air tight lids	marker pen	2 beakers 250cm^3
pond water	ruler	

Method

1. Cut up a handful of straw into small pieces about 1cm long. Place the pieces of straw into the bottom of the glass jar.

Warning: Sharp instrument, handle with care

2. Collect 200cm^3 of mud in a beaker and add a heaped spatula of calcium sulphate, mix these together. Pour the mixture into the jar up to a depth of at least 10cm. Mix the components thoroughly to eliminate as much air as possible.
3. Complete the preparation by adding more of the mixture of mud and chopped straw, that has not had calcium sulphate added to it, until the jar is completely full. Screw on the lid tightly. Label your jar with your name(s) and leave it near a window where it will receive sunlight.
4. Over the following weeks, observe the ecosystem regularly and observe any changes.



5. Determine what you think are the most important variables for the growth of the microbes. Set up a second jar to investigate the influence of one of these variables on the development of the microbial community.

Two zones have been created by the addition of calcium sulphate to the bottom layer mud and not to the top layer. The lower zone will rapidly produce anaerobic conditions. The sulphate provides a substrate for chemolithotrophic bacteria.

Microbes will appear as stains growing on the sides of the glass. Their colour and their position can be used to identify them.

Coloured stain	Microbe	Conditions in the column
Green	Alga or cyanophyta (photolithotrophs)	Aerobic clear water zone
Purple, red or orange	Purple non-sulphur bacteria (photo-organotrophs)	Upper mud zone: anaerobic and poor in sulphur
Purple	Purple sulphur bacteria (photolithotrophs)	Middle mud zone: anaerobic and rich in H_2S
Green	Green sulphur bacteria (photolithotrophs)	Lower mud zone: anaerobic and rich in H_2S and insoluble sulphur

Anaerobic heterotrophic bacteria, such as *Clostridium*, decompose the straw. This produces complex organic compounds, such as fatty acids, which can be used by chemolithotrophic sulphur bacteria. These bacteria produce hydrogen sulphide by the reduction of sulphates. The hydrogen sulphide, in turn, acts as a source of hydrogen for the green or purple photosynthetic sulphur bacteria.

To investigate further

- Different environments could be tried, e.g. light, dark, daylight, artificial light, filtered light.
- Different mixtures, e.g. with/without straw, more or less mud, different amounts of calcium sulphate.
- Top up the jar with pond water instead of mud and chopped straw.
- Mud or pond water from different locations could be compared.
- Such a mesocosm can also be setup in a tall cylinder to show various coloured microbial colonies and stages of succession. You could search the internet for 'Sergei Winogradsky' or the 'Winogradsky column'.

A Winogradsky column

Source: http://upload.wikimedia.org/wikipedia/commons/0/0b/Winogradsky.jpg?uselang=en-gb

Syllabus reference: Sub-Topic 4.1 Skill

Species living in the same habitat will have similar requirements. Their niches will overlap. To see if there is evidence of a significant overlap a test of association can be carried out using the χ^2 test.

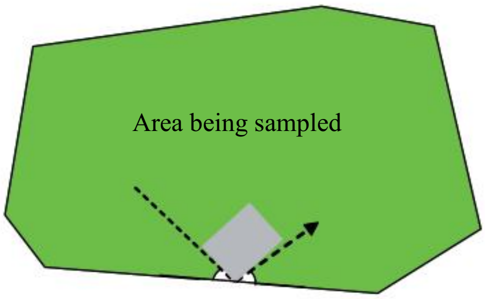
Materials

$1m^2$ quadrat

pen or pencil

random number tables (or notebook TI calculator with Prob Sim.)

Method



1. Identify the species to be recorded and set the limits of the sampling area. Spin your pencil and, heading in the direction that the pencil points, pace out the number of steps indicated by the first number on the random number tables. At the boundaries of your sampling area bounce off (diagram)and keep pacing.
2. Once the correct number of paces has been made, place the quadrat on the ground and record the presence or absence of the species. Look carefully in between the other plants in case they are hidden.
3. Repeat the exercise enough times so that the class results will give at least 100 quadrats.
4. Collect the class results and calculate the totals for:
    - the quadrats where both species are present
    - the quadrats where both species are absent
    - the quadrats where species A is absent but species B is present
    - the quadrats where species B is absent but species A is present

5. Compare the species using the the χ^2 test

Set up the Null Hypothesis (NH) and the Alternative Hypothesis (AH). In this case the Null Hypothesis would be that there is no association between the species.

Set up a 2 × 2 table of the observed frequencies (O).

		Species A		
Species B		Present	Absent	Row total
	Present			
	Absent			
	Column total			Grand total

There is no hypothesis that can lead us to a particular expected frequency we can calculate expected values from the observed values. According to the null hypothesis this should be what we get if there is no association.

Calculate the expected frequencies (E) for each of the boxes in the table = $\frac{\text{Row total} \times \text{Column total}}{\text{Grand total}}$

Now calculate the χ^2 value = $\Sigma \frac{(O - E)^2}{E}$

This is best done in a new table.

	O	E	(O-E)	$(O-E)^2$	$(O-E)^2/E$
Sp A present Sp B present					
Sp A present Sp B absent					
Sp A absent Sp B present					
Sp A absent Sp B absent					
				Total (χ^2)	

Calculate the degrees of freedom = (number of rows – 1) × (number of columns – 1) which will always be 1 in these cases.

Compare the calculated χ^2 value with the critical levels in the probability table.

Degrees of Freedom	Probability levels for χ^2						
	0.950	0.900	0.500	0.100	0.050	0.010	0.001
1	0.0039	0.016	0.455	2.71	3.84	6.63	10.83

← The difference is not significant. The NULL Hypothesis is accepted.

→ The difference is significant. The NULL hypothesis is rejected.

The critical value (χ^2_{crit}) is taken to be p = 0.05 (or the 5% level).

Do your results reject or accept the Null Hypothesis?

State the highest value of p for which the results are not significant.

Discuss and criticise the investigation.

Some points for consideration

- Do the species show any particular adaptations that may help them to compete easily with other species?
- Criticise this method of estimating the association between two species of plant in a habitat.
    - (i) Would it make any difference if the species were clumped, uniform or random in their distribution?
    - (ii) Would it make any difference if the species chosen were large or small?
    - (iii) How could you improve the method to make the quadrats more randomly distributed?

Syllabus reference: Sub-Topic 5.3 Application and Skill

Keys are a systematic way of identifying an organism. Dichotomous keys work by asking two mutually exclusive statements. The organism being observed should fit in one category or the other.

Materials

hand lens

type specimens of moss, fern, conifer and flowering plant

unidentified specimens

Method

1. Observe the specimens and divide them into two groups (they do not have to be equal sized groups).

Example

(1) (a) Possesses true leaves	go to (2)
or (b) Only possesses leaf scales	Bryophyta (Moss plant)

2. Repeat the process until each type specimen has been identified.

3. Test your key using the unidentified specimens.

Points to remember

- Do not use size as it is variable. However, the presence of strengthening tissue (e.g. wood) permitting tall growth forms (e.g. tree habit) to develop is permitted.
- Do not use colour, it can be very variable.
- Avoid using the absence of a feature as a principal characteristic. However, it can be used to separate into two groups: So the following is acceptable: (a) possesses seed bearing structures OR

  (b) does not produce seed bearing structures

Euphorbia
Angiospermophyta

Moss
Bryophyta

Pine tree - Coniferophyta

Fern - Filicinophyta

Syllabus reference: Sub-Topic 6.1 Application

A model of the digestive system can be made from a piece of dialysing tubing. Digestion is the breakdown of large molecules into small molecules so that they can be absorbed by the wall of the intestine.

Materials

starch solution (2%)	syringe 2cm^3	test tube holder
amylase solution (2%)	large test tube	stop watch/clock
2 small beakers	electronic water bath	wash bottle of distilled water
15cm of dialysing tubing	dropping pipette	Fehlings or Benedicts solution
glass rod	Pasteur pipette	iodine solution
cotton thread	6 test tubes in a rack	safety glasses

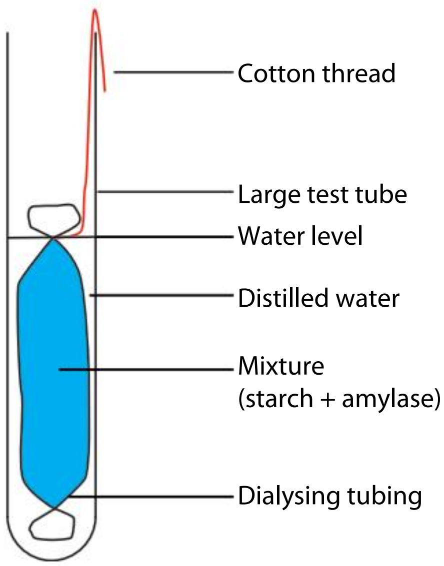
Method

1. Wet the piece of dialysing tubing and open it using a round-ended glass rod. Tie a secure knot in one end of the tubing. Inject the mixture of starch into the open end of the dialysing tubing using the dropping pipette.

2. Using a syringe, add 2cm^3 of amylase to the tube and tie a thread of cotton tightly around the open end. Wash the outside of the tubing thoroughly under the tap.

3. Suspend the dialysing tubing in the large test tube and fill it with distilled water up to the level of the knot (as shown opposite). Immediately start your stop clock and, using the Pasteur pipette, take one small sample from the distilled water in the large test tube. Pipette the samples into the three test tubes.

4. Test the samples for the presence of starch using iodine solution and for glucose by adding an equal volume of Fehlings or Benedicts solution to the sample in a test tube. Heat this mixture gently in a water bath at 80°C for 2 minutes. Positive result = green, yellow or red, depending upon the amount of sugar present.



Warnings: Hot water can scald, use a test tube holder. Fehlings solution is caustic. Wear safety glasses. Wash off spills with water.

5. Leave the mixture for two minutes, take a second sample and place it in a clean test tube. Repeat the test at 2-minute intervals for 24 minutes.

6. Record your results for the experiment and give your observations and conclusions.

Some points for consideration

- What are the sources of error that you may have encountered?
- How does the model resemble the process of digestion?
- How could the model digestive system be improved upon?

Syllabus reference: Sub-Topic 6.2 Skill

The heart of mammals is found in the thoracic cavity between the two lungs. It is the pump of the blood circulatory system. The heart is connected to the other organs of the body by blood vessels. The structure of the heart of a lamb (or a pig) is comparable to the structure of a human heart.

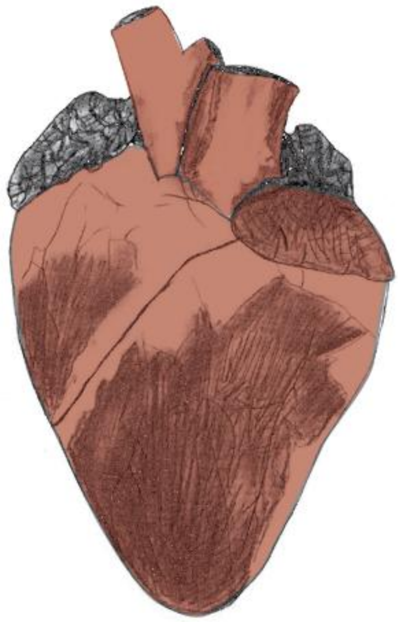
Materials

An intact heart of a sheep (or pig)	Large scissors	Rubber tube attached to a tap
Dissecting dish	Probe	Gloves
	Paper towel	

Method

PART A: Observations on the heart and the vessels attached to it

1. Record the external appearance of the heart, its shape and colour.
2. On the top of the heart are two atria. Note the colour of the atria. Locate the blood vessels on top of the heart and identify the arteries and the veins. The cross section of the arteries remains open and their walls are thick, yellowish and elastic. The thin, pinkish walls of the veins flatten and collapse where they are cut.
3. An oblique shallow groove runs across the lower part of the heart. This identifies the ventral side of the heart (See diagram opposite). What do you observe in this groove?
4. Make an annotated drawing of the ventral surface of the heart. Add any other observations that you have made (e.g. colour, distribution of fat).



Ventral view of the heart

PART B: The examination of the interior of the heart

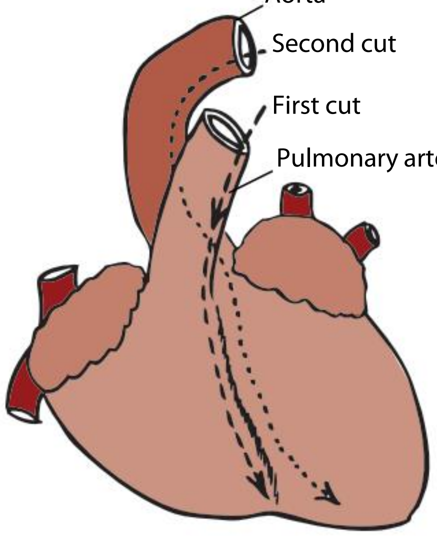
1. Reach into the interior of the heart by inserting your index finger (or a probe) successively into each blood vessel. Push against the heart wall in different places. How many cavities can you count? Do these cavities communicate with one another? Explain your answer.
2. The pulmonary artery is connected to the right ventricle and the aorta to the left ventricle. The superior vena cava and the inferior vena cava irrigate the right atrium, the pulmonary veins run into the left atrium. The right atrium receives blood from the two vena cava, which bring all the blood from the organs to the heart. The pulmonary veins bring blood from the lungs to the left atrium.

PART C: Using water to simulate the flow of blood in the heart

1. Slide the rubber tube from the tap into the right atrium. Flush water through the right atrium. Note your observations. Now do the same for the left atrium.
2. Next, slide the tube into the pulmonary artery. Let water flow gently into this vessel whilst you observe from above the vessel. What do you observe? Now do the same for the aorta.

PART D: Looking for structures which direct the blood flow through the heart

The dissection of the heart



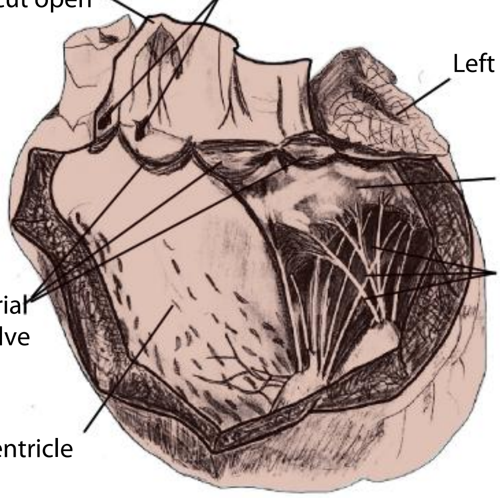
1. Put the heart in the dissecting dish with the ventral surface facing upwards and the base of the heart pointing towards you.
2. **First cut:** using a pair of scissors, cut the wall of the pulmonary artery along its entire length. Then cut the wall of the right ventricle a few millimetres from the groove that lies between the two ventricles, as indicated by the broken line (- - -) on the diagram opposite. Open the flap of the ventricle wall to observe the inside of the cavity.
3. **Second cut:** Cut the wall of the aorta and then the left ventricle. Follow the dotted line (........) on the diagram opposite as far as the apex of the heart, staying very close and parallel to the groove between the ventricles.

Warning: Sharp instrument, handle with care

4. Three pockets are found at the beginning of each artery. These are the arterial (semilunar) valves.
5. Each of the openings between the atria and the ventricles is guarded by a valve made of flaps. These flaps are attached by fibres to the ventricle walls. These valves are called the atrioventricular valves, (the valve on the left side of the heart is called the bicuspid and the valve on the right side of the heart is called the tricuspid).

Some points for consideration

- Compare the structure of the two sides of the heart.
- From your observations using water, deduce the pathway of the blood through the each side of the heart.
- Explain the role of the valves in the circulation of the blood.
- The entrance to the coronary arteries can be found just after the arterial (semilunar) valve in the aorta. Use a probe to explore this artery.



A ventral view of the heart with the left side opened

Some points for consideration

- How can you explain the difference in thickness between the walls of the right and left ventricles?
- How do the arterial valves (semilunar valves) differ from the atrioventricular valves? At what points will they function in the cardiac cycle?
- Describe the difference in the appearance of the arteries (e.g. aorta) and the veins (e.g. pulmonary vein).

Research

- What is the difference in the composition of the blood leaving the right ventricle and leaving the left ventricle?
- What are the respective functions of the veins and the arteries.
- The beating of the heart is a dynamic process.
- This is best observed in animations. A number of useful URLs exist:

A clear explanation with some useful animation of the heart beat.

<http://www.sumanasinc.com/webcontent/animations/content/human_heart.html>

Some interesting animation and interaction with this site from Nova. You can alter the heart rate.

<http://www.pbs.org/wgbh/nova/body/map-human-heart.html>

Syllabus reference: Sub-Topic 6.4 Skill

Spirometers measure the volume of air breathed in and out.

Materials

- data logger
- spirometer sensor
- disposable breathing tubes or antiseptic wash
- bacterial filter
- exercise bike
- nose clip
- consent form (see Appendix)
- Optional: gaseous oxygen sensor
- Optional: heart rate sensor

Method

Collecting the data

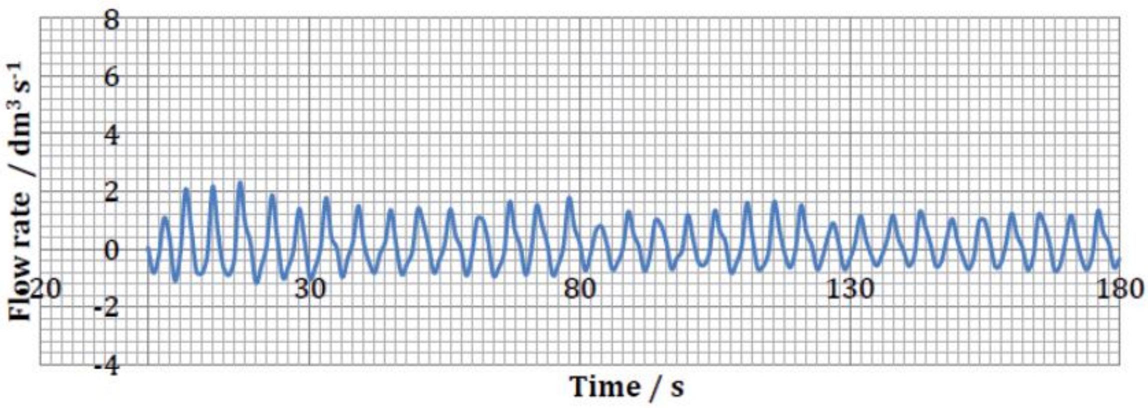
Warning: Ensure that the subject is healthy and able to participate in the exercise. Explain the nature of the experiment and use a consent form.

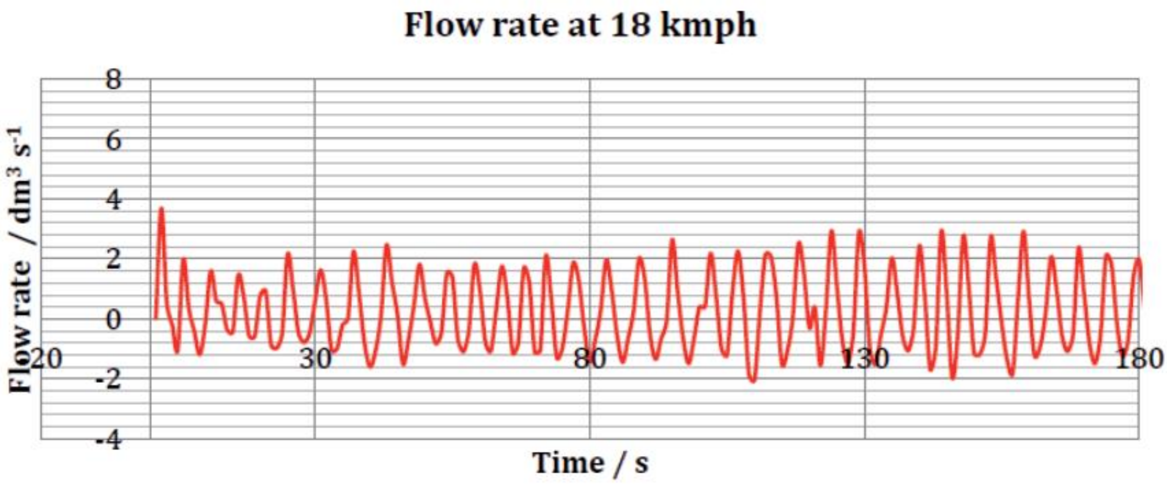
1. Set the subject comfortably on the exercise bike. Attach a nose clip.
2. Connect the spirometer sensor to the data logger and set the data logger to collect data at one measurement per second over 180s (3min).
3. Attach a disposable mouth piece to the spirometer and if available, a bacterial trap.
4. Take the subject's pulse rate at rest.
5. Ask the subject to breath in and out regularly through the disposable mouthpiece. Once a regular ventilation rhythm is obtained, start recording the ventilation using the data logger. Continue until the 180s are completed. Save the trial.
6. Ask the subject to pedal the bike at a regular speed (e.g. about 10km per hour), repeat the recording and save the trial data.
7. Check the subject's pulse rate until it returns to the resting rate.
8. Perform the experiment again pedalling at a faster speed (e.g. about 20km/h) and save the trial.

Analysing the data

The flow rate recorded by the spirometer will show an oscillation. The frequency of the oscillation is the ventilation rate and the range of the oscillation gives the volume breathed in and out per breath. Two examples are shown below.

Flow rate at 0 kmph





Sample the oscillations over the 3 minute period at 1 sample per second to obtain an average for the volumes and frequencies.

To investigate further

- Comparing ventilation rates at different speeds.
- Comparing ventilation rates at the same speed but different loads.
- Comparing males and females of the same age.
- Comparing subjects of different ages.
- Comparing breathing rates and pulse rates.
- Calculating recovery time after a given period and intensity of exercise.
- Measuring the oxygen content using a gaseous oxygen probe.
- Calculation of the VO_2 max

Syllabus reference: Sub-Topic A.2 Application

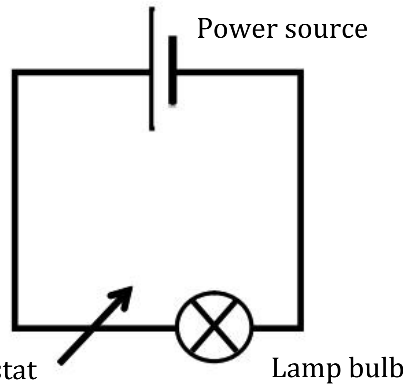
The pupil reflex is a standard test used in medicine to verify the state of the brain.

Materials

4V battery or power supply
3 cables and connectors
3.5V bulb in holder
rheostat (potentiometer)
small mirror
room with blackout
mobile phone or video camera
computer

Method

1. Build a simple electrical circuit (shown adjacent)
2. Position the circuit so that the lamp is out of line of sight (below your eyes.)
3. Turn out the lights in the room and turn the rheostat so that the lamp glows bright. Using the mirror, observe what happens to the pupils of each eye. Do they both behave in the same way?
4. Slowly turn the light down and pause at different intensities. Note how the iris reacts. When the light intensity is constant does the pupil stay the same diameter?
5. Move the lamp so it is in front of the right eye. Shade the left eye by placing your hand in front of your nose. Now increase the light and observe what happens to the irises of the two eyes.



6. Repeat this the other way round. Put the lamp in front of the left eye and shade the right eye from the light.
7. Try this on your partner. You may find it useful to film the reaction of the pupil using a mobile phone and analyse the response more carefully on screen.

Warning: Ensure that the subject has no eye problems and is able to participate in the exercise. Explain the nature of the experiment and use a consent form. Do not use LEDs or lasers as a light source for this investigation.

Some points for consideration

- How can the response of the iris be explained in terms of negative feedback?
- How can you explain the response of the shaded eye?
- Brain structure and detecting anomalies

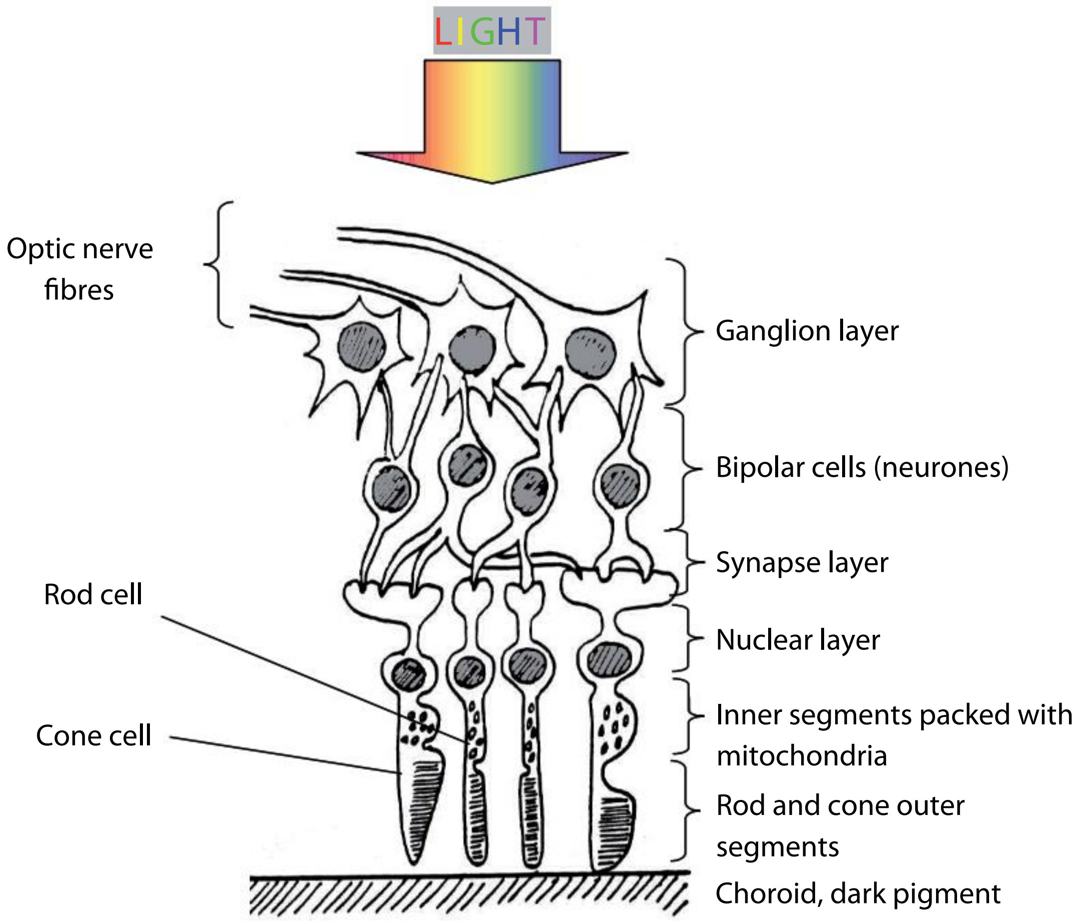
Research

- Find out which sensory and motor nerves are involved in the pupil reflex.
- Draw a diagram of the reflex arc involved in this reflex.

Syllabus reference: Sub-Topic A.3 Application

The retina is made of several layers of cells. Light passes through the neurones and stimulates the rod and cone cells next to the pigmented choroid layer.

The structure of the retina



The photoreceptors in the retina are the rod and cone cells. These are excitable cells that are stimulated by particular wavelengths of light.

Cell	Wavelength / nm	Peak Colour Sensitivity
Rod cells	500	Yellow-green
L cone cells	564-580	Orange
M cone cells	534-555	Green
S cone cells	420-440	Blue-violet

The photoreceptors contain pigments that absorb these different wavelengths. When the pigments absorb light they are bleached and need to be reactivated.

The rod cell pigment (rhodopsin) is very easily bleached but the pigments in the cone cells are less easily bleached.

What kind of cells are used in daylight vision and low light night vision?

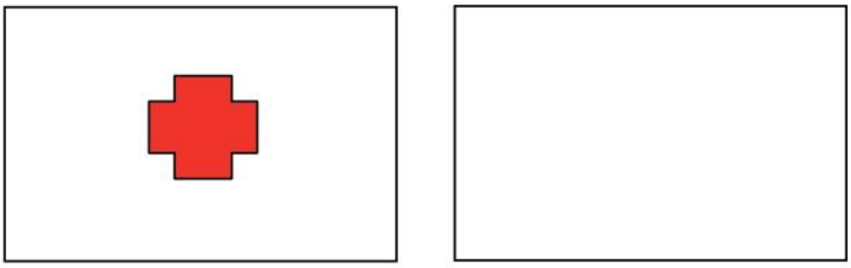
The colours of light that can be sensed are said to lie in the visible spectrum. Pure red, blue and green wavelengths are sensed by the different cone cells.

Afterimages and complementary colours

bench lamp
stop watch
sheets of thick, white paper
coloured marker pens: bright red, green and blue

Method

1. On one of the sheets of paper draw a large clear cross using the marker pen.



2. Shine the bench lamp onto the paper with the cross and concentrate on it for a full minute.
   - After one minute, your partner should place the other sheet of white paper covering the cross.
   - Concentrate on the new piece of paper.
   - Describe what you see.
   - You should see an afterimage of a cross. The colour of the cross is important.

3. Try the other colours.

   Which cones are stimulated when you look at a red cross?

   Which cone cells are being stimulated when you are looking at a white sheet?

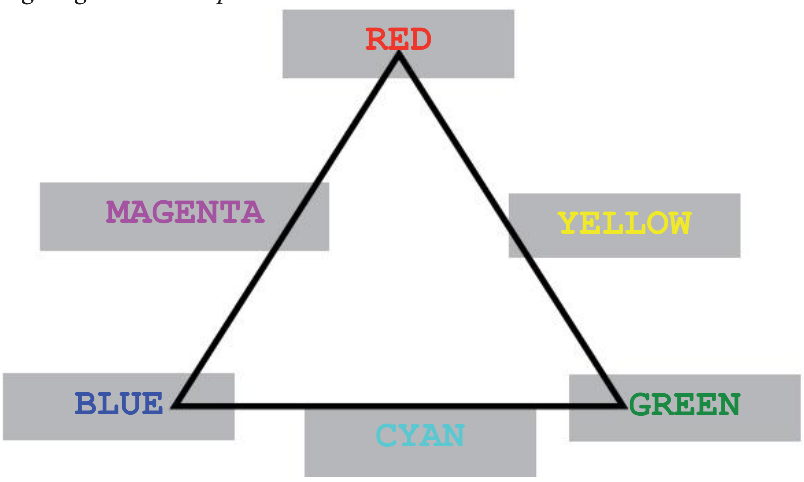
   Which cones would be stimulated if you were looking at a black object?

   How can we explain the presence of an afterimage? What is happening in the cone cells?

   Remember the pigments in the cone cells are bleached when light of the right wavelength falls on them.

   How can we explain the colour of the afterimages?

The following diagram will help.



The primary colours are at the corners of the triangle and the complementary colours are along the sides.

White is the presence of all colours and black is the absence of all colours.

Afterimages from complementary colours

Fluorescent highlight markers are especially good at giving afterimages.

Predict what you will get if you stare at a yellow, cyan and magenta crosses for a minute.

You might not get quite what you expect.

Though cyan is clearly a mixture green and blue and magenta is a mixture of red and blue, what about yellow?

We do not perceive reddish-green nor bluish-yellow as colours. How can we explain this?

Theories on colour vision

Two complementary theories try to explain colour vision are the **Trichromatic Theory** of *Young*, *Maxwell* and *Helmholtz* and the **Opponent-Process Theory** of *Ewald Hering*.

The trichromatic theory explains colour vision by the presence of three types of cone each sensitive to a different part of the visible spectrum. Colour vision is a simple mixture of the sensitivity of these three wavelengths. This works for most colour combinations.

However, some of the afterimage results can also be explained by the interaction between the signals coming from the three cells in the brain. These signals (red v green and blue v yellow) are stimulatory or inhibitory. In other words signals from different cones may oppose one another as well as add to one another. So when both L and M cones are stimulated but not S cones, we can see yellow as a distinct hue and not a greeny-red.

To investigate further

Assessing the state of an image to a colour blind person is important for safety, education and advertising. A number of sites dedicated to colour blindness awareness exist where colour blindness is simulated. Some of these sites even permit you to upload and try out an image as it would appear to people with different types of colour blindness.

Colour blindness simulations

<http://www.color-blindness.com/coblis-color-blindness-simulator/>

<http://www.vischeck.com/daltonize/>

<http://www.etre.com/tools/colourblindsimulator/>

<http://www.colourblindawareness.org/colour-blindness/colour-blindness-experience-it/>

Investigation 13A – Do disinfectants kill bacteria?

Syllabus reference: Sub-Topic B.1 Skill

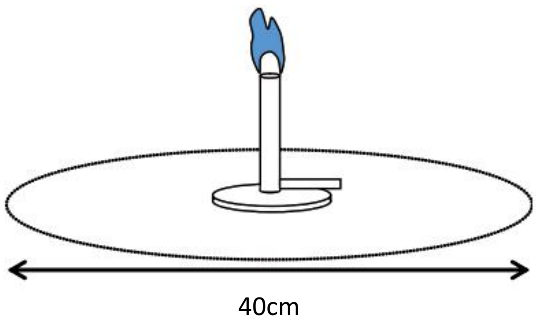
Disinfectants are chemicals that can kill a population of bacteria, although total sterility may not be achieved, **antiseptics** are chemicals that simply inhibit bacterial growth. The purpose of the following exercise is to demonstrate the effect of disinfectants on a population of bacteria.

Materials

bleach solution	beaker of alcohol	wire inoculating loop
cotton wool	test tube and stand	Bunsen burner
soap	bacterial culture (e.g. Lactobacillus)	matches
disinfectants in a beaker	2cm^3 syringe	forceps
discs of filter paper	nutrient agar plate	marker pen
glass spreader		self-adhesive tape

Working in a sterile zone

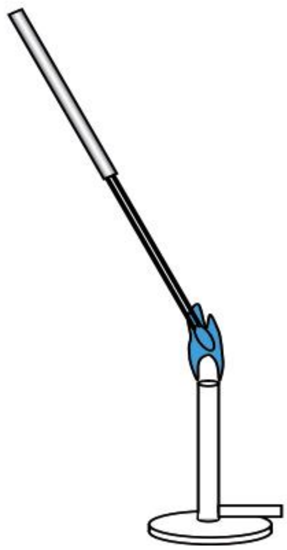
- Clean the bench with bleach using a cotton wool swab.
- Set up a Bunsen burner in the centre of the bench and leave it on a yellow flame. As soon as any sterile material is placed on the bench the Bunsen must be turned to a blue flame. The blue flame will ensure a sterile zone of about 40cm in diameter around the Bunsen burner.
- Material can now be installed in the sterile zone. Any manipulation must be carried out within this sterile zone.
- Whilst manipulating, avoid talking or excess movement as this will lead to air movement.
- Wash your hands before and after any manipulation.



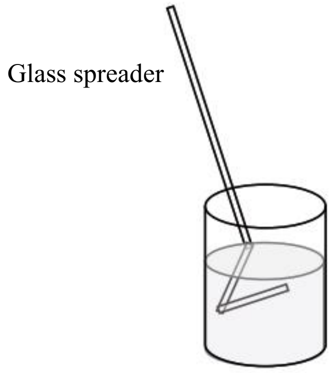
Warning: Use goggles with Bunsen burners

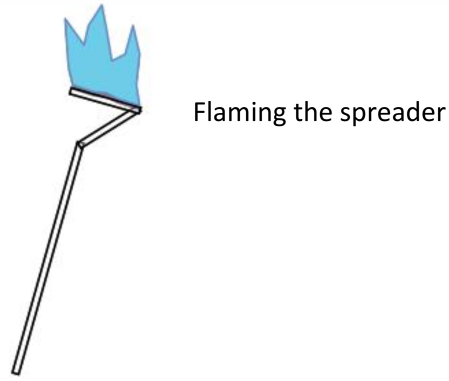
Method

1. Measure out 0.5cm^3 of distilled water into a test tube using a syringe. Using a flamed loop, transfer a loopful of culture to the water and mix well.
2. Label the base of the Petri dish A, B, C and D. Place the Petri dish next to the Bunsen flame and lift the lid up a little. Pour the water and bacteria onto the surface of the agar. Using the alcohol, sterilise the glass spreader or use a disposable spreader. Spread out the bacteria as evenly as possible over the agar surface. Place the spreader in the alcohol and flame it to sterilise it and replace the lid of the Petri dish.

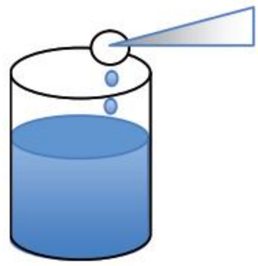


Sterilising a wire loop in a Bunsen burner flame





Warning: Do not put the beaker of alcohol near the Bunsen burner

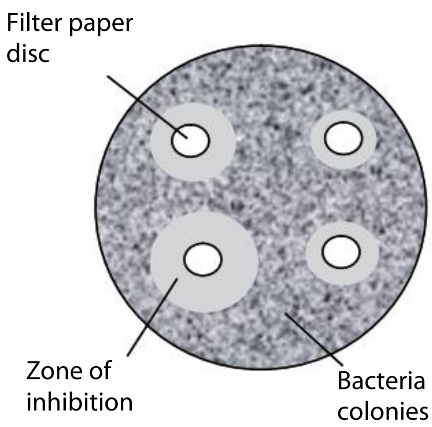


3. Dip the end of the forceps into the alcohol and pass it through the Bunsen flame. Use the forceps to pick out a filter paper disc from the beaker of disinfectant. Drain off the disk on the side of the beaker. Lift the lid of the Petri dish up a little and place the disk on one side of the agar plate over the label A.
4. Repeat the exercise with discs soaked in two other disinfectants and one disc soaked in sterile water only. Place them over labels B, C and D.
5. Label the dish (disinfectants, your name, class, date), tape down the lid, turn over the dish (so the agar is in top half) and incubate at 30°C.
6. Examine and measure any zone of inhibition through the base of the dish.

Warning: Never open a Petri dish that has colonies growing in it.

Inhibition of bacterial growth by disinfectant

Zones of inhibition (i.e. regions where bacterial growth has been prevented) may be seen. Different disinfectants will give different results, as will different strengths of the same disinfectant. It is therefore possible to determine the effect of the same disinfectant on several different species of bacteria.



To investigate further

- The bacteriocidal action of different soaps
- The effectiveness of cleaning agents at different dilutions
- The bacteriocidal action of spices and herbs
- Comparing hand sanitizers to standard hand soaps.

Syllabus reference: Sub-Topic B.1 Skill

The Gram stain is probably one of the most widely used stains for identifying bacteria. It was invented by the Danish microbiologist *Christian Gram* in 1884.

The technique requires four solutions:

- A basic stain (e.g. crystal violet)
- A mordant (e.g. Lugol's iodine solution)
- A decoloriser (e.g. acetone-alcohol)
- A counterstain (e.g. safranin or basic fuchsin)

Gram positive bacteria stain with crystal violet.

Gram negative bacteria stain red with safranin stain.

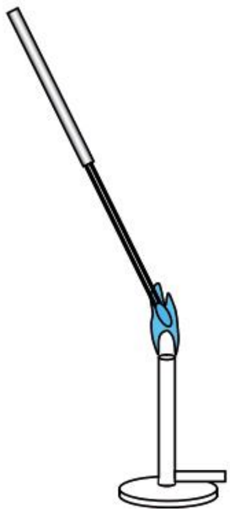
At the time it was not understood how it worked. We now know that the Gram staining properties of bacteria depend upon the nature of their cell walls.

Materials

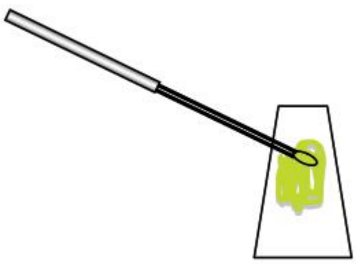
microscope (with ×100 objective)	sterile water	Crystal violet stain
immersion oil	Bunsen burner	Lugol's iodine
glass slides	matches	acetone-alcohol
wire loop	forceps	Safranin
stop watch	staining rack and dish	natural yoghurt
filter paper	rubber gloves	distilled water wash bottle

Method

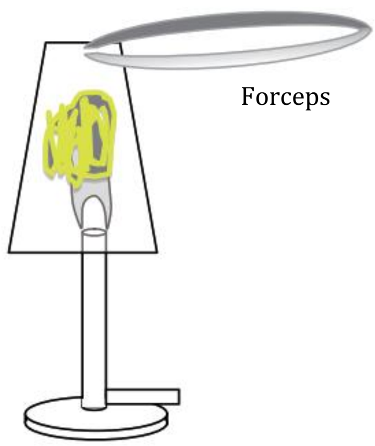
1. Flame the wire loop until red hot then let it cool. Dip the loop in sterile water (tap water will do here) and place two loopfuls on the slide.
2. Dip the loop into the yoghurt and mix with the water on the slide. Spread the mixture over 2cm of the slide. Leave the slide to air dry.
3. Hold the slide in forceps and pass it three times through a blue Bunsen flame. Do not overheat the slide. Switch off the Bunsen when complete. This heat treatment fixes the bacteria to the slide so they do not get washed off during the staining.



Sterilising a wire loop in a Bunsen flame



Spreading a bacterial culture on a slide



Heat fixing a smear in a Bunsen flame

Warning: Take care when handling hot objects

DANGER

4. Place the slide on a staining rack and flood it with crystal violet stain. Leave for 30s. Lift the slide with the forceps so the stain runs off the slide and wash it in a stream of Lugol's iodine. Replace the slide on the rack and flood with Lugol's for 30s then lift up the slide and wash it off in distilled water.

Warning: Wear gloves when handling biological stains.

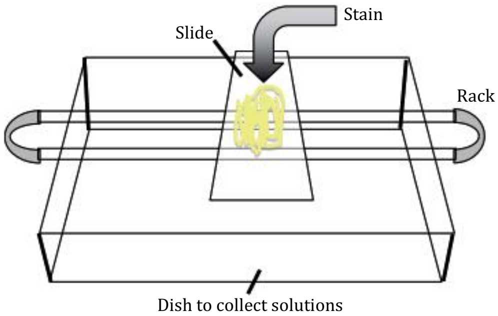
GLOVES

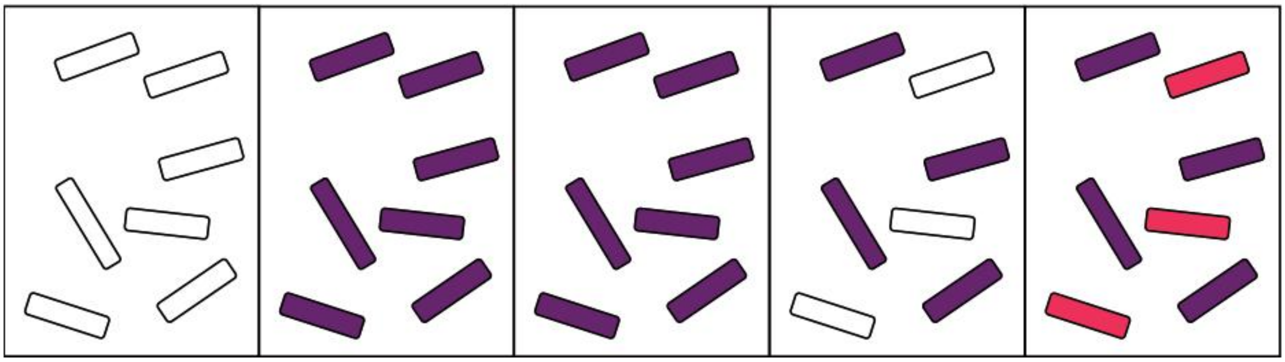
5. Add the acetone-alcohol until no more colour is seen to come off the slide (about 3s) then immediately wash with distilled water again.

Warning: acetone-alcohol is inflammable do not leave this near the Bunsen.

FLAMMABLE

6. Flood the slide with basic safranin and leave for 1 min. Wash off the safranin with distilled water and dry the slide gently between sheets of filter paper and let it dry in the air.
7. Add a drop of immersion oil to the centre of the slide and observe using the oil immersion objective lens on a microscope.
8. When your observations are complete, clean off the oil immersion objective using a lens tissue.
9. Dispose of the stains according to local regulations. *Do not pour them down the sink.*





The sequence for Gram stain

Research

What is the relationship between the Gram staining characteristics of a bacterium and its virulence when it has infected a patient?

Syllabus reference: Sub-Topic C.1 Skill

A transect is a series of observations, or measurements, taken at regular intervals along a line.

The aim is to use a transect to determine the change in a community of plant species as we progress from one habitat to another. In this exercise an interrupted belt transect will be used to study changes in vegetation with various abiotic factors.

Materials

- tape or string marked in metre intervals
- $1m^2$ quadrat
- 2 ranging poles marked at 10cm intervals
- spirit level
- 20cm ruler
- data logger
- temperature probe
- light probe
- humidity probe
- Identification guide or species Check list

Method

1. Lay out the tape or string across the transition zone so that it is straight and taught. Tie it down at both ends.

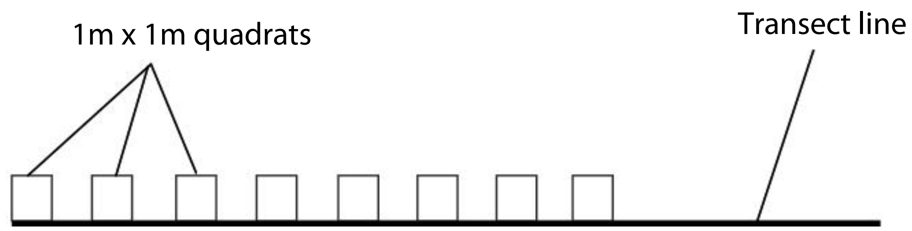
Environmental impact: Be careful to trample the vegetation beside the line as little as possible. Leave the site as you found it.

2. Note the orientation of the line (north-south, east-west etc.) and the major features along the transect. Use the prepared data table to record your results. Sketch the transect in your notebook. Take photographs of the site to support your observations.

3. Starting at one end, lay your quadrat by the side of the line and identify the species of plants present. Estimate their abundance using the scale given in the table below:

Scale	Percentage Cover	Abundance Rating
Trace	<1%	Single individual
1	1 - 5%	Rare
2	6 - 25%	Occasional
3	26 - 50%	Frequent
4	51 - 75%	Abundant
5	76 - 100%	Dominant

4. Record each species and their abundance in a table. Repeat this every other metre along the transect (*see diagram below*). Add new species to the bottom of the list.



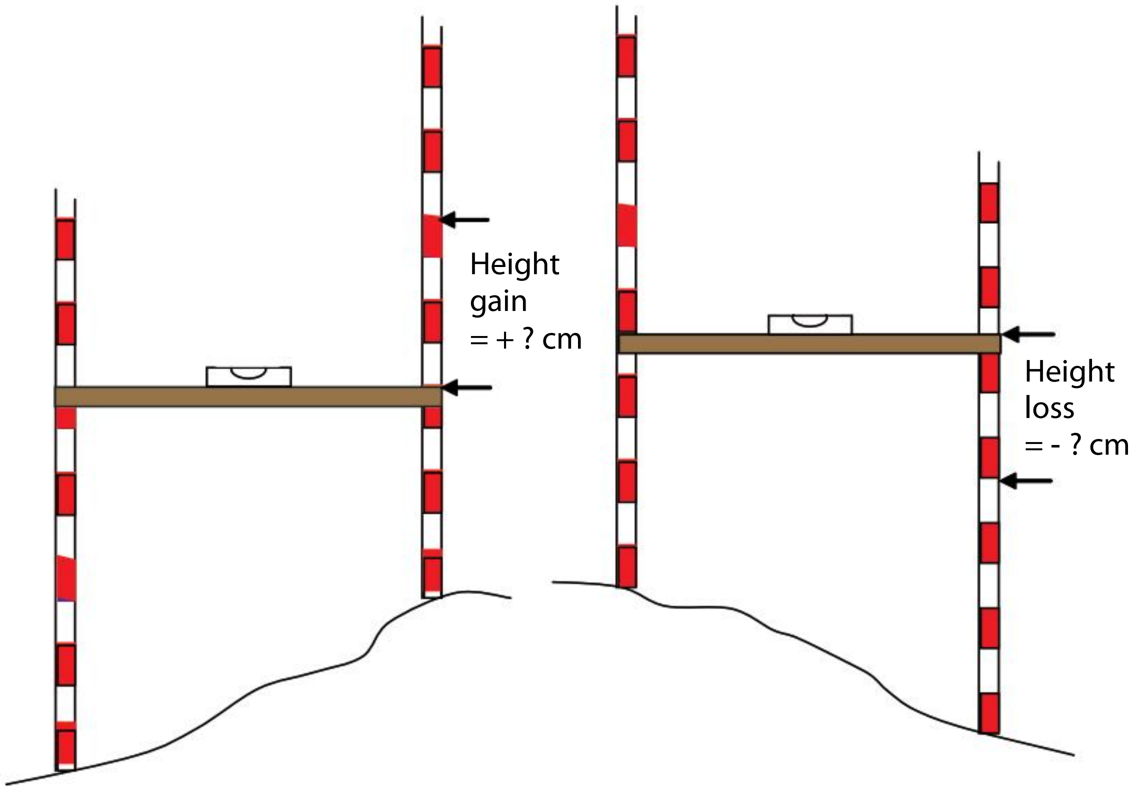
5. Record the abiotic factors measured every other metre along the transect using the data logger and probes. Measure these within a relative short space of time so that variations in the weather do not influence the readings too much.

6. Record the height profile of the transect using the ranging poles and the spirit level. Place the first pole at the beginning of the transect and the second pole at 1 metre. Using the spirit level, measure the difference in height of the two poles. *See the diagram on the next page.*

7. Record an increase as a positive value and a decrease as a negative value. Where there is a sudden change in height, place the poles at shorter intervals. On soft ground be sure NOT to push the poles into the soil, they should be gently resting on the ground.

8. This data can be entered on a spreadsheet. Calculate the cumulative change in height. When this is plotted against distance along the transect it gives the height profile. Profiles of the changes in abiotic factors and the changes in the flora can be compared with this data.

9. Discuss the results and evaluate the method. Are there any correlations apparent between the distribution of the species and the abiotic factors?



Syllabus reference: Sub-Topic C.1 Skill

Traps can be used to sample invertebrate populations. Sampling in this way provides an idea of distribution and abundance, but it does not provide an estimate of absolute density.

Materials

25m string marked at 1m intervals
6 traps
trowel
soil auger
white sorting tray
white plastic teaspoon
hand lens
data logger
temperature probe
humidity probe
light probe
Key to the orders of soil invertebrates

Method

1. Lay out the string marked at 1m intervals along the area to study. For example covering an area that lies partly in a field, across the field to woodland boundary, then entering into the wood.
2. At every fifth meter place a pitfall trap as follows:
   - Using the soil auger, drill out a core of soil as deep as the trap. Using the trowel, clean out the hole large enough to push in the trap. Then using the soil that you have just removed, make sure that the top of the trap is level with the soil surface.
   - Set up the trap cover.

3. Using data logging every metre over the 25m measure: the soil temperature and at one metre above the ground measure:
   - the air temperature,
   - the light intensity
   - the relative humidity.
4. Leave the traps for an interval of 24 hours. Sketch the transect in your notebook. Take photographs of the site to support your observations.
5. After 24 hours, repeat the abiotic measurements and take the traps in one by one. Identify the invertebrates found using a key as far as Order. Record your data in an organised way. Use a white sampling tray to separate out the similar looking invertebrates into the different parts of the tray before identification.
6. Note anything else that may have ended up in your traps.

Environmental impact: Release the animals in the areas where they were trapped. Remove all the traps and wooden sticks. Collect them in a rubbish sack to be disposed of in a suitable manner. Take in the string and look around to make sure that you have left nothing behind.

7. Present the results in a suitable way.
8. Discuss any trends that are seen in the distribution and abundance of the different invertebrate groups found along the transect. Relate them to the abiotic factors that have been recorded.

Data table

Order	Common names, examples	Trap 1	Trap 2	Trap 3	Trap 4	Trap 5	Trap 6
Class Arachnida							
Pseudoscorpiones							
Opilones	harvestman						
Acarina	mites						
Araneae	spiders						
Class Crustacea							
Isopoda	woodlice						
Class Insecta							
Diplura	two-pronged bristletails						
Protura	coneheads						
Collembolla	springtails						
Thysanura	silverfish						
Orthoptera	grasshoppers & cockroaches						
Demaptera	earwigs						
Psocoptera	barklice						
Thysanoptera	thrips						
Hemiptera	true bugs, aphids, leaf hoppers						
Lepidoptera	butterflies & moths						
Diptera	true flies including mosquitos						
Hymenoptera	bees, ants, wasps						
Coleoptera	beetles						
Others							
Class Oligochaeta	earthworms						
Class Nematoda	roundworms						
Class Diplopoda	millipedes						
Class Chilopoda	centipedes						
Order	Common names, examples	Trap 1	Trap 2	Trap 3	Trap 4	Trap 5	Trap 6

Syllabus reference: Sub-Topic C.2 & C 2.4 Skills

Many organisms living in water are sensitive to pollution. Pollution creates stress on an environment. When an ecosystem is under stress it can result in a loss of biodiversity. Biodiversity can be measured using the *Simpson's diversity index.*

An assessment of the quality of an environment, using living organisms, is called a *Biotic index.* This is an estimate of the quality of the water by sampling organisms that show a range of sensitivity to organic pollution.

Materials

- net
- 1m folding metre ruler
- 10m string marked at 1m intervals or tape measure
- white plastic bowl
- sorting tray
- white plastic teaspoon
- dropping pipette
- hand lens
- data logger
- temperature probe
- oxygen probe
- pH probe
- flow rate sensor
- Identification key to fresh water invertebrates

Method

1. Place the net firmly on the bottom of the riverbed, with the opening facing up stream and measure an area in front of the net of $0.5m^2$.

2. Remove any large stones in the sampling area causing the minimum disturbance possible. Displace the organisms from the stones by vigorous rubbing. Collect any organisms in a white plastic bowl containing a small amount of water. Leave the stones on the riverbank whilst the kick sample is carried out. Then carefully replace the stones where they were found.

The 'Kick Sampling' technique

3. The sand and gravel in the sampling area is disturbed for at least a minute by foot. The net is held downstream and moved accordingly to catch any organisms that may be displaced. Drain most of the water out of the net before turning the net inside out into the collecting bowl.

4. Take the organisms out from the white bowl and put them into the sorting tray. Sort the organisms into groups that have similar looking characteristics. Note any particular adaptations that these organisms have to allow them to live in the habitat that you are studying.

- Only organisms of more than 0.5cm are used here.
- To calculate the Biotic Index you will need to know the number of different groups of organisms present in the sample, that is, how many different Families are present.
- If time allows, key out to Species where this is possible. To calculate *Simpson's Index* each species needs to be counted though not necessarily identified. Once they have been counted and identified, return to the sampling area. Release the animals gently into the water.

5. Measure the following abiotic factors of the river: the depth, width, velocity of the current, temperature, oxygen concentration and pH.

6. If the Biotic Index is measured at a series of sites down a river it can be used to locate points that are having an impact on the community. In your notebook sketch a map of the river with important points marked on it. Take photographs of the site to support your observations.

Field results

Benthic Organisms: Order*	Family	Genus/Species	Numbers found
Plecoptera	Leuctridae Nemouridae Perlidae		
Ephemeroptera	Baetedae Ecdyonuridae Ephemerellidae Ephemeridae Leptophlebidae		
Trichoptera With cases	Limnephilidae Glossosomatidae Odontoceridae Sericostomatidae		
Without cases	Hydropsychidae Philopotomidae Polycentropedie Rhyacophilidae		
Odonatasub-orders Zygoptera Anisoptera			
Megaloptera	Sialidae		
*Crustacea	Gammaridae Asellidae		
*Gastropoda	Ancylidae		
Diptera	Simulidae Chironomidae		
Coleoptera	Dytiscidae Hydrophilidae Elminthidae		
*Hirudinea	Glossiphonidae Hirudinae		
*Oligochaeta	Tubificidae		
Other			

N.B. *Class is sometimes stated instead of Order.

The stonefly Perla is an indicator of very clean water

Table of abiotic results

Abiotic factor	Measurement
Depth / m	
Width / m	
Current /ms^{-1}	
Volume /m^3s^{-1}	
Temperature /°C	
O_2 /mg dm^{-3}	
pH	

Description of the site(s) studied:

Calculating the Simpson's diversity index

$$D = \frac{N(N-1)}{\sum n(n-1)}$$

Where:

- D is diversity
- N is the total number of all the animals sampled
- n is the numbers of each different species

A worked example for two sites on a river:

Species	Site A	n(n-1)	Site B	n(n-1)
Perla	10	90		0
Baetis	45	1980		0
Ecdyonurus	20	380		0
Rhyacophila	6	30		0
Gammarus	38	1406	6	30
Ascellus	15	210	498	247506
Ancylus	8	56		0
Limnea	7	42		0
Simulium		0	256	65280
Oulimnius	10	90		0
Hirudinea		0	211	44310
Dugesia	18	306		0
Hydracarina	30	870		0
Totals	**N = 207**	**Σn(n-1) = 5460**	**N = 971**	**Σn(n-1) = 357126**
N(N-1)	**42642**		**941870**	
D	**7.8**		**2.6**	

Calculating the Biotic index

In the table the invertebrates are ordered by their sensitivity to organic pollution. Those at the top are the most sensitive to pollution and those at the bottom are the most tolerant to pollution. Only those organisms that are known to be sensitive to pollution (as indicators) are present in the table.

A worked example

Select the organism that you have found that appears highest up the biotic index table.

Suppose that you have found two different species of *Plecoptera*.

At this station suppose 13 different families were found.

On the table below proceed to the row of *Plecoptera* and follow the line 'more that one species present' along to the column 11-15 groups found.

The Biotic index found is 9.

Biotic index: *10 = clean water* *< 5 = polluted water*

Organism		Total number of families present				
		0-1	2-5	6-10	11-15	16+
Plecoptera present	More than one species present	-	7	8	9	10
	One species only	-	6	7	8	9
Ephemeroptera present	More than one species present	-	6	7	8	9
	One species only	-	5	6	7	8
Trichoptera present	More than one species present	-	5	6	7	8
	One species only	4	4	5	6	7
Gammaridae present	All above species absent	3	4	5	6	7
Asellidae present	All above species absent	2	3	4	5	-
Tubificidae Tubificid worm and/or Chiromomidae (red worm larvae)present	All above species absent	1	2	3	4	-
All above organisms absent	**Eristalis tenax* that does not require oxyen, may be present	0	1	2	-	-

**Diptera; Syrphidae*

Some points for consideration

- Biotic indices are based on the sensitivity of benthic organisms to organic pollution. How does organic pollution effect the quality of the water and the organisms living in it?
- Diversity indices are based on the variety of species present and their abundance, relative to one another. How would the diversity of an environment under stress change?
- What are the potential sources of organic pollution in your sampling area? How would you test to see if this source does influence the Biotic index?
- How would the following factors influence the biotic index and diversity index found in the river?
  - geographical region
  - season
  - current
  - distance from the source of pollution
- Comment on each of these points and suggest how the problems incurred by such variations could be overcome.
- What other factors may influence the Biotic index or the Diversity index?
- How could the evolution of pollution in the river be studied?

Syllabus reference: Sub-Topic D.1 Skill

Our food provides us with the energy that we need. The purpose of this investigation is to compare the amount of energy that is released when different types of dried food are burned. This gives a rough estimate of their energy content.

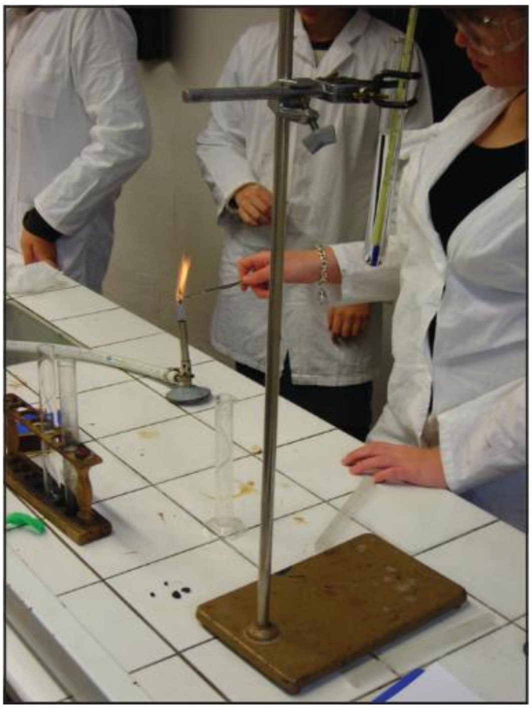
Materials

data logger	matches	measuring cylinder
temperature probe	mounted needle	stand and clamp
electronic balance	6 large test tubes	dry food samples
Bunsen burner	test tube holder	

Method

1. Plug a temperature probe to the data logger. Set the recording frequency to one reading per second for five minutes.
2. Measure out exactly 25cm^3 water into a large test tube. Clamp the tube upright in a retort stand. Put the temperature probe attached to a data logger in the test tube and leave it there while you go on with the next two steps.
3. Collect and weigh a piece of food. Write down its mass and the name of the food in your results table.

Warning: As nut allergies are quite common, foods containing nuts and their products should be avoided.



4. Light a Bunsen burner but keep it well away from the test tube.
5. Start the Data Logger.
6. Stick a mounted needle firmly into the piece of food. Set fire to the food by holding it in the flame of the burner. As soon as it is alight, hold or place the burning food under the test tube. Keep it there until it stops burning.
7. When the food stops burning, stir the water with the temperature probe until it stops recording.
8. From the temperature recorded, determine the initial temperature of the water and the highest temperature it rises to. The sample should not be so big that the water starts to boil.
9. Repeat the procedure at least once for each type of food that you use. Use fresh water and a clean test tube each time.

Analysing the results

- 1 cm^3 of water weighs 1 gram. How many grams of water did you put in the test tube?
- Energy is measured in joules (J) or kilojoules (kJ). One kilojoule = 1000 joules.
- Energy produced (kJ) = $\frac{\text{(mass of water used)} \times \text{(temperature rise)} \times 4.2}{1000}$
- Some pieces of food appear to give more energy than other pieces of the same kind of food. There is a simple reason for this. What is it?
- It would be simple to compare the results if everyone had used exactly the same amount of food. Is this a practical solution?
- What must be done to each result in order to compare your results with the results of others?

Scientists have used very accurate methods to work out exactly how much energy different foods contain. Find out the literature values for your food samples. These may be available on the packets of foods.

You will probably find that they are not absolutely the same as yours.

- Consider the procedure that you used. To get an accurate value for the amount of energy, the food sample must be completely oxidised to carbon dioxide and water and all of the heat produced must go to heat the water.
- Evaluate this method and suggest improvements.

Research

Find out how a bomb calorimeter works and compare it to your method.

Kilojoules and calories explained

Energy values can be confusing, several systems of notation are used.

- A kilojoule (kJ) is a unit of measure of energy and it is the accepted scientific unit.
- Previously, energy was measured in calories. It takes 1 cal of energy to raise 1g of water by 1°C.
- Food energy can also be measured in terms of the 'nutritional' or 'large' Calorie. There are 1,000 (small) calories in one (large) Calorie, which is why it is also sometimes known as a 'kilocalorie'. The terms 'calorie' and 'Calorie' are often used interchangeably, which can be very confusing.
- One Calorie (Cal) or kilocalorie (kcal) has the same energy value as 4.184 kilojoules (kJ).
- Therefore: 4.184 kilojoules = 4 184 joules = 1 Calorie = 1 kilocalorie = 1 000 calories

Syllabus reference: Sub-Topic D.4 Skills

Heart rate and blood pressure are important physiological indicators of health and fitness.

Materials

stethoscope	paper towel	Heart rate monitor
ECG sensor and electrodes Data logger	stop watch	Blood pressure monitor

Method

Heart Rate

There are several ways the heart rate can be measured:

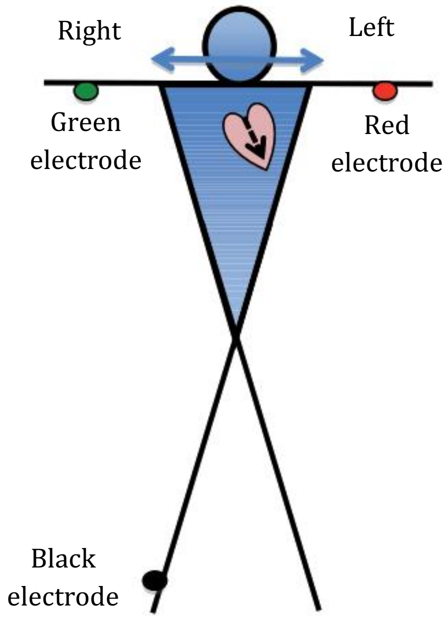
- by auscultation using a stethoscope
- electronically using an electrocardiogram or heart rate monitor
- indirectly, by palpation of the pulse in an artery

Do different ways of measuring the heart rate give the same result?

Warning: Use a consent form for each one of your subjects

ETHICS

1. Sit your subject, or yourself, down in a quiet room and allow the subject to settle down for a few minutes.



2. **Auscultation** requires the stethoscope to be positioned, by your subject, a little to the left of centre of the chest. The double beat of the heart can be heard and counted directly for 10s. Multiply by 6 to calculate the heart rate.
3. **Palpation** requires you to use your fingers, not the thumb, to feel for the pulse at a pressure point, either the radial artery in the wrist or the carotid artery in the neck. A light pressure will be needed to feel the pulse when the subject is at rest. Count the beat for 10s. Multiply by 6 to calculate the heart rate.
4. The **electrocardiogram** detects the electrical activity of the heart.
   - Three electrodes must be attached as follows.
     - Attach an electrode to the right wrist and clip the green lead (negative) to it.
     - Attach the red lead (positive) to an electrode stuck to the left wrist.
     - Finally attach the black lead (reference) to an electrode stuck to the inside of the right ankle.
   - Clean the skin where the electrodes are to be attached with soap and water then dry thoroughly using a paper towel.
   - Attach the ECG probe to a data logger and set to record every 0.01s for 10s. Multiply by 6 to calculate the heart rate.
5. **Electronic heart rate monitors** can be gripped by the hand. They need to be cleaned to give a good contact with the skin. Monitors signalling to data loggers will have default values for the sampling frequency and the duration of sampling. The duration for this exercise should be set to 1 min. This will provide enough data for the monitor to calculate the pulse rate.
6. Compare the results from the different methods of calculating the pulse rate at rest.

Does posture make a difference to the heart rate?

1. Using a convenient method for recording heart rate, record the heart rate or pulse

- Sitting
- Lying down
- Standing

2. The subject must adjust to the posture for a few minutes before measurement.
3. Collect data from the rest of the class. Calculate the average, maximum, minimum and range of heart rates for your population.
4. Collect the heart rate before and after a period of exercise.

Blood pressure

Blood pressure is the force exerted by the blood on the blood vessels. This varies depending on the stage in the cardiac cycle. When the heart is fully contracted (systole), the pressure is the highest. When the heart is fully relaxed (diastole), the pressure is the lowest. The pulse pressure is the difference between these two values.

Blood pressure is measured by using a pressure cuff and listening for the sounds of the pulse at the pressure point in the elbow joint. Measuring the blood pressure using a pressure cuff requires paramedical training, however, it is possible to measure the blood pressure safely using an electronic sensor. Electronic blood pressure monitors work by sensing the pulse after compression of the arteries in the arm using a programmed pressure cuff.

Measuring the blood pressure at rest

The blood pressure is measured sitting down in a quiet environment.

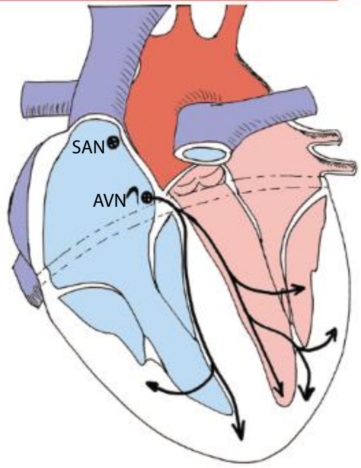
1. Attach the pressure cuff and allow the subject, or yourself, 2-3 minutes to rest. To neutralise the effect of gravity on the blood pressure, the pressure cuff must be at the same level as the heart. Attach it to the upper arm or if it is a wrist model attach it to the wrist and hold the wrist over the position of the heart in the chest. Make sure the pulse sensor is over the pressure point. The subject should not talk or move during the measurement.
2. Start the pulse reading. The pressure cuff will inflate until the sensor no longer detects the pulse. Then is when you will slowly relax sensing for the pulse. Usually this is indicated by a beeping sound. When the cuff is deflated the reading can be recorded.

Exploring blood pressure under different conditions

- Repeat the measurement standing up and lying down.
- Try the left arm and the right arm.
- Try taking the blood pressure before and after some light exercise.
- If the subjects permit it, try subjects of different:
  - size
  - gender
  - ages

Syllabus reference: Sub-Topic D.4 Skill

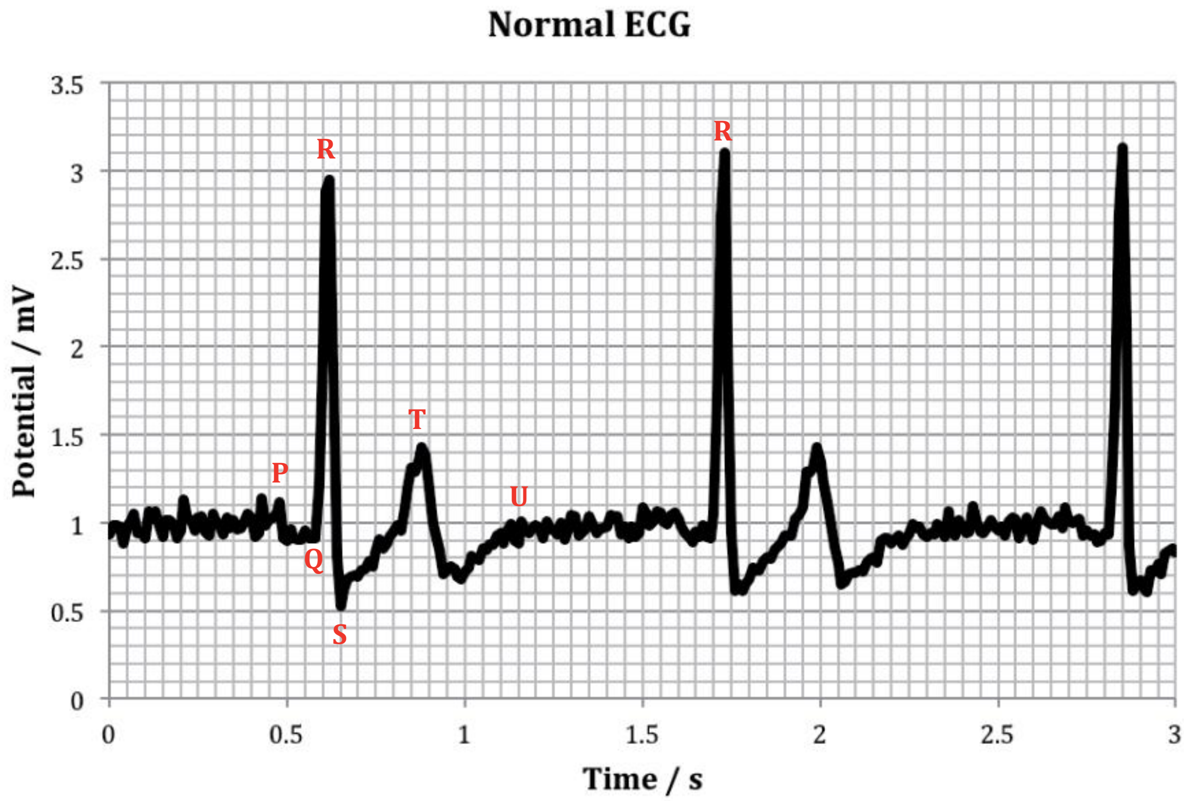
An electrocardiogram (ECG or EKG) is a recording of the electrical activity of the heart. The heart is mainly made of muscle tissue, composed of muscle cells. Muscle cells are excitable cells, that is they can become depolarised causing them to contract.



The heart has a built in rhythm. The heart starts to contract at the pacemaker or sinu-atrial node (SAN). This is found on the wall of the atrium near where the superior vena cava enters. The contraction crosses the atria to a point between the atria and the ventricle called the atrio-ventricular node (AVN). Once across the AVN, the contraction spreads rapidly across the ventricles.

The spread of the contraction across the heart can be seen in the electrocardiogram (ECG or EKG)

Observations from the ECG



- The cardiac rhythm from R to R
- The conduction of the wave of contraction from the atria to the ventricles P to R. This should be < 0.2s at rest.
- The QRS complex represents the contraction of the ventricles, which should be about 0.1s.
- The T wave represents repolarisation of the ventricles. Q-T represents the whole of the ventricular activity. It should be less then half of R-R at rest.

Note: The wave of atria repolarisation is hidden by the huge wave of ventricle depolarisation, so it cannot be seen on the ECG.

Materials

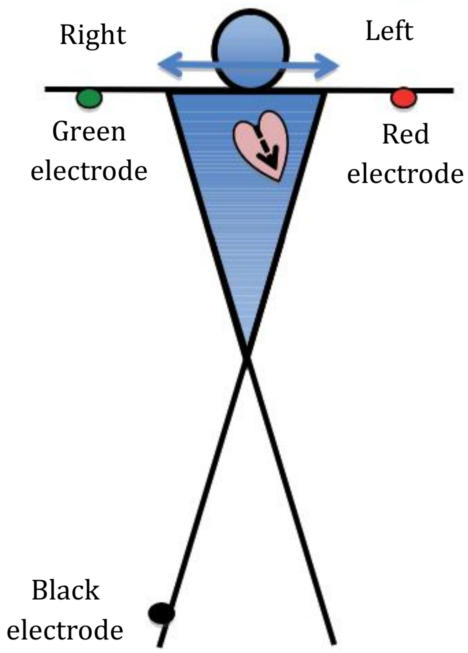
Data logger
ECG sensor
adhesive electrodes
paper towel
soap

Method

Taking an ECG

Warning: Make sure your subject reads and signs a consent form

1. You will need to attach three electrodes to the body to pick up the ECG. These can be attached to the limbs.
2. Clean the skin with soap and water, where the electrodes are to be attached, then dry thoroughly using a paper towel. Attach an electrode to the right wrist and clip the green lead (negative) to it. Attach the red lead (positive) to an electrode stuck to the left wrist. Finally, attach the black lead (reference) to an electrode stuck to the inside of the right ankle.
3. Attach the ECG probe to a data logger and set to record every 0.01s for 3s.
4. Try recording the ECG in different postures: sitting, standing, lying down.
5. Try a little exercise (e.g. knee bends) and record the ECG before and after.



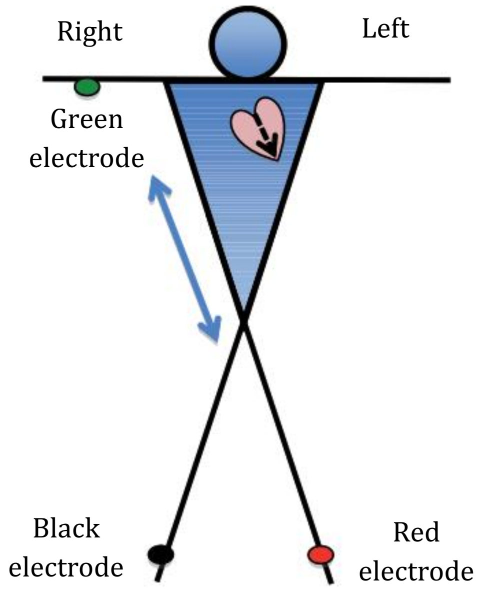
Analysing the axis of the heart

The orientation of the heart can be determined using the ECG. The heart can rotate on three axes:

- Left-right
- Forwards and backwards
- Around its vertical axis

The ECG can detect the orientation of the heart. This can be determined by attaching the electrodes in different positions.

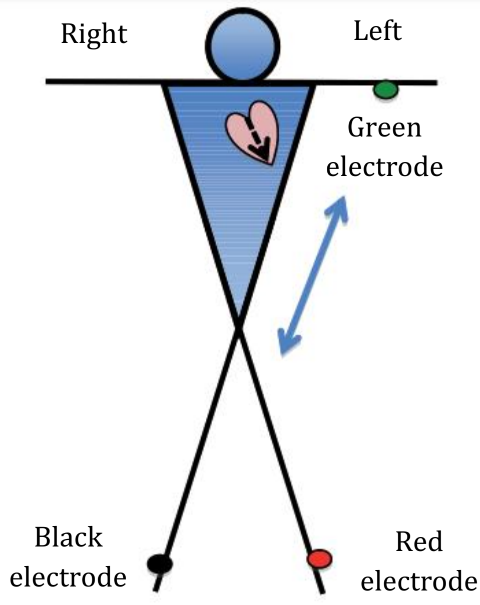
1. The diagram of the subject shown on the previous page is the basic position that gives the classic ECG. A line crossing between the red and green will follow the shoulders, as shown on the diagram on the previous page.
2. Try two other positions (as shown) and see what happens to the ECG.



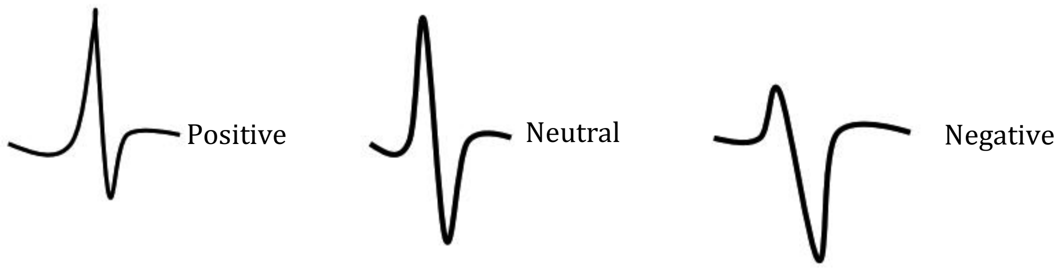
Green on the right wrist, red behind the left ankle and black behind the right ankle.

Green on the left wrist, red behind the left ankle and black behind the right ankle.

The lines crossing between the red and green electrodes are not in the same direction. These three positions form a triangle across the body called 'Einthoven's' triangle.



Which one gives the highest QRS wave amplitude? To work this out you need to consider both the rise at R and the drop at S.



The overall height of the QRS wave gives an indication of the angle of the axis of the heart.

- If the highest QRS amplitude is at position 1 the angle is about 0° to the norm
- If the highest QRS amplitude is at position 2 the angle is about 60°
- If the highest QRS amplitude is at position 3 the angle is about 120°

Note: Even though the norm is taken to be a heart apex pointing towards the left hip, the orientation of the axis of the heart varies with the individual. For example the age of your subject may make a difference to the results.

I B Biology Investigations

Volume 1 (Standard Level)

TEACHING NOTES

(For use with the IB Diploma programme)

(Fourth edition)

Author: Paul Billiet

Series editor: David Greig

Core Topic	Practical Number	Sub topic	Syllabus reference	Title
1	1A	1.1	**Skill:** Use of a light microscope to investigate the structure of cells and tissues, with drawing of cells. Calculation of the magnification of drawings and the actual size of structures and ultrastructures shown in drawings or micrographs. (Practical 1)	CELLS
	1B	1.4	**Skill:** Estimation of osmolarity in tissues by bathing samples in hypotonic and hypertonic solutions. (Practical 2)	ESTIMATING THE WATER POTENTIAL OF PLANT TISSUES
	1C		**Aim**: Dialysis tubing experiments can act as a model of membrane action. Experiments with potato, beetroot or single-celled algae can be used to investigate real membranes.	MEMBRANE INTEGRITY IN CABBAGE LEAF CELLS
	1D	1.6	**Skill:** Identification of phases of mitosis in cells viewed with a microscope or in a micrograph. **Skill:** Determination of a mitotic index from a micrograph.	THE CELL CYCLE IN PLANT TISSUES
2	2A	2.1	**Skill:** Identification of biochemicals such as sugars, lipids or amino acids from molecular diagrams.	DIALYSIS: SEPARATING MOLECULES BY SIZE
	2B	2.2	**Application:** Use of water as a coolant in sweat.	WATER AS A COOLANT
	2C	2.3	**Skill:** Use of molecular visualization software to compare cellulose, starch and glycogen.	POLYSACCHARIDE MOLECULES
	2D	2.5	**Skill:** Experimental investigation of a factor affecting enzyme activity. (Practical 3)	MEASURING THE RATE OF REACTION OF AN ENZYME CONTROLLED REACTION
	2E	2.5 & 2.8	**Application:** Use of anaerobic cell respiration in yeasts to produce ethanol and carbon dioxide in baking.	IMMOBILISING YEAST ENZYMES
	2F	2.8	**Skill:** Analysis of results from experiments involving measurement of respiration rates in germinating seeds or invertebrates using a respirometer	RESPIRATION RATES OF AN INVERTEBRATE
	2G	2.9	**Skill:** Separation of photosynthetic pigments by chromatograph. (Practical 4)	LEAF PIGMENTS, THEIR EXTRACTION AND SEPARATION
	2H		**Skill:** Drawing an absorption spectrum for chlorophyll and an action spectrum for photosynthesis	USING SPECTROSCOPY TO STUDY LEAF PIGMENTS
	2I		**Skill:** Design of experiments to investigate the effect of limiting factors on photosynthesis.	METHODS TO MEASURE THE RATE OF PHOTOSYNTHESIS
3	3A	3.1	**Skill:** Use of a database to determine differences in the base sequence of a gene in two species.	USING A PROTEIN DATABASE
	3B	3.5	**Skill:** Design of an experiment to assess one factor affecting the rooting of stem-cuttings.	FACTORS AFFECTING ROOTING IN PLANTS
4	4A	4.1	**Skill:** Setting up sealed mesocosms to try to establish sustainability. (Practical 5)	ECOLOGICAL SUCCESSION IN A MICROBIAL ECOSYSTEM
	4B		**Skill:** Testing for association between two species using the chi-squared test with data obtained by quadrat sampling	PLANT ASSOCIATION TEST
5	5A	5.3	**Application:** Recognition features of bryophyta, filicinophyta, coniferophyta and angiospermophyta **Skill:** Construction of dichotomous keys for use in identifying specimens	DICHOTOMOUS KEY FOR PLANT PHYLA
6	6A	6.1	**Application:** Use of dialysis tubing to model absorption of digested food in the intestine.	DIALYSING TUBING GUT
	6B	6.2	**Skill:** Recognition of the chambers and valves of the heart and the blood vessels connected to it in dissected hearts or in diagrams of heart structure.	THE ANATOMY OF THE HEART
	6C	6.4	**Skill:** Monitoring of ventilation in humans at rest and after mild and vigorous exercise. (Practical 6)	VENTILATION AND EXERCISE

Option Topic	Practical Number	Sub topic	Syllabus reference	Title
A	12A	A.2	**Application:** Use of the pupil reflex to evaluate brain damage.	THE PUPIL REFLEX
	12B	A.3	**Application:** Red-green colour-blindness as a variant of normal trichromatic vision.	THE RETINA AND COLOUR VISION
B	13A	B.1	**Skill:** Experiments showing zone of inhibition of bacterial growth by bactericides in sterile bacterial cultures	DO DISINFECTANTS KILL BACTERIA?
	13B		**Skill:** Gram staining of Gram-positive and Gram-negative bacteria	THE GRAM STAIN FOR BACTERIA
C	14A	C.1	**Skill:** Use of a transect to correlate the distribution of plant or animal species with an abiotic variable	STUDYING CHANGES IN A PLANT COMMUNITY USING A BELT TRANSECT
	14B			USING INVERTEBRATE PITFALL TRAPS ON A LINE TRANSECT
	14C	C.2 &4	**Skill:** Investigation into the effect of an environmental disturbance on an ecosystem Skill: Analysis of the biodiversity of two local communities using Simpson's reciprocal index of diversity	MEASUREMENT OF A DIVERSITY INDEX AND A BIOTIC INDEX
D	15A	D.1	**Skill:** Determination of the energy content of food by combustion	ENERGY FROM FOOD
	15B	D.4	**Skill:** Measurement and interpretation of the heart rate under different conditions	HEART RATE AND BLOOD PRESSURE
			Skill: Interpretation of systolic and diastolic blood pressure measurements	
	15C		**Skill:** Mapping of the cardiac cycle to a normal electrocardiogram (ECG) trace	TAKING AND READING AN ELECTROCARDIOGRAM

APPENDICES	1	6, 11 and D	The IB animal experimentation policy and the biology course safety guidelines.	INFORMED CONSENT FORM
	2	4.1	**Guidance:** Sampling should be based on random numbers. In each quadrat the presence or absence of the chosen species should be recorded.	TABLE OF RANDOM NUMBERS
	3			USING A TI CALCULATOR TO GENERATE RANDOM NUMBERS

1A CELLS

Syllabus reference: Topic 1.1

Skill: Use of a light microscope to investigate the structure of cells and tissues, with drawing of cells. Calculation of the magnification of drawings and the actual size of structures and ultrastructures shown in drawings or micrographs. (Practical 1)

Required knowledge: Use of the microscope, preparation of a temporary mount, characteristics of animal and plant cells, the test for starch using iodine solution.

1A.1 ANIMAL CELLS

Time: 1 hour

Materials

The bleach: This could be a household cleaning product containing sodium hypochlorite.
e.g. *Domestos* or sodium hypochlorite at 1% (1g per 100cm^3 of distilled water)
Methylene blue stain: 1%
1g + 0.6g NaCl in 100cm^3 distilled water
Other sources of epithelial cells could be:

- Moulted skin from a frog
- Cells removed with self-adhesive from the inside of the wrist

Data

An average cell size of 20μm may be measured.

Liver cells have a tendency to be bi-nucleate. This could encourage a discussion on the limits of the cell theory.

1A.2 PLANT CELLS

Time: 1.5 hours

Materials

For good results the *Elodea* should be freshly cut. The cut *Elodea* branches may be kept in an aquarium for a few days. The young leaves surrounding the terminal bud at the end of the branch give good results. If *Elodea* is not available the leaf scales of moss plants and leafy liverworts are usually sufficiently transparent though cyclosis may not be observable.

Avoid taking onion scales from the outside of the bulb, inner scales provide better results.

The banana should be ripe for the observation of starch grains, however, as the fruit ripens the cell wall is partially digested and becomes less visible.

One red onion and one banana should provide enough material for six groups.

Other plant material suitable for observation might include: potatoes (*Solanum tuberosum*) or rice (*Oryza*) for starch grains and tomatoes (*Solanum lycopersicon*) for pigmented chromoplasts.

The rice grains need to be soaked in water for a few minutes before crushing.

Iodine solution: Dissolve 1g of potassium iodide in a small amount of distilled water, mix whilst adding 0.5g of iodine crystals. Make the solution up to 100cm^3 with distilled water. Store the solution in dark brown bottles.

Sucrose solution: 20g of sucrose for 100cm^3 of distilled water. This is sufficient to shrink the vacuole by osmosis.

Method

As the *Elodea* leaf is several cells thick, the students may be confused over the limits of individual cells and their contents. This provides a good exercise in using the fine focusing control.

To observe the sap vacuole clearly it is possible to make it visible using fresh 0.1% aqueous neutral red stain. Transparent, colourless tissues such as the inner epithelium of onion scales or from leeks can be used.

Make sure the pH of the water used to make up the neutral red is 7 to 7.5. If it is slightly acidic, the stain will not be taken up. *Evian* water could be used here as it has a pH of 7.4.

Squares 1cm × 1cm squares can be cut from an onion scale or a leek leaf. Using fine forceps, peal off the epidermis from the inside surface of each square. Place the squares of epidermal tissue in a Petri dish containing neutral red stain at pH 7.4. Leave the tissues to soak for 3 minutes. Remove the squares of epidermis and transfer them to a Petri dish of buffer at pH 7.4. Wash them thoroughly four times in clean buffer. Mount a square of tissue on a microscope slide in a drop of the buffer. The sap vacuoles should stain up brick red.

1A.3 DRAWINGS

All drawings must be done in pencil and on drawing paper. They must have a title and be fully labelled. Neatness is very important. Avoid excess detail. The magnification used must be stated below the drawing (e.g. ×400).

Observations

The students should be encouraged to pose questions on observations made for each tissue looked at.

For example:

Canadian pond weed (*Elodea*): The Elodea leaf provides good observations of chloroplasts and their cytoplasmic streaming (cyclosis).

- Why are the chloroplasts so abundant?
- Why do the chloroplasts move?

Onion (*Allium*)**:** The red onion epidermal cells should be fully turgid in the distilled water and partly plasmolysed in the sucrose solution. The plasmolysed cells will permit the observation of the plasma membrane and the cell wall as separate structures.

- Why are nuclei not seen in each cell?
- Why are no chloroplasts observed here?
- Why was sucrose used to shrink the cell vacuole?

Students do not need to know about osmosis for this, though they may question the phenomenon they are observing. The point is to get them to distinguish between the cell wall and the plasma membrane which are usually stuck together..

- Banana (Musa): The banana cells contain starch grains which are clearly visible and they stain black with the iodine solution:
- Why are the starch grains so abundant here?

Some points for consideration

Table comparing the structures seen in animal and plant cells as seen with the light microscope.

Structure	Animal Cell	Plant cell
Cell wall	-	*
Cell surface membrane	*	*
Cytoplasm	*	*
Cell sap vacuole	-	*
Tonoplast	-	*
Chloroplasts	-	*
Starch grains	-	*
Nucleus	*	*
Nuclear envelope	*	*
Nucleolus	*	*

NB Plasmodesmata may be observed clearly in red pepper epidermal cells.

To investigate further

- A project could be carried out comparing starch grains in different tissues: e.g. potato (*Solanum tuberosum*), rice (*Oryza*), banana (*Musa*) and maize (*Zea*).
- What determines the speed of movement of chloroplast cyclosis? This could be investigated using a micrometer eye piece and stop watch whilst varying environmental conditions (e.g. temperature, light intensity, light colour/ wavelength or the composition of the solution bathing the leaf).
- The changes in the appearance of cells in ripening bananas.
- The effect of a range of sucrose solutions on red onion epithelial cells.

1A.4 THE MICROMETER EYEPIECE

This accessory will turn the microscope into a quantitative measuring tool.

Materials

Micrometer eyepieces

Various models of micrometer eyepieces exist. Eyepieces with built-in scales are the easiest to use. Graticules can be purchased and inserted into the standard eyepiece of the microscope. However, be careful, these graticules may not lie exactly in the plane of focus, they may fall in upside down and are liable to get fingerprints on them.

Micrometer slides

These are expensive. A class set should not be necessary. One slide per two or three microscopes is convenient. These slides are easily confused with ordinary slides. To avoid this confusion, a coloured self-adhesive label may be stuck to the slide. This has the advantage of informing the student which way up the slide is. A common error by students is to place the micrometer slide on the microscope stage upside down. The graduations cannot be brought into focus on high power when the slide is like this.

Microscopes

Numbering the microscopes in the laboratory is useful. The calibration of the eyepiece will not have to be repeated if the student uses the same microscope in subsequent investigations.

1B ESTIMATING THE WATER POTENTIAL OF PLANT TISSUES

Time: 1 hour for preparation + 1 hour for measurement.

Syllabus reference: Topic 1.4

Skill: Estimation of osmolarity in tissues by bathing samples in hypotonic and hypertonic solutions. (Practical 2)

Required knowledge: Osmosis, plant cell structure.

Materials

An electronic balance sensitive to 0.1g

Any plant tissue which is easily cut into chips or cylinders.

A potato chip cutter significantly speeds up the preparation time.

Flat bottomed specimen tubes (25mm diameter) rather than test tubes are very useful for this investigation.

Fruits (apple) or other roots, (carrot, swede, sweet potato, yam, cassava etc.) could also be tried.

Method

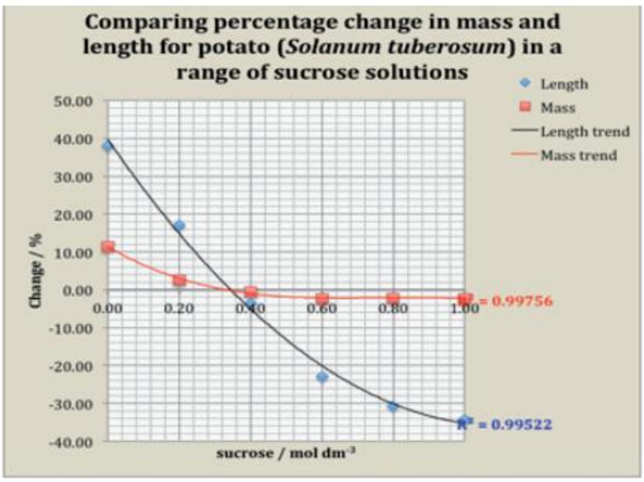
The strips should be left for at least several hours in the solutions before re-measuring. The tubes can be left for up to one or two days in a refrigerator and then re-measured.

For the method involving changes in density of the liquid results can be obtained in as little as 20 minutes.

Data

The following results were obtained for apple fruit, potato tuber and sweet potato tissues:

Sucrose solution /mol dm^{-3}	Potato			Apple		
	% change in mass	% change in length	Movement of methylene blue	% change in mass	% change in length	Movement of methylene blue
0	18.2	19.0	Up	16.0	5.0	Up
0.2	4.1	8.0	Up	15.4	8.0	Up
0.4	-13.0	-1.0	Down	15.0	3.5	Up
0.6	-28.6	-6.0	Down	4.1	-0.5	Stable
0.8	-34.3	-8.0	Down	-4.6	-2.5	Down
1.0	-36.8	-10.0	Down	-16.6	-3.5	Down



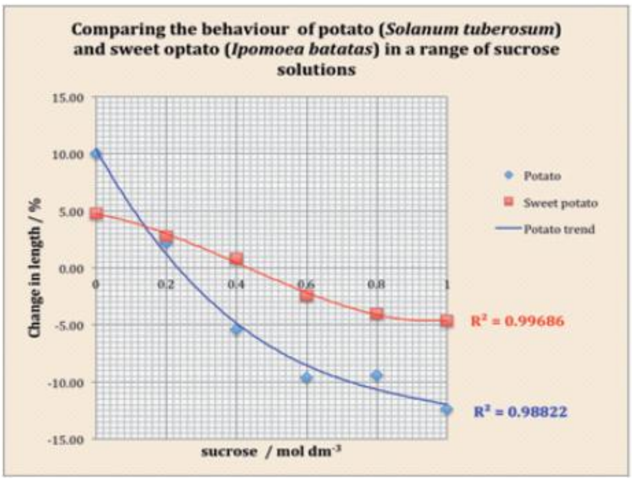


Table to show isotonic sucrose solution found with various plant tissues:

Tissue	Isotonic Sucrose Solution /mol dm^{-3}
Potato	0.19
Apple (Granny Smith)	0.35 - 0.55
Apple (Golden Delicious)	0.53
Celeriac	0.32
Sweet potato	0.50
Taro or Dasheen	0.20
Turnip	0.35 - 0.45

Evaluation of the three methods

- The gravimetric method is the easiest to carry out and produces results that are least likely to be affected by experimental error. The pieces of tissue, however, must be blotted dry to remove surplus liquid.
- The method involving a change in dimension is likely to produce errors due to unequal cutting or inaccurate measurement of the lengths of the pieces of tissue. The tissues used must be homogeneous in texture to ensure even expansion or shrinkage. Preparing the tissues is time consuming.
- The method involving a change in density (Chardokov's method) is rapid and easy to perform but the results are not always clear. They are less quantitative. A series of solutions with smaller increments would be necessary to pinpoint the isotonic solution.

Some points for consideration

- An increase in length or mass indicates that the cells in the tissue absorbed water by diffusion to become more turgid. The cells are in a solution that has a higher water potential than their cell sap (hypotonic). If the drop of methylene blue rises, the liquid bathing the tissue has become denser and water has been absorbed by the tissue.
- A decrease in length or mass indicates the opposite. The cells have become flaccid and may even be plasmolysed. These cells are in a solution which has a lower water potential than their cell sap (hypertonic). If the drop of methylene blue falls, the liquid bathing the tissues has become less dense and water has been lost by the tissues.
- In an isotonic solution the strips remain the same length or mass because there is no movement of water in or out of the cells. The drop of methylene blue will stay at the same level. The solution surrounding the cells has the same water potential as the cell sap.
- In the isotonic solutions no changes in length or mass will be seen and the drop of methylene blue will stay at the same level. The isotonic solution for the length and mass could also be found if the results are analysed graphically (sucrose concentration against change in length or mass), the isotonic solution for the tissue will be the point where the curve is equivalent to the original length or mass.

Other changes

- When placing the tissues in the solution, the pieces of potato tend to float in the denser sucrose solutions with a lower water potential. They sink in the solutions with a higher water potential.
- The tissues become flaccid in the solutions with a lower water potential and firmer (turgid) in the solutions which have a higher water potential. The degree of flexibility could be used as a measure of water uptake, or water loss.

1C MEMBRANE INTEGRITY IN CABBAGE LEAF CELLS

Time 1.5 hours

Syllabus reference: Topic 1.4

Aim: Dialysis tubing experiments can act as a model of membrane action. Experiments with potato, beetroot or single-celled algae can be used to investigate real membranes.

Required knowledge: The chemical composition of cell membranes. The chemical properties of lipids and proteins.

Materials

1. Raw beetroot can be used instead of raw red cabbage. The cylinders of beetroot must then be cut into discs about 3mm thick. This will make them more uniform than the red cabbage discs but the preparation is longer. Beetroot also has the advantage of producing more intense colour. Other plant tissues containing anthocyanins could be tried.
2. To cut the discs, a cork borer should be used of a diameter a bit smaller than the test tubes.
3. To wash the discs, they are best placed in a sieve and dipped in a bowl of water. Change the water periodically until it stops changing colour (about 3 hours). Discs can be washed the day before and left in soak over night. If they must be left over the weekend then leave them soaking in a refrigerator.

Method

The leaf discs tend to float in water. So when the students are heating them in the water bath make sure that the water in the water bath is high enough to cover the leaf discs in the tubes. The water level will drop as the tubes are successively removed.

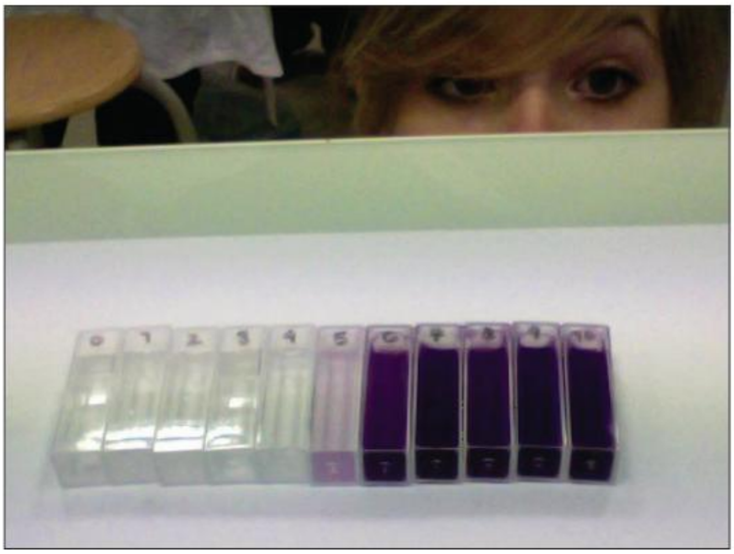
To make qualitative observations the colour depth of the tubes the students should place them, altogether, in front of a white piece of paper, in a uniformly illuminated part of their bench. Photographs should be taken.

Results

Observation and explanation

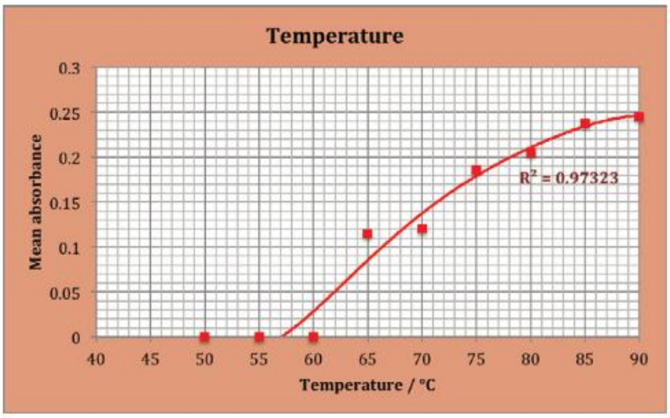
No leakage at 40°C. Slight leakage of pigment above 55°C. Maximum leakage from 65°C. The membranes are damaged by high temperatures. The proteins of the cell membranes are being denatured.

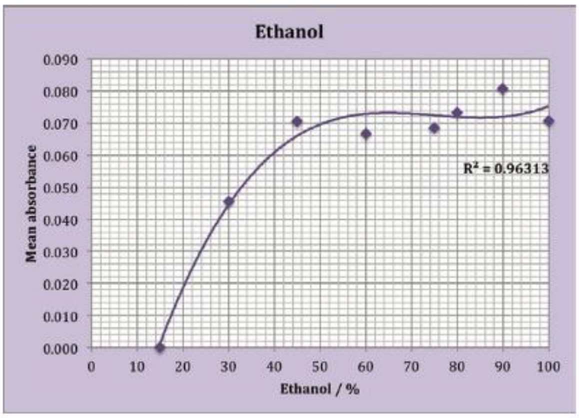
Ethanol dissolves the phospholipid bilayer and precipitates proteins. In ethanol the pigment leaks and gathers at the bottom of the tube.



A series of colorimeter cuvettes showing the leakage over the range of temperatures.

The effect of temperature and ethanol on the permeability of pigment from leaf epidermal cells of red cabbage (*Brassica oleracea var. capitata f. rubra*). Absorbance at 565nm





To investigate further

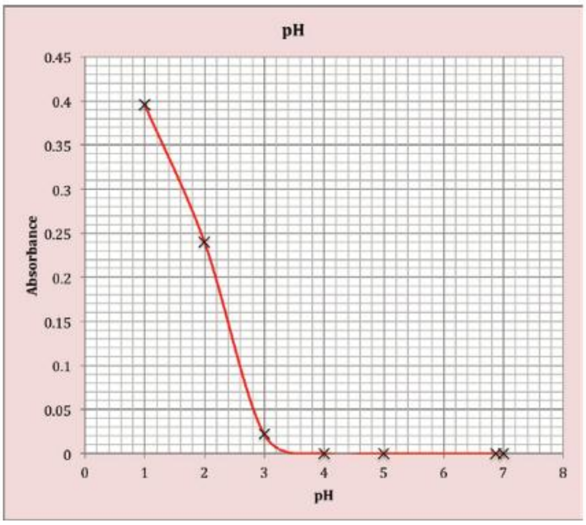
- A range of buffers at different pH values can be tried

In buffers below pH7 the pigment leaks and changes colour: from purple to bright red. A colorimeter set on a green or yellow light would be appropriate.

Above pH 7 the colour of the pigment becomes yellow and a different colour should be used in the colorimeter.

At pH 1 extreme pH damages the cell membranes, they leak freely. The proteins of the cell membranes are denatured. This pigment (anthocyanin) is also a natural pH indicator.

- Other organic solvents: e.g. propanone (acetone). Unfortunately acetone dissolves the plastic of plastic colorimeter cuvettes, glass ones will be needed. Benzene, toluene (methyl benzene) or aniline (phenylamine) should be avoided because of their carcinogenic nature.
- Alcohols have an increasing effect on membrane permeability as their polarity decreases. The series methanol, ethanol, propan-1-ol and butan-1-ol can be tried.
- The effect of freezing and thawing the leaf discs.



1D THE CELL CYCLE IN PLANT TISSUES

Time: 1 hour

Syllabus reference: Topic 1.6

Skill: Identification of phases of mitosis in cells viewed with a microscope or in a micrograph.

Skill: Determination of a mitotic index from a micrograph.

Syllabus reference: Topic 3.2

Aim: Staining root tip squashes and microscope examination of chromosomes is recommended but not obligatory.

Materials

Prepared slides of root tips all from the same species of plant.

If computers linked to internet are available, a shared class bulletin board can be set up which the students can access. A pre-prepared spread sheet will show data collection in real time.

A spreadsheet can be set up in Google Drive or in Dropbox. For Google Drive, it is useful if the students all have gmail addresses, this is not necessary in Dropbox.

Other online share sites exist in the Cloud, most have a small volume starter pack that is free.

Method

Systematic sampling is best. This can be discussed with the students after an initial observation of the slide. Either all the cells in one field of view, or as the cells come in lines, counting along lines in a field of view. This way the students will not be 'attracted' to cells with mitotic figures inside.

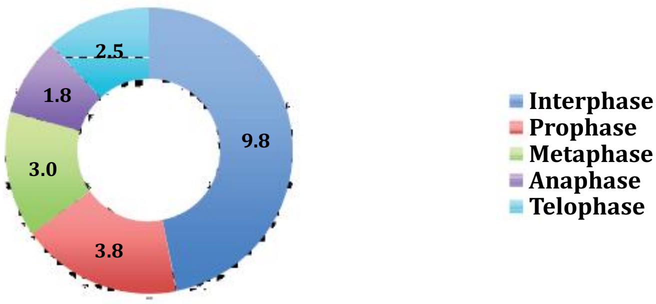
Data

Data from a class observing prepared slides of onion root tips (*Allium cepa*). Where research online indicated the average length of the cell cycle was 21h.

Stage	Number of Cells per 100 cells										Total	%	21h
Interphase	20	56	35	30	88	48	48	37	51	68	481	46.9	9.8
Prophase	24	8	15	35	5	32	30	17	16	6	188	18.3	3.8
Metaphase	20	4	15	25	2	11	8	17	19	24	145	14.1	3.0
Anaphase	9	14	18	18	2	6	3	11	9	0	90	8.8	1.8
Telophase	27	18	17	17	3	3	11	18	6	2	122	11.9	2.5
Total	100	100	100	125	100	100	100	100	101	100	1026	100	

The above data presented as a ring chart as follows overpage:

The phases of the cell cycle in onion *(Allium cepa)* root tips.
Figures show estimated duration in hours.



The **Mitotic Index** for these cells = 545/1026 = 0.53 or 53%

Problems encountered are mainly the identification of the different stages. For example, distinguishing between Interphase and Prophase. This will influence the calculation of the mitotic index.

Using freshly prepared root tip cells

It is possible to carry out the same exercise on freshly prepared slides of root tip squashes. The following method is recommended.

Materials

A microscope eyepiece with a pointer is a very useful instrument for this practical.

Fixative: 99 parts of 70% ethanol to 1 part of pure ethanoic acid.

Stains for chromosomes include

- **Acetocarmine stain:** Add 1g of carmine stain to 45cm^3 of pure ethanoic acid. Mix and add 55cm^3 of distilled water. Boil, cool and filter.
- **Toluidine blue:** 0.05% aqueous
- **Iodine green:** 1% aqueous
- **Acetic orcein:** Dissolve 3.3g of orcein in 100cm^3 of pure ethanoic acid. Reflux for six hours in a water-cooled condenser. Filter to obtain a stock solution. For use in staining, dilute 10cm^3 of stock solution with 12cm^3 of distilled water.

Warning: Care should be taken whenever using reagents that react specifically with nucleic acids. Avoid contact with the skin, wear gloves.

GLOVES

Method

Grow the roots to 1.5 cm long, cut off the last 0.5 cm from the growing tip and fix them in a mixture of ethanol and ethanoic acid (fixative) for at least two hours.

The development of the root tips is very important. The root should not be longer than 1.5cm. The length of time for the bulbs to sprout roots seems to vary a lot, depending on the variety of the bulb and the condition that it is in. Furthermore, some suppliers of onions and garlic seem to be using inhibitors to prevent the bulbs from producing roots. If roots have not appeared in three days, throw them away and try another source. The author has found garlic bulbs are more reliable than onion bulbs.

Other bulbs, such as shallot, tulip and hyacinth can be tried, but hyacinth is known to provoke allergies. Suspend the bulb over a beaker of water so that the bottom of the bulb is just touching the water. The roots should start growing in 24 hours. Garlic will produce roots of the desired length in 2 to 3 days. One bulb will produce more than enough roots for a class, but to be sure of success, two or three bulbs should be prepared.

The root tips can be cut off using scissors and dropped into freshly prepared fixative. They should be left, soaking in the fixative in a stoppered flask, for at least two hours. Once they are fixed, the root tips can be kept for several days, or even weeks, in the refrigerator.

Before observation, the root tips are hydrolysed to soften them in 1 mol hydrochloric acid for 6 to 7 minutes at 60°C. The acid is pipetted off and distilled water added to rinse the root tips. Do this twice. The root tips are then poured out into a Petri dish placed on the square of black paper. This helps to see the root tips easily.

The fixing and the hydrolysis in the acid make the root tips semi-transparent. The area of dividing cells can be observed as a dense yellow-green patch near the end of the root tip.

To increase the chances of success several slides can be prepared at once. It is better not to put more than one or two root tips on each slide. Too much tissue produces uneven staining of the cells.

One root tip is placed on a clean slide and one or two drops of stain added. This should be done on the piece of black paper. The root tip tissues are teased apart using two needles, then covered with a coverslip. The coverslip is tapped with the handle of the needle to spread the tissues out underneath.

Warning: Wear gloves whilst staining nuclei

GLOVES

The slide is covered with two layers of filter paper and firmly squashed using the thumb on the coverslip.

N.B. Pressure should be applied vertically, any side-ways movement of the coverslip will rupture the cells.

The cells may be observed under medium power on the microscope. The dividing cells are cube-shaped with a relatively large, conspicuous nucleus (as shown above). The colour of the chromosomes will depend upon the stain used. They can then be observed and counted under high power.

To investigate further

- Mitosis is a dynamic process, a fact which is easily forgotten when looking at these fixed cells. This practical investigation could be accompanied by a film or video showing the process.
- Investigating the cell cycle. It has been suggested that the incidence of mitotic divisions vary during the day. Some sources suggest 01.30 hours is a good time to harvest root tips. Other sources suggest harvesting after 15.00 hours.
- Forcing the bulbs has also been suggested, placing the bulbs in the refrigerator for 24 hours as soon as the roots reach the desired length. Then place the bulbs into an incubator at 30°C for half an hour.
- Using a micrometer eye piece it is possible to count the cells at know distances from the root tip. The mitotic index of the cell sample will fall as the distance increases from the root tip.

Websites

This process of mitosis is dynamic and viewing it using time-lapse photography of living cells and phase-contrast microscopy reveals this:

Plant cells are large and quite easy to film:
<http://www.youtube.com/watch?v=SlgV_zoHQxE>
Growing root tip marked with fluorescent stain:
<http://www.youtube.com/watch?v=o_dGBDjWW-A>

Animals cells are smaller and so the behaviour of the chromosomes is a little more difficult to observe:
<http://www.youtube.com/watch?v=YJP-egujAm0>
<http://www.youtube.com/watch?v=9A3jZYnzlpQ>
<http://www.youtube.com/watch?v=NVfqzSKa_Bg>

2A DIALYSIS

Time 1 hour

Syllabus reference: Topic 1.4

Aim: Dialysis tubing experiments can act as a model of membrane action. Experiments with potato, beetroot or single-celled algae can be used to investigate real membranes.

Syllabus reference: Topic 2.1

Skill: Identification of biochemicals such as sugars, lipids or amino acids from molecular diagrams.

Materials

5% starch, 5% glucose and 5% sodium chloride. 'Soluble' starch should be avoided, it tends to leak easily, ordinary laundry starch is best.

Dialysing tubing pore size: equivalent to a protein MW 12000.

Fehling's solution

Solution 1. Dissolve 34.6g of hydrated copper (II) sulphate ($CuSO_4$. 5 H_2O) in distilled water to give a final volume of 500cm$^{3.}$ Add a drop of concentrated sulphuric acid if the solution remains cloudy.

Solution 2. Dissolve 77g of sodium hydroxide together with 175g of sodium potassium tartrate in distilled water to give a final volume of 500cm^3.

Keep solution 1 and 2 separately for storage. For use, mix equal volumes of solutions 1 and 2.

Benedict's solution can be used instead of Fehling's solution.

Benedict's solution

Dissolve 173g of hydrated sodium citrate together with 100g of hydrated sodium carbonate in 800cm^3 of warm distilled water. Filter and add distilled water to a final volume of 850cm. Separately, dissolve 17.3g of hydrated copper (II) sulphate in 100cm^3 of cold distilled water. Add the copper (II) sulphate solution to the citrate-carbonate solution, stirring constantly. Add distilled water to a final volume of 1dm^3.

Iodine solution

Dissolve 1g potassium iodide in a little water. Mix whilst adding 0.5g iodine crystals. Make up to 100cm^3. Store in dark brown bottles.

Silver nitrate

0.1 mol dm^{-3} $AgNO_3$

Method

Completely wet the dialysis tubing may help to open it up. There may be problems with leaking dialysing tubing. Insist that the knot in the bottom of the tube is tied tightly but be careful not to tear the tubing with long fingernails. Use enough tubing so that the upper end is well clear of the water (as shown in the diagram on the student's guide).

Make sure that the results for time zero are all negative, though the chlorides may appear extremely quickly. If glucose and/or starch appear in the first samples it would be best to start again.

There should be enough distilled water surrounding the dialysing tubing to last for six samples but warn the students not to take large samples. Furthermore, the samples should not be "drowned" with reagent when being tested.

Tests for substances which have already appeared outside the dialysing tubing can be discontinued.

Data

Chloride	0 to 5 min
Glucose	10 to 20 min
Starch	Should not diffuse out of the tubing.
Iodide	Diffuses into the tube quickly, within 5 min the "sausage" of dialysing tubing should be black (*photograph opposite*).

Some points for consideration

The bigger the particle is, the slower its rate of diffusion through the dialysing membrane.

N.B. For the sake of simplicity, solutions of 5% concentration have been used, as the tests used are qualitative. If necessary, however, a mixture containing sodium chloride, glucose and starch of the same molarity could be used to give similar results.

From the additional information: Starch molecules are too big to diffuse through the pores in the membrane. Therefore, these pores must be less than 17500 nm wide. Glucose just manages to pass through. Therefore, the pores must be greater than 70 nm wide.

The addition of the iodine solution shows that diffusion of different particles can proceed in both directions at once. Diffusion depends upon the concentration gradient of each particle dissolved in the water. Furthermore, adding the iodine solution acts as a control; it shows that the starch is still present, trapped inside the membrane. The direction of the diffusion of a molecule or ion depends upon its relative concentration on either side of the membrane.

Errors may be encountered due to leaking membranes, badly tied knots or insufficient washing of the tube after filling. Beware of sharp rings and fingernails damaging the membrane.

To investigate further

Other molecules of different sizes could be tried:

- **Fructose, galactose, maltose, lactose.** These are all reducing sugars that will react with Fehlings reagent or Benedicts reagent.
- **Glycogen**. This turns chocolate brown with iodine solution.
- **Protein**. Proteins and peptides turn purple with the biuret test.

2B WATER AS A COOLANT

Time: 1 hour

This investigation is a relatively simple and quick one that will give the students experience in data logging.

Syllabus reference: Topic 2.2

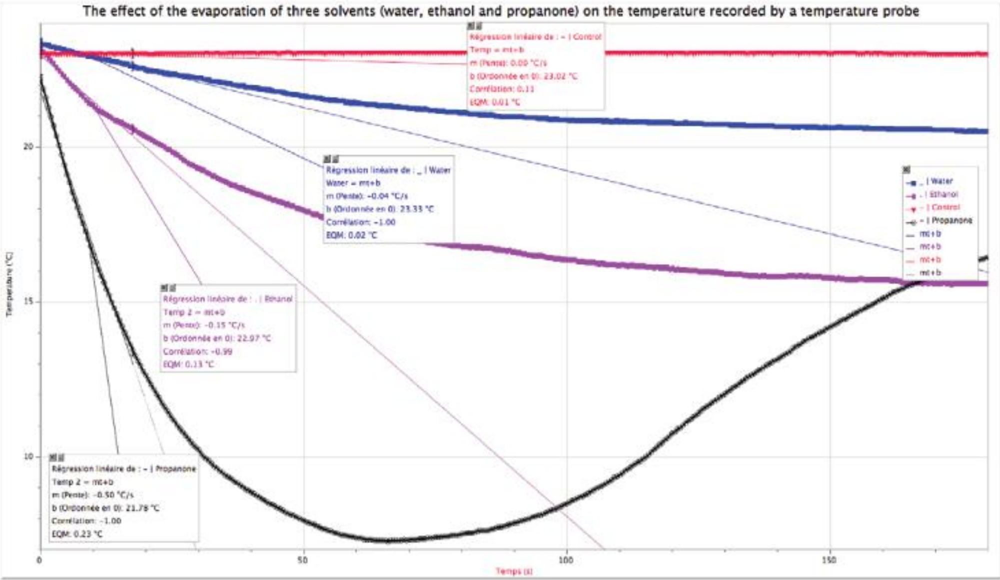
Application: Use of water as a coolant in sweat.

Materials

The material used for the trials is from Vernier but all other data logging systems have temperature probes that could be used.

Method

Air currents in the room need to be controlled or at least the temperature probes should be too far apart from one another and positioned in the same way, such that both the experimental and control probe are affected to the same degree.



Data

The graph above shows the cooling effect of the three solvents. The linear analysis is carried out to estimate the initial rate at which the temperature drops over the first 20s.

Water = 0.4 °C s^{-1}

Ethanol = 0.15 °C s^{-1}

Propanone = 0.50 °C s^{-1}

Some points for consideration

The temperature drop will depend upon the ambient temperature in the room but water should only drop by about 3°C, ethanol by 7 to 8°C and propanone by over 20°C. The propanone curve is interesting in that it rises back up again. This solvent is so volatile that all of it evaporates from the filter paper and the cooling effect is lost.

Water (H_2O) is the smallest molecule (MW 18), ethanol (CH_3CH_2OH) the next (MW 46) and propanone {$(CH_3)_2CO$} is the largest (MW 58). Curiously in this experiment the larger the molecular mass the faster the rate of evaporation cooling the temperature probes. There must be another reason.

Water has a very high polarity compared to ethanol and propanone and so it forms hydrogen bonds easily. Ethanol's alcohol group (-OH) gives it some polarity so it takes more energy to evaporate ethanol than propanone, which has no -OH group. Propanone's carbonyl group (=O) gives it a weak polarity.

Water is usually abundant in the environment or in the bodies of animals and plants. Having a high polarity it takes a lot of heat energy to evaporate a little water (compared to ethanol for example) so it is quite efficient as a thermal regulator. Ethanol and propanone are toxic to living systems because both are organic solvents that will disrupt cell membranes.

Adaptations include

Physiological e.g. reduced subcutaneous insulation, increased radiation of heat through increased blood flow to body surface, conservation of water released through urine or breath.

Morphological e.g. taller and/or slimmer body shape has an increased surface area to volume ratio.

Behavioural e.g. searching for shade, inactivity during hot parts of the day or year (aestivation).

2C POLYSACCHARIDE MOLECULES

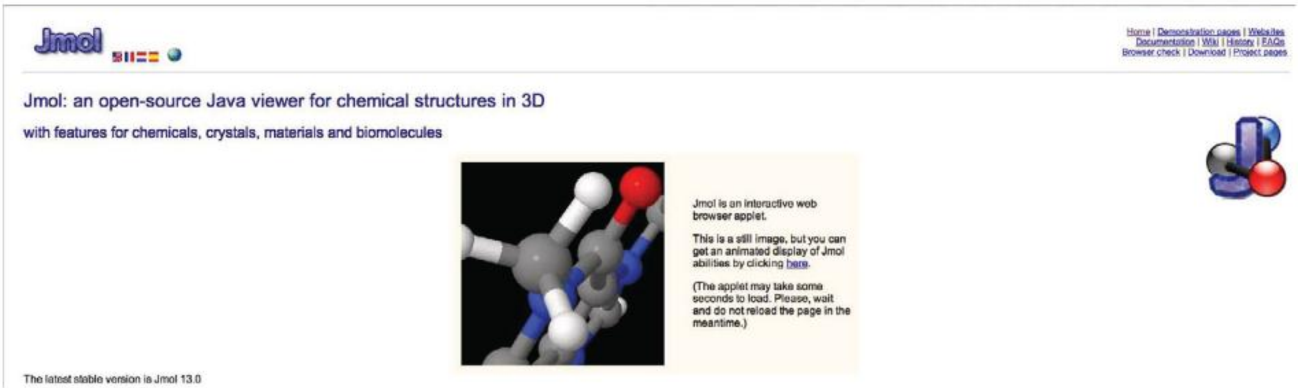
Time: 30 min

Syllabus reference: Topic 2.3

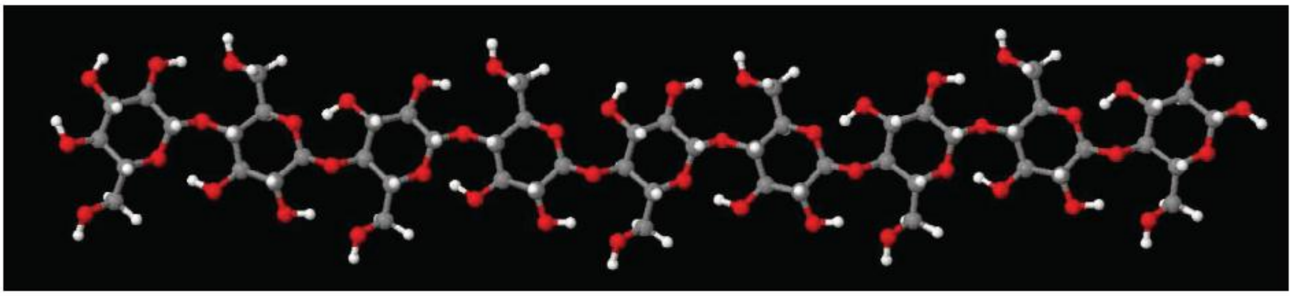
Skill: Use of molecular visualisation software to compare cellulose, starch and glycogen.

Materials

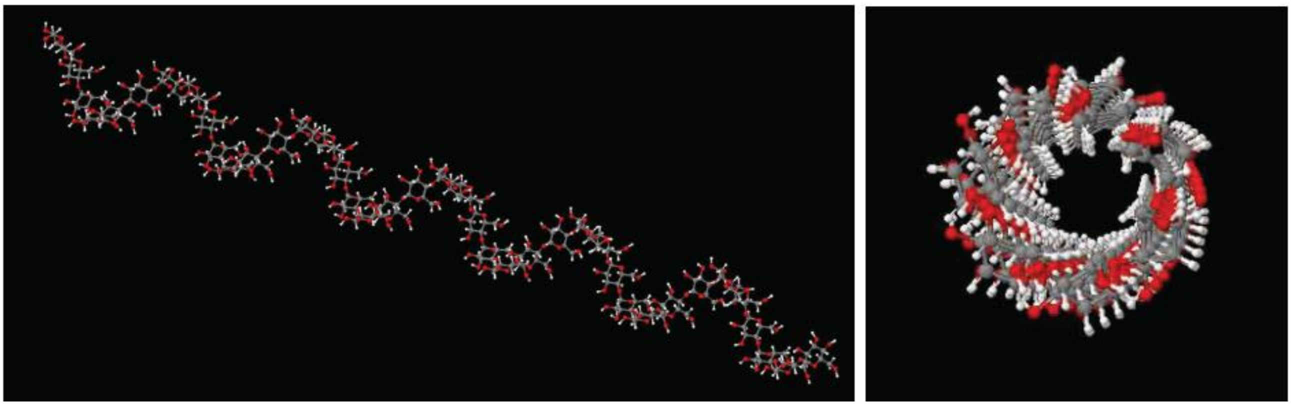
The JMol applet will need to be installed on the computer.



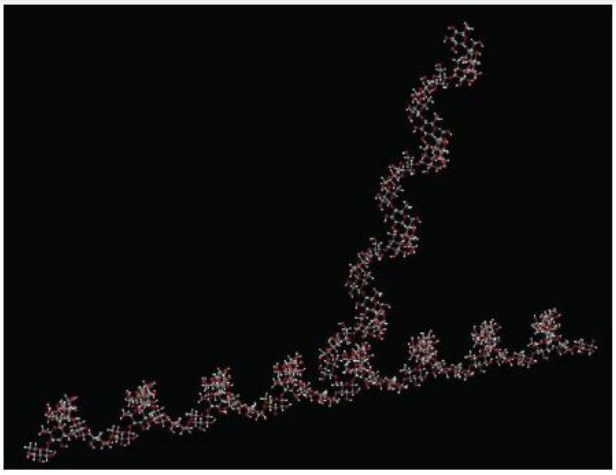
Images



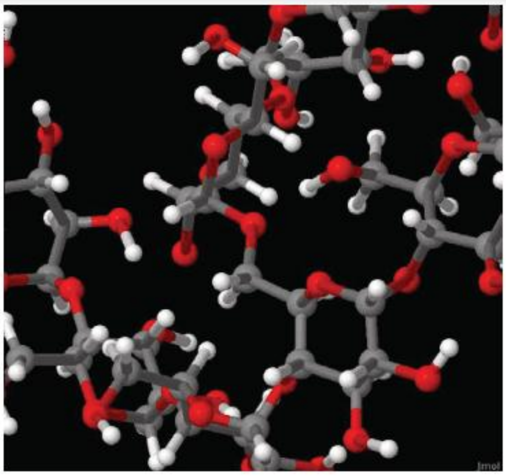
Cellulose



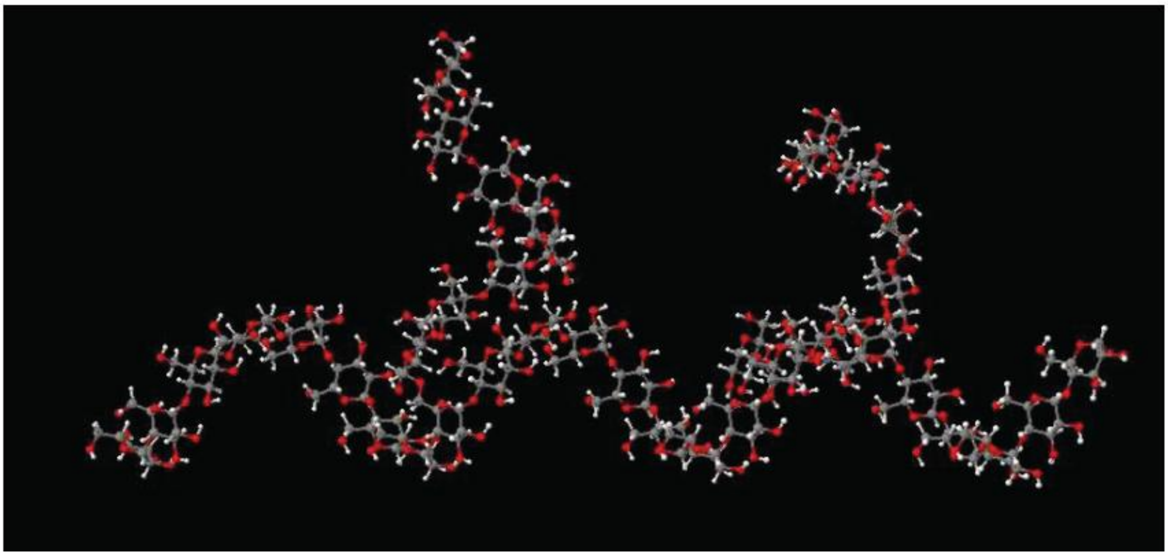
Amylose *Side view* *End view*



Amylopectin showing its branched structure



Close up view of the branch point in amylopectin



Glycogen showing it is more highly branched than amylopectin

Observations

- All of the molecules shown are made of glucose monomer units.
- Cellulose and amylose are unbranched molecules.
- Amylopectin has a branch in it (in fact the complete molecule is highly branched and much bigger, 2 000 to 200 000 glucose monomers), glycogen has more branches (again the complete molecule is bigger, 2000 to 600 000 glucose monomers, very highly branched).

Some points for consideration

- Glycogen and the starch are used as energy stores. They are easily built up from glucose monomers and broken down again when needed. The more branched the molecule is the more compact the storage. So animals, being motile, use the branched glycogen molecule as an energy store that is more compact.
- Weight for weight, fats contain twice the energy of polysaccharides. Animals being motile need an energy store that is compact and light. The same is true for the seeds of many plants. They often use oils as an energy store for the future seedling, as fats are less bulky than carbohydrate making dispersal easier.
- The simple sugars are soluble in water. A build up of these in membrane bound structures would create osmotic problems. Polysaccharides, being insoluble, can be stored in large quantities without osmotic problems.
- Cellulose is a long unbranched chain of glucose monomer units. The image here only shows a short chain but a cellulose molecule is usually made of 2 000 -14 000 glucose monomers. They align themselves into microfibrils held together by hydrogen bonds.

2D MEASURING THE RATE OF REACTION OF AN ENZYME CONTROLLED REACTION

Time 1.5 hours

Syllabus reference: Topic 2.5

Skill: Experimental investigation of a factor affecting enzyme activity. (Practical 3)

Materials

Small kitchen blenders are very useful as they can homogenise a sample of tissue rapidly.

Hydrogen peroxide concentrations are measured in a number of different ways. The term 'volumes' is used here.

To covert:

10 vol = 3.03 % = 0.83 mol dm^{-3}

Sterile water: Any bottled mineral water can be used. However if sterile water needs to be made, place distilled water heater in an autoclave to 120°C for 20 min.

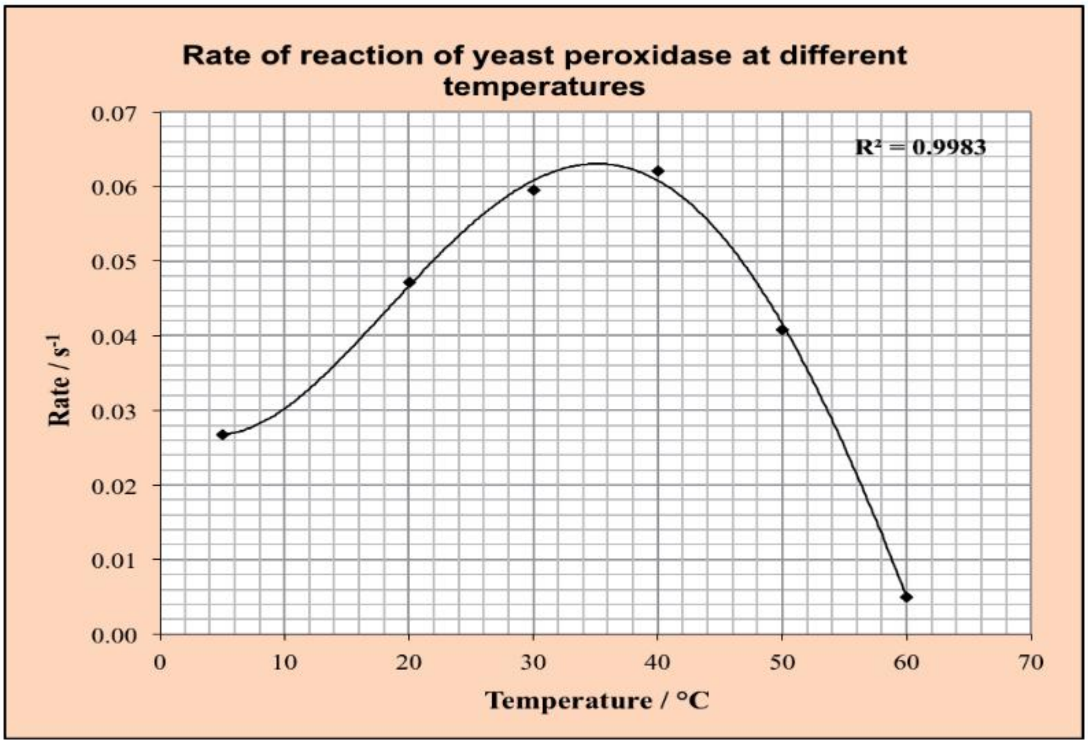
Method A: Using flotation to measure enzyme activity

This method could be adapted to investigate the influence of all the factors that affect the activity of catalase.

Materials

Large test tubes or 100 ml measuring cylinders are useful reaction chambers.

Example data



Method B: Using foam production to measure the enzyme activity

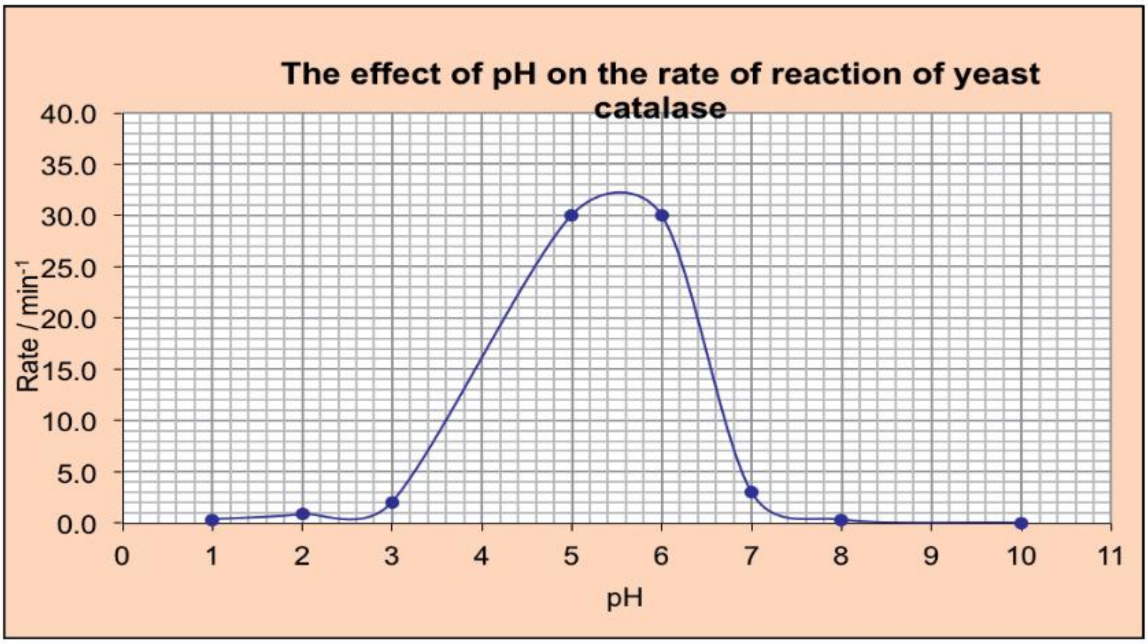
This method could be used for all factors that influence enzyme activity, except temperature.

Materials

A web cam or even the camera on a portable computer can be used to record the rate. To avoid parallax error, the camera needs to be positioned with care in front of the measuring cylinder at the level of the meniscus.

The measuring cylinder needs to be sufficiently large to be able to distinguish the graduations on it clearly.

Example data

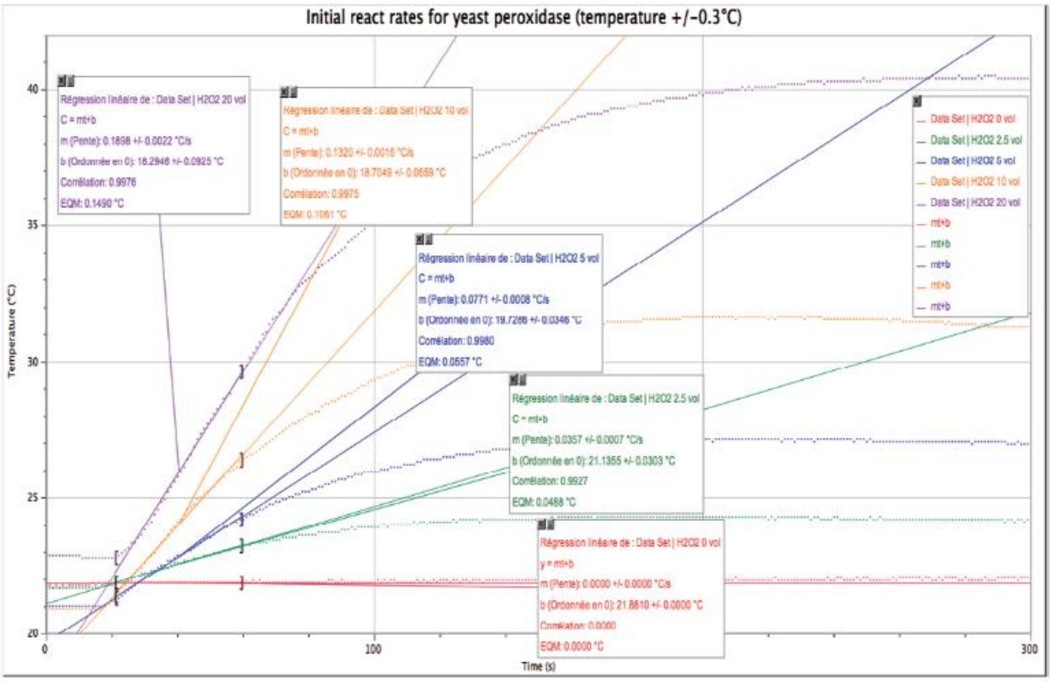


Methods C and D: Using the heat released as an indicator of the reaction

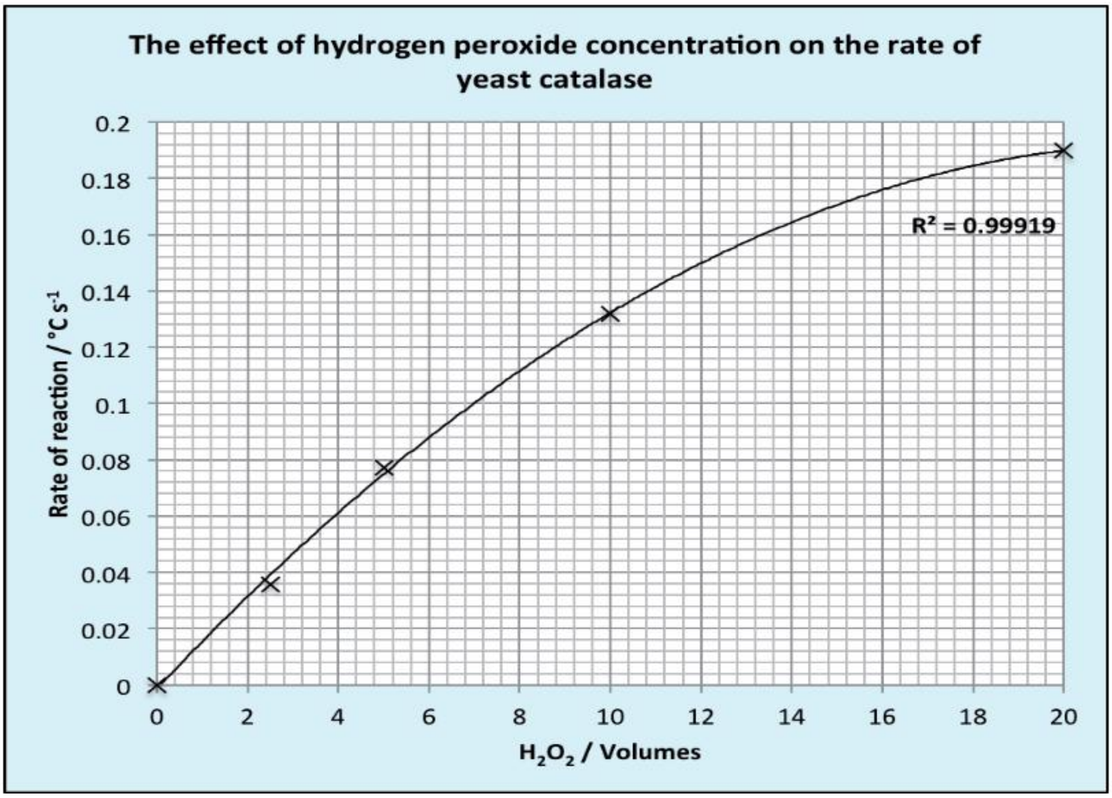
Temperature would be a difficult factor to investigate using these methods. At low temperatures the exothermic nature of the reaction itself would raise the temperature of the reaction mixture. At high temperatures the rise in temperature due to the reaction would not be recorded.

Example data

Calculating the initial reaction rates from the raw data.

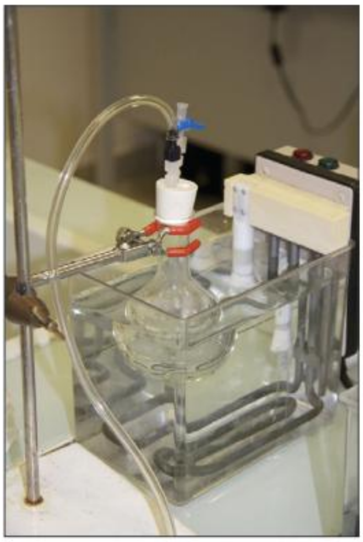


Plotting the reaction rates against the concentration of hydrogen peroxide approximates to the typical Michaelis-Menten curve.



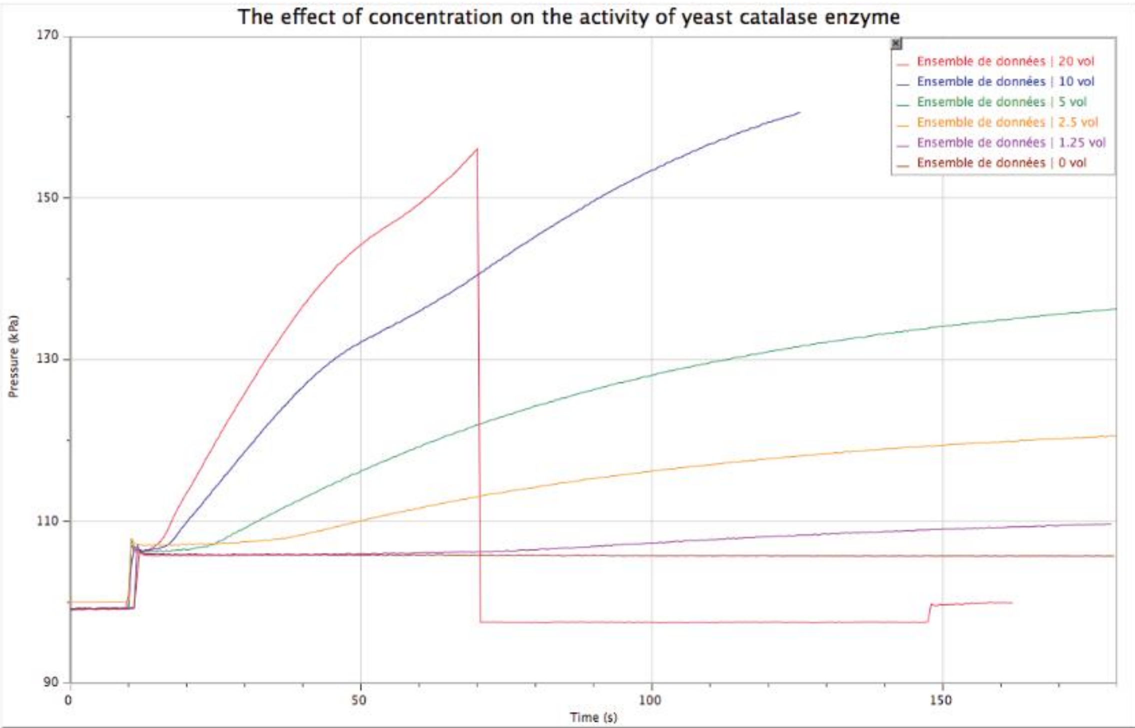
Method E: Using data logging and a pressure sensor

It is worth having the reaction chamber in a water bath to reduce fluctuations in the ambient temperature.

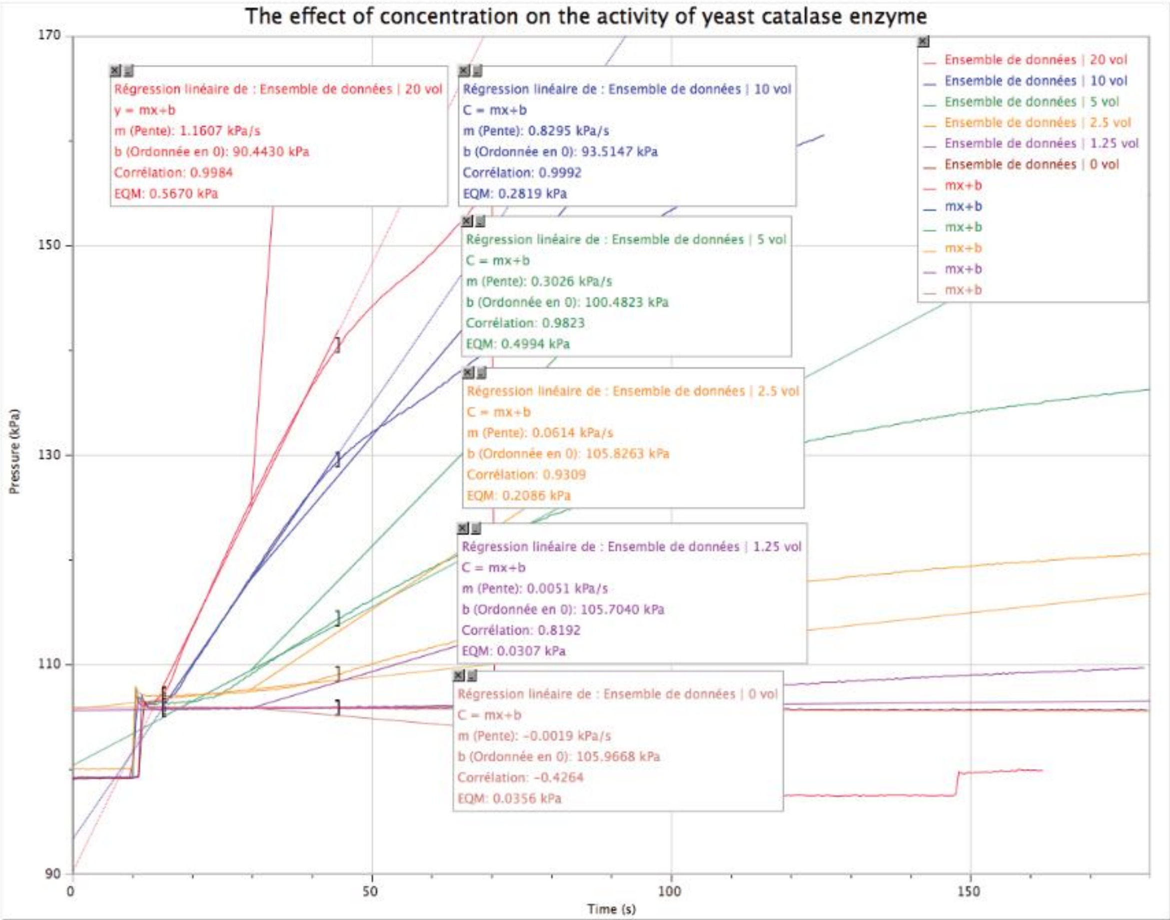


Example data

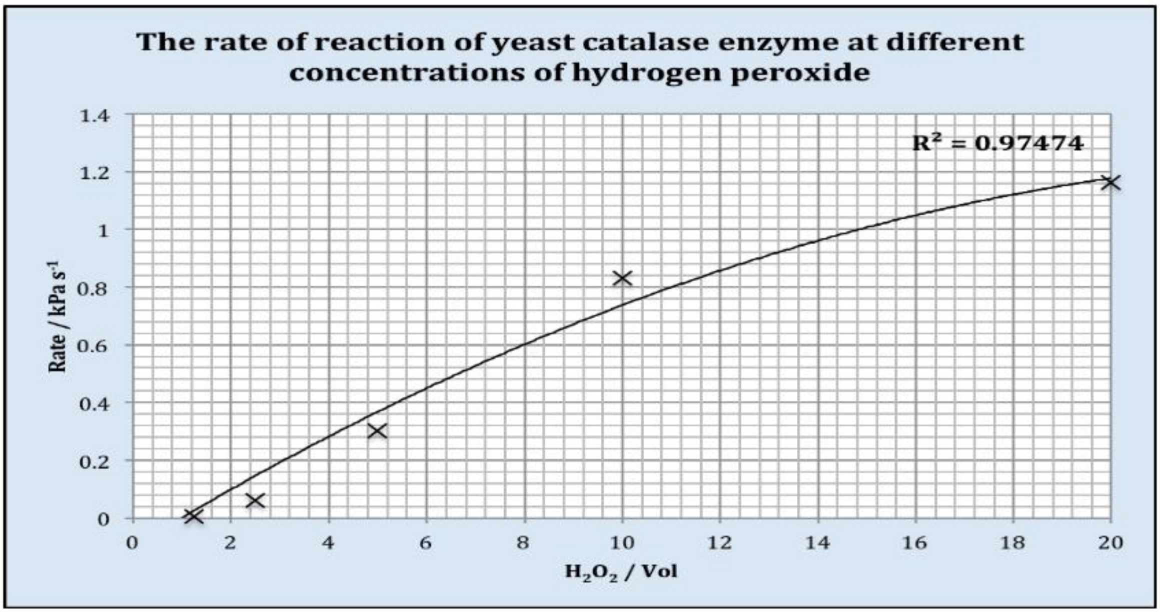
The sudden drop for the 20 vol solution is where the bung popped off the flask under the excess pressure. Nevertheless, the initial reaction rate can still be determined.



Using linear plot, the initial reaction rates are calculated.



Plotting the reaction rates against the concentration of hydrogen peroxide, the curve approaches the expected Michaelis-Menten curve.



2E USING SODIUM ALGINATE TO IMMOBILISE YEAST ENZYMES

Time: 2 hours

Syllabus reference: Topic 2.5

Understandings: Immobilised enzymes are widely used in industry.

Syllabus reference: Topic 2.8

Application: Use of anaerobic cell respiration in yeasts to produce ethanol and carbon dioxide in baking.

Required knowledge: A basic understanding of the properties of enzymes, a knowledge of the different types of microbes available is useful.

Materials

Fresh yeast works best. Dried yeast needs to be mixed with water first to allow it to rehydrate. Otherwise, it forms lumps in the alginate.

The number of glucose strips that are consumed can be reduced by cutting the plastic strips lengthwise.

Adding a needle to the syringe can make pellets of different size. Investigate surface area to volume effect.

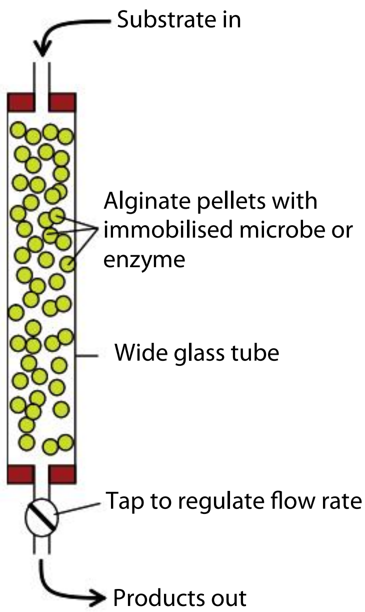
Warning: Sharp instrument, use with care

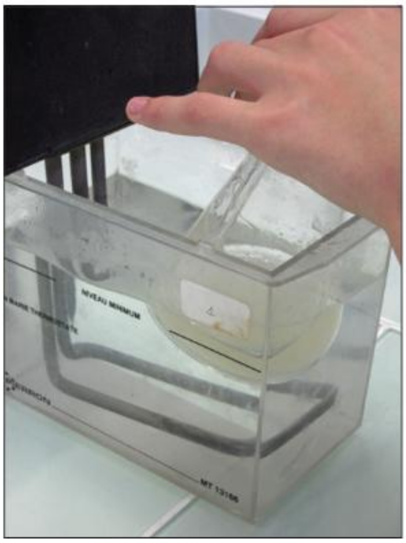
Method

Large-scale production of pellets can be made using a burette.

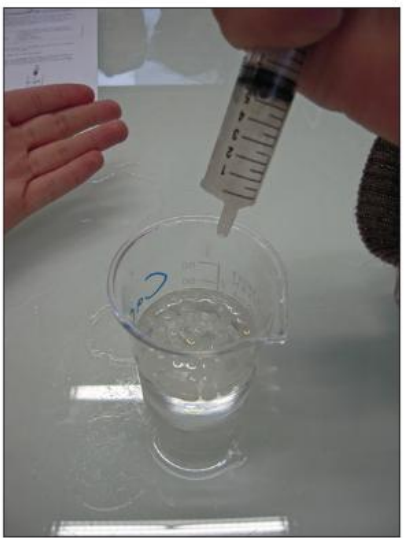
To investigate further

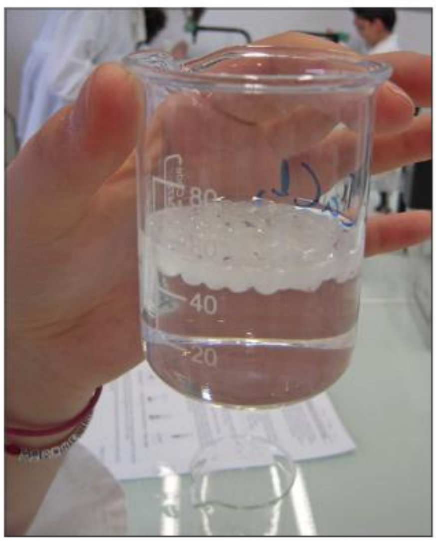
- Compare pellets of different sizes from different nozzle diameters.
- Investigate surface area to volume ratio effect.
- Compare pellets of different concentrations of alginate (try 1% and 0.5%).
- Compare different concentrations of yeast suspension.
- Compare yeast immobilised in pellets with yeast free in suspension.
- Try algae-alginate for nitrate or phosphate absorption (water treatment).
- Try homogenised liver for catalase activity.
- Try enzymes instead of yeast cells e.g. amylase, sucrase (invertase), lactase.
- Set up pellet columns with through-flow of substrate. (*See opposite*)





Mixing alginate and yeast





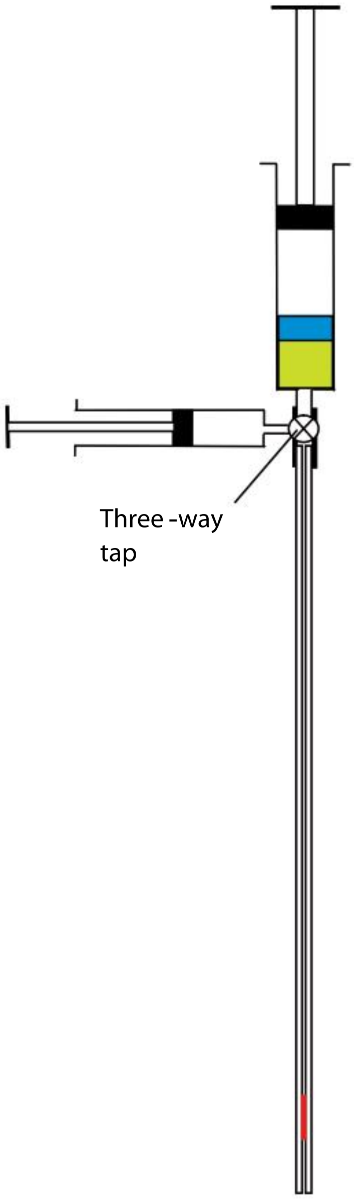
Producing pellets by flocculating alginate in calcium chloride solution

2F RESPIRATION RATES OF INVERTEBRATES

Time: 1.5 hours

Syllabus reference: Topic 2.8

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



Materials

Soda lime: Calcium oxide with sodium hydroxide.

Sodium hydroxide or potassium hydroxide could be used instead of soda lime. However, these compounds are hygroscopic and very caustic, especially in their solid form, and therefore, present an additional safety risk.

The piece of **sponge** should be carefully cut so that it is not so loose that the animals can burrow through or around it, nor too tight so that it prevents airflow.

A **three-way tap** can be introduced into the plastic tubing to "start" and "stop" the experiment when desired. A second, smaller, syringe can be attached to this tap to push the coloured liquid back down the capillary tube.

The **capillary tubing** needs to be kept clean. A build up of lime scale in hard water regions can lead to irregular results, the liquid does not flow smoothly in the tube.

Ensure that the **animals** are fresh and in good health.

Method

A respirometer without the mealworms permits the calculation of any change in volume due to other factors such as temperature or air pressure. It is properly called a **thermobarometer**. The apparatus without animals is a suitable control. The result of the control respirometer should be added or subtracted from the results for the respirometer that contains the mealworms.

After 10 to 15min the apparatus may fail to give further results. It is possible that the animals are no longer respiring aerobically, or their metabolism is slowing down. Changing the animals for fresh specimens can solve this problem.

Data

Average readings of about 12mm^3 O_2 g^{-1} min^{-1} have been observed for mealworms.
Variations in results can be quite large however. Example results obtained in a class are given below:

Mass of meal worms / g ±0.01g	Movement of bubble / mm min^{-1} ±0.05 mm min^{-1}	Volume of oxygen absorbed / mm^3 g^{-1} min^{-1}
3.28	60.0	14.34
5.00	93.5	14.70
3.16	76.5	19.06
3.04	36.5	9.40
3.54	36.5	8.05

These results are corrected for thermo-barometric fluctuations. They were taken using a 1mm bore capillary tube and the students were all working on the same batch of insects.

Alternative material

A data logger can be used with gaseous CO_2 or O_2 probes. Though they are expensive, they do extend the possibilities with additional safety.

Some points for consideration

- If this apparatus were used to measure the rate of respiration of a green plant it would need to be kept in the dark to inhibit photosynthesis. Photosynthesis produces oxygen and absorbs carbon dioxide.
- If the metabolism of the animal drops there may be no change recorded. If the animal's metabolism increases the rate of oxygen consumption will increase.
- If the organisms undergo anaerobic metabolism, then no movement will be recorded in the capillary tube.

Research

- The Respiratory Quotient (RQ) is the ratio of the amount of carbon dioxide given out per unit time divided by the amount of oxygen taken in per unit time. It allows you to determine the metabolites being used for respiration and to see if the organism is respiring aerobically or anaerobically.
- The above apparatus gives the rates of oxygen consumption only. The RQ could be calculated if it is assumed that only carbohydrates are being consumed.
- Using similar apparatus with animals (but without the soda lime) will permit the measurement of the difference between the volume of oxygen absorbed and the volume of the CO_2 produced. From this the Respiratory Quotient can be calculated.
- For carbohydrate consumption, where the amount of oxygen consumed is the same as the amount of carbon dioxide being given out, then the RQ = 1.
- If proteins or fats are metabolised, then the volume of oxygen consumed and the carbon dioxide being given out will differ. RQ for lipids = 0.7 and RQ for proteins is variable but below 1.0.

To investigate further

- If available, try comparing the respiration rates of mealworm larvae, pupae and adults, other invertebrates (e.g. blowfly larvae, woodlice) or germinating seeds.
- Using the above apparatus for measuring the RQ
- Design an apparatus to overcome the problems of the variation of temperature throughout the experiment.
- Assuming mealworms use only carbohydrate as their respiratory substrate, it may be possible to estimate how much carbohydrate they consume.

For example

If 14.34mm^3 g^{-1} min^{-1} of oxygen is being consumed.

1 mole of oxygen occupies 22.4dm^3 at stp

Using the equation for respiration, it is known that 6 moles of oxygen (6 × 22.4dm^3) are consumed for every mole (180g) of glucose consumed.

14.34 × 1 / 1000000 = 1.434 ×10^{-5}dm^3 g^{-1} min^{-1} of oxygen

We know that for every 6 × 22.4dm^3 of oxygen 180g of glucose are consumed.

So, for 1.434 × 10^{-5}dm^3 of oxygen(1.434 × 10^{-5}/134.4) × 180 = 0.0192mg of glucose g^{-1} min^{-1} was used.

2G LEAF PIGMENTS, THEIR EXTRACTION AND SEPARATION

Time: 2 hours.

Syllabus reference: Topic 2.9

Skill: Separation of photosynthetic pigments by chromatograph. (Practical 4)

Required knowledge: A basic knowledge of photosynthesis, absorption and action spectra.

Materials

Solvent = 90% petroleum ether + 10% acetone

It is best to use leaves that are dark green and that are not too hard.

Alternative source of leaf material: spinach beet

A large test tube could be used instead of a measuring cylinder.

Method

As fine a spot of pigment as possible should be made, at the most 3-4mm in diameter.

Thin layer chromatography give results faster and the sport of colour remain more condensed. In paper chromatography the spots do tend to smear on the paper.

Some points for consideration

- The leaves were ground to release the chlorophyll from the cells and to allow the pigments to dissolve in the alcohol. Some pigments may not be soluble in the alcohol.
- Four pigments are seen with this method. If parsley is used two traces of carotene may be seen.
- The different pigments separate out due to their different molecular masses, the affinity for the solvent and the affinity for the water molecules adsorbed to the paper.
- Some pigments may not be seen, due to being too dilute, or a similar colour to the paper, or have not separated out sufficiently to be observed. In the latter case, two-way paper chromatography could be used.
- Rf values are good if the chromatogram has been set up under the same conditions as those given in the table used. (e.g. same extraction, same solvent, same paper). Colour is misleading, as the way in which colour is perceived and described can vary greatly, but, it gives a simple guide.

Research

- The leaf is green despite the orange and yellow pigments that it contains. Each pigment absorbs all the wavelengths of light except that corresponding to their colour. There is more chlorophyll present than the other pigments. The chlorophyll masks the other pigments. This is not always the case: e.g. brown seaweeds, red seaweeds and copper beech.
- Plants have different pigments to absorb different parts of visible spectrum and maximise light energy absorbed.

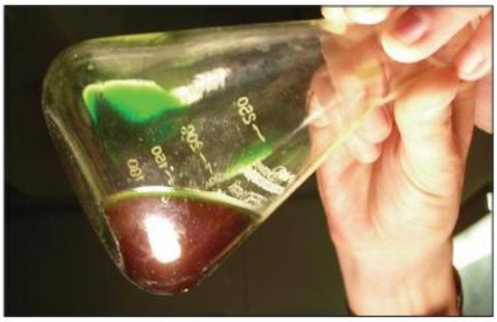
2H USING A SPECTROSCOPE TO OBSERVE LEAF PIGMENTS

Time: 1 hour

Syllabus reference: Topic 2.9

Skill: Drawing an absorption spectrum for chlorophyll and an action spectrum for photosynthesis.

Materials



The author has successfully used a diffraction grating of 140 line mm^{-1} others may be suitable. Diffraction gratings greater than 500 line mm^{-1} give wider spectra that are not intense enough for use on an OHP.

Plant material: Parsley extract works particularly well for the fluorescence part of the investigation. Spinach may give a turbid solution which hinders the reaction. Different species of plants, or leaves of the same plant in different states, can be compared (as shown in the results from the spectrometer below).

Fluorescence: This can be observed with greater intensity if a UV light of 365nm frequency is used.

Warning: UV light is harmful, wear protective spectacles.

Data

Absorption spectrum

The blue and the red ends of the spectrum are seen to be absorbed by the chlorophyll solution.

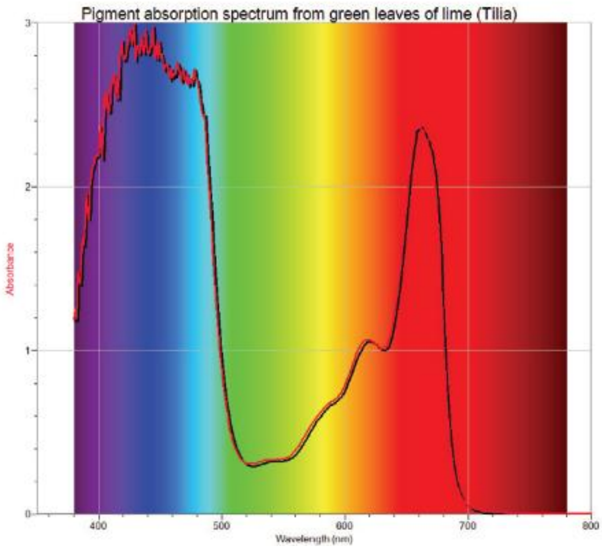
Fluorescence

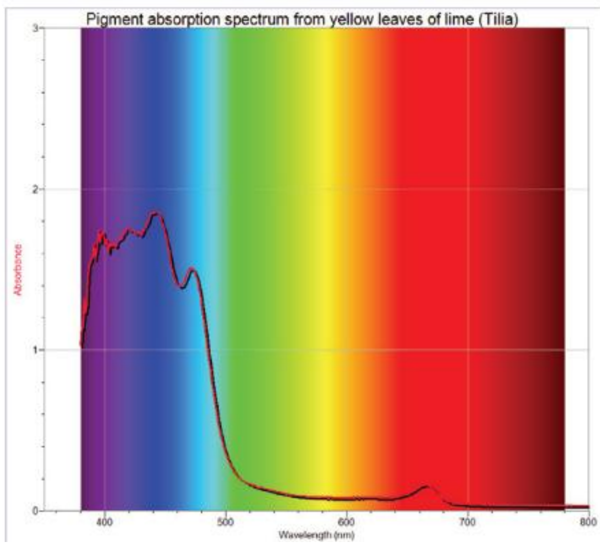
Under bright light the chlorophyll extract gives out red light. In the absence of electron acceptors, the electrons are exited out of their orbitals by the light. They fall back to their lowest energy levels, giving out light energy.

Some points for consideration

The leaf pigments trap light energy. They absorb certain wavelengths (parts of light). This could be called the absorption spectrum.

To see if all parts of light absorbed are used in photosynthesis, different wavelengths of light are used and starch or oxygen production is measured accordingly. This gives the action spectrum.





Absorption spectra of autumnal leaves of lime (Tilia)

2I METHODS TO MEASURE THE RATE OF PHOTOSYNTHESIS

Time 1.5 hours

Syllabus reference: Topic 2.9

Skill: Design of experiments to investigate the effect of limiting factors on photosynthesis.

Materials

Water enriched with carbon dioxide: Diluted carbonated water can be used if the concentration of the dissolved carbon dioxide is not critical. Alternatively, a 0.1 mol dm^{-3} solution of $NaHCO_3$ can be used. A serial dilution of this would produce an independent variable for dissolved carbon dioxide.

Lamp with 60 Watt bulb: A lamp with LEDs is best, they give the same intensity of light but they do not give out a lot of heat energy so the temperature can be controlled more easily.

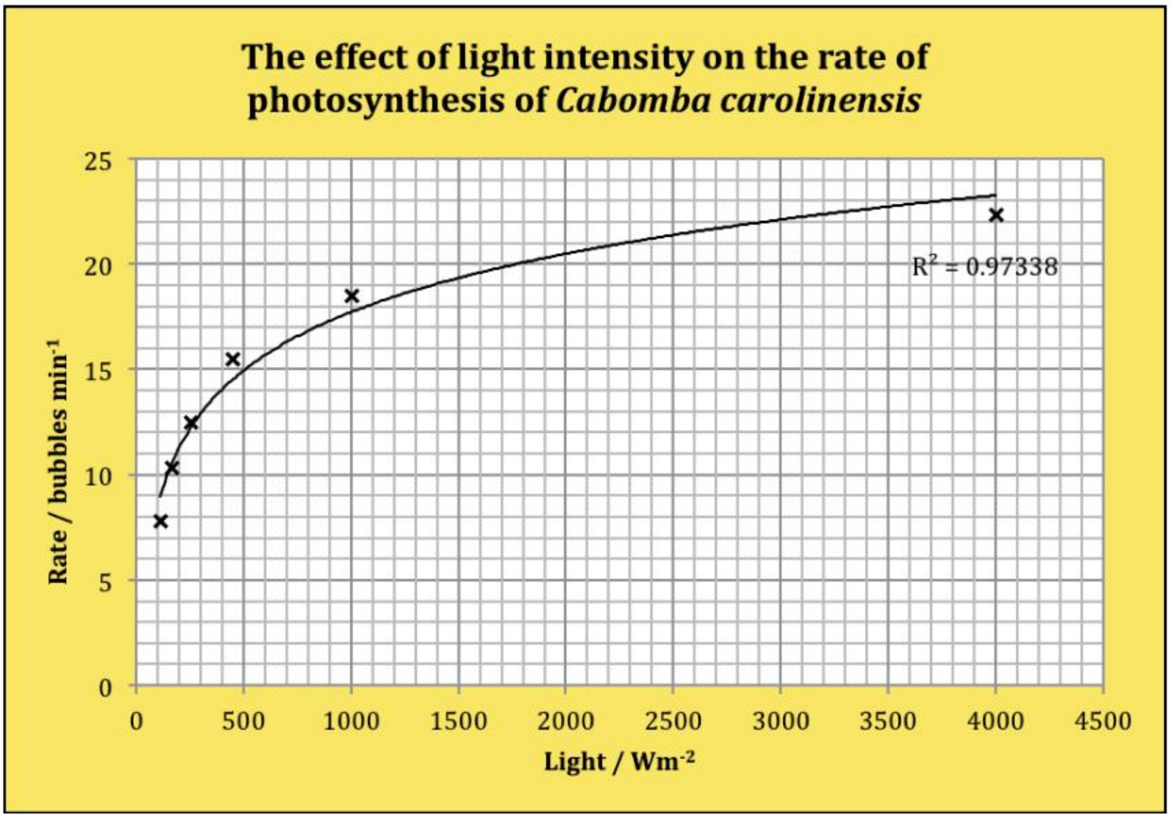
Water plant: Many species are possible, *Elodea, Cabomba, Mryiophyllum*. However, a number of these species are regarded as invasive aliens in certain parts of the world suitable alternatives should be used.

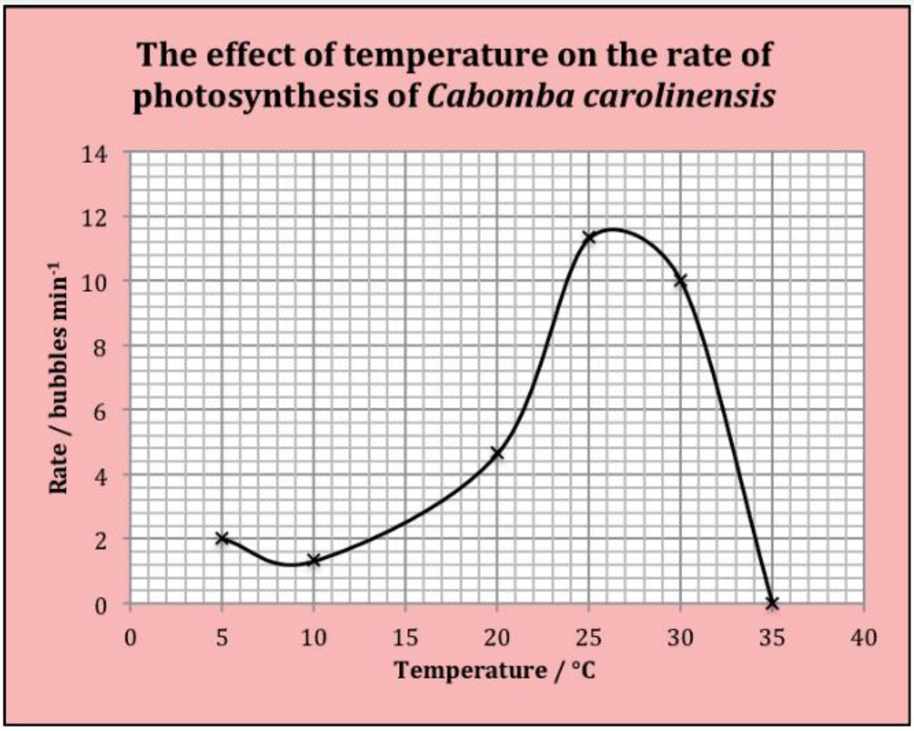
Using a water bath to control temperature is best but it may be possible for the plant to operate at room temperature. Nevertheless the room temperature should be monitored to see if it varies significantly, especially if a bench lamp is close to the apparatus.

Method A: Determining oxygen production in a water plant directly

This could be used to explore the effects of dissolved carbon dioxide, light intensity, light wavelength and, with a water bath, temperature.

Example Data





Method B: Determining oxygen production by the displacement of a liquid

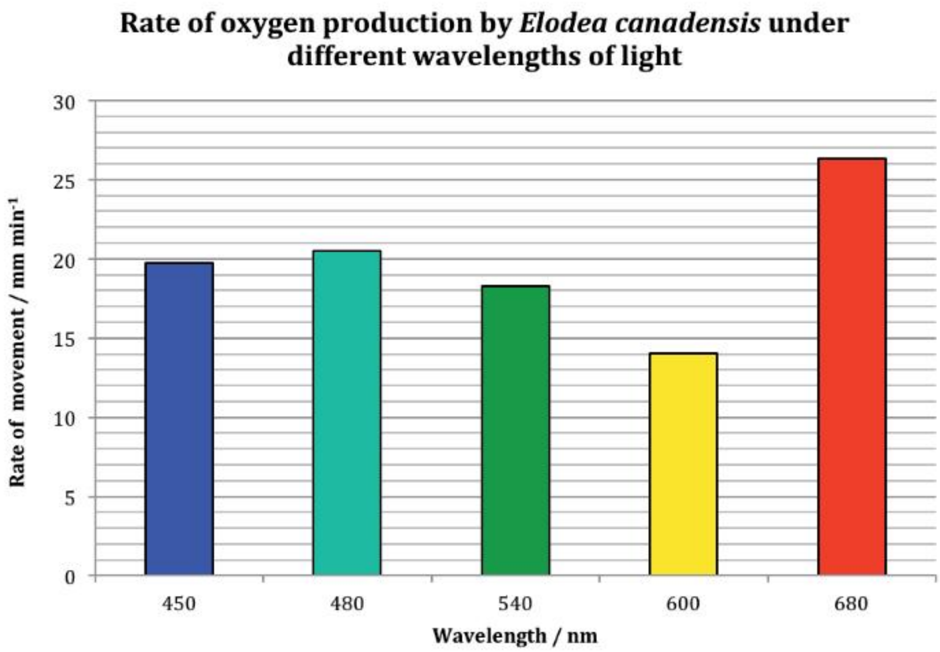
The temperature of this apparatus cannot be adjusted in a controlled way but it can be used to investigate the effect of anything dissolved in the water, the effect of light intensity or the effect of light quality.

A second apparatus will need to be set up as a control (a thermobarometer) as small changes in ambient temperature will tend to cause large changes in the level of the liquid in the capillary tube.

Materials

The capillary tubing needs to be kept clean, a build up of lime scale in hard water regions can lead to irregular results because the liquid does not flow smoothly in the tube.

Example Data

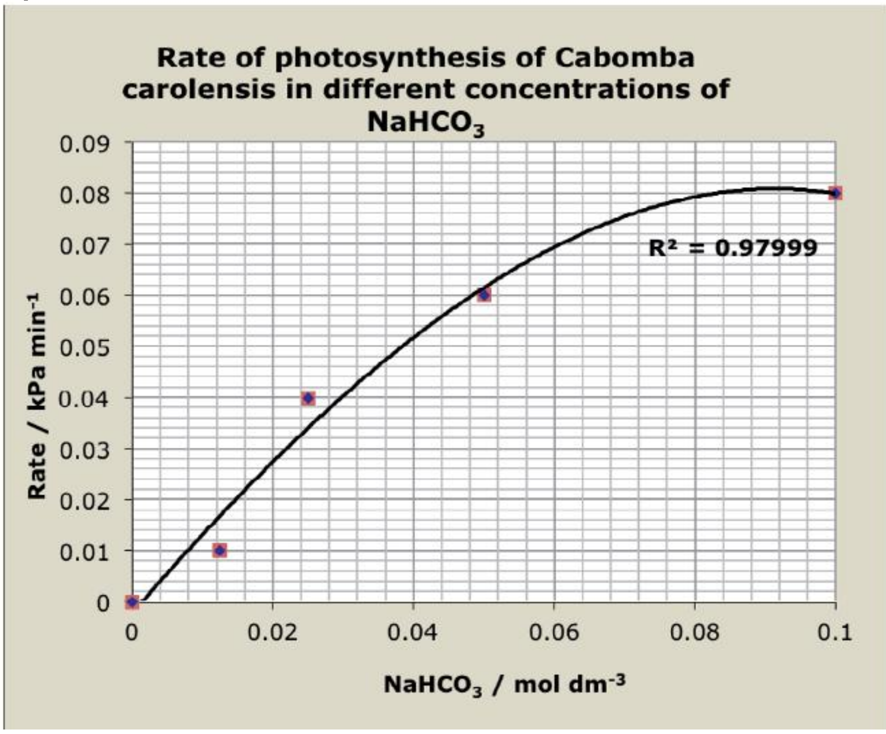


Method C: Determining oxygen production using a pressure sensor

This apparatus is useful for investigations involving the effect of light intensity, light quality or levels of dissolved carbon dioxide.

Varying temperature of this apparatus is not really a practical option as this would result in a change in pressure inside the reaction chamber.

Example Data



Method D: Determining the consumption of carbon dioxide by a water plant using a pH indicator

This method only provides a qualitative impression of the changes due to photosynthesis. It is possible to combine it with the use of a colourimeter, sampling the liquid around the plant periodically. Alternatively, it can be used as a visual check on the progression of the reaction with a pH probe. However, the change in colour of the indicator may have an impact on the wavelength of light reaching the plant.

Method E: Determining carbon dioxide absorption using a data logger and pH probe

This apparatus could not be used to investigate the effect of different levels of dissolved carbon dioxide as it would interfere with the dependent variable.

Other probes can be exploited for the measurement of photosynthesis. Dissolved oxygen probes for water plants and gaseous carbon dioxide or gaseous oxygen probes for terrestrial plants. If terrestrial plants are to be used, they should not be planted in soil as the microbial activity in the soil will influence the results.

3A USING A PROTEIN DATABASE

Time: 1 hour

Syllabus reference: Topic 3.1

Skill: Use of a database to determine differences in the base sequence of a gene in two species.

UniProt database <http:/www.uniprot.org> is an open access site.

It operates on most servers, however it would be advisable to trial the use of the site on the computers that are to be used for the exercise in case some of the programs are incompatible.

The amino acid sequence

Search programs using FAST-A (A for all)) can compare proteins by aligning them side-by-side. This permits searches for homology. Here, the FAST-A format is uses the amino acid sequence (sometimes referred to as FAST-P, P for protein). It uses the single letter abbreviations for the amino acids.

Amino acid	Abbreviation
Alanine	A
Arginine	R
Asparagine	N
Aspartate	D
Cysteine	C
Glutamate	E
Glutamine	Q
Glycine	G
Histidine	H
Isoleucine	I
Leucine	L
Lysine	K
Methionine	M
Phenylalanine	F
Proline	P
Serine	S
Threonine	T
Tryptophan	W
Tyrosine	Y
Valine	V

The Nucleotide Sequence

This also uses FAST-A format, though this time it is the nucleotide sequence of the four letters, A, T, G and C (sometimes called FAST-N, N for nucleotide).

3B FACTORS AFFECTING ROOTING IN PLANTS

Time: 1 hour to set up and 3 weeks to run

Syllabus reference: Topic 3.5

Skill: Design of an experiment to assess one factor affecting the rooting of stem-cuttings.

Liquid media

Although covering the flask with aluminium foil will prevent growth of algae, it will not prevent other microbes from growing. These may infect the medium. To reduce this risk it is best to make up the media with sterile water.

Investigating the effect of mineral deficiency on rooting using Sachs Solution

Showing the composition of the complete medium and the media deficient in one of the elements. In some cases the compound used in the complete medium (in blue) needs to be replaced by another compound (in red).

	The amount of mineral / g dm^{-3}						
Mineral content	**Complete**	**Minus Ca**	**Minus Fe**	**Minus N**	**Minus P**	**Minus S**	**Minus K**
KNO_3	0.70	0.70	0.70	KCl 0.52	0.70	0.70	$NaNO_3$ 0.59
$Ca_3H_4(PO_4)_2$	0.25	$NaH_2PO_4.2H_2O$ 0.71	0.25	0.25	$CaNO_3$. $4H_2O$ 0.16	0.25	0.25
$MgSO_4.7H_2O$	0.25	0.25	0.25	0.25	0.25	$MgCl_2$. $6H_2O$ 0.17	0.25
$CaSO_4$	0.25	K_2SO_4 0.2	0.25	0.25	0.25	$CaCl_2$ 0.16	0.25
NaCl	0.08	0.08	0.08	0.08	0.08	0.08	0.08
$FeCl_3$ $6H_20$	0.005	0.005	0	0.005	0.005	0.005	0.005

Safety

- Potassium nitrate (V): an oxidising agent and dangerous with some metals and flammable substances
- Iron (III) chloride-6-water: harmful as a solid
- Calcium nitrate (V)-4-water: an oxidising agent and an irritant
- Calcium chloride: irritant as a solid
- Sodium nitrate (V): an oxidising agent and harmful as solid. Dangerous with some metals and flammable materials.

4A LIFE IN AN ENCLOSED ECOSYSTEM

Time: About 1 hour to set up and clean up. At least 6 weeks for observations.

Syllabus reference: Topic 4.1

Skill: Setting up sealed mesocosms to try to establish sustainability. (Practical 5)

Required knowledge: An understanding of the principles of ecological succession is helpful but not essential.

Materials

Straw: Straw used for the bedding material of pet animals is a useful source. Shredded filter paper can be used instead of straw.

Mud: Ensure that the source is not contaminated with pathogens, e.g. sewage effluent. Always treat with care, use rubber gloves.

Water: Neutral or slightly alkaline pond water should be used. The pH could be adjusted by adding sodium hydrogencarbonate. Fresh tap water is not to be used, it is chlorinated. It should be left to stand for 24 hours.

Jar: The ideal jar would be a food preserving jar (e.g. Kilner jar) but any tall jar (> 20cm) with a tight fitting lid would do.

Method

Mapping the progressive changes in the microbial community can be carried out by using an acetate sheet wrapped around the jar. The limits of the colonies and their colours can be indicated using fine indelible marker pens.

Useful information

<http://en.wikipedia.org/wiki/Sergei_Winogradsky>

<http://en.wikipedia.org/wiki/Winogradsky_column>

4B THE ASSOCIATION BETWEEN TWO PLANT SPECIES

Time: 45 min

Syllabus reference: Topic 4.1

Skill: Testing for association between two species using the chi-squared test with data obtained by quadrat sampling.

Materials

Quadrats

These can be made of a number of different materials. Wood, metal or plastic frames can be constructed. They should be robust. Metal frames should be solid enough so they do not distort; wooden frames will need their corners reinforcing with metal right-angle brackets.

Large quadrat frames should preferably be collapsible. One tried and tested model can be made from 1.5cm or 2cm diameter plastic piping with right-angle joints for the corners. This could be made into 1m × 1m quadrat frames. One right angle joint should be firmly glued onto each 1m sidepiece. Four of these can then be slotted together in the field.

Small quadrats can be drawn or scratched on plastic Petri dishes.

Method

Work in the field has its risks. The teacher should have visited the site first, obtained permission from the owner to work there and verified any safety risks. The weather forecast for the day needs to be consulted. Adequate protection from cold or wet conditions must be provided for. Excessive sunlight and heat must be also considered.

Data

It is perhaps easy to count up for small sample sizes but using a spread sheet, such as MSExcel, the data can be filtered to give the totals. The example below is for a field of pasture grazed by cattle. It was sampled 30 times using 1×1m quadrats randomly placed. The two species recorded were Yarrow, *Achillea millefolium*, and Dandelion, *Taraxacum officinale*. They are recorded as 1 (present) or 0 (absent) on an MSExcel spread sheet. The 'Filter' function for the columns is selected from the Data menu.

The filters are adjusted to select 1 for both the Yarrow column and the Dandelion column. It will reveal the samples where both are present.

	A	B	C
1	Quadrats	Yarrow	Dandelion
2	1	1	1
3	2	0	1
4	3	0	1
5	4	1	1
6	5	1	1
7	6	0	1
8	7	1	1
9	8	0	1
10	9	0	0
11	10	0	1
12	11	1	1
13	12	1	1
14	13	0	1
15	14	0	1
16	15	1	1
17	16	1	1
18	17	0	1
19	18	1	1
20	19	0	1
21	20	1	1
22	21	1	1
23	22	1	1
24	23	1	1
25	24	1	1
26	25	1	1
27	26	1	0
28	27	1	1
29	28	1	1
30	29	0	1
31	30	1	1

	A	B	C
1	Quadrats	Yarrow	Dandelion
2	1	1	1
5	4	1	1
6	5	1	1
8	7	1	1
12	11	1	1
13	12	1	1
16	15	1	1
17	16	1	1
19	18	1	1
21	20	1	1
22	21	1	1
23	22	1	1
24	23	1	1
25	24	1	1
26	25	1	1
28	27	1	1
29	28	1	1
31	30	1	1
35	Total Both	18	
36			

Then the filters are changed to 1 for Yarrow and 0 for Dandelion.

	A	B	C
1	Quadrats	Yarrow	Dandelion
27	26	1	0
34	Total Yarrow not dandelion	1	
35			

Next, 0 for Yarrow and 1 for Dandelion.

	A	B	C
1	Quadrats	Yarrow	Dandelion
3	2	0	1
4	3	0	1
7	6	0	1
9	8	0	1
11	10	0	1
14	13	0	1
15	14	0	1
18	17	0	1
20	19	0	1
30	29	0	1
35	Total Dandelion not Yarrow		10
36			

Next, 0 for both filters.

	A	B	C
1	Quadrats	Yarrow	Dandelion
10	9	0	0
36	Total neither	1	
37			

Finally, return the filters to select all cells for both columns and the results required for the 2×2 table of the observed frequencies (O) will be shown.

	A	B	C
1	Quadrats	Yarrow	Dandelion
2	1	1	1
3	2	0	1
4	3	0	1
5	4	1	1
6	5	1	1
7	6	0	1
8	7	1	1
9	8	0	1
10	9	0	0
11	10	0	1
12	11	1	1
13	12	1	1
14	13	0	1
15	14	0	1
16	15	1	1
17	16	1	1
18	17	0	1
19	18	1	1
20	19	0	1
21	20	1	1
22	21	1	1
23	22	1	1
24	23	1	1
25	24	1	1
26	25	1	1
27	26	1	0
28	27	1	1
29	28	1	1
30	29	0	1
31	30	1	1
32	Total Both	19	
33	Total Yarrow not Dandelion	1	
34	Total Dandelion not Yarrow		10
35	Total neither	1	
36			

Observed frequencies (O)

		Dandelion		
		Present	Absent	Row total
Yarrow	Present	19	1	20
	Absent	10	1	11
	Column total	29	2	31

Calculating the χ^2 value

	O	E	(O-E)	(O-E)2	(O-E)2/E
Yarrow present Dandelion present	19	18.71	0.29	0.0841	0.0045
Yarrow present Dandelion absent	1	1.29	-0.29	0.0841	0.0651
Yarrow absent Dandelion present	10	10.29	-0.29	0.0841	0.0082
Yarrow absent Dandelion absent	1	0.71	0.29	0.0841	0.1185
				Total (χ2)	0.1963

The calculated χ2 value is $p<0.5$. This is well below the critical value of $p=0.05$.

Therefore, there is no association between these species.

Some points for consideration

- Do the species show any particular adaptations that may help them to compete easily with other species?
- Growth forms may vary from tall vertical stems to rosettes at ground level. Tall plants will rise above competitors and shade them out but low growing forms will perform better where there are grazers.
- Criticise this method of estimating the association between two species of plant in a habitat.

(i) Would it make any difference if the species were clumped, uniform or random in their distribution?

Clumped species tend to develop from asexual propagation through runners or rhizomes. This can be a very competitive strategy.

(ii) Would it make any difference if the species chosen were large or small?

Large species will tend to out compete smaller species.

5A CONSTRUCTING A DICHOTOMOUS KEY FOR PLANT PHYLA

Time 1 hour

Syllabus reference: Topic 5.3

Application: Recognition features of Bryophyta, Filicinophyta, Coniferophyta and Angiospermophyta

Skill: Construction of dichotomous keys for use in identifying specimens.

Materials

Environmental impact

If the specimens are taken from the wild, care must be taken not to harvest protected or vulnerable species. In any case, the number of specimens must be limited to a minimum. For large organisms e.g. conifer trees specimens should be cut from the tree, preferably using secateurs, to limit wounding.

Plant classification

This is greatly simplified below. The Tree of Life Project <http://tolweb.org/tree/phylogeny.html> has been set up online to provide information from international sources about biodiversity and the characteristics of the different taxonomic groups. This will provide much more detailed classification.

PHYLA	CLASSES	Characteristics
Bryophyta		Mosses. Small terrestrial plants restricted to damp habitats. Erect stems with leaf scales +/- rhizoids Show an alternation of generations between a haploid gametophyte and a diploid sporophyte. The gametophyte is the dominant generation in the life cycle. Water is needed for fertilisation. The sporophyte generation is represented by the spore capsule.
Vascular plants The sporophyte generation is dominant in the life cycle. It is differentiated into: leaves, stem, roots and rhizome. Lignified tissue (wood) may be present.		
Filicinophyta		Water is needed for fertilisation.
	Pteridopsida	Ferns. Leaves (fronds) often open by uncurling.
	Sphenopsida	Horsetails. Needle-like leaves radiating from a single stem.
Seed plants The sporophyte generation dominates, it is the plant. Water is no longer needed for fertilisation. The gametophyte generation is very reduced. The female gametophyte becomes a seed that contains a food store and protective coat.		
Coniferophyta		Conifers. Naked seeds born in cones.
Angiospermophyta		Flowering plants. Seeds protected by an ovary which becomes a fruit
	Dicotyledons	Plants with two seed leaves.
	Monocotyledons	Plants with one seed leaf.

Summary

Feature	Bryophyta	Filicinophyta	Coniferophyta	Angiospermophyta
Common name	Mosses	Ferns	Conifers	Flowering plants
Leaves	No, scales	Yes	Yes	Yes
Roots	No rhizoids	Yes	Yes	Yes
Vascular system	No	Yes	Yes	Yes
Woody tissue	No	Yes	Yes	Yes
Waxy cuticle	No	Yes	Yes	Yes
Water for fertilisation	Yes	Yes	No	No
Seeds	No	No	Yes	Yes
Cones	No	No	Yes	No
Fruit	No	No	No	Yes

6A THE DIGESTION OF STARCH IN A MODEL GUT

Time 1 hour

Syllabus reference: Topic 6.1

Application: Use of dialysis tubing to model absorption of digested food in the intestine.

Materials

Dialysing tubing pore size: equivalent to a protein MW 12000.

Fehling's reagent

Solution 1. Dissolve 34.6g of hydrated copper (II) sulphate ($CuSO_4$. 5 H_2O) in distilled water to give a final volume of 500cm^3. Add a drop of concentrated sulphuric acid if the solution remains cloudy.

Solution 2. Dissolve 77g of sodium hydroxide together with 175g of sodium potassium tartrate in distilled water to give a final volume of 500cm^3.

Keep solution 1 and 2 separately for storage. For use, mix equal volumes of solutions 1 and 2.

Benedict's solution can be used instead of Fehling's solution.

Benedict's reagent

Dissolve 173g of hydrated sodium citrate together with 100g of hydrated sodium carbonate in 800cm^3 of warm distilled water. Filter and add distilled water to a final volume of 850cm. Separately, dissolve 17.3g of hydrated copper (II) sulphate in 100cm^3 of cold distilled water. Add the copper (II) sulphate solution to the citrate-carbonate solution, stirring constantly. Add distilled water to a final volume of 1dm^3.

Iodine solution

Dissolve 1g potassium iodide in a little water. Mix whilst adding 0.5g iodine crystals. Make up to 100cm^3. Store in dark brown bottles.

Starch solution

'Soluble' starch should be avoided, it tends to leak through the pores easily, ordinary laundry starch is best.

Amylase solution

Commercial preparations of amylase are available. Salivary amylase should NOT be used.

Method

Completely wetting the dialysis tubing may help to open it up. There may be problems with leaking dialysing tubing. Insist that the knot in the bottom of the tube is tied tightly but be careful not to tear the tubing with long fingernails. Use enough tubing so that the upper end is well clear of the water (as shown in the diagram on the student's guide).

There should be enough distilled water surrounding the dialysing tubing to last for six samples but warn the students not to take large samples. Furthermore, the samples should not be "drowned" with Fehling's solution when being tested.

Some points for consideration

- *What are the sources of error that you may have encountered?*

The tests for glucose are not specific, they are general tests for reducing sugars. It is possible the maltose molecules are giving positive results.

The red precipitate indicating a positive test may not be easy to see in the small samples when the concentration is quite low.

- *How does the model resemble the process of digestion?*

Food is processed in the lumen of the digestive system before being absorbed by the wall of the gut, principally the small intestines. The dialysing tubing represents the digestive system and the distilled water surrounding the tube represents the blood.

- *How could the model digestive system be improved upon?*

Glucose uptake will be by active transport, it would be difficult to represent this in a non-living model.

The blood carries the glucose away (to the liver), this could be simulated by periodically circulating the blood or changing the water surrounding the tubing.

Food is a mixture of molecules, perhaps proteins and proteases, such as trypsin, could be added to the mixture.

The pH of the gut is controlled by secretions of acid or alkali digestive juices. Amylase works in the small intestines where the conditions are alkaline. These alkaline conditions could be simulated by adding sodium hydrogencarbonate to buffer the gut contents.

6B ANATOMY OF THE HEART AND ITS PHYSIOLOGY

Time: 2 hours

Syllabus reference: Topic 6.2

Skill: Recognition of the chambers and valves of the heart and the blood vessels connected to it in dissected hearts or in diagrams of heart structure.

Required Knowledge: The characteristics of muscle tissues, the basic anatomy of the circulatory system, use of dissecting instruments.

Materials

A lamb's heart is smaller than a human heart (it weighs approx. 150 g compared with 300 g for a human heart).

A butcher should be able to supply whole hearts which are not cut and with the vessels still attached. If not, this can be a major problem in the activities to follow.

It would be interesting to observe an intact heart-lung set from a lamb or a pig to show the relationship between these organs.

Part A: Observations on the heart and the vessels attached to it

1. The heart appears as a conical muscle, red where it is uncovered and yellowish or white where it is covered with fat.
2. The atria are reddish brown and they have an uneven surface covered in ridges of muscles.
3. Blood vessels (the coronary arteries and coronary veins) are visible in this groove that marks the division between the two ventricles. A second set of coronary vessels are found on the dorsal side of the heart.

Part B: Examination of the inside of the heart

The heart is a hollow muscle with elastic walls. It contains four cavities which are connected two-by-two. In each part (the lefthand side of the heart and the righthand side of the heart) the atrium is connected to a ventricle via the atrioventricular valve. There is no communication between the ventricles or the atria.

The wall of the left ventricle forms the apex of the heart.

Part C: The circulation of blood in the heart

During the experiment the water replaces the blood which normally circulates in this organ. A sheep's heart is better than a pig's heart for this. It is small and fills with water more rapidly.

1. When water is injected into the right atrium, it escapes from the pulmonary artery via the right ventricle. When water is injected into the left atrium it escapes from the aorta via the left ventricle.
2. When the water is introduced into the arteries under pressure, it tends to be rejected back out as the valves close. Some water does enter the ventricles and the atria due to leaking valves. The arterial valves in the arteries operate very well even in a dead heart. These may be observed closing to stop the back flow of water when it is poured into the arteries. The water will leak from the ventricle to the atrium however, because the flaps of the atrioventricular valves are no longer held taught by the fibres which are attached to dead cardiac muscle.

NB For the valves to close well the pressure of water flowing into the vessels needs to be increased gently or the water spurts back up. It might be a good idea to demonstrate this before the students try it by themselves.

Part D: Looking for the structures which direct the blood flow through the heart

- Both sides of the heart have the same basic structure (atrium, ventricle and valves).
- The wall of the left ventricle is much thicker than the wall of the right ventricle (two to three times thicker).
- The tricuspid valve, on the right, has three flaps and the bicuspid valve, on the left, has two flaps.
- The aorta has a thicker wall than the pulmonary artery.
- The vena cava (if attached) has a much bigger internal diameter (lumen) than the pulmonary vein.

(*NB The volume of the two ventricles is the same*).

The contraction of the atria and then the ventricles pushes the blood into the arteries. The atrioventricular valves and the arterial valves stop the back flow of the blood into the cavity that it has just left.

Therefore, the blood flows into the heart through the veins and collects in the atria. From there, it flows into the ventricles and out via the arterial valves into the arteries.

The students should conclude that each side of the heart has a one-way circulation of the blood.

Probing the coronary artery shows how it forms a ring around the heart between the atria and the ventricles. Branches of the artery can be seen spreading over the ventricles, especially down the groove between the left and the right sides. The coronary arteries distribute oxygen and nutrients to the cardiac muscle cells.

Some points for consideration

The right ventricle pumps blood towards the nearby lungs which surround the heart. The left ventricle must send the blood, via the aorta and its branches, to all the organs, including the most distant, in the head, as well as to the ends of the limbs. Thus, the muscular wall of the left ventricle is much thicker than that on the right in order to produce enough force to propel the blood all through the body. The relative thickness of the aorta can be explained by the high pressure which it must contain after each contraction, (about five times more pressure on the left than the pressure exerted by the right ventricle).

Research

- The blood leaving the right ventricle is deoxygenated the blood leaving the left ventricle is oxygenated.
- Arteries carry high pressure, high velocity blood away from the heart and veins carry low pressure, slow velocity blood towards the heart.

To investigate further

- A transverse cut above the apex of the heart, carried out at the end of the dissection, allows the different thickness of the ventricular walls to be observed clearly.
- Investigating the heart sounds (corresponding to the movement of the valves) using a stethoscope.
- Investigating the circulation of the blood in the vessels. Measuring the pulse and measuring the blood pressure using a sphygmomanometer after or during different activities, or in different groups of people, or at different times of the day.

6C ANALYSING BREATHING USING A SPIROMETER

Time: 1.5 hours

Syllabus reference: Topic 6.4

Skill: Monitoring of ventilation in humans at rest and after mild and vigorous exercise. (Practical 6)

Materials

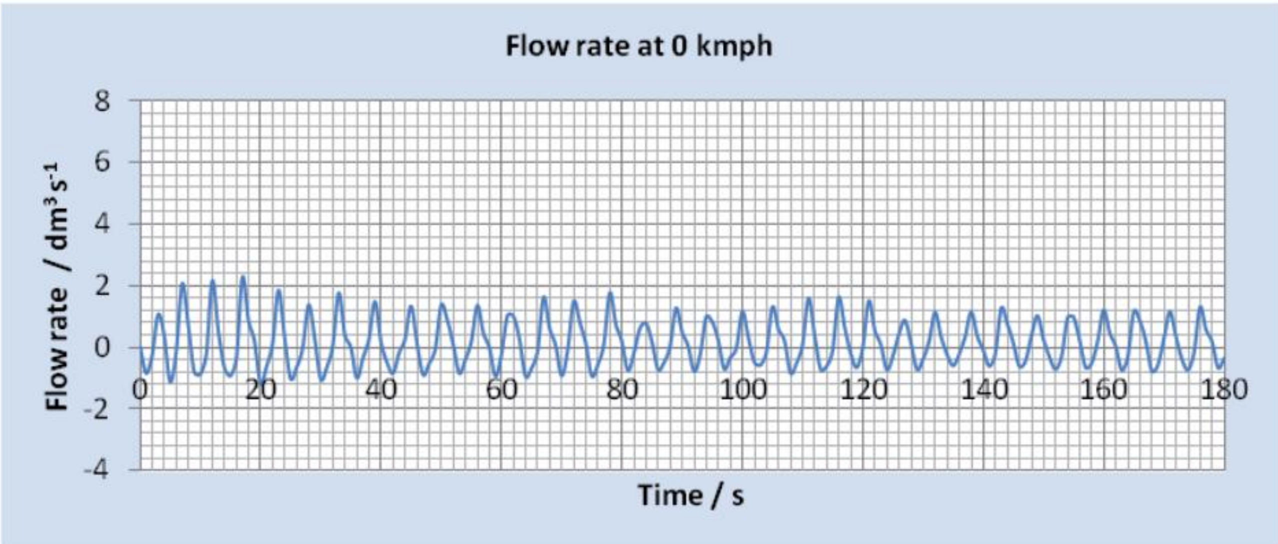
The data recorded here was obtained using Vernier ® data logging materials.

Data

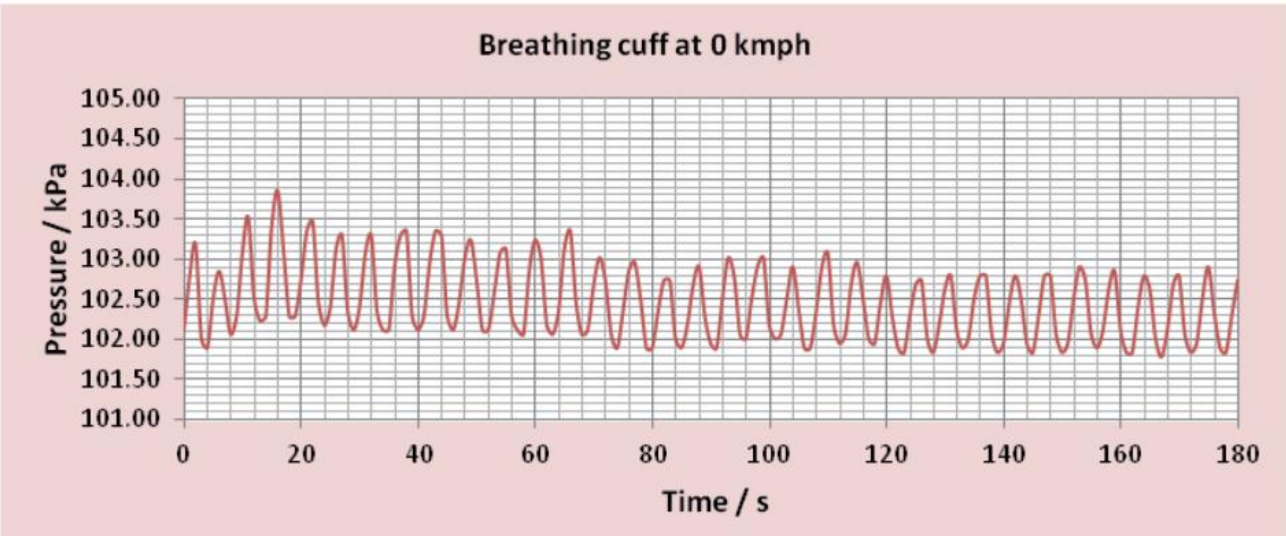
Using the breathing cuff attached to a pressure sensor, the frequency of the breathing can be determined and a relative measurement of the volume breathed is possible.

Using the spirometer the frequency and the volume of the breaths can be measured.

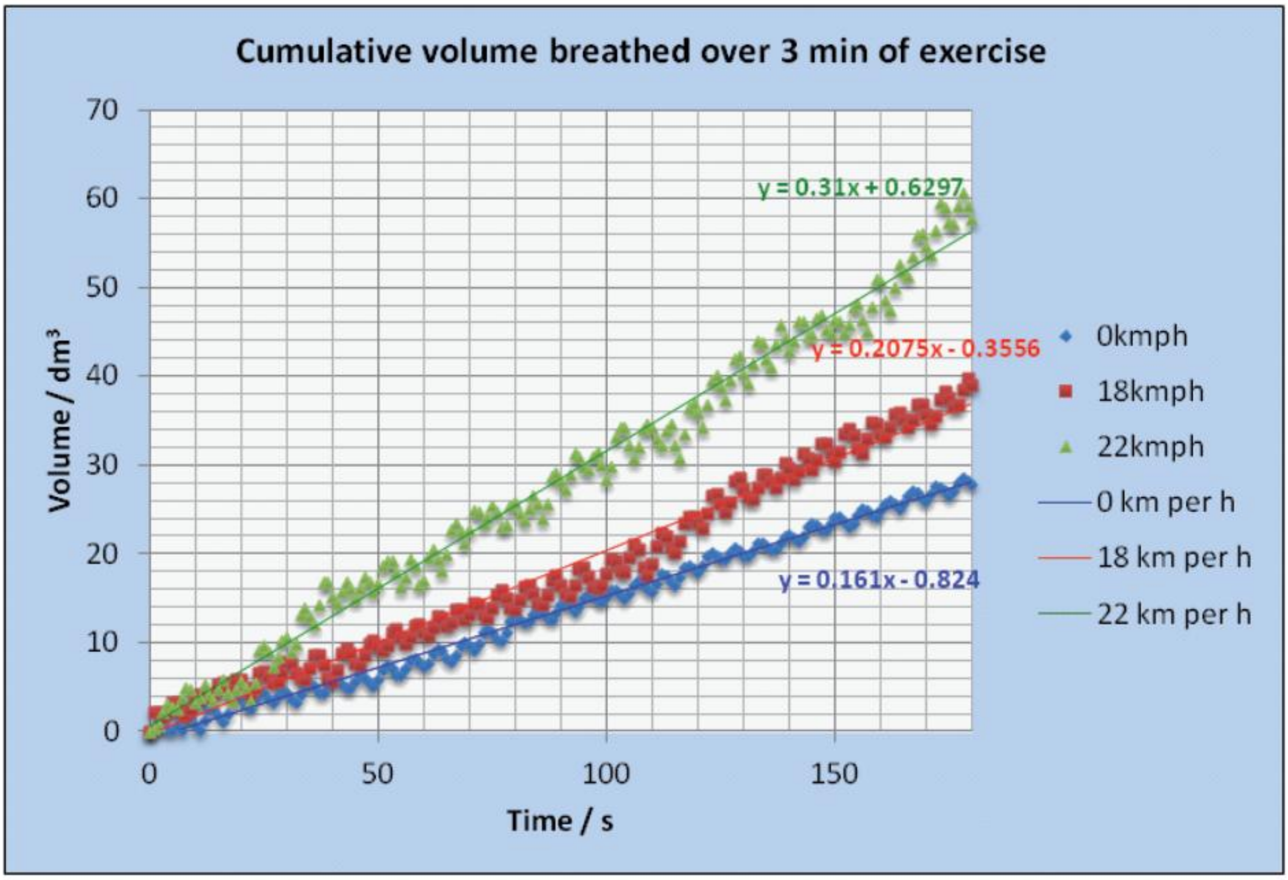
Spirometer data



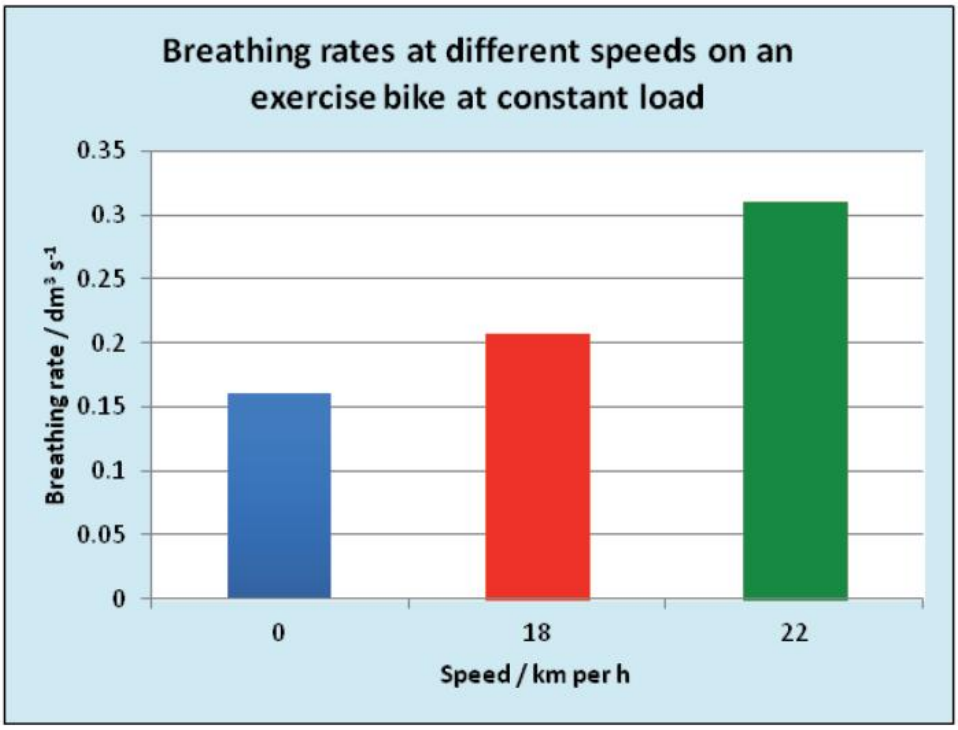
Breathing cuff data (on the same person at the same time)



With the spirometer, it is also possible to calculate the cumulative volume of air breathed. This can give a clear idea of the changes with the increase in activity.



Giving the following breathing rates:



OPTIONS

12A THE PUPIL REFLEX

Time: 1 hour

Syllabus reference: Option A.2

Application: Use of the pupil reflex to evaluate brain damage.

Method

The electric circuit set up will give adequate results, however, a pen light will give a clearer response and it is easier to manipulate if a subject is used.

Warning: The light should be placed below the line of sight and the subjects should not stare at it for long periods of time.
LEDs should not be used as a light source for this investigation.

UNDER NO CIRCUMSTANCES SHOULD A LASER POINTER BE USED

Observations

Direct reflex

The irises of both eyes should behave in the same way. Constriction of the pupil in bright light and dilation in the dark.

When the light intensity is constant, careful observation will reveal a small oscillation of the pupil diameter, even if the eye is perfectly steady. What is being observed is the effect of negative feedback from the brain.

The control of the pupil diameter is regulated by two pathways. One, using the parasympathetic system constricts the pupil. The other, from the sympathetic system dilates the pupil. The iris is a sphincter muscle with circular and radial muscles. The radial muscles pull the pupil open when they contract. The circular muscles pull the pupil shut when they contract.

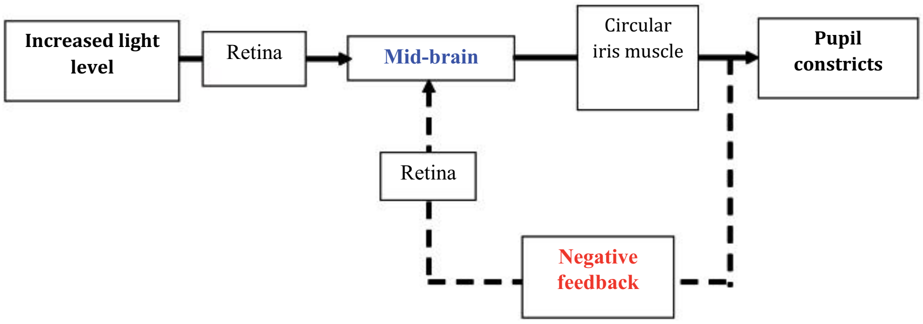
Note: Pupil diameter will also vary with gender, eye colour, emotional state, myopia and the distance of an object being observed from the eye.

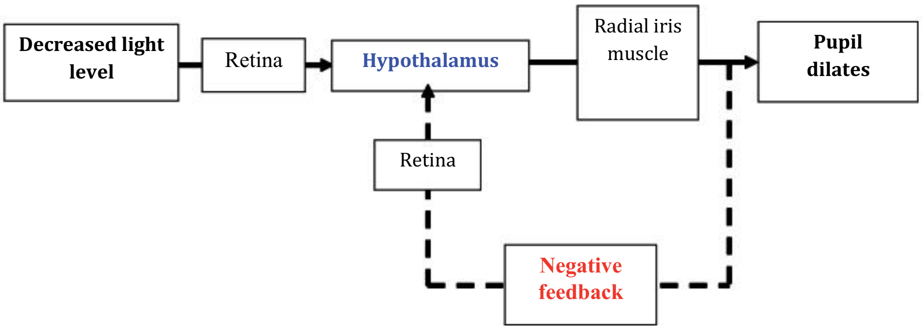
The consensual reflex

When one eye is illuminated, both pupils constrict.

Some points for consideration

How can the response of the iris be explained in terms of negative feedback?





In fact, there are two reflexes that interact with one another.

The pupil light reflex that operates when there is too much light, causing the pupil to constrict by contracting the circular muscles until the signals from the retina are reduced and the pupil stops constricting further.

The pupil dark reflex operates when the light levels are too low. It opens the pupil by contracting the radial muscles.

When the pupil dilates to the point where the signals from the retina arrive at the brain, the pupil constriction reflex with stop the pupil down. In this way, eyes observed under low light conditions tend to oscillate the size of the pupil a little.

- How can you explain the response of the shaded eye?

The pupil reflex applies to both eyes. Some neurones from the left eye pass to the midbrain on the right and vice versa. The information from the left visual field of the left eye passes to the left visual cortex as does the left visual field of the right eye and vice versa. So, the corresponding part of the visual cortex should be receiving information from both eyes.

Research

- Find out which sensory and motor nerves are involved in the pupil reflex. Draw a diagram of the reflex arch involved in this reflex.

The pupillary light reflex is a parasympathetic reflex

Signals from the retina pass to the optic nerve and to centres in the midbrain, then via the occulomotor nerve back to the pupil, constricting the circular muscles.

The pupillary dark reflex is a sympathetic reflex

Some of the neurones of the optic nerve innervate the hypothalamus, this takes a signal down the spinal cord to sympathetic ganglia at the level of the lungs in the thorax. A sympathetic nerve then returns to the head through the neck and innervates the radial muscles passing beside the trigeminal nerve.

12B THE RETINA AND COLOUR VISION

Time: 1 hour

Syllabus reference: Option A.3

Application: Red-green colour-blindness as a variant of normal trichromatic vision.

The structure of the retina

What kind of cell will be used in daylight vision or low light night vision?

Cone cells are used in daylight vision; the rod cells operate in low light.

Afterimages and complementary colours

Lamps with LEDs give a more intense light. Care must be taken not to look directly at the LEDs.

Data

When a white object is observed all three types of cones are being stimulated whereas a black object stimulates none of the cone cells.

As the complementary colour chart indicates:

- A red cross should give a cyan (turquoise) afterimage. L cones are mostly stimulated and bleached, so when the white sheet is observed the M and S cones are the only ones working giving a mixture of green and blue-violet while the L cones are regenerating.
- A blue cross gives a yellow afterimage. The L and M cones being the ones that are not bleached.
- A green cross green gives magenta. The L and S cones are not bleached.

Afterimages from complementary colours

- Predict what you will get if you stare at yellow, cyan and magenta crosses for a minute. You might not get quite what you expect.

The classic yellow highlighter usually gives a violet afterimage though blue would be logical. The reason being the nature of the pigments and the wavelength of the light source used.

Colourblindness simulations

Example images from the Colblindor simulator Coblis:

13A DO DISINFECTANTS KILL BACTERIA?

Time: 1 hour to set up and 30 min for observations

Syllabus reference: Topic B.1

Skill: Experiments showing zone of inhibition of bacterial growth by bactericides in sterile bacterial cultures.

Materials

Bleach solution: Use commercial strength bleach.

Method

Filter paper discs

The discs used here can be cut using a hole punch. Do not fold the paper to cut several disks at once. This tends to make the discs stick together and they are difficult to separate. Wrap the discs in an envelope made from aluminium foil and sterilise them in an autoclave.

Using the autoclave

All glass material and growth media to be used should be sterilised for 20 minutes at 120°C.

All open material should be either plugged with cotton wool and the cotton wool plug covered with aluminium paper before sterilisation (e.g. test tubes, conical flasks); or, the openings should be covered with aluminium foil before sterilisation (e.g. beakers).

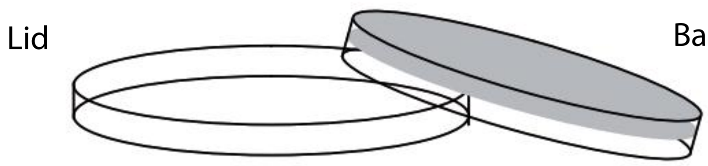
After sterilisation the material should be transferred to a working zone and used as soon as possible.

If material is to be kept, ensure that plugs and or aluminium paper covers are not removed and that the material is kept in a dry place.

Preparing media for growth of the micro-organisms

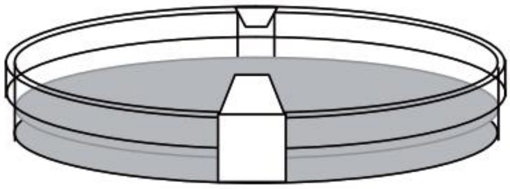
Different organisms have different growth medium requirements. Whichever medium is being used, it must be sterilised in the autoclave before use. Liquid media can be sterilised in closed conical flasks or in closed test tubes. For a medium that is to be solidified after sterilisation in plastic Petri dishes, the medium can be poured into opened dishes within the sterile zone. The dishes used should come from an unopened pack. The medium must be left to cool in the sterile zone with the lids on. Once the agar has set, the dishes can be opened and turned upside down to allow any condensation to dry for about 30 minutes.

The dishes should then be closed and kept upside down, until use, in a dry place (the solid medium upward).

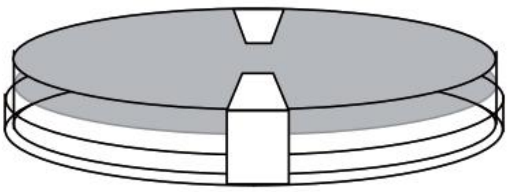


Use self-adhesive tape to hold on the lid of the Petri dish. Do not seal the dish completely as this may encourage the growth of anaerobic bacteria.

Use self-adhesive tape to hold on the lid of the Petri dish



Transfer the dish to the oven and leave for the required growth time and at the required temperature. The Petri dishes must be placed upside down (the solid medium towards the top)



The Petri dishes must be placed upsidedown

Examination of the micro-organisms

Warning: Do not open the dishes containing cultured microbes

It is preferable to keep microscopic investigation and staining to media that already contain bacteria which are not pathogenic, for example bacteria already present in fresh yoghurt. Even so when organisms are grown, the dishes must not be opened.

Disposing of material at the end of manipulation

Ideally, all the material should be autoclaved at 120° C for 20 minutes. Including plastic Petri dishes, which will melt in the process, so they must be placed in a suitable container before autoclaving. For any material that is to be thrown away after autoclaving, autoclavable plastic bags can be used, these should be tied before autoclaving. All material to be kept, such as glassware and slides, should be autoclaved and then washed in the normal way.

An alternative is to leave all the material (except metal tools) to soak in bleach for 24 hours.

Clean the working area once again with bleach. Wash your hands again after handling any of the experimental materials.

13B THE GRAM STAIN FOR BACTERIA

Time: 1 hour

Syllabus reference: Topic B.1

Skill: Gram staining of Gram-positive and Gram-negative bacteria.

Materials

Crystal violet stain (0.5% aqueous) Add 0.5g crystal violet to 100cm³ distilled water. Dissolve and filter.

Lugol's iodine Add 1g iodine to 2g potassium iodide in 25cm³ distilled water. Dissolve this mixture then add 75cm³ distilled water.

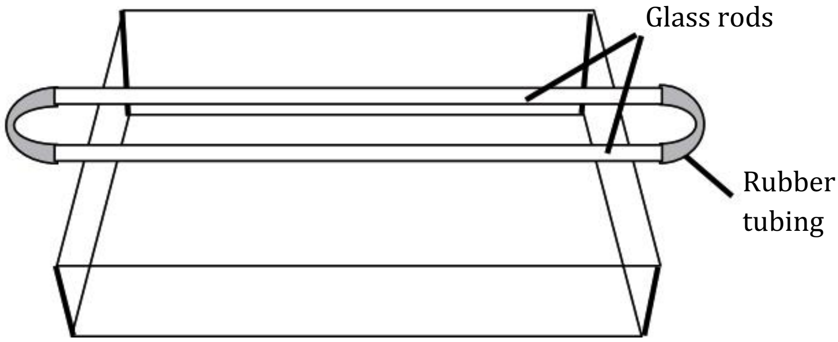
Acetone-alcohol (50% acetone + 50% pure ethanol) Mix 50cm³ acetone with 50cm³ 100% ethanol.

Safranin (1% aqueous) or Add 1g safranin to 100cm³ distilled water. Dissolve and filter.

Basic fuchsin (an alternative to Safranin). Dissolve 0.5 g basic fuchsin in 20 cm³ 95% ethanol. Then dilute to 100 cm³ with distilled water and filter the solution.

Making a staining rack

This can be made quite simply from two 0.7cm diameter glass rods about 30cm joined by two pieces of rubber tubing about 15cm long.



Cleaning off immersion oil

Immersion oil should always be cleaned off the lens after use. If necessary, the lens can be cleaned using a lens tissue soaked in ethanol.

Some oils recommend using solvents such as xylene for cleaning. These can be very toxic. Check the safety procedures before using them.

Research

What is the relationship between the Gram staining characteristics of a bacterium and its virulence when it has infected a patient?

Gram stain will help to identify bacteria. Even though more precise molecular techniques for identifying pathogens, (such as PCR), are used, Gram stain is often the first step. The cell wall characteristics of Gram negative bacteria is often associated with the presence of an endotoxin that stimulates an immune reaction that causes inflammation.

14A STUDYING THE CHANGES IN A PLANT COMMUNITY USING A BELT TRANSECT

Time: 3 hours in the field

Syllabus reference: Option C.1

Skill: Use of a transect to correlate the distribution of plant or animal species with an abiotic variable.

Required knowledge: Use of identification guide or key if it is to be used.

Materials

Quadrats: See investigation 4B.

Ranging poles: These can be made cheaply from 2m lengths of wood marked at 10cm intervals using bright insulating tape.

Data sheet: An example of a data sheet is given (see following page) which was used by students studying a lake-woodland transect in Europe.

The abiotic factors temperature, light intensity and humidity were recorded directly on a data logger. Height change can be recorded on a graphic calculator which can give a readout of the profile as the survey progresses.

Method

This method presents an interrupted belt transect over 25 metres. Shorter distances could use a continuous transect or even smaller units (e.g. 0.25 m^2). To get an idea of any changes in the transition zone, at least 10 results would be needed along a transect.

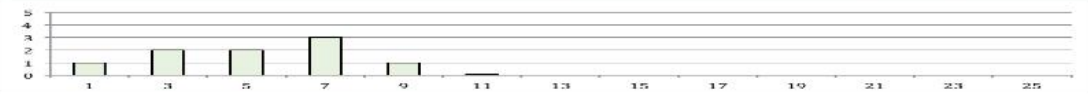
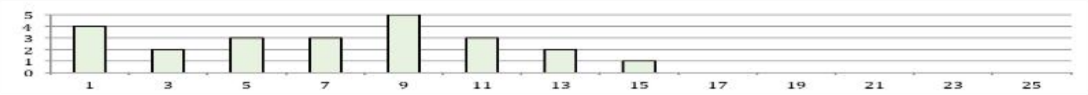
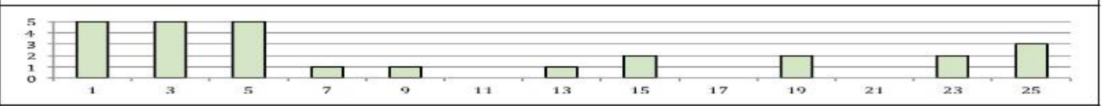
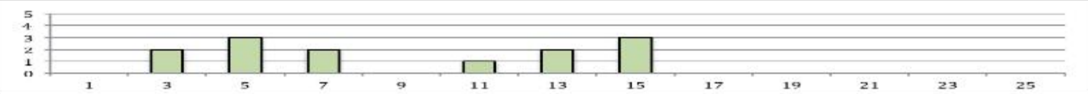
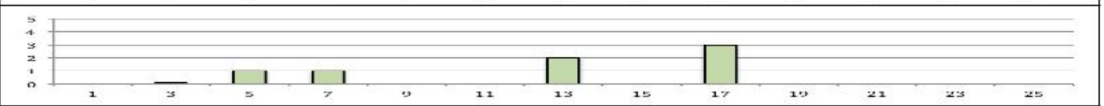
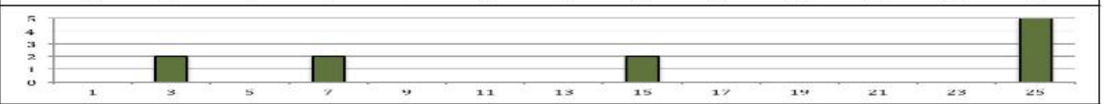
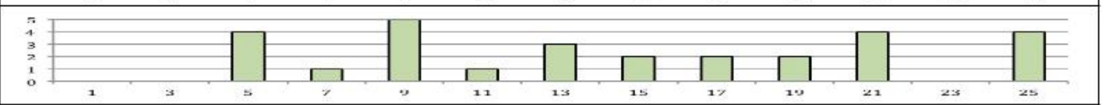
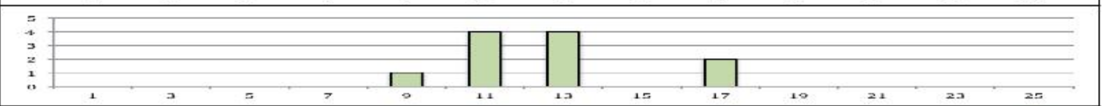
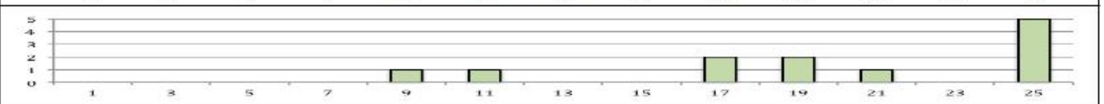
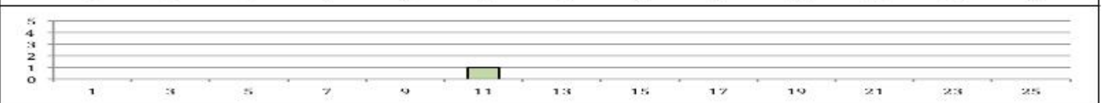
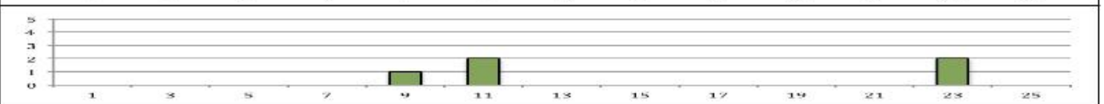
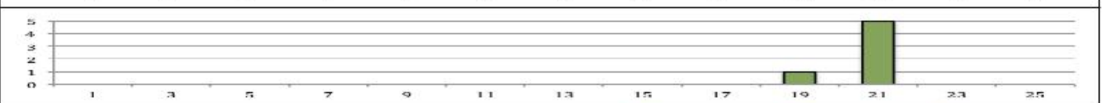
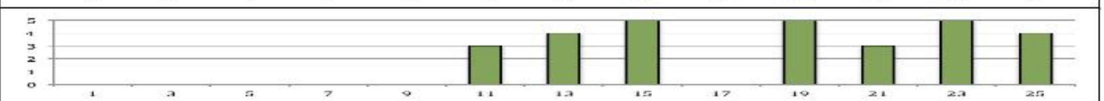
The problem of identifying species can be simplified by providing the students with a species check list which has been established for the area being investigated. This, of course, requires some prior investigation by the teacher. An example of a species checklist for a N.W European lake-woodland boundary is given.

Any transition zone could be studied using this method: lake margins, field to forest boundaries, seashore, sand dunes.

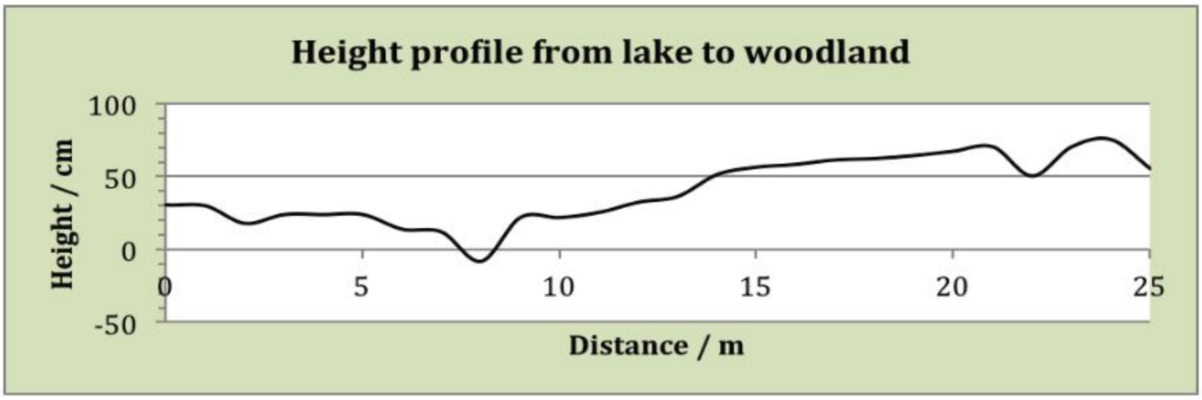
Rocky seashore habitats, where there are sessile animals such as barnacles and molluscs as well as algae (sea weeds) can use this method too.

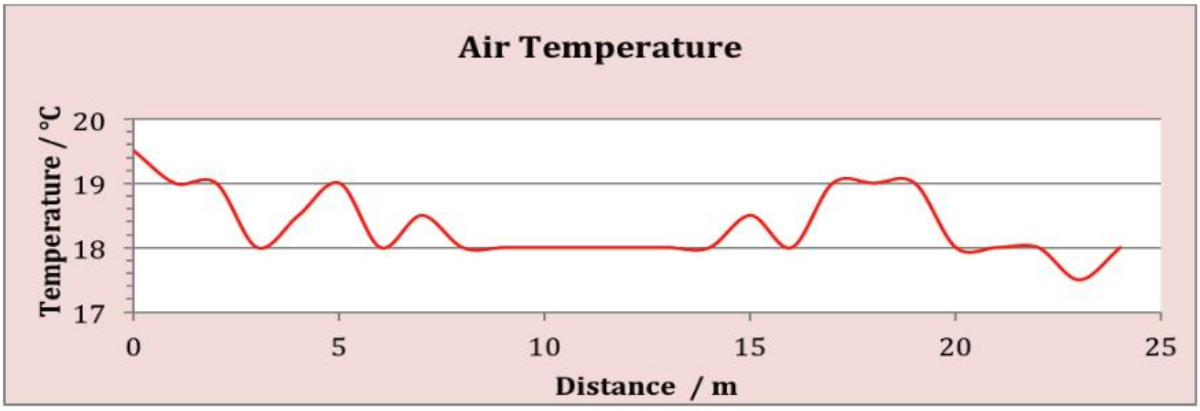
ZONATION OF LAKE SHORE VEGETATION – DATA SHEET

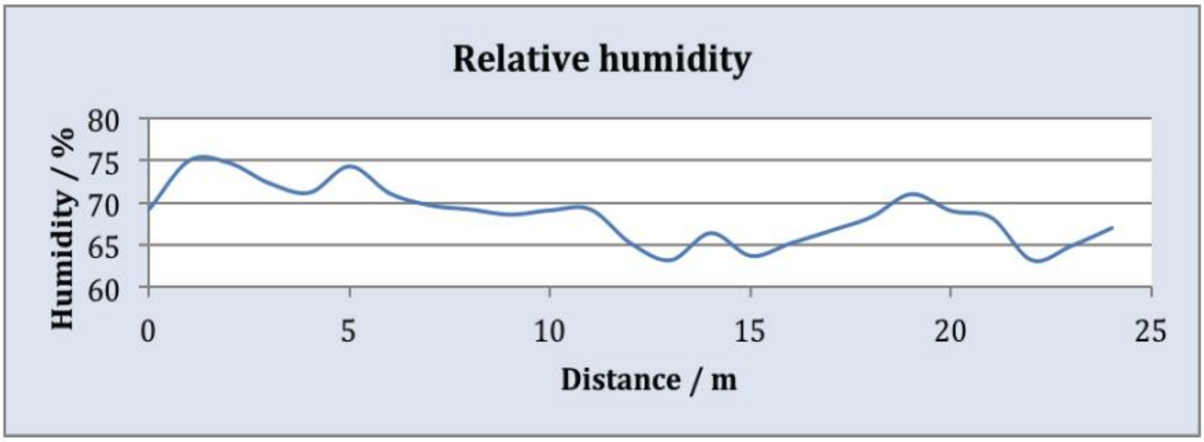
	QUADRAT/m along transect																								
SPECIES	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Open water																									
Equisetum palustre																									
Carex panicea																									
Lycopus europeus																									
Hydrocotyle vulgaris																									
Galium palustre																									
Filipendula ulmaria																									
Lotus corniculatus																									
Lysimachia vulgaris																									
Epilobium palustre																									
Aethus cynapium																									
Viola palustris																									
Poa serotina																									
Molinea caerulea																									
Betula pubescense																									
Alnus glutinosa																									
Salix alba																									
Pinus sylvestris																									
Juncus inflexus																									
J. conglomerata																									
Spagnum spp.																									
Stellaria sp.																									
Mysotis sylvatica																									
Urtica dioica																									
Rubus fruticosus																									
Cirsium sp.																									

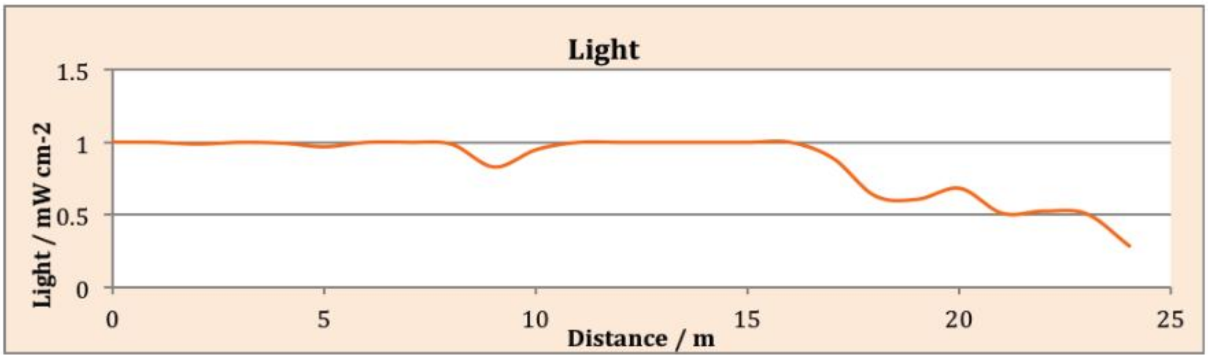
Species	Distance along transect / m
Open water	
Equisetum palustre	
Carex panicea	
Filipendula ulmaria	
Comarum palustre	
Lysimachia vulgaris	
Salix alba	
Sphagnum spp	
Epilobium palustre	
Viola palustris	
Lycopus europeus	
Angelic silvestris	
Lotus corniculatus	
Anthyrium filix-femina	
Poa serotina	
Molinia caerulea	

Abiotic data





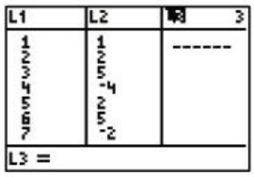




It is now possible to see if there are any trends that suggest a relationship. For example, a relationship with light intensity can indicate shade tolerant species. However, as the abundance scale is a very approximate scale of the importance of a species (it is only 0-5 scale), a scatter plot is not very realistic.

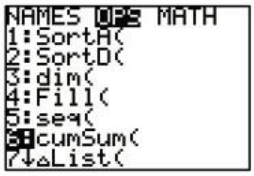
Using the Ti graphic calculators *to process the height profile* and Graph data

Press **STAT** then select **1: Edit…** to open a spread sheet.



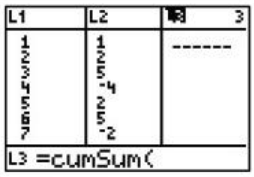
Just like MSExcel or other spread sheets you enter the data in columns or lists **L1**, **L2** etc. Type in your distance along the transect in **L1** and the height change for each metre along the transect in **L2**.

Use the cursor to get to the top of **L3**. Press **ENTER** then **2nd** and **LIST** and select **OPS** (use the cursor to move one to the right). This opens up a menu of functions rather like in Excel.

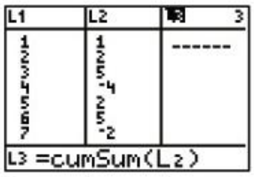


Select **6:cumSum(** This is the **cumulative sum function.**

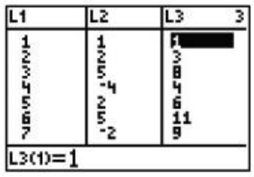
Press **ENTER** and the function will appear at the bottom of the screen
L3 = cumSum(



Press **2nd** and **L2** then **)**. Press **ENTER** and you will see the spread sheet calculate the cumulative sum of all your samples. This is your **height profile**



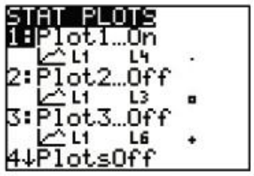
You can store other data for abiotic factors (e.g. light, temperature) along the transect in subsequent columns.



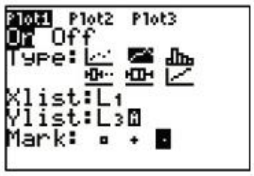
Graphing the data

To show the height profile plot a graph using **STAT PLOT**

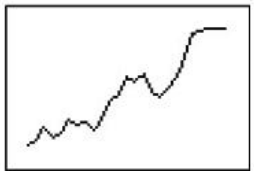
Press **2nd** and **STAT PLOT**. Open **Plot 1** by pressing **ENTER**



Using the cursors and **ENTER**
Select **ON**
Select the line graph plot
In **Xlist:** Press **2nd** and **L1**
In **Ylist:** Press **2nd** and **L3**



Then to see the graph Press **ZOOM** and select **9:ZoomStat**



You should be able to see a scaled graph showing the height profile of your transect.

14B USING INVERTEBRATE PITFALL TRAPS ON A LINE TRANSECT

Time: 1 hour setting up and 1 hour to identify

Syllabus reference: Option C.1

Skill: Use of a transect to correlate the distribution of plant or animal species with an abiotic variable

Materials

Trap line: String can be marked at five metre intervals with marker tape.

Making pitfall traps: To make the traps, collect plastic bottles such as *Evian* water bottles. Using a cutter, cut off the base of the bottle about 14cm high. Using scissors, make cuts 2cm deep at 4cm intervals around the cut edge and fold this inward.

Warning: Sharp instruments. Take care to cut away from yourself.

Cut off the coned top from what is left of the bottle and discard this. Cut out a panel, about 10cm wide t remaining sleeve and discard this also. The remaining section can be stretched over pegs (wooden barbecue skewers), placed around the trap, acting as a roof. Leave a 1cm space between the roof and the soil surface.

Method

The line transect could be set up to look at relative abundance in a number of situations:

For example: Field-transition zone-wood.

Along a succession zone lake to woodland.

The traps could be left for various intervals of time: 24 hours, 12 hours, morning/afternoon.

The Key

This will have to be relevant to your local habitat. Here, the following were used:

Insects of Britain and Northern Europe: Michael Chinery; Collins ISBN 0 00 219216 0

Grassland studies: Juliet Brodie; George Allen and Unwin ISBN 0 04 57 40208

Animals under logs and stones: C. Philip Wheater & Helen J. Read; Naturalists Handbook #22 Richmond Publishing ISBN 0 85546 301 5

Record sheet

In the record table, Order has been given as the lowest level of classification for most groups. However, a few groups have only been classified to the level of Class where further identification is difficult.

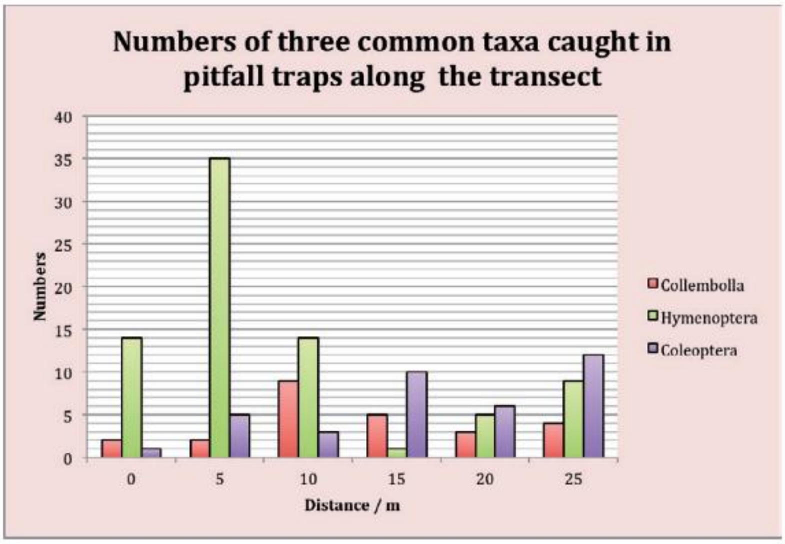
Warnings: *Work in the field has its risks:*
The teacher should have visited the site first, obtained permission from and owners to work there and verified any safety risks.
The weather forecast for the day needs to be consulted.
Adequate protection from cold or wet conditions must be provided for.
Excessive sunlight and heat must be also considered.

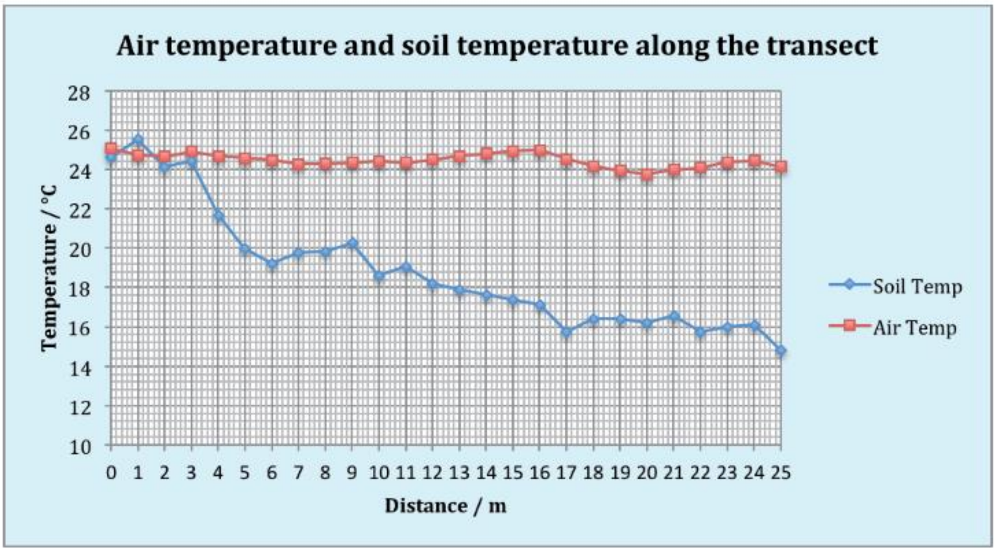
Data

Data from five parallel transects, placed from a lawn to a woodland the transition being at 10-15m.

The data logging material used to obtain these results is from Vernier ®.

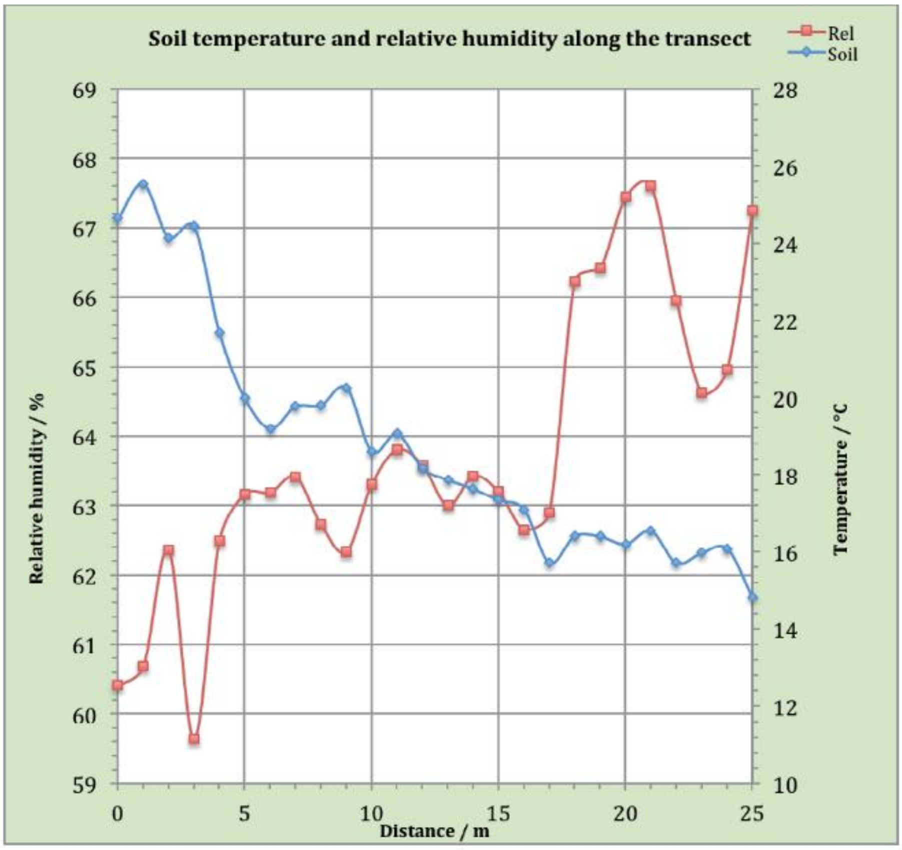
Habitat	Distance / m	Acarina	Araneae	Isopoda	Diplura	Protura	Collembola	Thysanura	Orthoptera	Demaptera	Diptera	Hymenoptera	Coleoptera	Class Diplopoda	Class Chilopoda	Class insecta	Class Mollusca	Totals
Lawn	0		7	1			2	1				14	1	3				29
	5		5				2	1		3		35	5		1	1		53
Transition	10		4		1		9		2	1		14	3		1			35
	15	3	8				5					1	10	1				28
Woods	20	1	7	1			3					5	6	1	1		1	26
	25	1	7				4			1	23	9	12					57



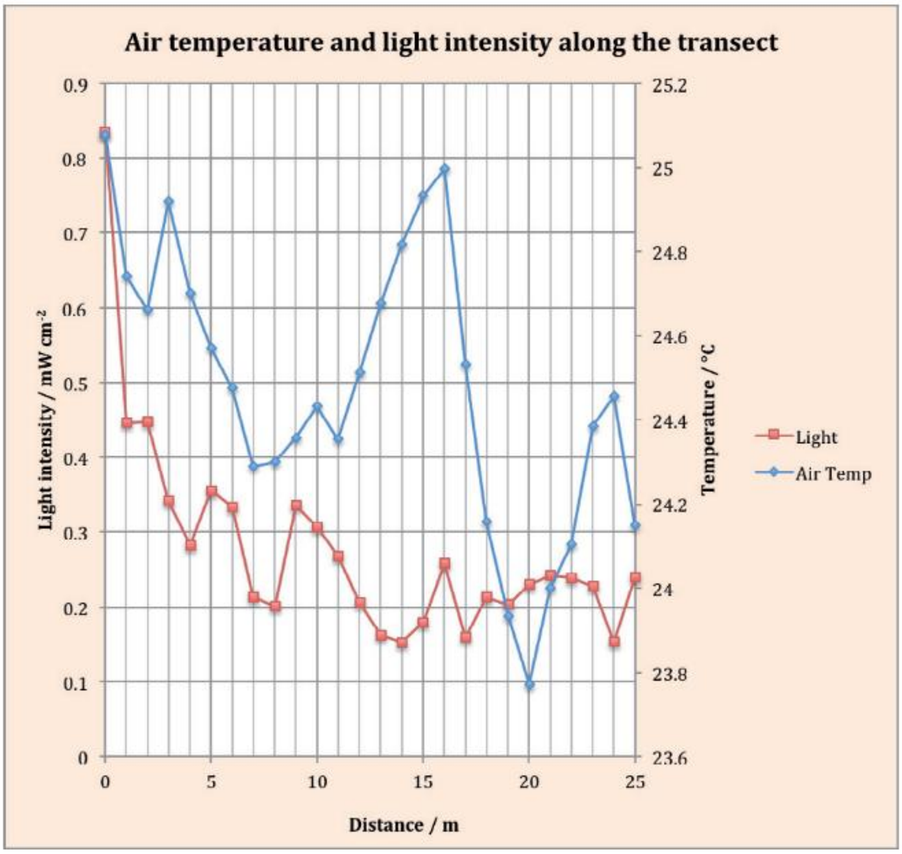


Profiles of various abiotic factors, measured at 1m intervals along the transect.

The air temperature can be seen to remain more stable across the transect than the soil temperature, which drops as the transect penetrates the woodland.



There is a clear inverse relationship between the soil temperature and the relative humidity.

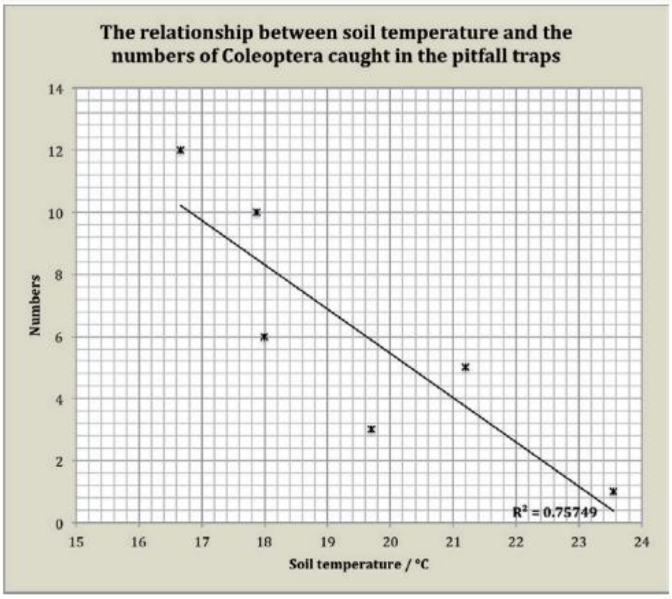


Here, the light intensity and the air temperature show a direct relationship.

Looking at these profiles and the distribution of the animal groups it may be possible to identify a relationship.

The Coleoptera numbers in the traps appear to rise as the soil temperature falls.

Plotting soil temperature values for the positions of the six traps gives a negative correlation, as expected. The coefficient of determination (R2) shows that the trend is quite a strong one.



Soil temp / °C	Coleoptera numbers
22.2	1
19.8	5
17.5	3
15.6	10
15.4	6
15.3	12
r =	-0.796

This suggests a negative correlation, though the sample size is very small.

This does not mean that the soil temperature directly affects the numbers of Coleoptera. It could be some other factor that influences the beetles, such as their food, which is in turn influenced by soil temperature. Furthermore, Coleoptera is a very large Order of the insects, each species will have its own niche.

To investigate further

- Measuring, for example, the number of Coleoptera per unit area could be carried out by sampling a given area or volume of soil or even by mark and recapture methods.
- Project work could be carried out on distribution and abundance at different time intervals, including seasonal influence.
- Investigating the other factors influencing the animals caught in the traps (e.g. the presence of bait in the traps, the position of the traps, other factors such as soil pH)

14C MEASUREMENT OF A DIVERSITY INDEX AND A BIOTIC INDEX

Time: 4 hours

Syllabus reference: Option C.2

Skill: Investigation into the effect of an environmental disturbance on an ecosystem.

Syllabus reference: Option C.4

Skill: Analysis of the biodiversity of two local communities using Simpson's reciprocal index of diversity.

Required knowledge: Biotic and abiotic factors. Agents bringing about the breakdown of organic material in water systems.

Materials and Method

A heavy, robust, long-handled (1m to 1.5m long) pond net is good, as the currents can be quite strong.

Sorting trays, such as the one seen in the photos are ideal for sorting the animals into groups, these trays are also used for presenting geological specimens.

White material: bowl, sorting tray and plastic teaspoons for sorting, small organisms are easier to observe against a white background.

Plastic pipettes are more practical than glass for use in the field.

Rubber gloves are also useful for warmth and possible protection against waterborne pathogens.

Hand lens: ×10 magnification should be sufficient for most field observations.

Tools can be marked with bright insulating tape or paint to prevent loss.

Keys

Much time is taken in working through the keys for precise identification of the organisms.

However, it is a good exercise to key out to species whenever this is possible.

Some observations for identifying specimens may require a binocular microscope, so a few specimen bottles would be useful in the field to collect difficult specimens for later identification in the laboratory.

White bowls, white plastic spoons and white geological sorting trays are useful when searching for animals.

A flow meter is a very useful tool when studying river systems. It measures the flow rate nearer to where the animals are living.

Measuring abiotic factors

Measurement	Method
Depth of the river (m)	Metre ruler
Width of the river (m)	Metre ruler or marked string
Water current (ms-1)	Flow meter or the time taken for a leaf or a small branch to float one metre distance
Temperature (° C)	Temperature probe or thermometer
Oxygen (mg dm^{-3})	Oxygen probe or oxygen test
pH	pH probe
Light (Wm^{-2} or lx)	Light meter at 1 metre above the surface

Oxygen

An oxygen probe is practical, however, they are expensive and they need to be calibrated before use if an absolute value is required. The percentage saturation of oxygen takes into consideration the water temperature. It can be calculated from the table below, that shows the oxygen concentration of fully saturated water:

Dissolved O_2 (mg dm^{-3})in saturated water at a given altitude / m

°C	0	200	400	600	800	1000	1500	2000
0	14.64	14.29	13.93	13.57	12.2	12.91	12.11	11.39
1	14.22	13.89	13.54	13.18	12.83	12.55	11.77	11.07
2	13.82	13.51	13.16	12.82	12.48	12.20	11.45	10.76
3	13.44	13.14	12.81	12.47	12.14	11.87	11.13	10.47
4	13.09	12.79	12.47	12.14	11.82	11.55	10.84	10.19
5	12.74	12.46	12.14	11.82	11.51	11.25	10.55	9.92
6	12.42	12.14	11.83	11.52	11.21	10.97	10.28	9.67
7	12.11	11.84	11.54	11.23	10.93	10.69	10.03	9.42
8	11.81	11.55	11.25	10.96	10.66	10.43	9.78	9.19
9	11.53	11.27	10.98	10.69	10.41	10.18	9.54	8.97
10	11.26	11.01	10.72	10.44	10.16	9.94	9.32	8.76
11	11.01	10.75	10.48	10.2	9.93	9.71	9.10	8.55
12	10.77	10.51	10.24	9.97	9.70	9.49	8.90	8.36
13	10.53	10.27	10.01	9.75	9.49	9.28	8.70	8.17
14	10.30	10.05	9.79	9.54	9.28	9.07	8.51	7.99
15	10.08	9.84	9.58	9.33	9.08	8.88	8.32	7.82
16	9.86	9.63	9.38	9.14	8.89	8.69	8.15	7.65
17	9.66	9.43	9.19	8.95	8.70	8.51	7.98	7.49
18	9.46	9.24	9.00	8.76	8.53	8.34	7.81	7.34
19	9.27	9.05	8.82	8.59	8.36	8.17	7.66	7.19
20	9.08	8.88	8.65	8.42	8.19	8.01	7.50	7.05
21	8.90	8.70	8.48	8.25	8.04	7.85	7.36	6.91
22	8.73	8.54	8.32	8.10	7.88	7.70	7.21	6.77
23	8.57	8.38	8.16	7.94	7.73	7.55	7.08	6.64
24	8.41	8.22	8.01	7.79	7.58	7.41	6.94	6.52
25	8.25	8.07	7.86	7.65	7.44	7.27	6.81	6.39
26	8.11	7.92	7.72	7.51	7.30	7.14	6.69	6.28
27	7.96	7.78	7.58	7.37	7.17	7.04	6.57	6.16
28	7.82	7.64	7.44	7.24	7.04	6.88	6.45	6.05
29	7.69	7.51	7.31	7.11	6.92	6.76	6.33	5.94
30	7.56	7.38	7.18	6.99	6.80	6.64	6.22	5.83

The river

Two or three different stations could be chosen for example:

(a) above and/or below a dam

(b) in tributaries and above and in the main river

(c) at a shaded area and unshaded area of the river

(d) in fast, lotic, and slow, lentic, areas

(e) in a stretch of the river running through a town and a stretch running through a country area

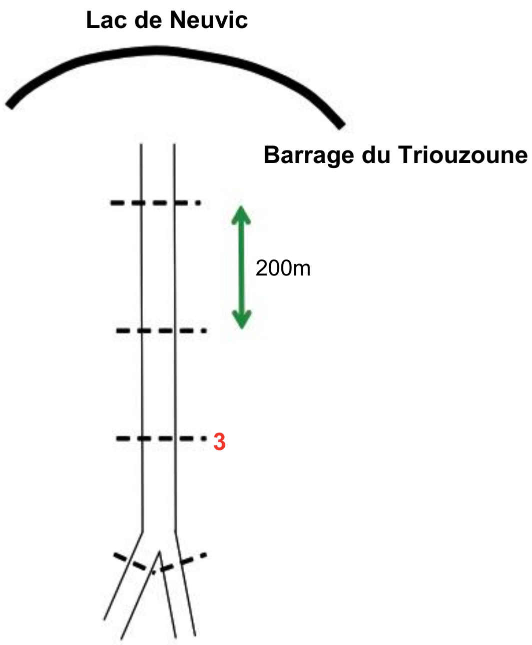
The water should not be too deep or too fast, it should be possible to cross the river (to measure the width). It should be fairly easy to clear an area of the large stones or small rocks for the kick sample. Check that you are allowed to work in this part of the river. Finally, ensure that there are no known sources of pollution in the vicinity, for health reasons.

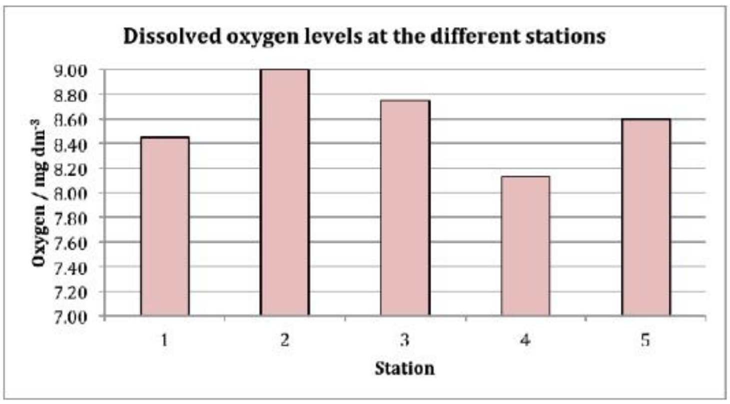
Warning: Polluted areas must be avoided for health reasons

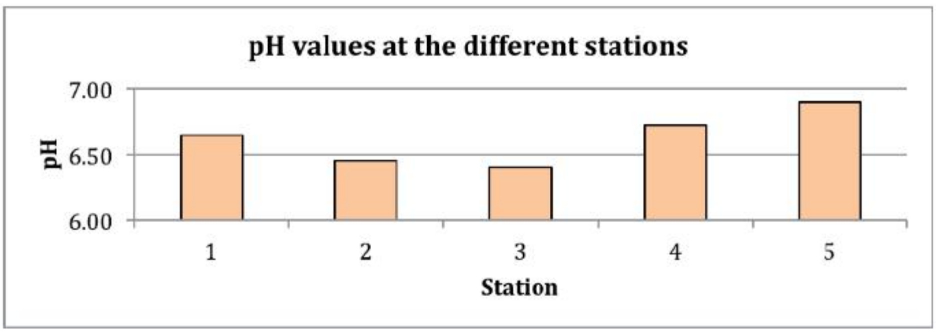
BIOHAZARD

Data

The following data were taken from a river flowing below a dam. Authorisation from the electricity generating agency was obtained prior to the visit.







Benthic animals captured by kick sampling in the River Triouzoune, Corrèze, France.

Organisms	STATION	1	2	3	4	5
Plecoptera	Leuctridae					
	Nemouridae					
	Perlidae		1			
Ephemeroptera	Baetedae					
	Ecdyonuridae					
	Ephemerellidae					
	Ephemeridae					
	Leptophlebidae					
Trichoptera						
With cases	Limnephilidae	1		2	4	
	Glossosomatidae					
	Odontoceridae					
	Sericostomatidae					
Without cases	Hydropsychidae	1				1
	Philopotomidae					
	Polycentropedie					
	Rhyacophilidae		5	12	1	4
Odonata	Zygoptera				1	
	Anisoptera					
Megaloptera	Sialidae					
Crustacea	Gammeridae					
	Asellidae		2			
Gastropoda	Ancylidae					
Diptera	Simulidae	2				
	Chironomidae	2	1			
Coleoptera	Dytiscidae					
	Hydrophilidae					
	Helodidae					
	Elminthidae					
Hirudinea	Glossiphonidae					
	Hirudinae		6	14	7	6
Oligochaeta	Tubificidae					
Others	Worm			5		
	SIMPSON'S INDEX	7.5	4.0	3.1	2.9	2.6
	BIOTIC INDEX	5	6	5	5	5

The Biotic index and the Diversity index

The Biotic index used here is based on that of Trent and Verneaux. For the purpose of this exercise, the above Biotic indices of Trent and Verneaux have been simplified, requiring identification to be carried out only as far as Family. It is considered that it will be fairly rare to find more than one species of the same Family in any one site. Also, considering the accuracy of the identification possible in the field in the time given, identification to Family is sufficient.

In the above-mentioned biotic indices, Trent excludes *Baetis rhodani* from the Ephemeroptera line, including it with the Trichoptera, Verneaux does not. Verneaux classes Ecdyonuridae with the Plecoptera line, Trent does not. A number of other differences arise between the biotic indices of Trent and Verneaux. In this exercise, for simplicity, all organisms will be classed within their major representative group.

Many biotic indices exist and all vary in detail. Ideally, each region should develop a biotic index to accommodate for local conditions and particularities of the organisms found. This simplified exercise does still give a good idea of the biotic index and compares well with results obtained using the indices of either Trent or Verneaux.

Biotic indices do not take into account abundance, so a unique appearance of 1 Plecoptera will considerably increase the index, even if a low diversity is seen. For a diversity index, the equitability between the species is important. One useful exercise is to compare the index measured with what it would be if all the species found were in equal numbers. This would give the theoretical maximum diversity index for a site (D_{max}).

The biotic index is an indication of organic pollution. Other forms of pollution, such as toxic pollution, may lead to a low diversity of organisms being present or high numbers of those groups tolerant to a particular pollutant. In the results table for this exercise only organisms more than 0.5cm are considered, so Hydrocarina and Platyhelminthes do not appear.

Some points for consideration

- Organic pollution reduces the oxygen content of the water, so those organisms most sensitive to oxygen, such as Plecoptera will be absent in poorly oxygenated water.
- Stress will reduce the species diversity. Species sensitive to the stress will fall in numbers or even disappear. Species that tolerate the stress will increase in numbers. The environment will fit the fundamental niche of the tolerant species more and more and their will be less competition.
- One possible source of pollution could be agricultural run off (this could include animal waste, other fertilisers and pesticides). This leads to organic pollution and low biotic indices. To see if agricultural run off has an effect on the river organisms, sampling could be carried out by a farm and compared to an area where there is no run off.
- Even in areas where human impact is quite low, dead leaves falling in the river from surrounding woodland can have their impact. The organic mater added to the river during the autumn leaf fall may contribute significantly to the energy imput in the river and at the same time its decomposition will consume dissolved oxygen.
- The biotic index and diversity index of the river should only be indicative of that particular site. Many factors influence the biotic index found.

Geographical region: Different representative groups will be found in different regions. An absence of a particular group may just be because it does not live here. Repeating sampling and collecting data over many years would reveal this.

Season: Some organism may be absent at certain periods in the year. Sampling should be carried out at different times of the year.

Current: Some organisms are better adapted to fast flowing water than others.

Sampling could be carried out in areas of the river with different currents.

Distance from a source of pollution: The Biotic Index would appear low near the source of pollution. Sampling could be carried out at various distances from a known source of pollution.

Other factors that may influence the biotic index or the diversity index: light, and temperature of the water, introduced alien species, such a pond weed.

To see the evolution of a section of river over the course of time it is important to always sample at the same area at regular intervals of time.

15A ENERGY FROM FOOD

Time: 1.5 hours

Syllabus reference: Topic D.1

Skill: Determination of the energy content of food by combustion.

Materials

Dry food samples: Potato chips/crisps, cocktail snacks e.g. *Curlie Wurlies*, and dried fruits can be used.

Warning: As nut allergies are quite common, foods containing nuts or products of nuts (e.g. peanut oil) should be avoided

Analysing the results

Some pieces of food appear to give more energy than other pieces of the same kind of food. There is a simple reason for this. What is it?

Not all pieces of food are the same size.

It would be simple to compare the results if everyone had used exactly the same amount of food. Is this a practical solution?

What must be done to each result in order to compare your results with the results of others?

Students may suggest cutting the food samples to be exactly the same mass, this is not realistic. Measuring the mass of the food samples, so the energy per unit mass of food is obtained, will permit a valid comparison. Nevertheless, the food samples need to be about the same size and not so large that the water reaches boiling point. If the water boils, the temperature will not rise any further.

Evaluate this method and suggest improvements

When the food samples are burned, ideally, ash is all that should remain. All the organic matter should have been oxidised. In reality, the food samples are rarely completely burned.

When the food sample burns, all the energy released should be transmitted to the water in the test tube. This is not the case. Energy is lost when the Bunsen ignites the food sample. When the burning food is transported to the test tube and while the burning food is under the test tube, energy radiates out in all directions, not exclusively into the test tube.

Enclosing the test tube in a chamber would limit the radiation of the energy from the burning food sample.

Instead of a test tube, a beaker with a larger flat bottom may give more realistic values.

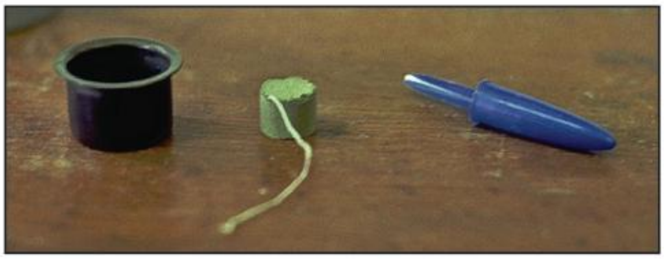
Research

Find out how a bomb calorimeter works and compare it to your method.

The bomb calorimeter works on the same principles as this experiment, however, the combustion is much shorter and the burning sample is enclosed in a vessel surrounded by an accurately measured volume of water.

The food sample is ground up and pressed into a pellet of given volume with a short cotton fuse.

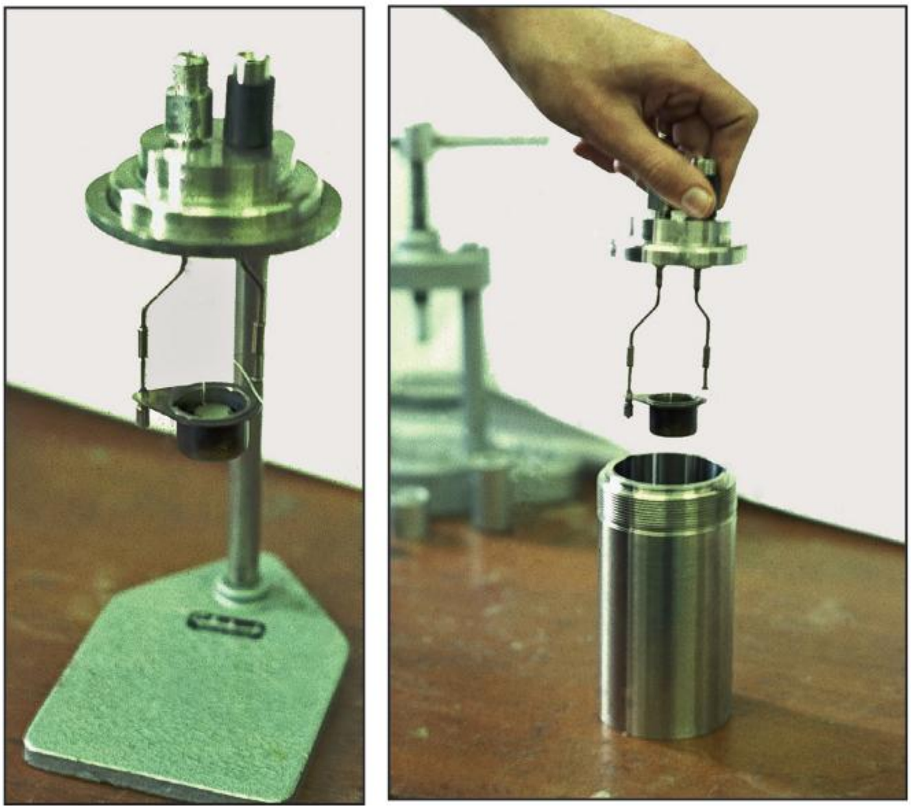
Bomb vessel (right) and the bucket that holds the water (left)



Pellet of dried food with the cotton fuse (centre)

It is massed and suspended in the bomb vessel. This vessel is made of a metal alloy that rapidly transmits the heat energy released. The bomb vessel is filled with pure oxygen and connected to an electricity supply. The whole vessel is immersed in an insulated bucket of water of known volume.

The cotton fuse lies across a wire connected to the electric terminals inside the bomb vessel. When the electricity is switched on, the wire heats, the fuse burns and the food sample burns very quickly in the pure oxygen (it explodes). The temperature of the water is measured before the explosion and during the ignition. Because the release of energy is so short, a more precise estimate of the energy content is obtained.



The bomb vessel being loaded with a food sample.

The bomb calorimeter needs to be calibrated to determine the specific heat capacity of the materials that is it made from.

15B HEART RATE AND BLOOD PRESSURE

Time: 1.5 hours

Syllabus reference: Topic D.4

Skill: Measurement and interpretation of the heart rate under different conditions

Skill: Interpretation of systolic and diastolic blood pressure measurements

Materials

Stethoscope: Make sure the ear pieces are properly cleaned before and after use. Certain models "switch" on and off by rotating the diaphragm head.

Heart rate monitor: Monitors that use a probe attached to the chest may need to be moistened or humidified on the surface facing the skin before they are attached.

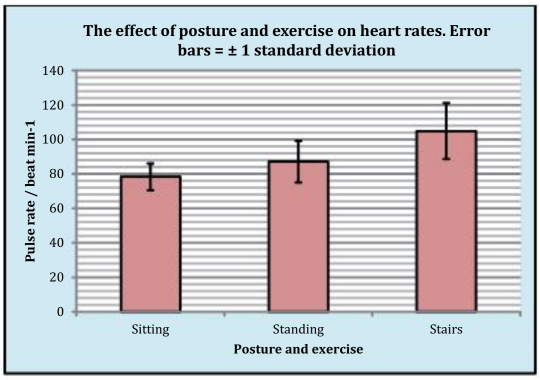
Method

Investigations on heart rates and blood pressure can be combined with investigations on the ECG and ventilation rates.

Data

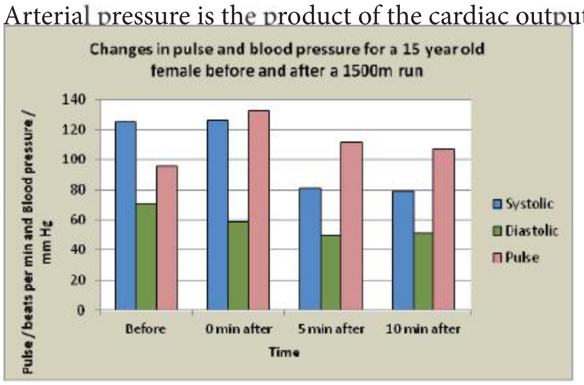
Heart Rate and Posture

The pulse increases slightly between sitting and standing. It increases a little more with a bit of light exercise.



Blood Pressure and Exercise

Arterial pressure is the product of the cardiac output and the peripheral resistance to blood flow.

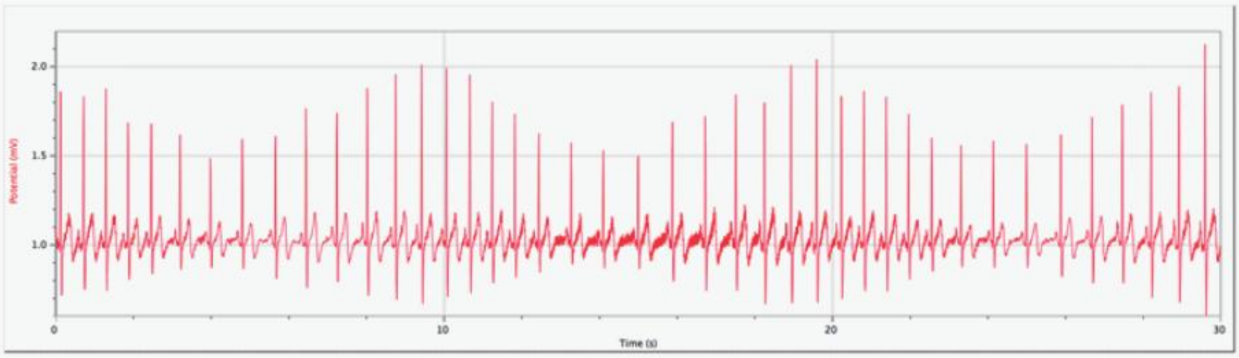


Increases in cardiac output will increase the systolic pressure, while an increase in peripheral resistance will increase the diastolic pressure. Vasoconstriction of blood vessels increases the resistance to blood flow and will increase the difference between systolic and diastolic pressure. After 1500m of running, the vasodilation of the arterioles in the muscles will reduce the resistance to blood flow so the diastolic pressure is lowered. The systolic pressure has not changed that much indicating this is probably a fit individual.

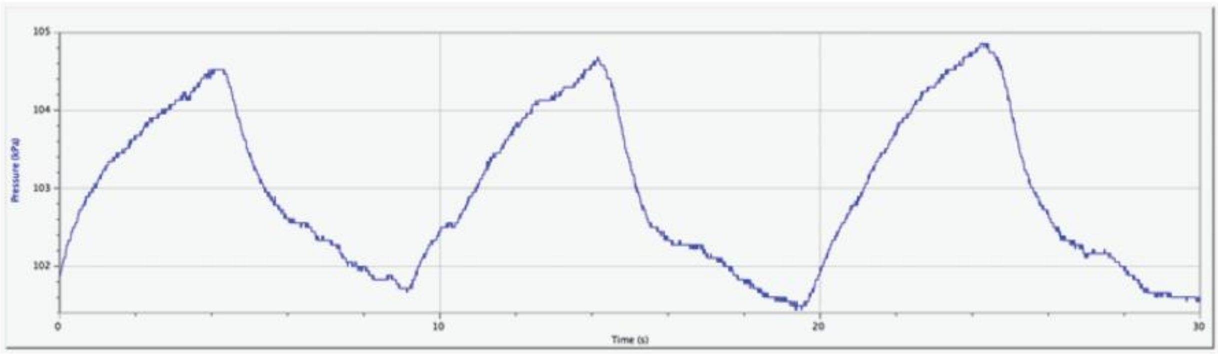
What is interesting is that the pulse pressure (the difference between the systolic and diastolic pressure) has increased. This is normal, the resistance to blood flow has dropped and the stroke volume of the heart (blood pumped per beat) has increased. This large difference in pulse pressure drops back within 5 minutes of resting.

The diastolic pressure may lower even further after running as the skin arterioles dilate further to radiate heat from the body. While running, the wind chills the skin by causing the evaporation of sweat. This effect stops when resting but there is still a lot of heat to be dissipated just after a run.

Heart Rate and Ventilation



ECG trace over 30s during 10s inspiration expiration cycle



Ventilation over 30s during 10s inspiration expiration cycle

Using the ECG sensor can reveal some interesting relationships. The heart rate tends to increase as a person breathes in. Here the intervals between the ventricular contractions (the spikes) are seen to get smaller during inhaling and wider during exhaling. Stretch receptors in the lungs inhibit the cardio-inhibitory centre of the brain, thus the heart rate speeds up during inhaling and slows during exhaling. This is normal and it is called sinus arrhythmia.

The amplitude of the ECG trace may also change as the subject breaths due to the change in orientation of the heart during breathing.

15C TAKING AND READING AN ELECTROCARDIOGRAM

Time: 1 hour

Syllabus reference: Topic D.4

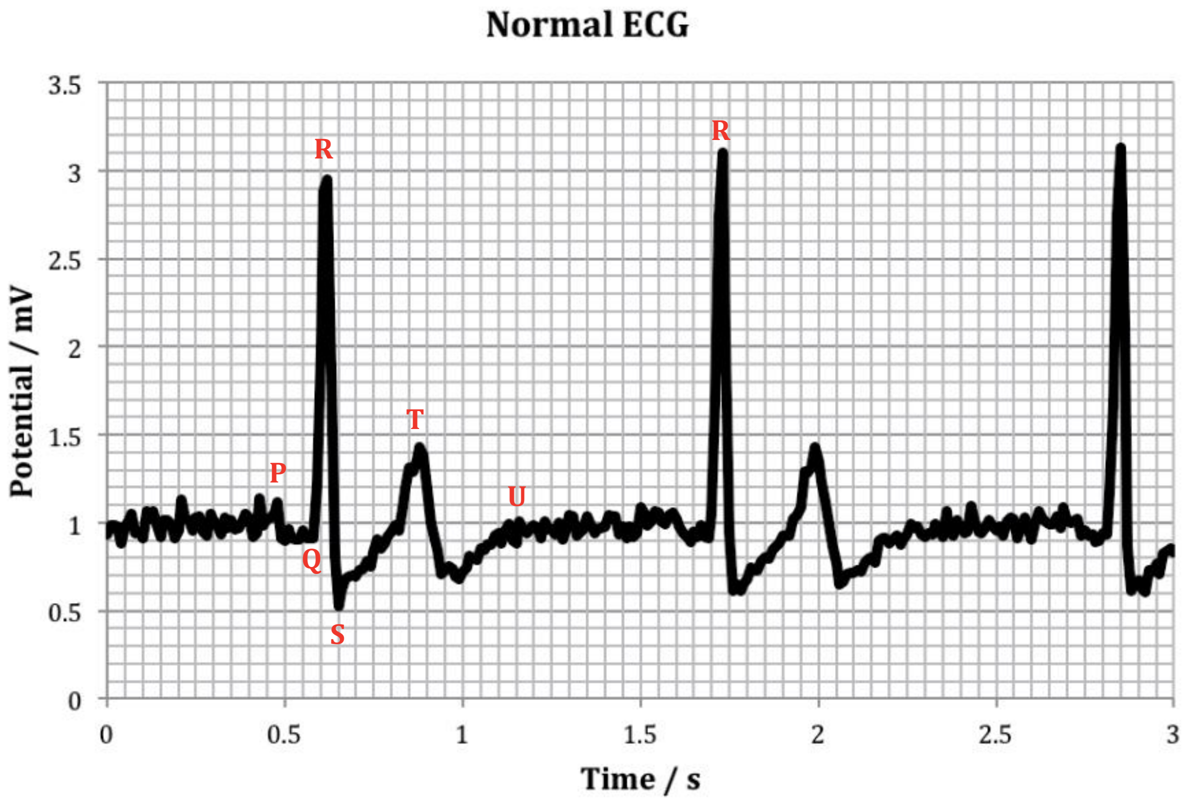
Skill: Mapping of the cardiac cycle to a normal electrocardiogram (ECG) trace

Data

The ECGs taken by an apparatus in school are not of the same quality and precision as those used by medical personnel. Doctors use more electrodes placed around the heart. Thus, though the readings can be used to investigate the electrical activity of the heart, they should not be relied upon for medical diagnosis.

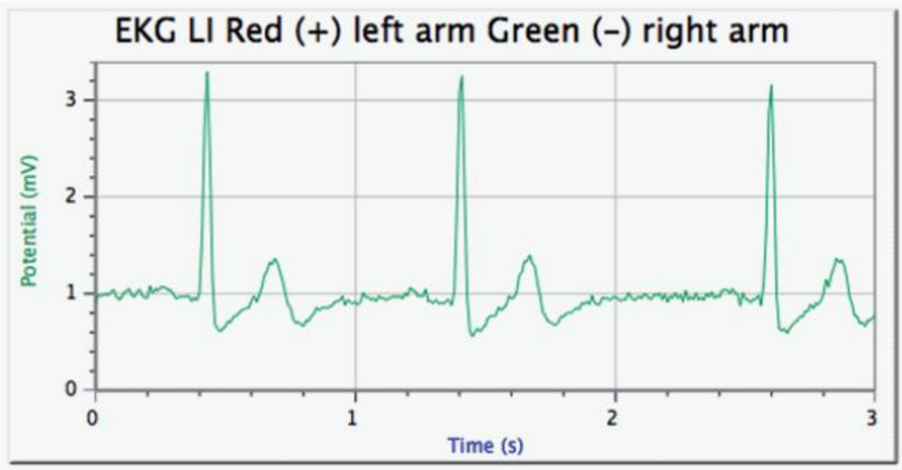
Reading an ECG

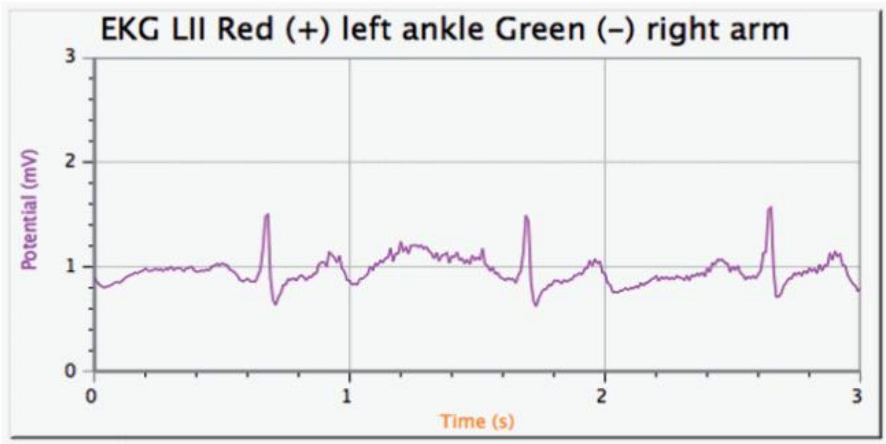
Observations from the ECG

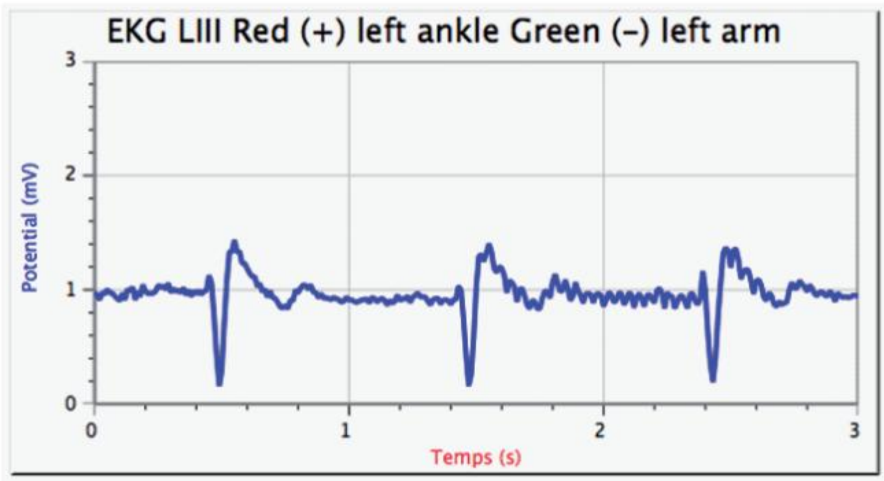


- The cardiac rhythm from R to R = 1.1s which gives a fairly slow heart rate.
- The conduction of the wave of contraction from the atria to the ventricles P to R = 0.15s
- The QRS complex = 0.075s
- The ventricular activity, Q-T = 0.3s R-R = 1.1s
- So, Q-T/R-R = 0.27 which is less than half of R-R, as it should be.

Analysing the axis of the heart







The highest QRS amplitude is at position 1 for this subject. Therefore, the angle of the heart is about 0° to the norm.

Appendix 1 Informed Consent Form

Read this form and listen carefully to the description of the tasks that you are asked to carry out. Feel free to ask as many questions as you wish that are relevant to the investigation.

You are free to ask questions at any time during the course of the experiment.

You may stop your participation in the experiment when you wish.

The data obtained from each volunteer will be made anonymous in all subsequent documents.

You may ask for your data to be deleted.

Investigation title: ______________________________

Name of experimenter: ______________________________

Location: ______________________________

Date: ______________________________

Description of the investigation and the role of the volunteer

Possible risks or discomforts

I have read and understand these instructions.

I have no known health problem that may be incompatible with this investigation.

I understand that I may withdraw from the experiment at any time

Signature ______________________________

Date ______________________________

Appendix 2 Table of random numbers

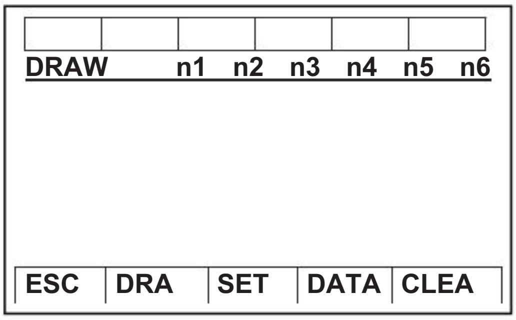
The numbers have been arranged in pairs of columns. These may be used as random co-ordinates (X and Y) or individual columns may be used as strings of random numbers.

X	Y	X	Y	X	Y	X	Y
71	5	52	83	11	40	36	51
53	41	77	82	100	46	49	39
58	86	5	59	68	49	16	11
29	79	59	99	2	21	47	78
30	37	47	91	58	33	26	46
77	96	30	23	10	10	63	75
1	87	62	70	10	59	54	60
76	6	65	98	80	17	16	83
81	95	26	24	28	93	94	2
71	36	28	53	5	10	65	21
30	41	91	56	51	55	44	79
38	41	26	69	46	92	27	30
30	71	79	91	35	54	87	24
95	33	38	83	40	41	75	48
98	63	29	2	27	85	27	25
40	21	92	54	6	83	67	34
28	19	63	92	24	67	26	4
16	58	63	43	98	72	9	48
16	8	43	68	6	100	3	21
65	46	10	50	39	34	32	86
36	51	7	19	65	21	8	76
49	39	11	68	44	79	59	92
16	11	33	45	27	30	75	62
47	78	13	36	87	24	93	35
26	46	0	15	75	48	33	15
63	75	54	70	27	25	54	48
54	60	66	93	67	34	8	22
16	83	54	53	26	4	63	99
94	2	83	9	9	48	41	13
65	21	8	76	3	21	96	3

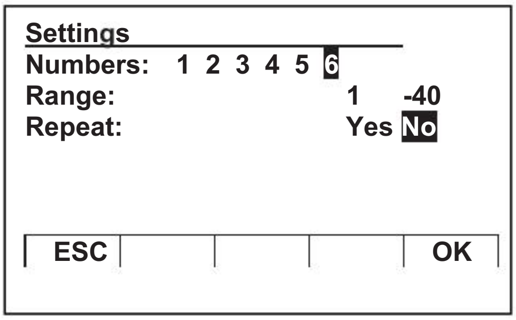
Appendix 3 Using a TI calculator to generate random numbers

Press **APPS** and select **Prob Sim**. This opens the 'Probability Simulation' program.
Press any key to open the menu.
(The **Probsim** program can be downloaded to the calculator from the Texas Instruments ® site.)

Select **6. Random Numbers**



Select **SET** (press the **ZOOM** key).

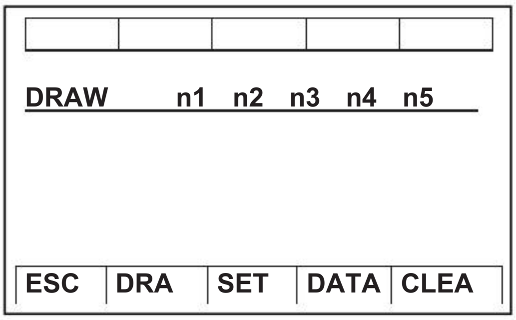


Numbers determines the set of random numbers drawn. Up to six can be drawn at one time.

Range sets the range of the numbers that can be chosen (limited between 1 and 99)

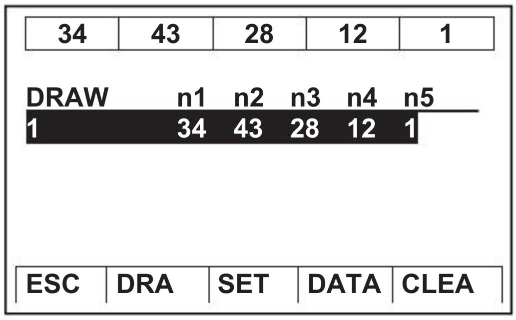
Repeat determines whether you want a number to appear more than once or not.

Make your choices and select **OK** (press the **GRAPH** key)



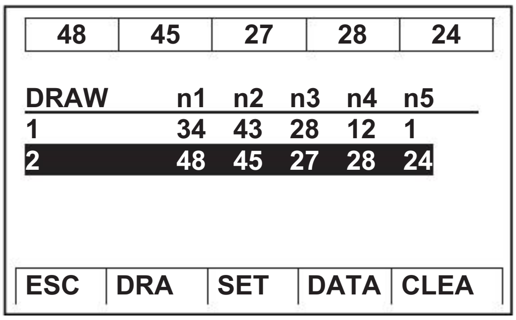
Example:
Settings
Numbers: 5
Range: 1-50
Repeat: No

Select **OK** (press the **GRAPH** key)



Select **DRAW** (press the **WINDOW** key)

If you want more random numbers with the same settings, select **DRAW** again.



To start again using the same settings select **CLEAR** (press the **GRAPH** key).
To change the settings select **SET**.

To quit **Random Numbers** select **ESC** (press the **Y=** key).