

## PRACTICAL EXAMINATION \# 4: ECOLOGY LABORATORY

This practical examination is composed of 2 Tasks:

Task 1: Plant responses to nitrogen nutrition and carbon dioxide levels (20 points)
Task 2: Interactions between two aquatic plants (16 points)

## GENERAL INSTRUCTIONS

Competitors are advised to read the examination before commencing.

It is recommended that Competitors proportion their time according to the allotted points for each task and question.

## IMPORTANT

All answers must be recorded on the answer sheets provided.

Ensure that your 3 digit code number is written and coded on the top of each page of the answer sheets.

Using the pencil provided, fill in the appropriate circle on the answer sheet.

TASK 1: Plant responses to nitrogen nutrition and carbon dioxide levels. (20 points)

## INTRODUCTION

Forty plants were grown in a mixture of fine sand and sandy loam topsoil, in controlled temperature enclosures in a glasshouse with natural illumination. The temperature regime $\left(25^{\circ} \mathrm{C}\right.$ day, $20^{\circ} \mathrm{C}$ night) was near the optimum for the species concerned and the relative humidity was maintained above $75 \%$. Nutrients were supplied as half-strength Hoagland solution which provided a sufficient and balanced supply of essential macro- and micronutrients except for nitrogen. Five nitrogen treatments were applied, containing nitrate at concentrations of $0,1,2,4$ or 8 mM . Nutrient solutions were supplied to the soil surface each day until solution drained from the base of the pot. Carbon dioxide concentrations in the chambers were regulated to either 350 or 700 parts per million by volume ( ppm ) by scrubbing $\mathrm{CO}_{2}$ from the air stream entering the chambers and then adding $\mathrm{CO}_{2}$ at the required rates.

The plants were grown for 20 weeks and the five youngest fully expanded leaves were harvested.

## TASKS

You have been provided with a picture of the five youngest fully expanded leaves from one plant at the final harvest of the treatment that supplied nitrate at 8 mM and a $\mathrm{CO}_{2}$ concentration of 350 ppm . Please do not write on or mark the photograph.

Question P4.T1.1 From the images of the leaves you have been given, indicate in your answer sheet the plant group to which this species belongs:
A. Monocotyledon (Monocot)
B. Gymnosperm (Conifer)
C. Pteridophyte (Fern)
D. Bryophyte (Moss)
E. Dicotyledon (Dicot)

Task P4.T1.2 For each leaf image, use the measuring scale to measure the length of the leaf from the apex to the point where the leaf is attached to the petiole, and the maximum width of the leaf measured at right angles to the direction of the measurement of leaf length. Record your measurement to the nearest 1 mm in your answer sheet.

| Leaf number | Maximum Length (mm) | Maximum Width (mm) |
| :---: | :--- | :--- |
| 1 |  |  |
| 2 |  |  |
| 3 |  |  |
| 4 |  |  |
| 5 |  |  |

Task P4.T1.3 For another plant from a different treatment, the leaf length and width is given in Table 1.

Table 1: Length and width of five leaves from one plant from another treatment in the experiment.

| Leaf number | Leaf length <br> $(\mathrm{mm})$ | Leaf width <br> $(\mathrm{mm})$ | Leaf area $\left(\mathrm{cm}^{2}\right)$ |
| :---: | :---: | :---: | :---: |
| 1 | 112 | 72 | Value a |
| 2 | 107 | 71 | Value $b$ |
| 3 | 104 | 68 | Value $c$ |
| 4 | 99 | 64 | Value $d$ |
| 5 | 86 | Value $e$ |  |
| Mean | Value $f$ | Value $h$ |  |

An estimate of the area of each leaf in Table 1, can be obtained by using the equation:

$$
\text { Area }\left(\mathrm{cm}^{2}\right)=[0.0079 \mathbf{X} \text { length }(\mathrm{mm}) \mathbf{X} \text { width }(\mathrm{mm})]-0.252
$$

Calculate the values $a$ to $h$ in Table 1 to the nearest 0.1 mm or $0.1 \mathrm{~cm}^{2}$ and record them on your answer sheet. The value $h$ should be calculated by taking the mean of values $a$ to $e$.

Task P4.T1.4 Calculate the mean leaf area of the plant in Task P4.T1.3 by using the mean values for leaf length (value $f$ ) and leaf width (value $g$ ) in the formula given for estimating the area of a leaf.

TASK P4.T1.5 Compare the values for mean leaf area as calculated by the mean of the area of each individual leaf with that caleulated by using the mean length and mean width values. Indicate which of the following statements is correct:
A. The values are equal
B. The values are unequal because of an unbalanced data set.
C. The values are unequal because of an error in calculation.
D. The values are unequal because of a normal distribution of leaf area data about the mean.
E. The values are unequal because of a symmetrical distribution of leaf length data about the mean.
F. The values are unequal because of an irregular distribution of values within the data sets.

TASK P4.T1.6 Table 2 shows the mean leaf area for the five youngest fully expanded leaves of plants grown for 20 weeks under two ambient carbon dioxide concentrations and five nitrate nutrition regimes.

Table 2: Mean leaf area $\left(\mathrm{cm}^{2}\right)$ for the five youngest fully expanded leaves on plants grown under two ambient $\mathrm{CO}_{2}$-eoneentrations and five nitrate concentrations.

| Nitrate (mM) | Ambient $\mathrm{CO}_{2}$ concentration (ppm) |  |
| :---: | :---: | :---: |
|  | 350 | 700 |
| 0 | 19.8 | 17.2 |
| 4 | 33.0 | 31.6 |
| $z$ | 44.4 | 45.6 |
| 4 | 50.2 | 51.5 |
| 8 | 39.5 | 53.2 |

On the graph paper provided, prepare a graph comparing mean leaf area against nitrate concentration in the nutrient solution for each of the two $\mathrm{CO}_{2}$ treatments. From these plots, which of the following statements is/are most likely to be correct?

I At beth 350 and 700 ppm CO zand at nitrate concentrations in the nutrient medium between 0 and 2 mM , leaf area is limited by nitrogen availability.

II At 700 ppm CO, , photon flux density is likely to limit leaf size at nitrate concentrations in the nutrient medium higher than 4 mM .

II This evidence proves that, at $350 \mathrm{ppm} \mathrm{CO}_{2}$, total plant leaf area is redureed when nitrate supply increases from 4 to 8 mM .
A. I only
B. III only
C. II and III only

Đ. I and II only
E. I, $H$ and $\Pi!$

## TASKS P4.T1.7 to P4.T1.10 relate to information in Tables 3a and 3b.

Tables 3 a and 3 b show the values of leaf mass of the five youngest fully expanded leaves from one plant grown at $350 \mathrm{ppm} \mathrm{CO} \mathrm{CO}_{2}$ and one plant grown at 700 ppm CO 2 . You are asked to determine whether the values for leaf mass at the two $\mathrm{CO}_{2}$ concentrations are significantly different using unpaired values (Table 3a) and paired values (Table 3b). The appropriate test is Student's $t$-test. Instructions for the use of this test and a table of values of $t$ can be found in Appendices A and B. Please also refer to the abbreviations given at the end of Table 3b. To assist, some of the data has been processed. You may write on the exam paper but ensure you provide the appropriate answers on the answer sheet.

Table 3a: Leaf mass for plants grown at 350 ppm and $700 \mathrm{ppm} \mathrm{CO}_{2}$. Calculations for unpaired values

| Leaf <br> Number |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |

Table 3b: Leaf mass for plants grown at 350 ppm and 700 ppm CO 2 . Calculations for paired values

| $\begin{gathered} \text { Leaf } \\ \text { number } \end{gathered}$ | Leaf mass (mg) |  | $\mathrm{D}=(\mathrm{Y}-\mathrm{X})$ | D- $\underline{D}$ | $(\mathrm{D}-\underline{D})^{2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\begin{aligned} & 350 \mathrm{ppm} \\ & \mathrm{CO}_{2}(\mathrm{X}) \end{aligned}$ | $\begin{aligned} & 700 \mathrm{ppm} \\ & \mathrm{CO}_{2}(\mathrm{Y}) \end{aligned}$ |  |  |  |
| 1 | 448 | 619 |  |  |  |
| 2 | 428 | 593 |  |  |  |
| 3 | 415 | 484 |  |  |  |
| 4 | 386 | 479 |  |  |  |
| 5 | 370 | 400 |  |  |  |
| $\operatorname{Sum}\left(\Sigma X_{i}\right.$, etc. $)$ |  | 2575 |  |  |  |
| Mean ( $\underline{X}, \underline{Y}, \underline{D}$ ) |  | 515.1 |  |  |  |
| Difference between means |  |  |  |  |  |
| $\mathrm{SD}=\left(\left[\left(\sum(\mathrm{D}-\underline{\mathrm{D}})^{2}\right) /(\mathrm{n}-1)\right]^{0.5}\right) /\left(\mathrm{n}^{0.5}\right)$ |  |  |  |  |  |
| $t=\left(\underline{D}-(\Sigma(D-\underline{D})) / S_{D}\right.$ |  |  |  |  |  |

## Abbreviations:

n , number of individuals in sample
S, sum of variates
t , Student's t
D, difference between pairs of variates
D, mean of differences between pairs of variates
df, degrees of freedom
$P$, probability of a significant difference
SD, Standard deviation

TASK P4.T1.7 Calculate the standard deviation about the mean leaf mass for the 350 ppm $\mathrm{CO}_{2}$ treatment in the unpaired value comparison and enter the value (to the nearest 0.1 mg ) in your answer sheet.

TASK P4.T1.8 Use the Student's $t$-test to determine the level of probability that the leaf mass at $350 \mathrm{ppm} \mathrm{CO}_{2}$ is not significantly different from the leaf mass at $700 \mathrm{ppm} \mathrm{CO}_{2}$ in the unpaired test (Table 3a) is:
A. Greater than 0.99
B. 0.95 to 0.99
C. 0.05 to 0.10
D. $\quad 0.01$ to 0.05
E. Less than 0.01

TASK P4.T1.9 Use the Student's $t$-test to determine the level of probability that the leaf mass at 350 ppm CO 2 is not significantly different from the leaf mass at $700 \mathrm{ppm} \mathrm{CO}_{2}$ in the paired test (Table 3b) is:
A. Greater than 0.99
B. 0.95 to 0.99
C. $\quad 0.05$ to 0.10

D: $\quad 0.01$ to 0.05
E. Less than 0.01

TASK P4.T1.10 Indicate which of the following statements is correct::
A. The two tests produce the same level of significance of the difference between the means.
B. The variation in significance of the difference between means in the two tests is due to an error in the formula.
C. The variation in significance of the difference between means in the two tests is due to random variation in one sample.
D. The variation in significance of the difference between the means in the two tests is due to non-random variation in one sample.
E. The variation in significance of the difference between the means in the two tests is due to non-random but matching variation in both samples
(1 point)

TASKS P4.T1.11 to P4.T1.12 relate to information in Table 4.

Table 4: Leaf mass of plants grown under two ambient $\mathrm{CO}_{2}$ concentrations and five concentrations of nitrate in the nutrient medium.

| Nitrate <br> $(\mathrm{mM})$ | Leaf mass (mg) |  |
| :---: | :---: | :---: |
|  | $\left[\mathrm{CO}_{2}\right] 700 \mathrm{ppm}$ |  |
| 0 | 118 | 103 |
| 1 | 214 | 221 |
| 2 | 310 | 365 |
| 4 | 401 | 514 |
| 8 | 316 | 532 |

On the graph paper provided, plot the mean leaf mass for each treatment against the nitrate concentration in the nutrient solution.

Compare the graphs you have plotted for:

1. leaf area vs. nitrate concentration, and
2. leaf mass vs. nitrate concentration.

TASK P4.T1.11 From the relationships between leaf area, leaf mass and nitrogen concentration in your graphs, indicate on your answer sheet which of the following statements is most likely to be correct:

I At nitrate supply concentrations between 0 and 2 mM , there is a positive interaction between nitrate concentration in the soil solution and $\mathrm{CO}_{2}$ concentration in the atmosphere in their effects on mean leaf mass.

II Climate change that results in a doubling of the ambient $\mathrm{CO}_{2}$ concentration is very likely to increase mean leaf mass significantly in this plant species at all nitrate concentrations in the root medium between 0 and 8 mM .

III At 700 ppm CO 2 , there is no change in leaf mass per unit area at nitrate supply concentrations between 4 and 8 mM .
A. I only
B. III only
C. I and II only
D. I and III only
E. I, II and III

TASK P4.T1.12 Indicate which of the following statements are correct:
I Between 0 and $2 \mathrm{mM} \mathrm{NO}_{3}{ }^{-}$, neither leaf area nor leaf mass is affected by the concentration of $\mathrm{CO}_{2}$ in the air.

II The evidence from this study indicates that climate change that results in a doubling of the ambient $\mathrm{CO}_{2}$ concentration is likely to result in more uniform plant growth over the surface of the earth

III Between 4 and $8 \mathrm{mM} \mathrm{NO}_{3}{ }^{-}$, there is a negative interaction between increasing $\mathrm{NO}_{3}{ }^{-}$and $\mathrm{CO}_{2}$ concentrations in their effects on leaf mass.
A. I only
B. II only
C. III only
D. I and III only
E. I, II and III

## Task 2: Interactions between two aquatic plants

## INTRODUCTION

Lemna is a monocotyledon plant and azolla is a fern. Both are floating plants and reproduce by vegetative means. Azolla has a cyanobacterium associated with its root system that fixes nitrogen in water with low nitrogen concentrations. The growth of colonies of these plants can be indicated by counting leaves (lemna) or fronds (azolla), or by measuring their dry weights at different times. Both of these procedures have difficulties, and a common ecological expression of the importance of species in a community is the proportion of land or water surface that they occupy. The following questions relate to the estimation of the area occupied by two species in a mixed population. In most ecological studies, it is impossible to measure every individual, and sampling must be used. The following problem requires you to evaluate a sampling procedure.

## TASKS

To complete TASKS P4.T2.1 to P4.T2.5, you have been provided with a photograph of an area of fresh water supporting lemna (small bright green leaves) and azolla (the darker green fern). For reference, we have provided you with a petri dish with samples of lemna and azolla. You are required to design in part a sampling procedure to estimate the percentage of area occupied by different components of this plant community. Sampling may be carried out by (1) random points, (2) randomly placed transect lines, or (3) randomly placed sampling areas. Please do not write on or mark the photograph.

Ten randomly located transect lines (each approximately 50 mm long) have been drawn on the photograph. Each line has a number that represents the order of its selection and corresponds to a row in Table 5. Make your measurements in the order indicated by the line number.

NOTE: You may not need to measure every line. Analyse your data progressively, so that after you have measured four lines, you calculate the mean and other values as indicated. With each additional line, your sample size increases, and you should complete another calculation.

For each transect line that you select, place your measuring scale on the line with the zero end closest to the line number. Make your measurements on the side of the line closest to the number. Measure the total length of the line that lies over:

1. lemna
2. azolla

Light coloured areas on the photograph represent open water. Dark coloured areas on the photograph represent submerged azolla, which should be measured.

Use these values to calculate the percentage of the transect line occupied by each species. Record the percentage cover values in your work sheet. For each sample size greater than three (that is four or more sample lines), calculate:
(1) the mean percentage cover of lemna
(2) the standard deviation and confidence limit (probability 0.05 ) of the mean percentage cover of lemna. Use the formulae in Appendix A.
(3) the coefficient of variation, which is the ratio (confidence limit)/(mean percentage cover of lemna).
(4) the mean percentage cover of azolla.
(5) the standard deviation and confidence limit (probability 0.05 ) of the mean percentage cover of azolla. Use the formulae in Appendix A.
(6) the coefficient of variation, which is the ratio (confidence limit)/(mean percentage cover of azolla).

Record these values in your work sheet (Table 5).
For each species, plot the coefficient of variation against the sample size.
Repeat the sampling process until the coefficient of variation for the percentage of area occupied by azolla is less than $\mathbf{0 . 2 5}$. When you have reached this point, enter the following values in your answer sheet:

TASK P4.T2.1 How many transect lines do you need to measure to obtain a coefficient of variation for percentage cover of azolla which is less than 0.25 ?

TASK P4.T2.2 The mean percentage of the area of the photograph occupied by lemna (to the nearest 1 per cent).

TASK P4.T2.3 The mean percentage of the area of the photograph occupied by azolla (to the nearest 1 per cent).

TASK P4.T2.4 The confidence limit $(P=0.05)$ of the mean percentage of the area of the photograph occupied by lemna (to the nearest 0.1 per cent).

TASK P4.T2.5 The confidence limit $(\mathrm{P}=0.05)$ of the mean percentage of the area of the photograph occupied by azolla (to the nearest 0.1 per cent).

TASK P4.T2.6 Indicate which of the following statements are correct:
I The confidence limit of the mean area of each species is independent of sample size.

II The confidence limit of the mean area for azolla decreases with increasing sample size

III For a given sample size, the coefficient of variation for the area of lemna is greater than the coefficient of variation for the area of azolla because lemna plants become separated and are redistributed during vegetative growth but azolla remains in larger colonies.

IV For a given sample size, the coefficient of variation for the area of lemna is greater than the coefficient of variation for the area of azolla because the area occupied by lemna is smaller than the area occupied by azolla.

V Sampling by random line transect is completely unreliable.
A. I only
B. V only
C. II and IV
D. II and III
E. II, III and IV

Table 5: Percentage A reas (A) of lemna and azolla in a mixture

|  | Lemna |  |  |  |  |  |  |  | Azolla |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Sample number | A rea <br> (\%) <br> (A) | M ean | $A^{2}$ | $\Sigma \mathrm{A}^{2}$ | $(\Sigma A)^{2}$ | SD | CL | CV | A rea <br> (\%) <br> (A) | M ean | $A^{2}$ | $\Sigma \mathrm{A}^{2}$ | $(\Sigma A)^{2}$ | SD | CL | CV |
| 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 3 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 4 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 5 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 6 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 7 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 8 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

A bbreviations: SD, standard deviation; CL, confidence limit ( $P=0.05$ ); CV, coefficient of variation
Formulae to describe these parameters are included in A ppendix A or in the text.

TASKS P4.T2.7 to P4.T2.10 Ecological studies are often undertaken in order to predict the future characteristics of plants or plant associations. The data in Table 6 describe the change in fresh biomass $(\mathrm{g})$ of a colony of lemna growing in a pond with a high nutrient concentration.

Table 6: Biomass of lemna on selected days after initiation of a culture.

| Time <br> (days) | Biomass of plants <br> $(\mathrm{g})$ |
| :---: | :---: |
| 0 | 10 |
| 8 | 40.2 |
| 12 | 78.5 |
| 16 | 159.7 |
| 20 | 325 |
| 24 |  |

TASK P4.T2.7 By graphing the data in Table 6 (after transforming the data if necessary), extrapolate the relationship to estimate the biomass of the colony on Day 24 of the experiment.

Enter the value (to the nearest gram) on the answer sheet.

TASK P4.T2.8 From the data in Table 6, calculate the Relative Growth Rate (RGR)of the lemna colony between Day 0 and Day 8, using the formula given below. Enter the value (to the nearest $0.001 \mathrm{~g} \mathrm{~g}^{-1} \mathrm{day}^{-1}$ ) in your answer sheet.

$$
\mathrm{RGR}=\frac{\left(\ln \mathrm{W}_{2}-\ln \mathrm{W}_{1}\right)}{\left(t_{2}-t_{1}\right)}
$$

where RGR is Relative Growth Rate $\left(\mathrm{g} \mathrm{g}^{-1}\right.$ day $\left.{ }^{-1}\right), \mathrm{W}_{2}$ is weight $(\mathrm{g})$ at time $t_{2}$ (days), and $\mathrm{W}_{1}$ is weight $(\mathrm{g})$ at time $t_{1}$ (days)

TASK P4.T2.9 From the data in Table 6, calculate the Relative Growth Rate of the lemna colony between Day 12 and Day 20. Enter the value (to thenearest $0.001 \mathrm{~g} \mathrm{~g}^{-1}$ day $^{-1}$ ) in your answer sheet.

TASK P4.T2.10 From the graph of lemna biomass vs. time, indicate which of the following statements are correct:

I Lemna relative growth rate increased between Day 8 and Day 20
II The lemna colony grew exponentially between Day 0 and Day 20
III Lemna growth was not limited by nutrient availability.
IV Lemna growth was limited by space.
A. I only
B. II and III
C. III only
D. II and IV
E. I, III and IV

TASKS P4.T2.11 to P4.T2.12 Lemna and azolla have different maximum growth rates, and when grown in mixture, one species or the other may dominate the site. The data in Table 7 indicates the initial biomass values for an experiment where different mixtures of lemna and azolla with a total mass of 10 g were distributed in samples of a medium with a constant level of nutrition.

Table 7: Distribution of biomass between lemna and azolla in three populations established as cultures.

| Per cent initial <br> Azolla biomass | Biomass (g) |  |
| :---: | :---: | :---: |
|  | Azolla initial | Lemna initial |
| $\theta$ | $\theta$ | 10 |
| 25 | 2.5 | 7.5 |
| 50 | 5 | 5 |
| 75 | 7.5 | 2.5 |
| 100 | 10 | 0 |

After three weeks of growth, the plants were separated and weighed, with the results presented in Table 8.

Table 8: Biomass of lemna and azolla grown for three weeks in three cultures as described in Table 7.

| Per cent initial | Final Azolla |  |  |
| :---: | :---: | :---: | :---: |
| Azolla biomass | Final Lemna |  |  |
| biomass (g) | Final total <br> biomass (g) <br> biomass (g) |  |  |
| $\theta$ | $\theta$ | 15.6 | 15.6 |
| 25 | 4.6 | 13.1 | 17.7 |
| 50 | 9.6 | 9.9 | 19.5 |
| 75 | 14.5 | 5.4 | 19.9 |
| 100 | 18.8 | 0 | 18.8 |

On the same graph, plot the final azolla biomass, finall lemna biomass and final total biomass after three weeks of growth against the percentage of initial azolla biomass.

TASKS P4.T2.11 Indicate which of the following statements most completely describes the evidence in the graph:

1 The growth of azolla is not affected by the presence of lemma
H A species that is less vigorous in monoculture should show a relative depression in growth when grown in mixture with a species that is more vigorous in monoculture.

III This experiment proves that azolla releases nitrate ions into the water.
IV The propertion of lemna in the pend may be expected to increase progressively with time.
A. I only
B. II only
C. I and IV
D. I, III and IV
E. I and IV

TASKS P4.T2.12 Indicate which of the following statements is correct:
$I \quad$ A positive interaction between species is indicated by a curve relating total biomass to different propertions of the two species (from Table 7) that is convex in shape.

H If a species is aggressive in a mixture its final proportion of biomass is greater than the initial proportion. This characteristic may be independent of the vigour of the species in a monoculture.

HI This experiment does not demonstrate that the two species interact in their use of resources during growth.

IV Lemma derives some benefit from the presence of azolla, but azolla does not derive any benefit from the presence of lemma.
A. III only
B. IV only
C. I and IV only
D. II and IV only
E. I, II and IV only

## APPENDIX A

## A1.1 Determination of standard deviation of a mean

The standard deviation is calculated from the following attributes of a sample:
$\sum X_{i}^{2}$, the sum of the squares of each value of the variable $X_{i}$ where i has values from 1 to $n$.
$n$ is the number of values of the variable $X$ in the sample
$\left(\sum X_{i}\right)^{2}$, the square of the sum of all values of the variable $X_{i}$ where i has values from 1 to $n$.
$S D=\sqrt{\frac{\sum X_{i}^{2}-\frac{\left(\sum X_{i}\right)^{2}}{n}}{(n-1)}}$

## A1.2 Confidence limit of a mean

The confidence limit (CL) is derived from the standard deviation by
$C L=\frac{t \times S D}{\sqrt{(n-1)}}$
Where $t$ is the value of the distribution of Student's $t$ (Appendix B) for the desired level of probability
$(\mathrm{P}=0.05)$ and the number of degrees of freedom $(d f)$ where $n$ is the number of samples in the analysis. The number of degrees of freedom is $(n-1)$.

## A1.3 Significance of difference between two sample means

Differences between two samples (variable $X$ and variable $Y$ ) can be tested using the Student's t-distribution, which is calculated from the differences between individual values ( $X_{i}$ and $Y_{i}$ ) and the mean values ( $\underline{X}$ and $\underline{Y}$ ) for the two samples where $\mathrm{n}_{\mathrm{x}}$ and $\mathrm{n}_{\mathrm{y}}$ are the numbers of individuals in the two samples. This can be done for either unpaired or paired values from the two sample groups. If the calculated value of $t$ is greater than the value of $t$ for a particular level of probability in the table for the degrees of freedom applicable to the sample, then it can be concluded that the difference between two mean values is real (that is, significant) at that level of probability.

## A1.3.1 Unpaired samples

If the collection of data for the two samples was completely independent, each value from one sample value must be compared with all values from the other sample. The procedure can be summarised as

$$
S=\sqrt{\frac{(X-\underline{X})^{2}+(Y-\underline{Y})^{2}}{\left(n_{x}+n_{y}-2\right)}}
$$

and
$t=\frac{(\underline{X}-\underline{Y})}{S \cdot \sqrt{\frac{2}{n_{x}+n_{y}}}}$

The value of $t$ is compared with the value in the Table of distribution of $t$ (Appendix B), for the level of significance that you want and the number of degrees of freedom, $d f$, where $d f=(n-1)$.

## A1.3.2 Paired samples

If the collection of data for the two samples was arranged so that pairs of variables were measured, the differences between the means can be assessed as follows. The difference (D) between the two values ( X and $Y$ ) of each pair enables the mean difference $(\underline{D}$ ) to be calculated. The algebraic sum of $(\mathrm{D}-\underline{\mathrm{D}})$ for all pairs will equal zero. The procedure can be summarised as

$t=\underline{\mathrm{D}}-\Sigma(\mathrm{D}-\underline{\mathrm{D}})$
S
The value of $t$ is tested in the same way as described above for unpaired samples.

## APPENDIX B

Table III. Distribution of $t$

|  |  |  |  |  |  |  | 仡 | ity. |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $d f$ | $\cdot 9$ | . 8 | $\cdot 7$ | - 6 | $\cdot 5$ | 4 | $\cdot 3$ | $\cdot 2$ | - I | . 05 | $\cdot \mathrm{O2}$ | - OI | $\cdot \mathrm{OOT}$ |
| I | - 158 | $\cdot 325$ | - 510 | $\cdot 727$ | 1.000 | I. 376 | I. 963 | 3.078 | $6 \cdot 314$ | $12 \cdot 706$ | $3 \mathrm{I} \cdot 82 \mathrm{I}$ | $63 \cdot 657$ | $636 \cdot 619$ |
| 2 | -142 | - 289 | - 445 | . 617 | .816 | I.061 | I. 386 | I. 886 | $2 \cdot 920$ | $4 \cdot 303$ | 6.965 | 9.925 | 31.598 |
| 3 | -137 | - 277 | . 424 | . 584 | $\cdot 765$ | . 978 | I. 250 | I. 638 | 2.353 | 3.182 | $4 \cdot 541$ | $5 \cdot 841$ | 12. 924 |
| 4 | - 134 | -271 | . 414 | $\cdot 569$ | -741 | -94I | 1.190 | I. 533 | $2 \cdot 132$ | 2.776 | 3. 747 | $4 \cdot 604$ | 8.610 |
| 5 | -132 | -267 | . 408 | - 559 | $\cdot 727$ | - 920 | I. 156 | 1.476 | 2.015 | 2.571 | 3.365 | 4.032 | 6.869 |
| 6 | 121 | - 265 | $\cdot 404$ | $\cdot 553$ | . 718 | - 906 | I• 134 | 1.440 | I•943 | 2.447 | 3. 143 | 3.707 | 5.959 |
| 7 | - I30 | - 263 | . 402 | - 549 | -711 | -896 | I•119 | I.415 | I. 895 | $2 \cdot 365$ | 2.998 | 3.499 | $5 \cdot 403$ |
| 8 | - 130 | . 262 | - 399 | - 546 | - 706 | . 889 | I. 108 | I. 397 | I. 860 | 2. 306 | 2.896 | 3.355 | 5.041 |
| 9 | -129 | - 261 | - 398 | . 543 | $\cdot 703$ | . 883 | I-100 | I. $3^{8} 3$ | I. 833 | $2 \cdot 262$ | 2.821 | 3. 250 | $4 \cdot 781$ |
| 10 | -129 | - 260 | - 397 | - 542 | $\cdot 700$ | . 879 | 1.093 | I. 372 | I.812 | $2 \cdot 228$ | $2 \cdot 764$ | $3 \cdot 169$ | 4.587 |
| 11 | -129 | 260 | $\cdot 396$ | - 540 | . 697 | . 876 | 1.088 | I. 363 | I•796 | $2 \cdot 201$ | 2.718 | 3.106 | $4 \cdot 437$ |
| 12 | - 128 | - 259 | - 395 | - 539 | - 695 | . 873 | 1.083 | I. 356 | I•782 | 2. 179 | 2.68 I | 3.055 | 4.318 |
| 13 | -128 | - 259 | - 394 | - 538 | - 694 | . 870 | I.079 | I. 350 | 1.771 | 2. 160 | 2.650 | 3.012 | 4.221 |
| 14 | -128 | - 258 | - 393 | - 537 | -692 | . 868 | I.076 | I•345 | I 761 | 2. 145 | 2.624 | 2.977 | 4.140 |
| 15 | -128 | $\cdot 258$ | $\cdot 393$ | . 536 | -691 | . 866 | $1 \cdot 074$ | I'34I | $1 \cdot 753$ | 2. I3I | 2.602 | 2.947 | 4.073 |
| 16 | - 128 | - 258 | $\cdot 392$ | . 535 | -690 | . 865 | 1.071 | I. 337 | I. 746 | 2.120 | 2. 583 | 2.921 | 4.015 |
| 17 | -128 | -257 | $\cdot 392$ | . 534 | . 689 | . 863 | 1.069 | I. 333 | I-740 | $2 \cdot 110$ | $2 \cdot 567$ | 2.898 | 3.965 |
| 18 | -127 | - 257 | - 392 | - 534 | . 688 | . 862 | I. 067 | 1.330 | I•734 | $2 \cdot 101$ | 2. 552 | 2.878 | 3.922 |
| 19 | -127 | -257 | -391 | - 533 | . 688 | -86I | I.066 | I• 328 | I. 729 | 2.093 | $2 \cdot 539$ | 2.861 | 3.883 |
| 20 | -127 | -257 | -391 | $\cdot 533$ | . 687 | . 860 | I. 064 | I-325 | I. 725 | 2.086 | 2.528 | 2.845 | 3.850 |
| 21 | -127 | $\cdot 257$ | -391 | . 532 | . 686 | . 859 | 1.063 | I* 323 | 1.721 | 2.080 | 2.518 | 2.831 | 3.819 |
| 22 | -127 | - 256 | -390 | - 532 | . 686 | -858 | I.06I | I•321 | 1.717 | 2.074 | 2.508 | 2.819 | 3.792 |
| 23 | -127 | - 256 | - 390 | - 532 | . 685 | . 858 | 1.060 | I-319 | I.714 | 2.069 | 2.500 | 2.807 | 3.767 |
| 24 | -127 | - 256 | - 390 | -531 | . 685 | -857 | I.059 | I-318 | r•71I | 2.064 | 2.492 | 2.797 | 3.745 |
| 25 | -127 | - 256 | - 390 | -53I | . 684 | -856 | I.058 | I. 316 | I. 708 | 2.060 | 2.485 | $2 \cdot 787$ | 3.725 |
| 26 | - 127 | - 256 | - 390 | -531 | . 684 | . 856 | I-058 | I.315 | I. 706 | 2.056 | 2.479 | 2.779 | 3.707 |
| 27 | -127 | - 256 | $\cdot 389$ | . 53 I | . 684 | . 855 | 1.057 | I.314 | I. 703 | 2.052 | 2.473 | $2 \cdot 771$ | 3.690 |
| 28 | -127 | - 256 | $\cdot 389$ | - 530 | . 683 | . 855 | I.056 | I. 313 | I. 701 | 2.048 | 2.467 | $2 \cdot 763$ | 3.674 |
| 29 | -127 | - 256 | $\cdot 389$ | - 530 | .683 | . 854 | I.055 | I.3II | 1. 699 | 2.045 | 2.462 | 2.756 | 3.659 |
| 30 | -127 | - 256 | $\cdot 389$ | - 530 | . 683 | . 854 | I. 055 | I. 310 | 1. 697 | 2.042 | 2.457 | 2.750 | 3.646 |
| 40 | - 126 | - 255 | $\cdot 388$ | $\cdot 529$ | . 681 | . 851 | I. 050 | $1 \cdot 303$ | I. 684 | 2.021 | 2.423 | $2 \cdot 704$ | 3.551 |
| 60 | -126 | - 254 | $\cdot 387$ | . 527 | . 679 | -848 | I. 046 | I. 296 | I. 671 | 2.000 | 2.390 | 2.660 | 3.460 |
| 120 | - 126 | - 254 | - 386 | - 526 | -677 | . 845 | I.041 | I-289 | I. 658 | I.980 | 2. 358 | 2.617 | 3. 373 |
| $\infty$ | -126 | - 253 | - 385 | . 524 | . 674 | $\cdot 842$ | I. 036 | I. 282 | I. 645 | I.960 | $2 \cdot 326$ | 2.576 | 3. 291 |

