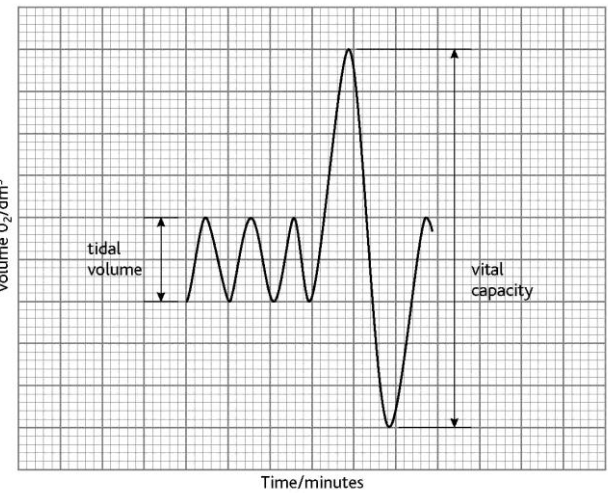
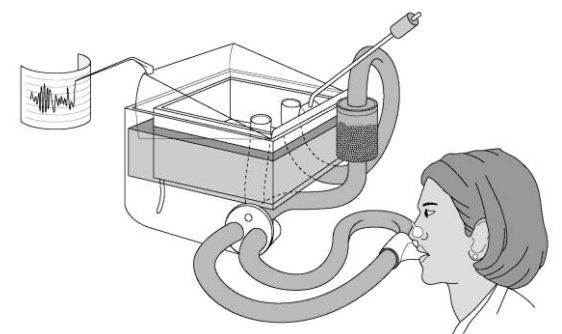
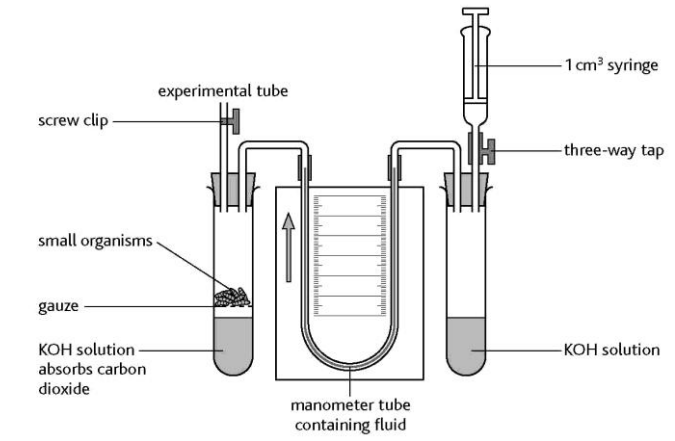
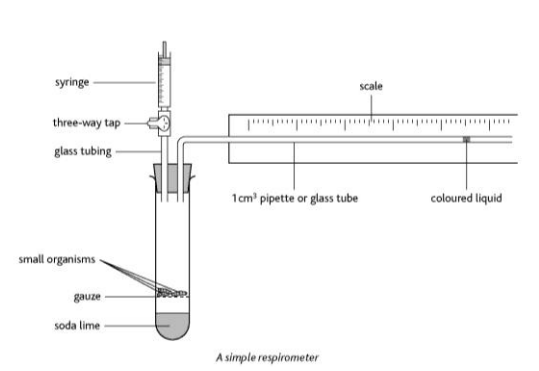


A2 BIOLOGY CORE PRACTICAL SUMMARY

Name of practical Independent & dependent variables	Other variables to be controlled	equipment	Method and outcome	Possible evaluation issues
<p>Observing patterns by Ecological sampling</p> <p>Random sampling Systemic sampling</p>	<p>Abiotic factors e.g. light, temperature, soil water, humidity, O₂ concentration, pH, aspect, slope angle</p>	<ul style="list-style-type: none"> • Gridded Quadrat • Tape measure • Point quadrat • Pitfall trap • Sweep Net • Pooter • Tullgren funnel • Baermann funnel 	<p><u>Several methods.</u></p> <p>1 <i>random sampling</i> = set up grid using tape measure, use random numbers to generate points to place quadrat to collect data.</p> <p>2 <i>systemic sampling</i> = line transect often used especially to study zonation. A tape measure is laid along several zones to be looked at and quadrats are used to record data at regular intervals</p> <p>3 <i>Measuring abundance</i></p> <p>Density = presence of organisms per quadrat</p> <p>Frequency = percentage of quadrat squares containing organism</p> <p>Percentage cover = percentage of ground covered with organism in a quadrat (usually for plants)</p> <p>Pitfall trap = to collect invertebrates</p> <p>Sweep net = to collect invertebrates in long grasses</p> <p>Pooter = to collect invertebrates into a container</p> <p>Tullgren funnel = to collect organisms from soil or leaf litter</p> <p>Baermann funnel = to collect living organisms from water</p>	<ul style="list-style-type: none"> • constant changing of abiotic conditions • Movement of organisms • Sampling taken within a small amount of time • Limitations of only 1 study • Consideration for safety of organisms • Disruption to normal habitat • Ethics of measuring wild organisms
<p>The effect of temperature on the hatching success of brine shrimp</p> <p>Independent = temperature</p> <p>Dependent = number of hatched shrimp</p>	<ul style="list-style-type: none"> • Light intensity • pH • salt content • presence of chlorine from tap water • oxygen concentration 	<ul style="list-style-type: none"> • Brine shrimp egg cysts • 2 g sea salt for each treatment • de-chlorinated water for each • treatment • beakers • Water baths or incubators • Forceps • Bright light • pipette 	<p>Decide on a range of temperatures from 5 °C to 35 °C to be tested. Place 2 g of sea salt into a 100 cm³ beaker. Add 100 cm³ of de-chlorinated water and stir until the salt completely dissolves. Label the beaker with sea salt and the temperature at which it will be incubated. Place a tiny pinch of egg cysts onto a large sheet of white paper. Wet the piece of graph paper using a few drops of salt water. Dab the paper onto the white sheet to pick up approximately 40 eggs. Use a magnifying glass to count the eggs. Put the paper with the 40 eggs into the beaker (eggs-side down). After 3 minutes, use a pair of forceps to gently remove the paper, making sure that all the egg cysts have washed off into the water. If possible replicate the treatments. Incubate the beakers at the appropriate temperatures, controlling exposure to light as far as possible. The next day count the number of hatched larvae in each of the beakers. To do this, place a bright light next to the beaker. Any larvae will swim towards the light. Using a fine glass pipette catch the brine shrimps and place them in a small beaker of salt water. Brine shrimps are very delicate and care must be taken when handling them. Record the number of larvae that have successfully hatched at each temperature.</p> <p>Outcome The majority of the shrimp should hatch at the optimum temperature between 25 and 30°C. (optimum at 28°C). Stats tests could be used to show evidence for data.</p> <p>Difference = student t test or mann whitney U</p> <p>Correlation = spearman's rank</p>	<ul style="list-style-type: none"> • ethics of hatching shrimp under different conditions • use of animals in experiments • effect of light intensity, may be a difference in light in each sample • fluctuating temperatures • not accurate salt measurements • may not have counted exactly 40 eggs • may miss seeing some of the baby shrimp • some eggs may not be viable anymore and wont hatch
<p>DNA gel electrophoresis</p>		<ul style="list-style-type: none"> • selected restriction enzymes • agar gel • gel tank • electrical supply • micropipettes • DNA sample • Loading dye • UV light • Camera • Buffer solution • DNA restriction ladder 	<p>Mix DNA with desired restriction enzyme and loading dye. Prepare agar and pour into electrophoresis mould. Once set, fill electrophoresis tank with buffer solution. Use micropipette to load restriction ladder into first well then DNA samples cut with restriction enzyme into the other wells. Connect to electrical supply, turn on and leave until the dye has moved to the opposite end of the gel tank. Switch off and disconnect electrical supply. Carefully remove the gel from the tank and view under UV light. Take picture if desired.</p> <p>Outcome DNA will be separated out through the agar gel, with the heaviest (biggest) DNA strands near the wells and the lightest (smallest) will be at the opposite end. The DNA restriction ladder can be used as a 'ruler' to measure the size of the different fragments.</p>	
<p>DNA amplification using PCR</p>		<ul style="list-style-type: none"> • Thermocycler • DNA sample • Taq polymerase • Nucleotides • primers 	<p>DNA sample is placed into tube in thermocycler with nucleotides, primers and polymerase. Step 1: denaturation = DNA heated for 1min at 94°C to denature it. This breaks the H bonds between nucleotides and makes the double stranded DNA, single stranded. Step 2: annealing = temperature reduced to 54°C. Bonds form between primers and the template strands. This will allow the polymerase enzyme to start to copy the template.</p> <p>Step 3: extension = carried out at 72°C. This is the optimum for taq polymerase enzyme. The bases are placed in their correct position, extending the strand from the primer.</p>	<ul style="list-style-type: none"> • The amount of DNA doubles each cycle (steps 1-3) therefore a considerably amount of copied DNA can be made for use in DNA fingerprinting etc. • 35 cycles (a few hrs) = 34 billion copies

<p>Effects of different antibiotics on bacteria</p> <p><i>Independent = antibiotic</i> <i>Dependent = diameter of inhibition zone</i></p>	<ul style="list-style-type: none"> • Concentration of antibiotic • Amount of antibiotic • Disc size • Bacterial species • Temperature • Ruler 	<ul style="list-style-type: none"> • Samples of different antibiotics on mast ring or filter paper discs • Petri dishes • Agar gel • Disinfectant • Bunsen burner • Forceps • Marker pen • Adhesive tape • incubator 	<p>Wash hands. For this practical you will need to work in sterile conditions (aseptic technique) i.e. you will need to flame the forceps in the Bunsen after every use. Prepare an agar plate seeded with bacteria. Label the Petri dish on the base at the edge with your name, the date and the type of bacterium it is inoculated with. Flame the forceps and then use them to pick up an antibiotic disc or Mast ring. Raise the lid of the Petri dish and place the Mast ring firmly in the centre of the agar; if individual discs are used they will need to be spaced evenly around the dish. Tape the dish securely with two pieces of adhesive tape (but do not seal it completely), then keep it upside down at 30°C for 48 hours. After incubation, look carefully at the plate but do not open it. Where bacteria have grown the plate will look opaque, but where the antibiotics have inhibited growth, clear zones called inhibition zones will be seen. Measure the diameter of the inhibition zones in millimetres and use this information to decide which antibiotic is most effective at inhibiting the growth of the bacterium.</p> <p>Outcome : dependent on bacterial species used and antibiotics used. E.g. E.coli is gram negative and not often susceptible to penicillin which is effective mainly with gram positive species. The larger the inhibition zone, the more effective the antibiotic against that species.</p>	<ul style="list-style-type: none"> • Ensuring that the discs are placed evenly on the Petri dish • Having good aseptic technique to prevent plate contamination • Age of antibiotic, if the antibiotic used is out of date it is likely to be less effective • Repeats • Accuracy of incubation temperature and time
<p>Measuring the rate of oxygen uptake</p>	<ul style="list-style-type: none"> • No of organisms • Temperature • Time • Amount of soda lime 	<ul style="list-style-type: none"> • Respirometer • Soda lime • Coloured liquid • 5g Organisms e.g. maggots, germinating peas, woodlice • Cotton wool • Stop clock • Marker pen 	<p>Place 5g of organism (maggots) into the tube and replace the bung. Introduce a drop of dye into the glass tube. Open the connection (three-way tap) to the syringe and move the fluid to a convenient place on the pipette (i.e. towards the end of the scale that is furthest from the test tube). Mark the starting position of the fluid on the pipette tube with a permanent OHT pen. Isolate the respirometer by closing the connection to the syringe and the atmosphere and immediately start the stop clock. Mark the position of the fluid on the pipette at 1 minute intervals for 5 minutes. 6. At the end of 5 minutes open the connection to the outside air. Measure the distance travelled by the liquid during each minute (the distance from one mark to the next on your pipette). If your tube does not have volumes marked onto it you will need to convert the distance moved into volume of oxygen used. (Remember the volume used = $\pi r^2 \times$ distance moved, where r = the radius of the hole in the pipette.) Record your results in a suitable table. Calculate the mean rate of oxygen uptake during the 5 minutes.</p> <p>Outcome: Oxygen molecules are absorbed by the organism and used in respiration. The same number of carbon dioxide molecules are released but these are absorbed by the soda lime. This reduces the pressure inside the test tube (fewer molecules = lower pressure). Atmospheric pressure pushes the liquid along the tube, until the pressure in and outside the tube is equal. Oxygen is the final electron acceptor, and it eventually combines with hydrogen to make water. The carbon dioxide comes from the carbon dioxide released in the link reaction and the Krebs cycle as the carbohydrate is broken down.</p>	<ul style="list-style-type: none"> • Simple respirometer – disadv. = does not allow you to reset; it needs a control tube used alongside it; no scale so measurements likely to be less accurate. Adv = very simple to set up; minimal number of connections makes a good seal easier to obtain. • U-tube respirometer – disadv. = tendency for the connections to leak in elderly school/college models (making the equipment useless); expense. Adv. = does not need to have an additional control as the second tube balances out the effects of changes in temperature or atmospheric pressure; the syringe allows you to move the liquid in the U to reset the apparatus.
<p>Effects of exercise on tidal volume and breathing rate</p>		<ul style="list-style-type: none"> • Spirometer • Kymograph • Disinfectant • Eye protection • Soda lime 	<p>The general principle behind a spirometer is simple. It is effectively a tank of water with an air-filled chamber suspended in the water. It is set up so that adding air to the chamber makes the lid of the chamber rise in the water, and removing air makes it fall. Movements of the chamber are recorded using a kymograph (pen writing on a rotating drum). Tubes run from the chamber to a mouthpiece and back again. Breathing in and out through the tubes makes the lid of the chamber fall and rise. The volume of air the person inhales and exhales can be calculated from the distance the lid moves. The apparatus can be calibrated so that the movement of the lid corresponds to a given volume. A canister containing soda lime is inserted between the mouthpiece and the floating chamber. This absorbs the CO₂ that the subject exhales. In which direction will the pen move when the subject inhales. After calibration, the spirometer is filled with oxygen. A disinfected mouthpiece is attached to the tube, with the tap positioned so that the mouthpiece is connected to the outside air. The subject to be tested puts a nose clip on, places the mouthpiece in their mouth and breathes the outside air until they are comfortable with breathing through the tube. Switch on the recording apparatus and at the end of an exhaled breath turn the tap so that the mouthpiece is connected to the spirometer chamber. The trace will move down as the person breathes in. After breathing normally the subject should take as deep a breath as possible and then exhale as much air as possible before returning to normal breathing. See trace example below.</p> <p>Outcome: The tidal volume is the volume of air breathed in and out in one breath at rest. The tidal volume for most adults is only about 0.5 dm³. Vital capacity is the maximum volume of air that can be breathed in or out of the lungs in one forced breath. Breathing rate is the number of breaths taken per minute. Minute ventilation is the volume of air breathed into (and out of) the lungs in one minute. Minute ventilation = tidal volume \times rate of breathing (measured in number of breaths per minute). Some air (about 1 dm³) always remains in the lungs as residual air and cannot be breathed out. Residual air prevents the walls of the bronchioles and alveoli from sticking together. Any air breathed in mixes with this residual air.</p>	

<p>Investigating habituation to a stimulus</p> <p>Independent variable = number of pokes</p> <p>Dependent variable = retraction time</p>	<ul style="list-style-type: none"> • Replication using snails of approx same size and age • Equal handling history • Drying out 	<ul style="list-style-type: none"> • 1 giant African land snail • 1 dampened cotton wool bud • Clean firm surface • Stop watch 	<p>Collect one giant African land snail, and place it on a clean, firm surface. Allow the snail to get used to its new surroundings for a few minutes until it has fully emerged from its shell. Dampen a cotton wool bud with water. Firmly touch the snail between the eye stalks with the dampened cotton wool bud and immediately start the stopwatch. Measure the length of time between the touch and the snail being fully emerged from its shell once again, with its eye stalks fully extended. Repeat the procedure in step 3 for a total of 10 touches, timing how long the snail takes to re-emerge each time. Record your results in a suitable table. Present your results in an appropriate graph.</p> <p>Outcome: spearman's rank stats test to look for correlation in data. There is a negative correlation – as the number of stimuli increase the time taken for the snail to re-emerge decreases. Students should make a reference to the data. With repeated stimulation, Ca²⁺ channels in the presynaptic membrane become less responsive. Less Ca²⁺ crosses the membrane into the presynaptic (sensory) neurone. As a result less neurotransmitter is released into the synaptic cleft. This means that an action potential across the postsynaptic membrane is less likely. Fewer action potentials are produced in the postsynaptic motor neurone so less of a response is observed.</p>	<ul style="list-style-type: none"> • Snails already handled before the experiment may not react in the same way • Determining when a snail has fully emerged • Lack of moisture may encourage snail to stay more in its shell • Measuring eye stalk length instead
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A sketch of a trace showing normal breathing and one forced breath in and out

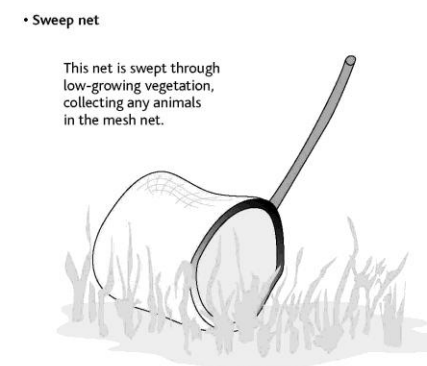
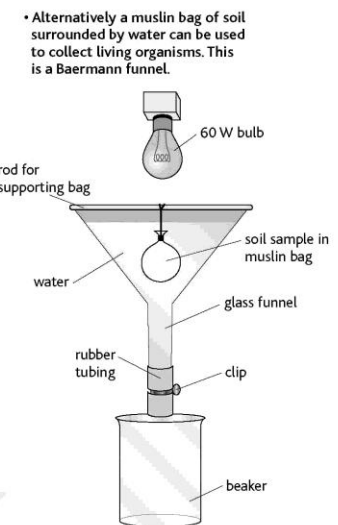
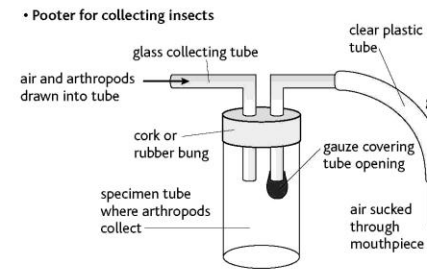
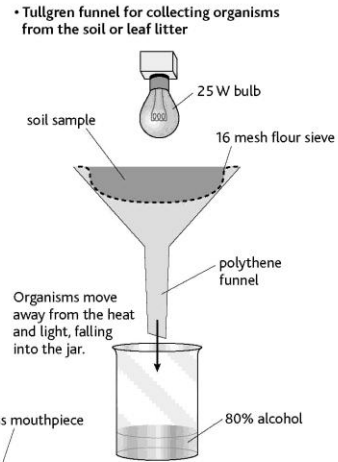
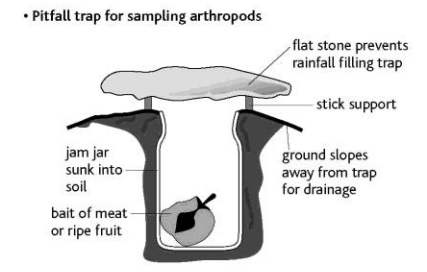


Figure 3 Net and traps for sampling animals.