

*The scientific investigation (the internal assessment, 20% of the grade) and the data-based questions of Paper 1B both mark the same habits: replicates instead of single readings, a measure of spread beside every mean, a statistical test where a difference is claimed, and a conclusion no stronger than the data.*

### The scientific investigation at a glance

About 10 hours of work, written up in at most 3,000 words, marked out of 24: four criteria of 6 marks each. Tables, graphs and calculations do not count towards the words.

**Research design** – a focused question naming the organism or tissue, both variables and the range; the biology behind it; and a method another student could repeat, including how each control is held and how many replicates

**Data analysis** – raw data with units and uncertainties, means with standard deviations, a graph with error bars, and a statistical test that matches the question

**Conclusion** – answers the question using the test result and the graph, explains the pattern with biology, and compares it with published findings

**Evaluation** – weaknesses that affected THIS data set – biological variation, uncontrolled variables, sample size – and improvements a school lab could carry out

Living material varies, so replication is not optional: at least three, and preferably five, replicates for each value of the independent variable.

Write the ethics into the method: no harm to animals beyond what normal handling causes, return organisms to where they were found, and get permission before working on private land.

### Statistics – which tool for which question

**Mean and standard deviation** – the mean summarises the replicates; the standard deviation (SD) says how much they spread. Report them together, with the SD to the same decimal place as the mean

**Error bars** – say in the caption what they show –  $\pm 1$  SD is usual. Bars that overlap widely suggest the difference may not be significant; bars that do not overlap suggest it may be. Only a test decides

**t-test** t – compares the MEANS of two groups. Null hypothesis: there is no difference between them. If  $p < 0.05$ , reject it: the difference is significant at the 5% level

**Chi-squared test** – compares observed COUNTS with expected ones – a genetic cross ratio, or whether two species occur together more than chance predicts. If  $\chi^2$  exceeds the critical value at the right degrees of freedom, reject the null hypothesis

**Correlation** – the correlation coefficient r runs from  $-1$  to  $+1$ ;  $R^2$ , the coefficient of determination, is the fraction of the variation in y that the line accounts for. A correlation, however strong, does not show that x causes y

State the null hypothesis before the test, the test you used and why, the value you got, the critical value

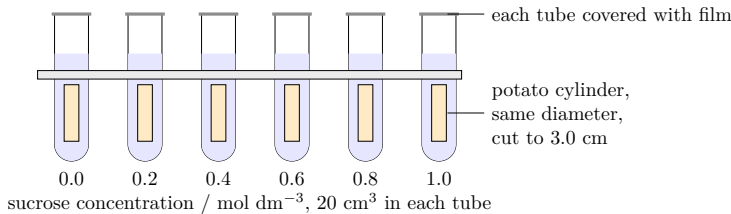
or p value, and the decision. ‘The t-test proved the hypothesis’ is wrong twice: tests reject or fail to reject a null hypothesis, and nothing is proved.

Failing to reject the null hypothesis does not show there is no effect. It shows this sample could not detect one, which is a point for the evaluation (more replicates, a wider range).

### Worked investigation: osmosis in potato tissue :

Research question: how does the concentration of a sucrose solution, from  $0.0$  to  $1.0 \text{ mol dm}^{-3}$ , affect the percentage change in mass of potato cylinders after 24 hours at  $20^\circ\text{C}$ ? Where the change is zero, the solution has the same solute concentration as the cells, so the graph’s x-intercept estimates the solute concentration of the potato tissue.

#### Apparatus



One tube per concentration is drawn; the investigation ran three cylinders at each concentration, each in its own tube.

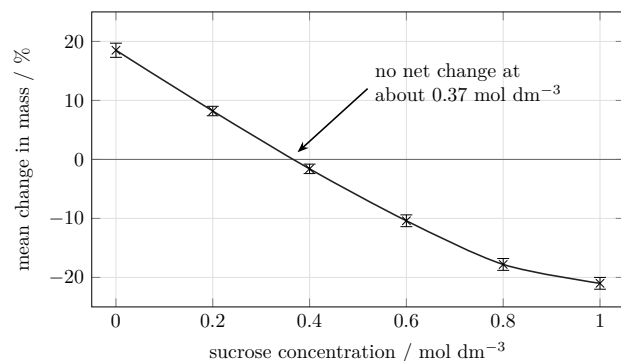
#### Method

1. Cut 18 cylinders from the same potato with one cork borer, and trim each to 3.0 cm with a scalpel on a tile. One potato and one borer keep the tissue and the surface area the same.
2. Blot each cylinder on paper towel in the same way, weigh it to 0.01 g, and record its mass against its tube.
3. Put each cylinder in  $20 \text{ cm}^3$  of its sucrose solution, three tubes per concentration, and cover the tubes to stop evaporation.
4. Leave them 24 hours at  $20^\circ\text{C}$ , then remove each cylinder, blot it the same way as before and reweigh it.
5. Calculate percentage change in mass =  $(\text{final} - \text{initial}) \div \text{initial} \times 100$ . Percentage change, not change in mass, because the cylinders do not start at exactly the same mass.

#### Results

| Sucrose / $\text{mol dm}^{-3}$ | Rep. 1 / % | Rep. 2 / % | Rep. 3 / % | Mean / % | SD / % |
|--------------------------------|------------|------------|------------|----------|--------|
| 0.0                            | +19.6      | +17.2      | +18.7      | +18.5    | 1.2    |
| 0.2                            | +8.9       | +7.4       | +8.3       | +8.2     | 0.8    |
| 0.4                            | −1.0       | −2.5       | −1.3       | −1.6     | 0.8    |
| 0.6                            | −9.6       | −11.5      | −10.1      | −10.4    | 1.0    |
| 0.8                            | −17.0      | −18.9      | −17.5      | −17.8    | 1.0    |
| 1.0                            | −20.1      | −22.0      | −20.9      | −21.0    | 1.0    |

Percentage change in mass, each calculated from masses read to  $\pm 0.01 \text{ g}$  on cylinders of about 2.5 g.



Means with error bars of  $\pm 1$  SD, and a smooth curve of best fit.

## Conclusion

Below about  $0.37 \text{ mol dm}^{-3}$  the cylinders gained mass and above it they lost mass: water moved by osmosis from the solution with the higher water potential to the one with the lower. The tissue's solute concentration is therefore about  $0.37 \text{ mol dm}^{-3}$ . The curve levels off above  $0.8 \text{ mol dm}^{-3}$  because cells that have lost most of their water, and are plasmolysed, have little more to lose.

The SD bars of neighbouring concentrations do not overlap anywhere, so each step in concentration changed the mass clearly; a t-test between any two neighbouring concentrations would test that.

## Evaluation

Blotting is the largest source of random error: a cylinder that keeps more surface water seems heavier. Improvement: roll each cylinder once on paper towel, the same way, for the same time.

The intercept depends on two points either side of it, 0.2 apart. Improvement: a second run from 0.30 to  $0.45 \text{ mol dm}^{-3}$  in steps of 0.05, with five replicates, to place it more precisely.

The result applies to this potato. Cylinders from several potatoes would show how much the tissue concentration varies between individuals – and whether one potato is a fair sample.

## Enzyme investigations

Measure the INITIAL rate: the gradient at the start of the product–time curve, before the substrate runs low. A rate averaged over the whole reaction mixes the effect you are studying with substrate depletion.

Temperature: equilibrate the enzyme and the substrate separately in the same water bath, check both with a thermometer, then mix. Record the bath temperature at the start and end of each run.

pH: use buffer solutions, one per pH, and keep the volume of buffer the same. Measure the pH of each mixture rather than trusting the label.

Controls: a tube with boiled (denatured) enzyme shows that the change needs active enzyme; a tube with water in place of enzyme shows how fast the reaction runs without it.

Above the optimum the rate falls because the active site changes shape and substrate no longer binds – the enzyme is denatured. ‘The enzyme dies’ is wrong: enzymes are not alive.

## Sampling in ecology

**Random quadrats** – lay two tapes at right angles and pick each quadrat's coordinates from a random-number generator, so the sampler's choice cannot bias where quadrats fall. Use for an area that is roughly uniform

**Transect** – quadrats at regular intervals along a line, for a habitat that changes along a gradient – up a shore, away from a path, into a wood

**Mark–release–recapture** – population size  $\approx$  (number marked in the first sample  $\times$  number in the second sample)  $\div$  number of marked animals recaptured – the Lincoln index

The Lincoln index assumes the marks do not come off or make an animal easier to catch or prey on, that marked animals mix back fully into the population, and that there is no birth, death or migration between the samples. Name the one most likely to fail in YOUR population.

Enough quadrats: plot the running mean (or the number of species found) against the number of quadrats, and stop once the line levels off.

## Paper 1B – data-based questions 1B:

Paper 1B sets an experiment you have not done and asks you to read it. Quote numbers from the graph or table in every answer: ‘the rate rose from 0.8 to 2.4 units between  $20^\circ\text{C}$  and  $40^\circ\text{C}$ ’, not ‘the rate increased’.

Describe the pattern first, then any change in it, then any anomaly. When asked to explain, give the biology for each part of the pattern you described.

When asked whether data support a claim, look at the error bars or the test result, not just the means. Means that differ with overlapping error bars may not differ at all.

A percentage change is calculated from the starting value:  $(\text{new} - \text{old}) \div \text{old} \times 100$ . State whether it is an increase or a decrease.

Read the axis scales and units carefully – two y-axes on one graph, a log scale, and an axis not starting at zero are all set on purpose.

## Evaluation – what scores and what does not :

Every weakness needs three parts: what it was, how it affected the data (more scatter, a value too high or too low), and an improvement you could actually carry out.

‘More repeats’ scores when tied to the data: ‘the SDs at  $0.0 \text{ mol dm}^{-3}$  were the largest, so five replicates there would make the mean more reliable’.

Biological variation is a real limitation – but say what varied (age of the leaves, the potato used, the individual organisms) and how you would control or sample it.

Never write ‘human error’ or ‘be more careful’. Name the step, what went wrong in it, and the change that would fix it.