CORE PRACTICAL GUIDE

For the Edexcel Biology A Level (SNAB)

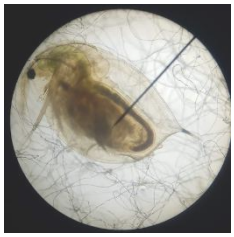
CONSIDERING:

- Control Variables
- Identification of Independent and Dependent Variables
- Any Calculations and Equations Required
- Explanation of Results Using Key Biological Principles
- Justification of Method/Equipment
- Sources of Error
- Ethical Considerations
- Safety Issues
- Use of Aseptic Technique
- Quantitative Measurement

Edexcel Biology A (SNAB) A Level: Core Practicals

CP1: Investigate the Effect of Caffeine on Heart Rate in *Daphnia*

As caffeine concentration increases, heart rate should also increase, as caffeine is a cardiac stimulant



Method

1. Place a few strands of cotton onto a cavity slide; *this will restrict the movement of the water flea*
2. Use a pipette to transfer a large water flea onto the slide. Remove the water around the flea then add one or two drops of the relevant caffeine solution. Use as much water as possible and do not use a cover slip – *these precautions maintain sufficient oxygen supply*
3. View the Daphnia under low power and focus on the heart. Allow acclimatisation time of four minutes under the light
4. Let one person use a stopwatch to time 15 seconds, whilst another counts the number of heartbeats in this time by making pencil markings every time a pulse is seen. Record the heart rate for three periods of 15 seconds *to calculate the reliable average*.
5. Ensure the study is blind, *so the person counting the heartrate is unaware of the caffeine concentration and the test is void of bias*
6. Repeat using other water fleas and caffeine solutions, with a control using distilled water (0% concentration)

Independent Variable	Concentration of caffeine in solutions, %
Dependent Variable	Heart rate of Daphnia, bpm
Control Variables	• Size of Daphnia used – approximate size should be large • Type of Daphnia – use *Daphnia Magna* • Volume of caffeine solution added – 3 drops using pipette • Acclimatisation time – 4 minutes • Temperature – always place over light microscope
Sources of Error and how this error was minimised	• Left for too long under microscope (random error) – exactly time four minutes • Daphnia moving around can make it hard to count heart beats (random error) – place Daphnia in cotton wool to limit movement • Water from Daphnia extraction may have diluted the caffeine concentration (random error) – use a paper towel to dry around the water flea to remove this water • Heart rate counted only for short time so may have missed a few heart beats when calculating beats per minute (systematic error) – take repeats and average
Justify Use of Organism	• Daphnia was used as it was small enough to be viewed under a microscope, and its transparent body allowed observation of the heart. Since it is an aquatic organism, it is able to take in caffeine from the solution • Invertebrates do not feel pain • However, they are still living organisms so should be handled with respect

CP2: Investigate the Vitamin C Content of Food and Drink

The lower the volume of juice required, the higher the Vitamin C concentration.

Method

1. Pipette 1cm^3 of blue DCPIP into a test tube.
2. Using a burette, add 1% Vitamin C solution dropwise. Shake the tube gently after each drop
3. Continue until the blue colour just disappears. Record volume needed for decolourisation.
4. Calculate the mass of Vitamin C required to decolourise 1cm^3 of DCPIP, using this measurement, given that 1cm^3 of 1% Vitamin C concentration contains 10mg of Vitamin C. *This will form the basis of later calculations*

5. Repeat using burettes filled with different orange juices. Record the volume required for decolourisation of 1cm^3 of DCPIP.

6. The concentration of Vitamin C can be calculated using the equation c = m ÷ v using the mass calculated in (iii)

Independent Variable	Type of fruit juice, each with varying Vitamin C concentrations
Dependent Variable	Volume of juice required for decolourisation of 1cm^3 of DCPIP
Control Variables	• Concentration of DCPIP – use from stock solution • Volume of DCPIP – measure exactly 1cm^3 using accurate pipette • End Point – ensure the blue colour has just disappeared • Shaking of test tube – continuously shake for all experiments
Sources of Error and how this error was minimised	• Inconsistent endpoint (systematic error) – use of white tile to identity colour change • Water residue in test tube (random error) – rinse then dry tubes between tests • Readings of juice (random error) – read from bottom of meniscus at eye level • Incorrect volume of juice from air gap in burette (random error) – run through with solution so only exact volume required is measured

CP3: Investigate Membrane Structure, including the Effect of Temperature on Membrane Permeability

Less light should be transmitted as temperature increases. As temperature increases, the phospholipid bilayer saw more destruction as the hydrogen bonds holding channel proteins in their tertiary structure weakened, increasing space for betalain to leak out of the cells. Therefore, a greater volume of betalain was able to diffuse out of the cell and the water became darker, meaning more light was absorbed by the purple hue. Rate of change is increasing as eventually the proteins denature and higher volumes of betalain are able to leak out of the cell

Method

1. Cut 1cm length cylindrical samples from a single beetroot using the same cork borer

2. Place these sections in a beaker of distilled water *to wash away excess dye*

3. Place boiling tubes, each containing 5cm^3 of distilled water, into water baths set to different temperatures, ranging from 0°C to 60°C. Add a beetroot sample to each boiling tube and leave for 30 minutes

4. Filter the water into a second boiling tube *without squeezing the beetroot.* Shake the water *to disperse the dye equally through the water*

5. Use a pipette to measure out 1cm^3 of the sample into a cuvette.

6. Place cuvette into a colorimeter set to 490nm, zeroed for clear water previously. Take a reading for absorbance and record in a table

Independent Variable	Temperature of water, °C
Dependent Variable	Absorbance of light, a.u.
Control Variables	• Volume of distilled water used – set at 5cm^3 • Time left in water – measure using a stopwatch, leave for 30 minutes • Beetroot piece – same size sample of 1cm using same cork borer, taking sample from the same part of the plant to ensure equal betalain concentration in vacuoles • Colour of filter – 490nm wavelength (green)
Sources of Error and how this error was minimised	• Different sized beetroot samples (random error) – carefully cut with cork borer • Varying concentration of betalain in sample (random error) – use same part of plant • Difficulty maintaining temperature (random error) – record average temperature • Inaccurate colorimeter (systematic error) – check calibration before use
Justify use of organism	Beetroot contains betalain pigment in vacuoles. Leakage of the pigment is affected by temperature, indicating the weakening of the membrane. The dye appears dark purple
Justify use of colorimeter	• Provides quantitative results which can be analysed, as they are more accurate than qualitative observations • Repeats can be taken due to quantitative scale for comparison

CP4: Investigate the Effect of Enzyme and Substrate Concentrations on the Initial Rates of Reactions

Milk protein casein is broken down by protease enzyme trypsin, meaning its opaque white colour becomes a clear solution. Absorbance therefore increases as more of the casein is broken down. As concentration of trypsin increases, the initial rate of reaction increases. This is because there is a higher frequency of successful collisions leading to the formation of enzyme-substrate complexes, meaning more transparent substrate is produced faster and therefore transmission of light through the solution increases more rapidly.

Method

1. Produce $10cm^3$ of 0.2%, 0.4%, 0.6% and 0.8% trypsin solutions using 1% trypsin stock solution and distilled water
2. Use a reference cuvette containing only water to set the colorimeter absorbance to zero

   Measure $2cm^3$ of milk suspension into a cuvette
3. Add $2cm^3$ of the 0.2% trypsin solution to the milk in the cuvette. Mix and place the solution into the colorimeter and start the stop clock
4. Measure transmission immediately and then every 15s intervals for 5 minutes
5. Rinse the cuvette with distilled water and repeat for each concentration.

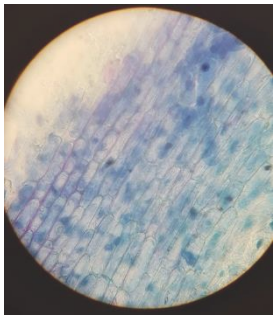
Independent Variable	Concentration of trypsin solution, %
Dependent Variable	Transmission of light, a.u.
Control Variables	• Volume of casein and trypsin solutions used – set at $2cm^3$ • Concentration of casein – always use from stock solution • Temperature – maintain using water bath • pH – maintain using buffer solutions • Colour of filter – 490nm wavelength (green)
Sources of Error and how this error was minimised	• Colorimeter may not have been tared (systematic error) – always calibrate using distilled water and trypsin only • Same cuvette may have been used without cleaning – rinse between trials with distilled water or use different, cleaned, dry cuvettes • Trypsin concentration is inaccurate (random error) – write out proportions of water and trypsin required

CP5: Observing Mitosis in Root Tips

If a group of cells is dividing rapidly, a high proportion of cells will be undergoing mitosis. The amount of cells undergoing mitosis is quantified using the mitotic index: $\text{Mitotic Index} = \frac{\text{Number of Cells in Field of View Undergoing Division}}{\text{Total Number of Cells in Field of View}}$

Method

1. Cut a thin slice from a root tip using fine scissors. Select white and firm tips as these will provide better results
2. Place into a glass block containing hydrochloric acid, then place on watchglass containing cold water. Dry carefully using filter paper. This helps make cells more visible
3. Transfer to a slide and macerate using a mounted needle. Add a drop of toluidine blue stain to make DNA visible
4. Cover with coverslip and blot. View under x400 and look for a group of cells with visible DNA. Photograph then calculate mitotic index



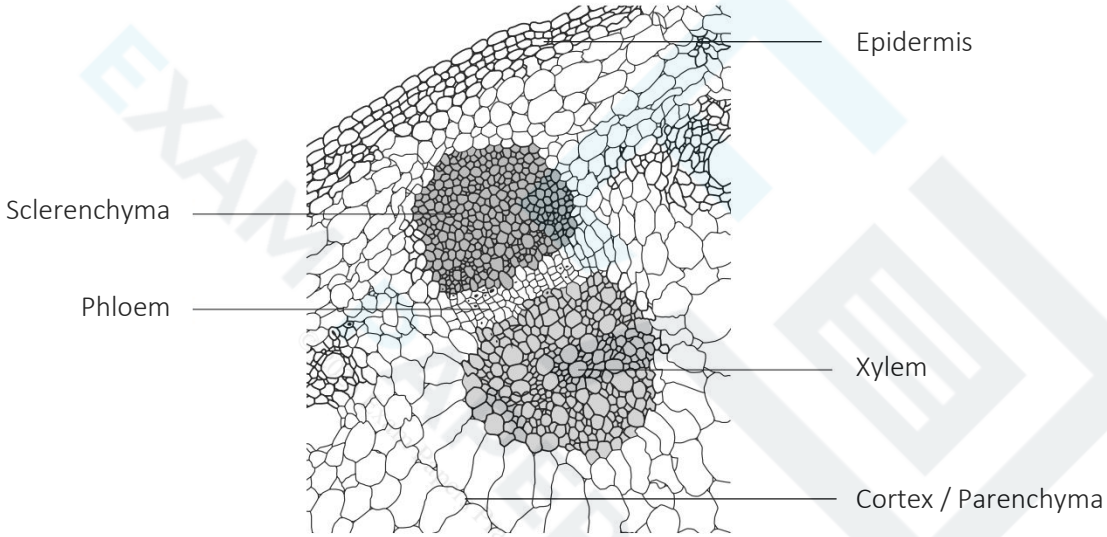
Sources of Error and how this error was minimised	• Low resolution of microscope (systematic error) – use a more detailed microscope with a greater magnification • Human error in counting numbers of cells (random error) – take a picture of the image in the lens as this should make counting easier • Inadequate maceration or staining (random error) – carry out for longer

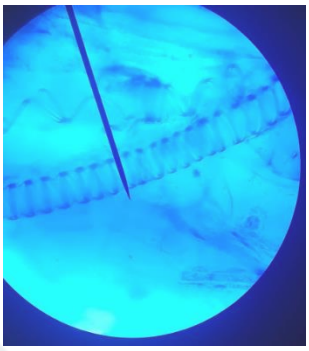
CP6: Identify Sclerenchyma Fibres, Phloem Sieve Tubes and Xylem Vessels and their Location within Stems through a Light Microscope

Sclerenchyma fibres and xylem vessels are both dead tissues which contain lignified walls, whilst phloem tissue is alive and has cytoplasm in the cells. The tissues are found next to each other in vascular bundles

Method

1. Place a piece of rhubarb on a watch glass. Use forceps to pick out a vascular bundle and place on slide
2. Use mounted needle to tease the bundle apart. Cover with methylene blue then leave to stain
3. Blot extra stain then add a drop of glycerol. Place on cover slip
4. Examine under the microscope at varying magnifications. Look for specific tissues:
    a. Xylem vessels are elongated tube-like cells, with lignin coils in the walls
    b. Phloem sieve tube cells are elongated with pores between cell
    c. Sclerenchyma fibres are hollow cells with lignified cell walls





Above: Lignin coils in xylem, viewed under ×400 magnification

CP7: Investigate Plant Mineral Deficiencies

Plants require minerals to grow: magnesium is needed for chlorophyll, calcium is needed for the middle lamella and controlling cellular activities, and nitrate is needed for DNA and amino acids.

Method

1. Add 10cm^3 of a solution containing all the nutrients to a test tube
2. Wrap the tube in black paper and cover the top of with cling film. Piece the top of the film
3. Record the initial mass and root length of a mung bean seedling and then push into test tube
4. Repeat these steps with solutions containing: no Mg^{2+}, no Ca^{2+}, no NO_3^- and distilled water. Leave the plants to grow in for a week in an area with equal exposure to light, CO_2 and temperature
5. Observe growth of the plantlets, comparing each solutions' effects by measuring percentage mass and root length change, in addition to comparing leaf surface area. Observe mineral deficiency symptoms (left to right from the 2nd plant):
    i. NO_3^- - leaves become yellow without chlorophyll
    ii. Mg^{2+} - leaves become yellow without chlorophyll
    iii. Ca^{2+}- stunted growth, curled leaves, lack up upright structure

Independent Variable	Minerals present in the growth solution
Dependent Variable	Any of: root length, mass, leaf area, stem turgidity (qualitative), deficiency symptoms
Control Variables	• Concentration of remaining minerals – use from stock solutions • Volume of solution added – exactly $10cm^3$ using pipette • Same species of the plant – mung bean • Size of plantlets used – roughly same length plantlet cuttings should be used • Amount of light received – place tubes on same sunny ledge • Same amount of time growing – leave for a week
Sources of Error and how this error was minimised	• Different volumes of each solution added (random error) – use a measuring cylinder first and measure $10cm^3$ of each solution • Microbes in mineral solution (random error) – heat each mineral solution first and allow to cool to innoculate • Insufficient time to see an effect (systematic error) – allow to grow for longer

CP8: Determine the Tensile Strength of Plant Fibres

Plant fibres are strengthened by the structure of the cells in the fibre. Cellulose is integral to cell walls its strength is due to hydrogen bonding forming microfibrils, binded in a matrix of pectin and hemicellulose which allows for greater strength and flexibility. Secondary thickening by lignin further increases strength. Middle lamella joins adjacent cells walls together with calcium pectate, further strengthening plant fibres.

Method

1. Remove plant fibres by retting or mechanically
2. Measure the diameter of the fibre using callipers
3. Connect between two clamp stands. Increase friction using sandpaper to ensure the fibre does not move
4. Add weights in 50g increments. Record the mass required for breaking of the fibre
5. Calculate tensile strength using force required for breakage ÷ cross-sectional area of the fibre: $\frac{\text{mass (kg)} \times 9.8}{\pi r^2}$

Dependent Variable	Tensile Strength ($N\ mm^{-2}$)
Control Variables	• Length of fibre – each fibre should be roughly the same length • Mass added – use 50g increments • Aim to keep cross-sectional area consistent
Sources of Error and how this error was minimised	• Large mass increments (systematic error) – add 10g masses instead for more accurate results • Difficult producing fibres (random error) – use retting techniques • Variation within fibres (random error) – use a large sample of fibres with repeats for reliability
Safety Precautions	• Use heatproof mat to allow weights to fall safely • Use G clamps to keep stands steady

CP9: Investigating the Antimicrobial Properties of Plants

Plants possess antimicrobial properties due to some of the chemicals they secrete. For example, the active ingredient of garlic, allicin, interferes with lipid synthesis and RNA production in bacteria, giving garlic a defence mechanism

Method

1. Pour sterile agar into a petri dish which has been sterilised in an autoclave
2. Flame neck of bacterial culture. Use a pipette then spread on bacterial culture. Divide the petri dish into five using a black marker

3. Prepare plant extracts by crushing plant material with industrial alcohol – plants could include mint, clove, garlic and tea tree extracts. Include control of distilled water
4. Place a sterile paper disk inside each plant extract and allow to soak for 2 minutes, then place into relevant section of the petri dish containing the bacteria
5. Tape with hazard tape and leave to incubate upside down, to allow collection of condensation
6. After incubation period, collect the petri dish and measure the diameter zone of inhibition for each plant and the control
7. Calculate the area of the zones of inhibition using πr^2
8. Take repeats then calculate the mean and standard deviation. If there is a significant difference between the control and the plant extract, then it is likely that the plant has antimicrobial properties

Independent Variable	Plant extract used
Dependent Variable	Area of zone of inhibition, mm^2
Control Variables	• Same concentration of plant material used – use constant volume of alcohol to prepare the extract • Type and amount of bacteria used – use E.coli evenly spread across the agar • Contamination of culture – aseptic techniques and sterile equipment used to avoid contamination of bacteria culture • Temperature of cultures – all Petri dishes should be incubated overnight at the same temperature
Sources of Error and how this error was minimised	• Contamination of microbes (random error) – use improved aseptic techniques. Clear and wash the area of work before and after with alcohol gel and wash hands before. Wear sterile gloves and set up Petri dishes under a naked flame. • Not shaking extract enough to ensure enough active ingredient (random error) – use a centrifuge to separate and mix the extract. • Uneven bacteria growth (random error) – ensure same lighting conditions used by keeping cultures under a lamp.
Aspetic Techniques Used	• Disinfect surfaces • Flame neck of bacterial culture • Sterilise inoculating loop by passing through roaring flame • Sterilise glassware in an autoclave • Work in updraught of a flame – warm air rise with any contaminating microbes
Justify Incubation Temperature	Bacteria is only cultured at 20-30°C to minimise the risk of growing human pathogens. The compromise is a lower growth rate

CP10: Carry out a study on the ecology of a habitat, using quadrats and transects to determine distribution and abundance of organisms, and measuring abiotic factors appropriate to the habitat

Population size changes with abiotic factors, as species occupy particular niches. A change is species along an environmental gradient is known as zonation.

Method 1: Transect Line – Systematic Sampling

1. Before the experiment, produce a species identification chart, procuring examples of each leaf type (seen on the right)
2. Peg out the transect line using a tape measure
3. Make measurements for species richness at regular intervals along the transect line by counting the number of different species present in a quadrat of constant size. Ensure that the quadrat's top right corner is placed at the correct distance along the transect line

4. Measure abiotic factors – using pH meters, soil moisture sensors, thermometer, luminous intensity meters
5. Conduct a Spearman's Rank Test to observe correlation between a particular abiotic factor and species richness

Method 2: Comparing Species Richness – Random Sampling

1. Use tape measures to lay out a grid. Use a random number generator to generate coordinates on the grid to sample
2. Place the quadrat at the correct location and measure species richness in each quadrat. Also measure abiotic factors as above
3. Take repeats for this location, then undertake an investigation in the second location using the same methodology and quadrat size
4. Conduct a t-test to compare the mean species richness at each location

Control Variables	• Where quadrat is placed – top right corner • Quadrat size e.g. 50cm by 50cm • Conditions e.g. rain may affect animal species presence
Types of Sampling	• Random Sampling: used to measure the distribution of in a large and fairly uniform area. A larger number of samples taken increases reliability of results. • Systematic Sampling: samples are taken at fixed intervals, usually along a line. This typically observes how populations change with an abiotic factor in a given habitat
Alternative Methods for Measuring Abundance	• Density – presence of organisms per quadrat • Frequency – percentage of quadrat squares containing organism • Percentage cover – percentage of ground covered with organism in a quadrat

CP11: Investigate Photosynthesis using Isolated Chloroplasts – The Hill Reaction

Photosynthesis occurs in two reactions – the light dependent reaction, which produces ATP and NADPH, and the light independent reaction (the Calvin Cycle), which produces glucose by carbon fixation using the products of the LDR.

Method

1. Crush the leaf material in a cold mortar using a chilled pestle, with the addition of some isolating fluid, of pH 7, containing a set 0.4M sucrose concentration
2. Filter through nylon sheets to produce a filtrate. Centrifuge the filtrate in a test tube
3. Pour the supernatant into a boiling tube. Resuspend the chloroplast pellet using a Pasteur pipette
4. Set up the following test tubes. Place test tube 3 in the dark, and 1, 2, 4 and 5 in front of a strong lamp

Tube	Leaf Extract	Supernatant	Isolation Medium	Distilled water	DCPIP
1					
2					
3					
4					
5					

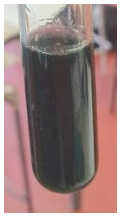
5. Observe carefully for any colour changes. Use a phone to film the colour changes. Results are found below

Tube	1	2	3	4	5
Observation	Light Green	Remains blue	Remains blue	Light Green	Remain Blue

Test Tubes 1 - 5 Before DCPIP Addition

Tubes 1, 2, 4 and 5 after exposure to light

3 (In Dark)



Control Variables	• Concentration of all solutions (Chloroplast and DCPIP) • Volumes of solutions used • Light intensity reaching test tubes 1, 2, 4 and 5 • Time tests tubes are left for
Why is equipment chilled	To preserve but slow enzyme activity.
Explain the use of the isolation medium	The pH of 7 ensures that the enzyme active sites maintain their shape. The sucrose concentration of 0.4M is equal to that inside the chloroplasts – the medium has no osmotic effect on chloroplasts, preventing their bursting.
Explain why DCPIP is used as an indicator	DCPIP is an oxidising agent; it accepts electrons from the ETC and changes colour from blue to colourless. If DCPIP is decolourised, then the light dependent reaction (LDR) is occurring
Justify the use of each test tube and explain its result	1 – Contains chloroplast in light, which photosynthesise. The LDR is occurring, producing electrons which are accepted by DCPIP, hence it decolourises 2 – Ensures that the isolation medium has no effect on DCPIP – any colour changes are due to the organelles found in the leaf. Since DCPIP is unaffected, the solution remains blue 3 – Kept in the dark, where the LDR cannot take place. No electrons are passing along the thylakoid membranes and DCPIP is not decolourised – the solution remains blue 4 – Distilled water indicates what a positive result with DCPIP looks like 5 – Ensures that the supernatant contains no photosynthesising organelles – as a result, the solution remains blue
Suggest some possible independent variables to compare tubes 1&3	Light intensity, Temperature, CO_2 or Chloroplast Concentration, Plant Species, Inhibitors Note – colorimeter or measurement of time for DCPIP decolourising provides quantitative results for rate of photosynthesis/LDR

CP12: Investigate the Effect of Temperature on the Initial Rate of an Enzyme-Catalysed Reaction, to include Q_{10}

As temperature initially increases, there is an increased rate of enzyme-substrate collision due to greater KE of molecules. If temperature continues above optimum temperature, the bond holding the enzyme in its tertiary structure break and the substrate no longer fits in the active site – the enzyme is denatured.

Q10 is a ratio which describes what happens when temperature is increased by 10°C. Most enzymatic reactions have a Q10 of 2-3; a value of 2 means that rate of reaction doubles with a 10°C rise in temperature.

$$Q_{10} = \frac{\text{Rate of Reaction at Temperature T + 10°C}}{\text{Rate of Reaction at Temperature T}}$$

Method

1. Set up 5 water baths with temperatures at 10°C intervals. Place a beaker of lipase in each water bath.
2. Label 5 test tubes. Add a set volume of milk, sodium carbonate and a few drops of phenolphthalein to each test tube. The solution will be pink.
3. Put the test tube into the relevant water bath and leave until the contents reach the correct temperature.
4. Use a syringe to measure out a set volume of lipase from the beaker. Add the lipase to the test tube and start the time. Stir the contents of the test tube using a glass rod. Record time for decolourising of the phenolphthalein.
5. Calculate rate of reaction for each temperature by 1/time. Calculate Q10 using the formula above.

Independent Variable	Temperature that the reaction takes place at, °C
Dependent Variable	Time for decolourising of phenolphthalein, seconds
Control Variables	• Volumes and concentrations of every solution used • Temperature of all solutions before combination • Definition of 'colourless' for end point

CP13: Investigate the Effects of Temperature on the Development of Organisms

Climate change is affecting development cycles of organisms. An example of the effect of temperature on life cycles and development can be seen in brine shrimp

Method

1. Collect a large sample of brine shrimp egg cysts and place at least 10 into each of 10 solutions containing 2g of salt in 100cm^3 of distilled water in a beaker. These beakers should be placed in water baths set to at least 5 different temperatures.
2. Allow each sample to grow for 6 hours
3. Count the number of hatched larvae by catching any that swim towards a bright light source. Repeat the counting daily. Calculate the hatching rate by total number of hatched larvae by number of days.
4. Conduct a Spearman's rank correlation coefficient test to see if there is any significance correlation between hatching rate and temperature

Control Variables	• Time allowed for hatching • Light intensity • Mass of salt added and volume of water in each solution • Number of shrimps in each beaker
Ethical Considerations	Brine Shrimp are not complex enough to suffer stress. However, there is debate over whether animals have rights. Since shrimps cannot provide consent, the method should minimise the amount of suffering by considering welfare. This can be done by: • Storing the shrimps in conditions that replicate their natural environment • Minimise stress by working quickly and not using extreme temperatures

CP14: Use Gel Electrophoresis to Separate DNA fragments of Different Length

Electrophoresis is used in forensics to profile DNA.

Method

1. Perform a restriction digest - mix the DNA with restriction enzymes and a blue dye
2. Melt agarose gel and pour it into an electrophoresis tank. Wells are made in the gel using a comb. Load the DNA fragments into the wells using a micropipette
3. Load the restriction ladder into the final well
4. Submerge the gel in a buffer and connect the electrodes are connected, producing a potential difference of 36V
5. The negatively charged DNA moves towards the anode. Smaller fragments with fewer STRs travel faster
6. View the sample under UV light if the dye is insufficient

DNA profiles are unique due to the number of STRs within intron sections of DNA.

CP15: Investigate the Effect of Different Antibiotics on Bacteria

Antibiotics are chemicals produced by microorganisms with the ability to inhibit growth of or destroy bacteria. There are two types of antibiotic: **bactericidal** antibiotics destroy bacteria, whilst **bacteriostatic** antibiotics prevent multiplication of bacteria, allowing the host's immune system to then destroy the pathogens. Effectiveness of antibiotics vary due to their action on bacteria.

Method

1. Pour sterile agar into a petri dish which has been sterilised in an autoclave
2. Flame neck of bacterial culture. Use a pipette then spread on bacterial culture. Divide the petri dish into five using a black marker
3. Split the agar into sections using a pen and label with a control and the different antibiotics. On the control, sector, place a sterile paper disk soaked in distilled water
4. In the other sectors, place paper disks containing antibiotic of equal concentration
5. Tape with hazard tape and leave to incubate upside down, to allow collection of condensation
6. After incubation period, collect the petri dish and measure the diameter zone of inhibition for each plant and the control
7. Calculate the area of the zones of inhibition using πr^2
8. Take repeats then calculate the mean and standard deviation. If there is a significant difference between the control and the antibiotic(s), then it is likely that the antibiotic is effective against this bacteria.

Independent Variable	Type of antibiotic
Dependent Variable	Area of inhibition, mm^2
Control Variables	• Concentration and volume of antibiotic used on each paper disc • Species of bacteria and concentration within agar • Use of aseptic techniques • Temperature for incubation – within range of 20-30°C • pH of agar (7pH)
Sources of Error and how this error was minimised	• Random Sampling: used to measure the distribution of in a large and fairly uniform area. A larger number of samples taken increases reliability of results. • Systematic Sampling: samples are taken at fixed intervals, usually along a line. This typically observes how populations change with an abiotic factor in a given habitat
Purpose of control	Tests whether bacteria die even if no antibiotic is being used

CP16: Investigate Rate of Respiration

Oxygen is taken in by the aerobically respiring organism, whilst any CO_2 produced is absorbed by the soda lime. As moles of gas reduces, pressure falls, drawing in the capillary fluid.

Method

1. Assemble a simple respirometer, using a boiling tube, capillary tube and a three-way tap attached to a syringe. Measure the radius of the capillary tube
2. Place soda lime or potassium hydroxide solution at the bottom of the tube to absorb CO_2. Place a piece of gauze in to prevent the organism coming into contact with the irritant
3. Place 5g of the respiring organism into the tube and replace the bung. Introduce a drop of orange fluid into the capillary tube. Open the connection to the syringe to move the fluid to a suitable position. Mark the starting position
4. Isolate the respirometer by closing the connection to the syringe. Record the position of the fluid every minute for 5 minutes and clearly mark the final position.
5. Calculate the mean metabolic rate by $\frac{\pi \times \text{radius of capillary tube}^2 \times \text{distance moved by fluid}}{\text{time} \times \text{mass of organism}}$ ($mm^3\ g^{-1}\ min^{-1}$)
6. If comparing metabolic rates, use a t-test to compare mean metabolic rates. If changing mass of organism or another variable, find the Spearman's Rank value before completing a hypothesis test

Dependent Variable	Metabolic rate ($mm^3\ g^{-1}\ min^{-1}$)
Control Variables	• Temperature, pH, original O_2 concentration, mass of organism

Factors Affecting Metabolic Rate	• Metabolic rate for endotherms is higher due to heat loss • Smaller animals tend to have higher basal metabolic rates per unit mass than larger animals, as SA:V ratio is higher • More active animals have a higher metabolic rate than less active animals • During torpor, e.g. hibernation, metabolism slows
Evaluating the Respirometer Used	• Advantages: syringe allows the dye in the apparatus to be reset, easy to set up, minimal number of connections makes a good seal easier to obtain • Disadvantages: no scale so measurements are less accurate

CP17: Investigate the effects of exercise on tidal volume, breathing rate, respiratory minute ventilation and oxygen consumption using data from spirometer traces

- Tidal Volume is the volume of air breathed in and out in one breath. This is found by measuring the average change in volume during a breath
- Vital Capacity is the maximum volume of air that can be inhaled and exhaled, found when the individual takes a very large breath in and out
- Breathing Rate is the number of breaths taken per minute, found by dividing 60 by the number of breaths recorded
- Minute Ventilation is the volume of oxygen inhaled in a minute, found by tidal volume × breathing rate
- Oxygen consumption is found by finding the change in volume between the troughs of the first and last breaths

Method

1. A spirometer is a tank of water with an air-filled chamber suspended in the water. It is set up so that the lid of the chamber rises and falls as a subject breathes
2. Calibrate the spirometer by adding in known volumes of oxygen into an empty tank. Place a canister containing soda lime between the mouthpiece and the floating chamber
3. Fill the spirometer is filled with oxygen. Attach a disinfected mouthpiece to the tube. The subject puts a nose clip on, to ensure all air in the lungs enters the spirometer, and places the mouthpiece in their mouth and breathes
4. A trace is produced on the kymograph.
5. Repeat after exercise, then analyse the traces using the methods above.

Explain why oxygen consumption increases after exercise	• Oxygen debt exists after exercise as skeletal muscle respires anaerobically • Tidal volume and breathing rate therefore increase to remove all lactic acid build up
Safety Measures	• Limit the time spent breathing through the spirometer and carefully observing each student should prevent problems • Soda lime is corrosive and should not be handled directly • Should not be used during exercise due to difficulty breathing • Avoid use if an individual has asthma or other breathing or circulatory problems

CP18: Investigate Habituation to a Stimulus.

Habituation is a loss of responsiveness to stimuli that convey little or no information. Habituation increases fitness by allowing an organism's nervous system to focus on stimuli that signal the presence of food or danger instead of wasting energy on other stimuli that do not affect the organism's survival.

- Habituation occurs as, with repeated stimulation, the calcium ion channels of the presynaptic neurone become less responsive to the changes in voltage associated with action potentials.
- Fewer calcium ions enter the presynaptic neurone and less neurotransmitter is released from the presynaptic neurone.
- Fewer sodium ion channels are opened in the postsynaptic neurone
- There is less depolarisation of the membrane, and no action potential is propagated.

Method

1. Collect one giant African land snail and place it on a clean, firm surface. Wait for a few minutes until the snail has fully emerged from its shell and has acclimatised to its new surroundings.
2. Dampen a cotton wool bud with water. Firmly touch the snail between the eye stalks with the 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.
3. Repeat for a total of ten touches, timing how long the snail takes to fully re-emerge each time.
4. Conduct a Spearman's Rank test for correlation at the 5% significance level

Independent Variable	Touch number
Dependent Variable	Time taken for full re-emergence (s)
Control Variables	• Handling history – all snails should have developed in the same habitat • Point where bud touches –poked between eye stalks • Pressure used when touching the snail – gentle • Texture of the cotton wool – moist • When to stop the stopwatch – should be when both eye stalks are fully extended • Time between touches – touch 10s after full re-emergence