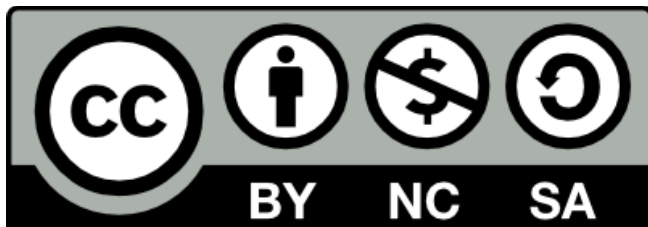




All IBO examination questions are published under the following Creative Commons license:



CC BY-NC-SA (Attribution-NonCommercial-ShareAlike) -
<https://creativecommons.org/licenses/by-nc-sa/4.0/>

The exam papers can be used freely for educational purposes as long as IBO is credited and new creations are licensed under identical terms. No commercial use is allowed.

25th INTERNATIONAL BIOLOGY OLYMPIAD

5 – 13 July, 2014

INDONESIA



PRACTICAL TEST 1 CELL & MOLECULAR BIOLOGY

Total points: **64.5**

Duration: 90 minutes

COUNTRY:
STUDENT:

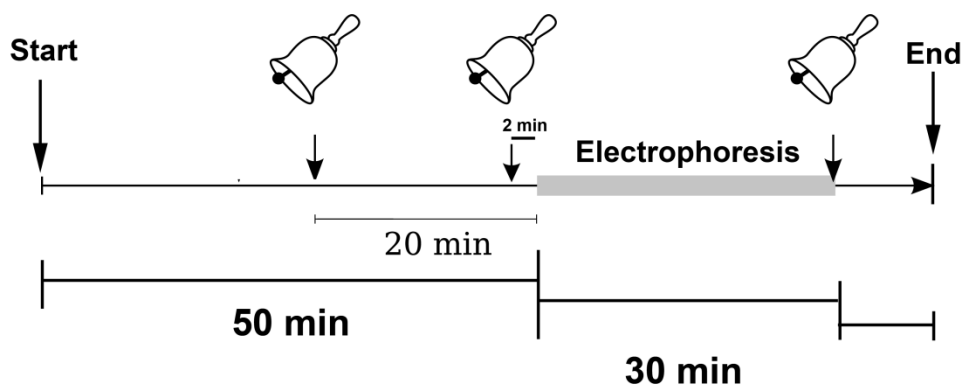
Dear Participants

- In this test, you have been given the following tasks:

Part A. Confirmation of plasmid samples X, Y, and Z by restriction enzyme analysis.
(40 points)

Part B. Cell reproduction and telomere analysis of *Paramecium*. (24.5 points)

- Answer all the questions in the **Answer Sheet** provided.
- The answers written in the **Question Paper** will **NOT** be evaluated.
- Write your answers legibly in ink.
- Please make sure that you have received all the materials and equipment listed for each task. **If any of these items are missing, please raise your hand immediately.**
- Use the gloves provided for the experiment.**
- Stop answering and put down your pen **immediately** when the final bell rings.
- At the end of the test, place the Answer Sheet and Question Paper in the envelope provided. Our Assistants will collect the envelope from you.
- Note!** All the DNA electrophoresis instruments will be turned on simultaneously by the assistants **50 minutes after the start of the test**. Make sure that you have placed your samples in the agarose gel in accordance to the instructions on the Question paper before the power supply is turned on. After this, you will **NOT** be allowed to run the gel. To remind you, the assistant will ring the bell three times. Bell 1 will remind you that the electrophoresis will be carried out in 20 minutes. Bell 2 is a warning that electrophoresis will begin in two minutes' time. Bell 3 will mark the end of electrophoresis. At that time you will be asked to remove your gel and place it into the box provided for assessment.



Materials and Equipment

Materials	Quantity	Unit
Restriction endonucleases FD EcoRI (kept on ice)	1 (8 μ L)	tube
Restriction endonucleases FD HindIII (kept on ice)	1 (8 μ L)	tube
10X Restriction buffer solution (labeled FD Buffer) kept on ice	1 (8 μ L)	tube
Plasmids 1, 2 and 3 kept on ice	3 (5 μ L)	tubes
Sterile water (labelled Deion) in zipper bag	1 (100 μ L)	tube
DNA staining solution, labeled Gel Red (in black tube) in zipper bag	1 (200 μ L)	tube
DNA ladder stock solution, labeled 1 Kb Ladder , kept on ice	1 (20 μ L)	tube
Ready-made agarose gel	1	piece
Running buffer (TAE)	1 (300 mL)	bottle

Equipment	Quantity	Unit
DNA electrophoresis gel tank per person	1	set
Power supply for four persons	1	unit
Micropipettes and tips in boxes (p10, p200)	2	sets
Stopwatch	1	piece
Tube rack	1	piece
Sterile microtubes (in zipper bag)	6	pieces
Plastic box	1	piece
Floating rack styrofoam	1	piece
Minicentrifuge	1	set
Sticker	1	sheet
Marker pen	1	piece
Tissue paper	1	box
Gloves	1	Pair

Note : Use given reagents properly! No additional reagents will be provided.

Part A (40 points)

Identification of Plasmids by Restriction Enzyme Analysis

Introduction

A scientist was surprised to find unlabelled tubes in the freezer. These tubes, containing Plasmids X, Y and Z, were arbitrarily labelled Plasmid 1, Plasmid 2 and Plasmid 3. These plasmids are indistinguishable by DNA electrophoresis because they are all 3750 bp long. Since the three plasmids differ in their restriction pattern when using *EcoRI* and/or *HindIII* (Figure 1), you will conduct restriction enzyme analysis to correctly identify the three plasmids.

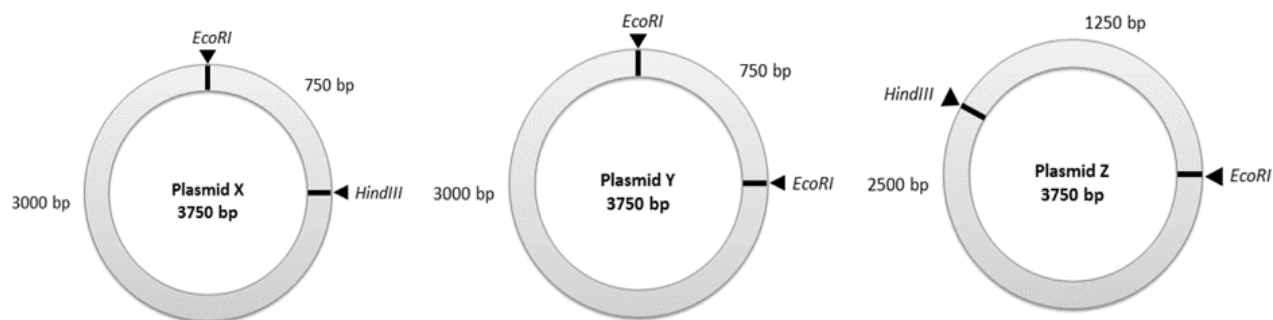


Figure 1. Restriction maps of Plasmids X, Y, Z.

Table I in the **Answer Sheet** shows the experimental design you will perform. The experiment is divided into Series 1 and Series 2, carried out simultaneously. In each series, all three plasmids should be cut with the same restriction enzyme(s). The final concentration of the restriction buffer solution should be 1X.

Question 1.1. Determine how these restriction enzyme(s) can be used to differentiate between the plasmids. Complete Table I on the **Answer Sheet** to indicate your chosen protocol for each series! (8 points). You must choose one or two enzymes for each series that are most informative based on Figure 1. If the enzyme is used in the reaction, add 1 μ L enzyme.

Question 1.2. Complete the figure on your **Answer Sheet** to show the expected molecular weight(s) of the completely digested plasmid for Plasmids X, Y and Z that you would observe using each of your two chosen Series. Indicate the restriction enzyme(s) used for Series 1 and Series 2 of your experimental design. (6 points – 1 points each lane).

Plasmid restriction and DNA electrophoresis protocol

Note: Electrophoresis will start 50 minutes after the test begins. After this, you will **NOT** be allowed to run the gel.

Note: Spin down all reagents in microtubes before pipetting (Be sure to balance the minicentrifuge by placing the microtubes opposite each other, even if there is only one tube to spin).

1. Label the microtubes S1 to S6, and prepare the reaction mixtures according to Table I in the Answer Sheet. Spin down the mixture by placing the microtubes in the minicentrifuge for 10-20 seconds.

2. Place the microtubes into the floating rack labelled with your bench number and incubate for at least 10 minutes at 37°C in the water bath, located at the end of your aisle in the direction of the arrow.

3. Add **ONLY 2 µL** of DNA staining solution into microtube labelled 1 Kb Ladder. Spin down the solution for 10 seconds.

4. After incubation, raise your bench number and your tubes will be returned to you. Add **ONLY 1 µL** of DNA staining solution into each of these tubes, mix well by pipetting and spin down any residual liquid.

5. Open the gel agarose package and place the gel with its tray in the electrophoresis chamber. Pour the entire TAE buffer from the bottle into the tank.

Note: Black plug is cathode (-) and red plug is anode (+).

6. Load 10 µl of samples S1 to S6 and molecular weight marker solution into the agarose gel as shown in **Figure 2**.



Figure 2

7. Close the lid of the gel chamber and notify the assistant by raising your hand that you are ready. The assistant will connect the cables to the power supply.

During the electrophoresis continue with Part B.

8. After the electrophoresis (indicated by Bell 3 ringing), the assistants will turn off the power supply. Carefully place the gel with the tray in the plastic box provided. **Pour the running buffer from the electrophoresis chamber into the plastic box.** Close the box, label the sticker with your **Student ID** and affix it to the side of the box. Leave the box on your bench. Later the gel will be documented and the picture will be attached onto your answer sheet by the assistants.

Part B (24.5 points)

Cell Reproduction and Telomere Analysis of *Paramecium*

Two of the phenomena displayed by *Paramecium tetraurelia* are binary fission (asexual reproduction) and conjugation (exchange of genetic materials), which are affected by the abundance of nutrients in the media. Figure 3 shows microscopic observations of two *P. tetraurelia* cultures grown in rich and poor media, respectively.

Question 2.1. (2 points) Which of the phenomena are shown by the two cultures in Figure 3A and 3B?

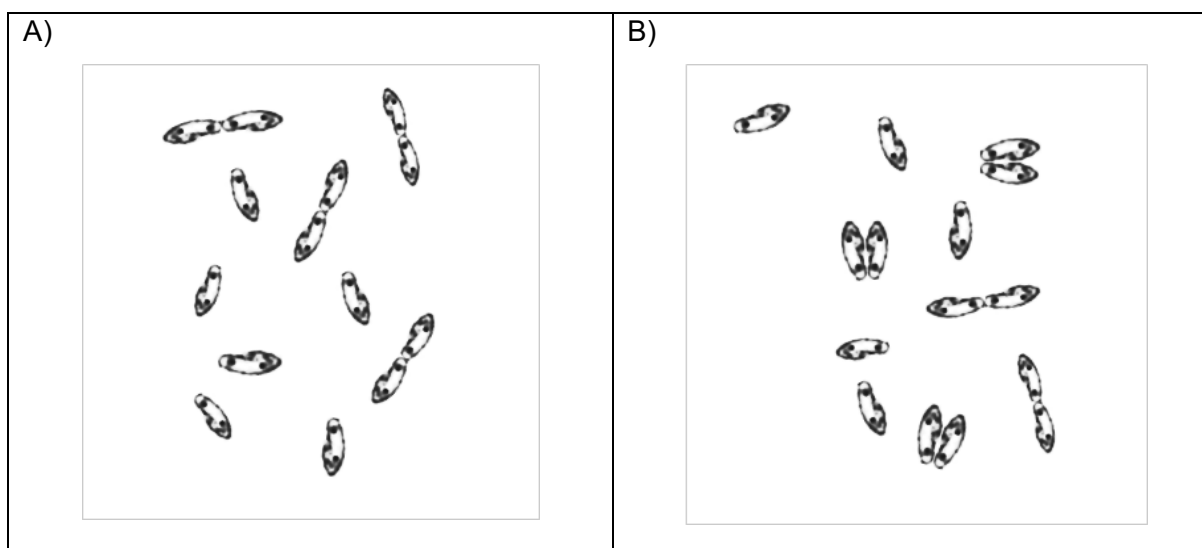


Figure 3. *P. tetraurelia* growing in rich (A) and poor (B) media.

Paramecium, which has just undergone conjugation, continues to asexually reproduce. In this instance, telomere shortening occurs for a certain number of divisions during logarithmic growth.

Table B-1 shows data for the growth of a strain of *Paramecium* in three replicates for four days. The cultures can be grown continuously with no significant change in the fission rate.

Table B-1. *P. tetraurelia* growth in the first four days.

Day	Cell Concentration (Cell/mL)		
	A	B	C
0	1	1	1
1	8	10	16
2	80	120	128
3	640	960	1024
4	5760	7680	10240

Question 2.2. (2.5 + 8 points) Calculate the average cell concentrations and using the logarithmic value, draw the growth curve of *P. tetraurelia* from Days 0 to 4 on the graph provided in your **Answer Sheet** (Precision: two places after decimal point).

Figure 4 shows the result of Southern blot analysis of *P. tetraurelia* telomeres for a total of 30 synchronized generations of cultivation. DNA from *P. tetraurelia* was digested at the beginning of the telomere region, blotted onto a membrane and then hybridized with telomere probes. Telomeres were seen as smears, the midpoints of which can be matched with molecular weight markers to determine the average telomere length.

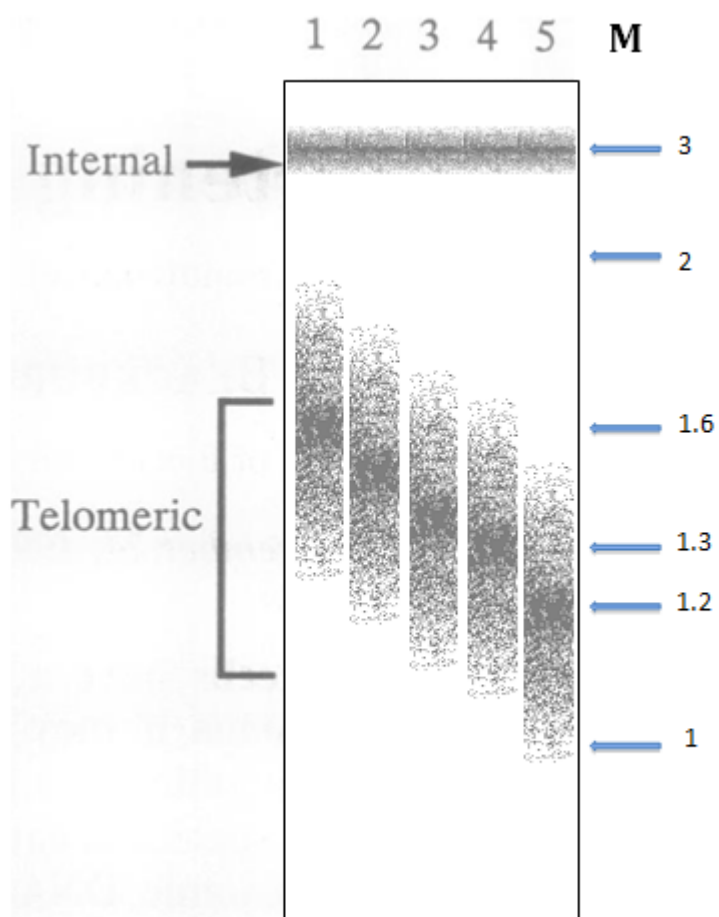
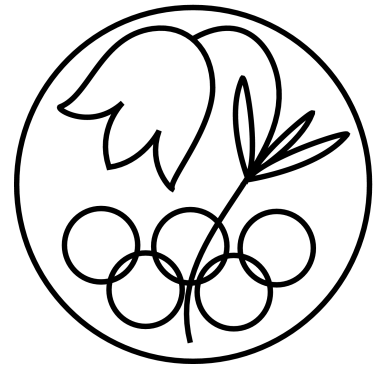


Figure 4. Southern blot of *P. tetraurelia* telomeres. Lanes 1 to 5 show telomeres after 4, 7, 17, 23, and 30 generations, respectively. DNA size markers (M) are shown next to Lane 5 (in kbp).

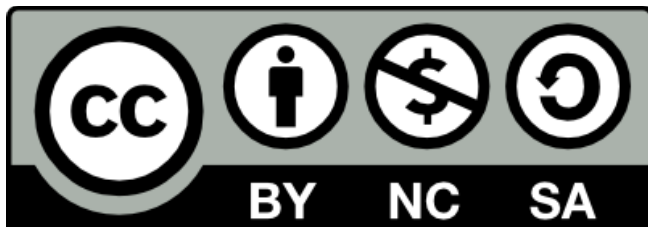
Internal: Internal part of the chromosomal DNA that contains telomeric sequence

Question 2.3. (12 points) Indicate in the answer sheet with a tick (✓) if the following statements are true or false

No.	Statement
a	The media can support the growth of <i>P. tetraurelia</i> to more than 10^4 cells/mL.
b	The Telomere probe used for Southern blot analysis is not specific for telomeric sequences.
c	Based on the Southern blot, telomeres of the cells from the same generation have the same length.
d	Paramecium can grow from 10^5 to 10^7 cells in less than three days, if enough medium is provided.
e	Based on telomere data from lane 4 and 5, telomeres shorten by 20-25 base pairs per day.
f	At beginning of the experiment (day 0), telomeres were likely between 1500 and 1700 base pairs long.



All IBO examination questions are published under the following Creative Commons license:



CC BY-NC-SA (Attribution-NonCommercial-ShareAlike) -
<https://creativecommons.org/licenses/by-nc-sa/4.0/>

The exam papers can be used freely for educational purposes as long as IBO is credited and new creations are licensed under identical terms. No commercial use is allowed.

25th INTERNATIONAL BIOLOGY OLYMPIAD

5 – 13 July, 2014

INDONESIA



PRACTICAL TEST 2

PLANT ANATOMY AND PHYSIOLOGY

Total points: **96**

Duration: 90 minutes

COUNTRY:
STUDENT:

Dear Participants

- This test consist of three tasks,

Task 1: Determination of plant pigment (36 points)

Part A: Qualitative identification of pigments by TLC

Part B: Quantitative measurement of pigments by using spectrophotometer

Task 2: Determination of starch in plant extract (21 points)

Task 3: Observation of structural adaptation in plants (39 points)

- Please write your country name and student code in the box on title page.
- **You have to write down your results and answers in the Answer Sheet. Answers written in the Question Paper will not be evaluated.**
- Write your answers legibly in ink.
- Please make sure that you have received all the materials and equipment listed for each task. **If any of these items are missing, please raise your hand immediately.**
- **Safety: Always wear goggle, mask and gloves for TLC experiment (Part A).**
- Stop answering and put down your pen **immediately** when the bell rings.
- At the end of the test, place the **Answer Sheet** and **Question Paper** in the envelope provided. Our Assistants will collect the envelope from you.

Materials and Equipment

Task 1

Plant material:

- 2 x 500 μL of leaf ethanol-extract labeled A and B (in 1.5 mL microtube)
- 2 x 1000 μL of leaf ethanol-extract labeled C and D (in 1.5 mL microtube)

Solution and reagents:

- 200 mL of hexane:acetone mixture
- 25 mL of alcohol (EtOH)

Technical material

- 1 TLC tank
- 1 Micropipette 20-200 μL and yellow tips (1 box)
- 1 Micropipette 100-1000 μL and blue tips (1 box)
- 2 Cuvettes
- 1 Cuvette rack
- 2 Falcon tubes (15 mL)
- 1 Timer
- 1 TLC plate (10 x 20 cm^2)
- 1 Filter paper (20 x 20 cm^2)
- 1 Forceps
- 1 Pencil and sharpener
- 1 Ruler
- 1 Calculator
- 1 Pair of gloves
- 1 Goggle
- 1 Mask
- 2 Cuvette label

Task 2

Plant material:

- 2 x 1000 μL of root extracts labeled C7 and C8 (in 1.5 mL microtube)

Solution and reagents:

- 1500 μL of 250 ppm starch stock
- A bottle of I_2KI reagent
- A bottle of H_2O

Technical material:

- 1 Micropipette 100-1000 μL and blue tips (1 box)
- 4 Cuvettes

- 2 Microtubes (Eppendorf) (1.5 mL)
- 1 Timer
- 1 Pair of gloves
- 1 Calculator
- 4 Cuvette labels

Task 3

Plant material:

- 3 x plant specimens labeled X, Y and Z (in Falcon 15 mL)

Solution and reagents:

- A bottle of H₂O
- A bottle of Aniline Sulphate – stain lignin – yellow color
- A bottle of Sudan III – stain lipid, i.e. suberin – red color

Technical material

- 1 microscope
- 2 razor blades
- 6 microscope slides
- 12 cover slips
- 1 forceps
- 1 dissecting needle
- Tissue paper

Note : Use given materials properly! No additional materials will be provided in this experiment.

Task 1: Determination of plant pigment

Several plants are resistant to flooding stress by various physiological and morphological defense mechanisms. Prolonged levels of water stress may cause several physiological symptoms resulting in death.

In this task, plants were grown for 2 weeks with 12/12 h (light/dark) photoperiod and exposed either to flooding treatment or normal watering as control.

To analyze the physiological conditions, leaf samples of stressed and normal plants were extracted and analyzed for pigments. These include qualitative observation by Thin Layer Chromatography (TLC) and quantitative measurement by spectrophotometry analysis.

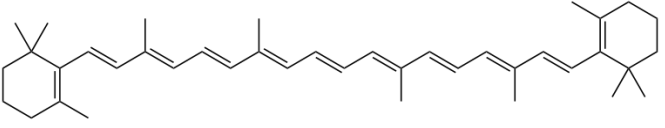
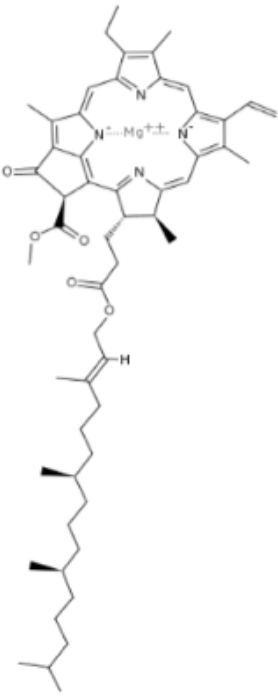
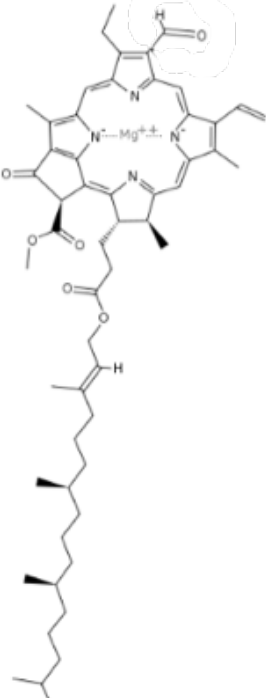
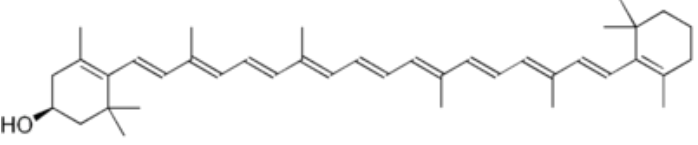
Part A Qualitative identification of pigments by TLC (20 points)

You will be provided with one extract from treated-plants and the other from control leaves, arbitrarily labeled A and B to run on TLC plate together. Prepare the task by:

- Attach the filter paper provided to one of the inside TLC tank walls.
- Add 200 mL of hexane:acetone (7:3) solution to the TLC tank, and close the lid.
- Use a pencil to lightly mark the edge of TLC plate 2 cm from bottom, top, left and right edges. Draw lines one on the bottom and one on the top of the TLC plate. Indicate A and B on the bottom line.
- Micropipette 100 μ L of Extract A and discharge the extract on the bottom line of TLC plate and repeat with Extract B. Make sure you concentrate the extract on one spot.
- Air-dry the plate for 5 minutes then place the plate into the TLC tank.
- As the eluent reaches the top line, remove the TLC plate immediately from the tank. Mark the end of the solvent front.
- Remove the TLC plate and place it on the sheet provided with your student code. Raise your hand. An assistant will take a photograph of your TLC plate for grading (**4 points**).

Question 1.1 Calculate the R_f values which are a ratio between distance of center of the spot to start line and distance of eluent from start to stop line and determine tentative name of pigments. Identify the pigments using the Table below (**12 points**).

Table 1. List of 4 major pigments to be identified

No.	Pigment	Chemical structure	
1	β -carotene		
2	chlorophyll a	chlorophyll a	
3	chlorophyll b	<div> <div>  </div> <div>  </div> </div>	
4	xanthophylls		

Question 1.2 Indicate with a tick (✓) if each of the following statements is True or False (4 points).

- A. R_f values of chlorophyll a and chlorophyll b are different due to their molecular weights.
- B. R_f values of chlorophylls and β-carotene are different due to their polarity.
- C. Flooding stress affects the concentration of chlorophyll b.
- D. Plant A was grown in flooded conditions.

Part B Quantitative measurement of pigments using spectrophotometer (16 points)

Quantitative measurement using spectrophotometer is essential to determine content of pigment in control and flooded plants. In this task, you will be given leaf extracts C and D.

1. Transfer 1 mL of each extract into two separate 15 mL Falcon tubes.
2. Dilute each extract to 10 mL with alcohol and mix the solution well.
3. Pipette the diluted Extract C into the cuvette and label with C and repeat for Extract D.
4. **Raise your hand. Lab assistants will take your cuvettes to measure** absorbance (A) (λ = 649 and 665 nm) and provide you with the readings.
5. Calculate the concentrations of chlorophyll a, b and total (mg/L) using the following equations (Wintermans & de Mots, 1965).

$$\text{Total chlorophyll (mg/L)} = 20.0 (A_{649}) + 6.1 (A_{665})$$

$$\text{Chlorophyll a (mg/L)} = -5.76 (A_{649}) + 13.7 (A_{665})$$

$$\text{Chlorophyll b (mg/L)} = 25.8 (A_{649}) - 7.7 (A_{665})$$

Question 1.3 Write down the absorbance values into the **Answer Sheet**. Calculate the concentration of total chlorophyll and chlorophylls a and b. (precision: two places after the decimal point) (10 points).

Question 1.4 Calculate the ratios of chlorophyll a to b for Extracts C and D (precision: two places after the decimal point) (2 points).

Question 1.5 Indicate with a tick (✓) whether each of the following statements is True or False (4 points).

- A. Plant C was grown in flooded conditions.
- B. Under the flooding stress, the degradation of chlorophyll b is higher than of chlorophyll a.
- C. Photosynthetic rate in Plant C is higher than in Plant D.
- D. Flood-stress plant has a lower nitrogen absorption than under normal conditions.

Task 2: Determination of starch content in root extract (21 points)

Under flooded conditions, the respiration of roots and microorganisms quickly deplete the remnant oxygen in the soil. As a result, flooded tissues will rely more heavily on anaerobic metabolic pathways. In this task, you will quantify the effect of the lack of oxygen on the recruitment of carbohydrate from starch reserves using spectrophotometry on extracts from flooded and control plants, arbitrarily labeled C7 and C8.

Part 2.1 Measurements

In order to quantify starch content using spectrophotometry, you will compare the absorbance of the Lugol-stained plant extracts with the absorbance measured for dilutions of 250 ppm and 100 ppm starch.

Question 2.1 You are provided with a stock solution of 250 ppm starch. Prepare an additional 1000 µL stock solution of 100 ppm. Begin by indicating the required volumes of the 250 ppm starch stock solution and H₂O in the **Answer Sheet**. Pipette these volumes into a fresh microtube and mix well (1 point).

Prepare now each of the four samples; starch dilutions of 250 ppm (C4), 100 ppm (C6), plant extracts (C7 and C8) as follows in cuvettes:

1. Pick one of the provided labels and stick it to a cuvette.
2. Pipette of 900 µL of the sample into the cuvette.
3. Add 100 µL of Lugol (I₂KI) solution and mix well.
4. Incubate for 4 minutes at room temperature.

Raise your hand when your samples are ready. The assistant will then collect your samples for measurement and return a print out of the obtained absorbance.

Question 2.2 Fill the table of absorbance values in your **Answer Sheet** (8 points).

Part 2.2 Data analysis

Under the assumption that absorbance is changing linearly with the starch concentration and is 0 for a starch concentration of 0 ppm, the relationship between starch concentration (x) and absorbance (y) is given by

$$y = ax$$

Question 2.3 Use a linear regression approach to estimate the **slope (a)** of the above equation as

$$a = \frac{\Sigma xy}{\Sigma x^2}$$

and report the obtained result with a precision of two places after the decimal point in the **Answer Sheet (4 points)**.

Question 2.4 Use your estimate of the **slope (a)** to calculate starch concentrations (ppm) in Samples C7 and C8 (integer, **4 points**).

Question 2.5 Indicate with a tick (✓) if each of the following statements is True or False (**4 points**).

- A. C7 was grown in flooded conditions.
- B. After flooding, the concentration of sucrose and other soluble sugars increase in the roots.
- C. Impaired carbon translocation facilitates starch accumulation in roots of flooded plants.
- D. Alcoholic fermentation in the root might increase as a result of flooding.
- E.

Task 3. Observation of structural adaptation in plants (39 points)

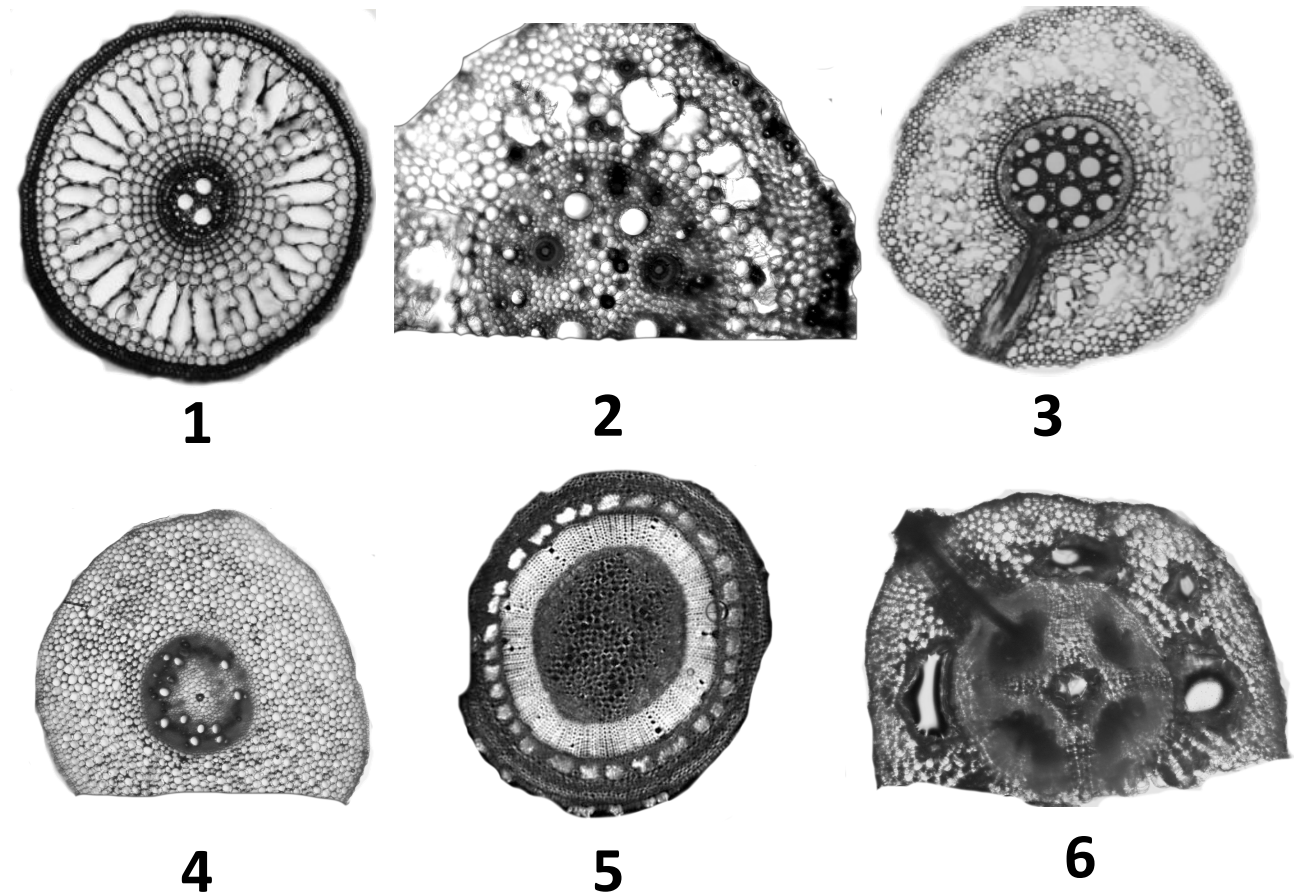
Anatomical and morphological changes in root and shoots usually occur as structural adaptation to waterlogged soils. The stress brought on by low O₂ concentrations in flooded soils leads to the formation of aerenchyma in some tolerant plant species, a process that can occur in roots, nodules, rhizomes, stems and submerged leaves.

Find three specimens in Falcon Tubes **X**, **Y**, and **Z**. These specimens were obtained from plants developed in two different soil conditions.

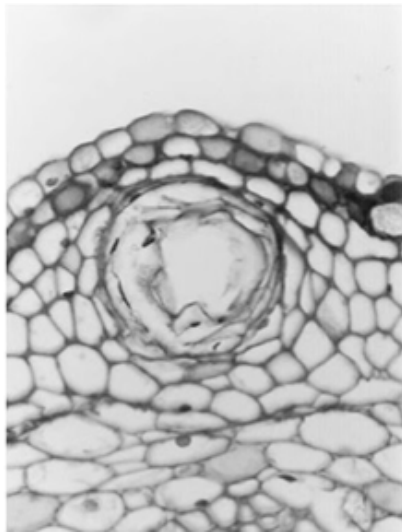
- Make a cross section of each specimen and stain with staining solutions provided.
- Observe the structure of stained specimens under the light microscope.

Question 3.1 Observe the sections carefully and complete the table on the **Answer Sheet** using symbols: "+" for presence, "-" for absence. **(6 points @0.5)**

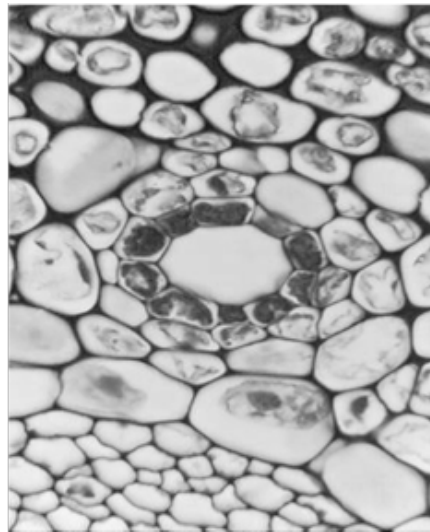
Question 3.2 Indicate the diagram number corresponding to the specimen **(9 points)**.



Question 3.3 Indicate with a tick (✓) the type of aerenchyma of each specimen (**9 points**)



Lysigenous

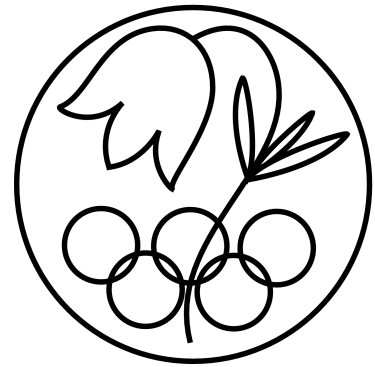


Schyzigenous

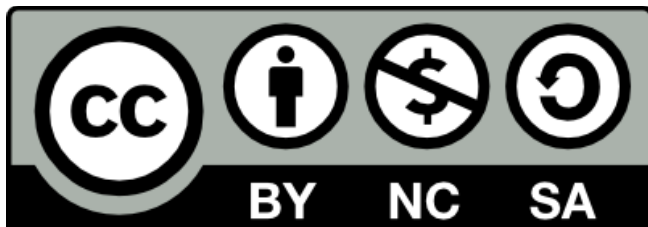
Question 3.4 Indicate with a tick (✓) link the corresponding specimens with particular plant organ(s) based on your observations (**9 points**).

Question 3.5 Indicate with a tick (✓) for each of the corresponding specimen with their growth condition (**6 points**)

End of the Practical Exam



All IBO examination questions are published under the following Creative Commons license:



CC BY-NC-SA (Attribution-NonCommercial-ShareAlike) -
<https://creativecommons.org/licenses/by-nc-sa/4.0/>

The exam papers can be used freely for educational purposes as long as IBO is credited and new creations are licensed under identical terms. No commercial use is allowed.

25th INTERNATIONAL BIOLOGY OLYMPIAD

5 – 13 July, 2014

INDONESIA



PRACTICAL TEST 3

ANIMAL ANATOMY, PHYSIOLOGY, AND SYSTEMATICS

Total points: **93.5**

Duration: 90 minutes

COUNTRY:

STUDENT:

Dear Participants

1. In this test, you have been given the following three tasks:
 - Part A.** Acute Response of Fish Larvae to Changes in Salinity (12 points)
 - Part B.** Calculating LC_{50} (19.5 points)
 - Part C.** Classification of Prawns (62 points)
2. Use the **Answer Sheet**, which is provided separately, to answer all the questions.
3. The answers written in the **Question Paper** will **NOT** be evaluated.
4. Write your answers legibly in ink.
5. Please make sure that you have received all the materials and equipment listed for each task. **If any of these items are missing, please raise your hand immediately.**
6. Stop answering and put down your pen **immediately** when the bell rings.
7. At the end of the test, place the **Answer Sheet** and **Question Paper** in the envelope provided. Our Assistants will collect the envelope from you.
8. **Note:** You are provided with a magnifier glass with lamp, and a desk lamp. Make sure both lamps are working prior to the test.
9. You may work on these tasks in any order. Each task is independent of each other.
10. You may use your question paper as note to do math work.

Materials and Equipment

Materials	Quantity	Unit
Prawns in tray	6	specimens
Equipment	Quantity	Unit
Dissecting kit	1	set
Magnifier glass with lamp	1	set
Desk lamp	1	set
Pencil	1	piece
Tissue paper	1	box
Tablecloth	1	piece
Gloves	1	piece
Mask	1	sheet
Ruler	1	piece
Calculator	1	piece
Sharpener	1	piece
Label	1	sheet

Part A

Acute Response of Fish Larvae to Rapid Changes in Salinity (12 points)

Introduction

In Indonesia, the salt concentration in brackish areas is strongly affected by the season. While heavy rain at headwaters and watersheds lower salt concentration during the rainy season, the lower water volumes during the dry season increase salt concentration.

Fish larvae are highly sensitive to changes in salinity of their environment, to which they can respond by osmoregulation if salinity remains within their **tolerance level**. When fish encounter salinity levels outside the tolerance level (either lower or higher), osmoregulation mechanisms fail and salt becomes toxic for these fish. The goal of this experiment is to assess how larvae of milkfish (*Chanos chanos* Lacepede) respond to rapid changes in salinity.



Experimental procedure

To determine the mortality of fish larvae as a result of rapid change in salinity, 10 fish larvae were each transferred from 0.4 M solution to different concentrations of salt and the number of fish that died in 5 minutes were recorded. The table below shows the results of three replicates of the experiment.

Salinity	Number of dead fish larvae			Position of dead fish larvae in the tubes		
	Replicate 1	Replicate 2	Replicate 3	Surface	Middle	Bottom
2 M	9	10	8	26	1	0
1.6 M	7	6	9	18	3	1
1.2 M	5	5	6	12	4	0
0.8 M	2	3	2	1	5	1
0.4 M	1	2	0	0	2	1
0 M	4	3	3	0	1	9

Data Analysis

Question 1.1 Draw a line graph showing the average mortality rate across replicates (label “M”) as a function of salinity (label “S”) in the **Answer Sheet (6 points)**.

Question 1.2 Mark on the graph the concentration that caused mortality of 50% of the fish larvae (**2 points**).

Question 1.3 What is the most probable cause of death for the majority of fish that died in this experiment? (**2 points**)

- A. Compared to the solutions, the fish are hypertonic and thus suffer from water entering their tissues.
- B. High concentrations of salt increase the viscosity of water, and hence increase the energy demand of the fish for movement and respiration.
- C. High concentrations of salt reduce the oxygen available to the fish.
- D. The fish suffer from anoxia due to damages in the gill tissues, caused by the high difference in water potential between their bodies and the environment.

Question 1.4 In this experiment, what is the most probable cause for the dead fish to float in the water with high salt concentration? **(2 points)**

- A. Their body weight is reduced because water diffuses from their tissues.
- B. Their body density is reduced because the tissues fluids are more dilute than the surrounding salt solution.
- C. Gas accumulates inside their bodies as a result of decomposition.
- D. They lose control over their swim bladders.

Part B:
Calculation of LC₅₀ (19.5 points)

Introduction

Indonesian aquaculture in brackish areas benefits from the natural inflow of fresh water from nearby rivers. Recently, these cultures have come under threat from various chemicals such as insecticides washed into the rivers. A common method to assess the toxicity of such substances is to determine the concentration at which 50% of the tested animals die. The corresponding concentration is named “lethal concentration 50%” or LC₅₀.

The table below shows the results of such an experiment to assess the toxicity of a common insecticide for fish larva. Determine the LC₅₀ of this insecticide for the fish larvae.

No	Concentration [μM]	Tested Animals	Number of Dead Animals
1	10.2	50	44
2	7.7	50	42
3	6.5	50	35
4	5.1	46	24
5	4.4	50	22
6	3.8	50	16
7	3.2	50	11
8	2.6	50	6

Probit Analysis

A powerful way to estimate LC₅₀ from such data is Probit (Probability Unit) analysis based on linear regression. The basic idea is to model relationship between the log₁₀ concentration of the insecticide (x) and the probit conversion value of mortality rate (y) as a linear function of the form $y = a + bx$. To ensure the relationship is approximately linear, the mortality rates are first transformed into probits, defined as the position of the value in the cumulative distribution of a standard normal. You can conduct this transformation on Finney's table (Finney, 1952) (Table 1). As an example on how to use this table consider a mortality rate of 17%, for which the corresponding probits of -0.95 is indicated in row “10” and column “7”. The probit of a mortality rate of 50% is 0.00.

Table 1.

%	0	1	2	3	4	5	6	7	8	9
0		-2.33	-2.05	-1.88	-1.75	-1.64	-1.55	-1.48	-1.41	-1.34
10	-1.28	-1.23	-1.18	-1.13	-1.08	-1.04	-0.99	-0.95	-0.92	-0.88
20	-0.84	-0.81	-0.77	-0.74	-0.71	-0.67	-0.64	-0.61	-0.58	-0.55
30	-0.52	-0.5	-0.47	-0.45	-0.41	-0.39	-0.36	-0.33	-0.31	-0.28
40	-0.25	-0.23	-0.2	-0.18	-0.15	-0.13	-0.1	-0.08	-0.05	-0.03
50	0.00	0.03	0.05	0.08	0.1	0.13	0.15	0.18	0.2	0.23
60	0.25	0.28	0.31	0.33	0.36	0.39	0.41	0.44	0.47	0.5
70	0.52	0.55	0.58	0.61	0.64	0.67	0.71	0.74	0.77	0.81
80	0.84	0.88	0.92	0.95	0.99	1.04	1.08	1.13	1.18	1.23
90	1.28	1.34	1.41	1.48	1.55	1.64	1.75	1.88	2.05	2.33

Question 2.1 For data points 2, 4 and 7, transform the concentrations with \log_{10} , calculate the mortality rates and transform them to probits. Report your results in the table in the **Answer Sheet (4.5 points)**.

Note: Use a precision of two digits after the decimal point for all calculations in this task. Except for calculation of mortality rate where no decimal required for calculation result.

Slope ***b*** and intercept ***a*** of a linear equation can be estimated from the *n* data points using the least squares approach as:

$$b = \frac{\sum_{i=1}^n x_i y_i - \frac{1}{n} \sum_{i=1}^n x_i \sum_{i=1}^n y_i}{\sum_{i=1}^n x_i^2 - \frac{1}{n} \left(\sum_{i=1}^n x_i \right)^2}$$

$$a = \frac{1}{n} \left(\sum_{i=1}^n y_i - b \sum_{i=1}^n x_i \right)$$

Question 2.2 Calculate x_i^2 and $x_i y_i$ for data points 2, 4 and 7 and report your results in the table in the **Answer Sheet (3 points)**.

Question 2.3 Calculate all required sums **(4 points)**.

Question 2.4 Use the equations provided above to estimate both slope and intercept **(4 points)**.

Question 2.5 Use your estimates of the slope and intercept to estimate the LC_{50} concentration of this insecticide for the fish larvae. Report your result in μM **(4 points)**

Part C (62 points)

Classification of Prawns

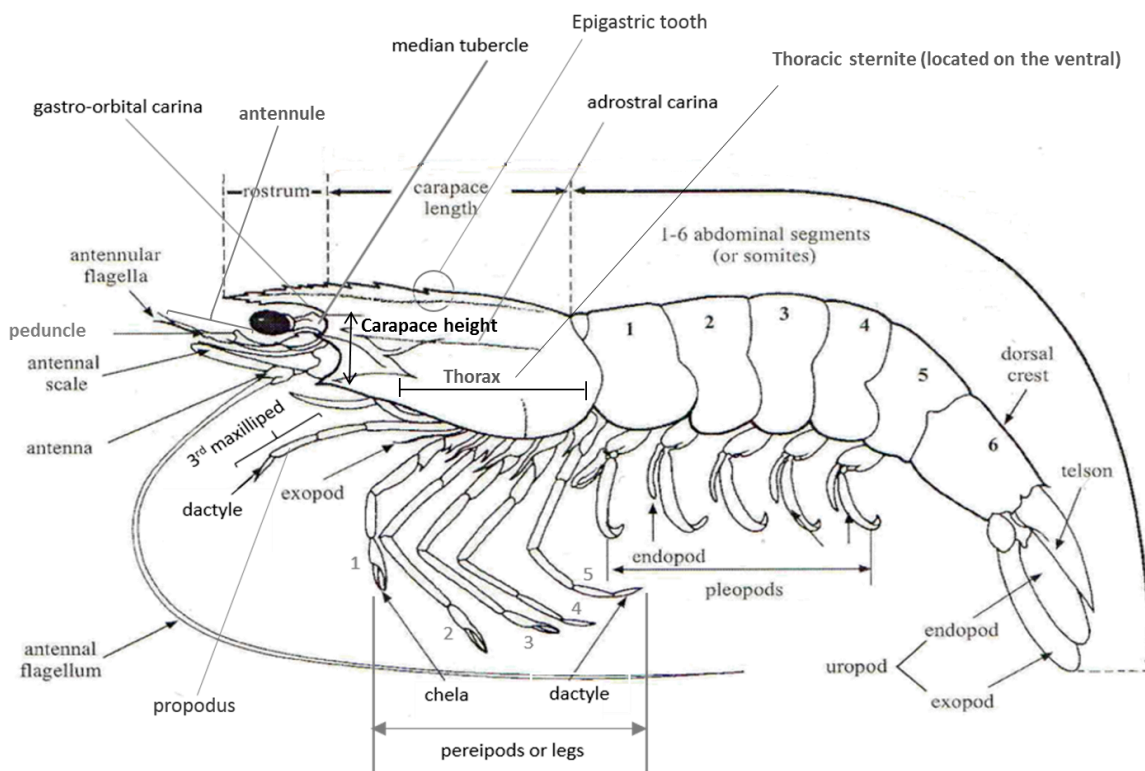
Introduction

Indonesia is one of the main producers of prawns with many small to large scale aquacultures along the long coastline. Due to differences in environmental conditions along the coast, different prawn species are cultured in various locations. The goal of this task is to identify specimens of Indonesia's famous prawn species and to study their phylogenetic relationships based on morphological characters. You may use the magnifying glass, dissecting kit, forceps and the needle provided.

Note: Damage to the specimens will not lead to point subtraction. In this practical work, two specimens actually belong to one species.

Identification of Prawn Specimens

Question 3.1 Identify all prawn specimens using the following identification key. Consult the figure below to identify the required morphological features **(15 points)**.



Identification Key for Prawns

Note: A dash “-” in b indicates the absence of the pattern described in a.

- | | | | | |
|-----|----|---|------|---|
| 1. | a. | Second pereopod bigger and longer than the other pereopods | (2) | |
| | b. | - | (4) | |
| 2. | a. | Antennal scale anterior margin strongly produced forward at or near mid-line | | <i>Macrobrachium rosenbergii</i> |
| | b. | - | (3) | |
| 3. | a. | Thoracic sternite 4 with distinct median suture | | <i>Macrobrachium australiense</i> |
| | b. | - | | <i>Macrobrachium koomboolomba</i> |
| 4. | a. | Rostrum with ventral teeth | (12) | |
| | b. | - | (5) | |
| 5. | a. | Telson with fixed subapical spines | (6) | |
| | b. | - | (9) | |
| 6. | a. | Telson with 1 pair of fixed subapical spines | (7) | |
| | b. | Telson with 3 pairs of fixed subapical spines | (11) | |
| 7. | a. | Maxilliped 3 and pereopod 2 with spine on inside of basis | | <i>Metapenaeopsis barbata</i> |
| | b. | - | (8) | |
| 8. | a. | Carapace with longitudinal suture (seam) | (10) | |
| | b. | - | | <i>Penaeopsis eduardoi</i> |
| 9. | a. | Pereopod 5 with exopod | | <i>Trachypenaeus curvirostris</i> |
| | b. | - | | <i>Metapenaeus monoceros</i> |
| 10. | a. | Rostrum as long as peduncle of antennule and antennal scale | | <i>Parapenaeus australiensis</i> |
| | b. | Rostrum shorter than peduncle of antennule and antennal scale | | <i>Parapenaeus sextuberculatus</i> |
| 11. | a. | Rostrum with 4-6 dorsal teeth | | <i>Funchalia villosa</i> |
| | b. | Rostrum with 10-13 dorsal teeth | | <i>Funchalia woodwardi</i> |
| 12. | a. | The height of the gastro-orbital carina is at least half of the height of the carapace at the same location | (13) | |
| | b. | - | | <i>Penaeus merguensis</i> |
| 13. | a. | Rostrum with 6-7 dorsal teeth and 3 ventral teeth | | <i>Penaeus monodon</i> |
| | b. | Rostrum with 8-9 dorsal teeth and 2 ventral teeth | | <i>Litopenaeus vannamei</i> |

Compiling Character and Similarity Matrices

In this task, you reconstruct the phylogenetic relationships among the six prawn specimens from morphological characters. You will first need to compile a character matrix, which is a list of the presence or absence of the morphological characters, listed below, for each specimen. Use the figure provided under Question 3.1 to identify the required morphological characters.

Number	Description
1	Second pereopod bigger and longer than the other pereopods
2	Distinct median tubercle
3	Upper antennular flagellum subequal to lower one, attached to apex of third antennular segment
4	Antennal scale anterior margin strongly produced forward at or near mid-line
5	The height of the gastro-orbital carina is at least half of the height of the carapace at the same location
6	Rostrum with ventral teeth
7	Blade of rostrum high, broadly triangular in shape
8	Telson with one pair of fixed subapical spines.
9	Adrostral carina and groove extending as far as, or slightly ahead of epigastric tooth
10	In adult males, dactyle of third maxilliped half (0.5 to 0.6 times) as long as propodus
11	Pereopod 5 without exopod and telson without fixed subapical spines
12	Body white yellowish, yellow reddish with black or green spots
13	Rostrum with 6-9 dorsal teeth and 2-3 ventral teeth
14	The movable finger of the chela has very short and fine spinules
15	Fifth pereopod without an exopod

Question 3.2 Compile the character matrix in the **Answer Sheet**. Use “1” for presence and “—” for absence (**22.5 points**).

Reconstructing phylogenetic relationships using UPGMA

UPGMA (Unweighted Pair Group Method with Arithmetic Mean) is considered the simplest method for reconstructing phylogenetic trees with assumption the data provide has constant rate of evolution. In this method, the pair of clusters with the shortest distance is combined into a higher-level cluster at each iteration. To illustrate this concept, consider the number of character differences between the Taxa A, B, C and D.

	A			
A	0			
B	6	0		
C	7	8	0	
D	8	9	4	0

Iteration 1: The pair of clusters with the smallest distance is the pair C and D, which is thus combined into a higher-order cluster (C,D). The **relative age** of the newly formed cluster is computed as half the distance between the two original clusters. In this case, the relative age of the cluster is 2.

Next, a new matrix of all distances is generated by computing the distance between clusters as the average distance between all taxa from one cluster to all taxa of the other cluster. The distance between Cluster A and Cluster (C,D), for instance, is computed as the average between $d(A,C)$ and $d(A,D)$, where $d(x,y)$ is a notation to indicate the distance between Clusters x and y.

	A		
A	0		
B	6	0	
(C,D)	7.5	8.5	0

Iteration 2: The pair of clusters with the smallest distance is now the pair of A and B, which is thus combined into a higher-level cluster (A,B) with a relative age of 3.

Again, a new matrix is constructed by calculating all distances as indicated above. The distance $d((A,B),(C,D))$ is defined as the average between $d(A,C)$, $d(A,D)$, $d(B,C)$ and $d(B,D)$.

	(A,B)	
(A,B)	0	
(C,D)	8	0

Iteration 3: In the last iteration, the two remaining taxa are combined into the new cluster ((A,B),(C,D)) and the relative age of this cluster is 4.

Question 3.3 Calculate the distance matrix based on the character matrix provide at **Table 2**. The distance between two specimens is defined as the number of characters at which the two specimens show different character states (one present and the other absent) **(7.5 points)**.

Table 2.

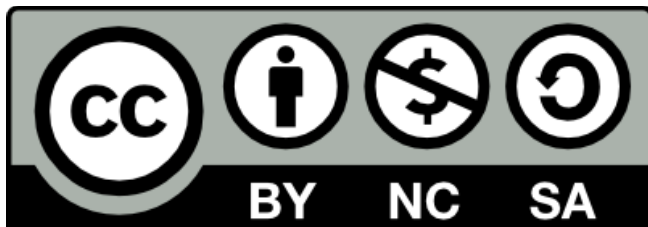
Character	I	II	III	IV	V	VI
1	-	-	-	1	-	-
2	-	-	-	-	1	-
3	-	-	-	1	-	-
4	-	-	1	-	-	1
5	-	-	-	1	-	-
6	1	-	1	-	1	1
7	1	-	1	-	1	1
8	1	-	1	-	-	1
9	-	1	-	-	-	-
10	-	-	1	-	-	1
11	-	1	-	-	-	-
12	1	-	-	-	1	-
13	-	-	-	1	-	-
14	-	-	-	1	-	-
15	-	1	-	-	1	-
16	1	1	1	-	1	1
17	-	1	1	1	-	1
18	1	-	1	1	1	1
19	-	1	-	-	-	-
20	1	-	-	-	1	-

Question 3.4 Resolve the phylogenetic relationships of all six specimens (I-VI), showed at table 2, iteratively using the UPGMA method and based on the distance matrix you already create based on data of table 2 (**Q.3.3**). Make sure to report the names of the clusters using the specimen codes I through VI (**12 points**).

Question 3.5 Draw a phylogenetic tree (dendrogram) based on the UPGMA results. Indicate the relative length of each branch by writing the correct number next to it (**5 points**).



All IBO examination questions are published under the following Creative Commons license:



CC BY-NC-SA (Attribution-NonCommercial-ShareAlike) -
<https://creativecommons.org/licenses/by-nc-sa/4.0/>

The exam papers can be used freely for educational purposes as long as IBO is credited and new creations are licensed under identical terms. No commercial use is allowed.

25th INTERNATIONAL BIOLOGY OLYMPIAD

5 – 13 July 2014

INDONESIA



PRACTICAL TEST 4

ECOLOGY AND ETHOLOGY

Total points: **100**

Duration: 90 minutes

COUNTRY:
STUDENT:

Dear Participants

- The theme of this test is “**Biogeography and Biodiversity**”. The test consists of three tasks:
 - Task 1 : Island Biogeography (17 points)
 - Task 2 : Primary Succession after volcanic eruption
 - Part A: Succession and plant community structure (22 points)
 - Part B: Dispersal biology of *Ficus* (30 points)
 - Task 3 : Speciation in song birds (31 points)
- Use the **Answer Sheet**, which is provided separately, to answer all the questions. Do not write or make any markings on the Question Paper.
- Answers written in the Question Paper will **NOT** be evaluated.
- Write your answers legibly in ink.
- Please make sure that you have received all the material and equipment listed for all tasks.
If any items are missing, raise your hand immediately.
- Materials such as photographs and model fruits for Task 1 and 2 will be used by your country team mates. Please do not mark, damage, or take them with you. You may, however, mark on spectograms provided for Task 3.
- Stop answering and put down your pen **immediately** when the bell rings.
- At the end of the test, place your Answer Sheet and Question Paper in the envelope provided. Our Assistants will collect the envelope from you.

Materials and Equipment

	Quantity	Unit
Material		
Aerial photographs, coded A, B and C	3	sheets
Model chips of <i>Ficus</i> fruits (in one plastic bag)	30	chips
Sample of whole and dissected <i>Ficus</i> fruit	1 ½	fruits
Bird song spectrogram and oscillogram	9	sheets
Blank paper for scrap paper	2	sheets
Equipment		
Calculator	1	piece
Ruler	1	piece
Vernier caliper	1	piece
Magnifying glass	1	piece
Color pencils	4	pieces
Pencil sharpener	1	piece
MP3/audio player with recording of bird song + earphone	1	set

BIOGEOGRAPHY & BIODIVERSITY

Indonesia is a megabiodiversity country consisting of over 17,000 islands, spanning from Sumatra island in the west, to Papua in the east (see aerial photograph coded A). The country's rich biodiversity can be attributed to its tropical setting, as well as its biogeographical history. The following three tasks concern several important concepts related to biogeography, colonization, succession, and speciation, using examples from Indonesia.

Task 1. Island Biogeography (17 points)

Introduction

The Equilibrium Theory of Island Biogeography (MacArthur and Wilson, 1963, 1967) suggests that the number of species on an island represents an equilibrium between immigration of new species (i.e., addition of species) and local extinction (i.e., loss of species). The rate of new species immigration is affected by the island's distance from the mainland as a colonization source, while the rate of local extinction is affected by island size.

- Study the aerial photograph provided for Halmahera islands (coded B), which are on the eastern part of Indonesia, and locate the following five islands :
 - Ternate (area: 111.80 km²)
 - Tidore (117.60 km²)
 - Mare (6.04 km²)
 - Moti (24.60 km²)
 - Makian (113.12 km²)
- Using the ruler provided, estimate the actual distance from the mainland of Halmahera to each island. Distance is measured as the shortest length connecting the outer edges of islands (example given in **Figure 1**).

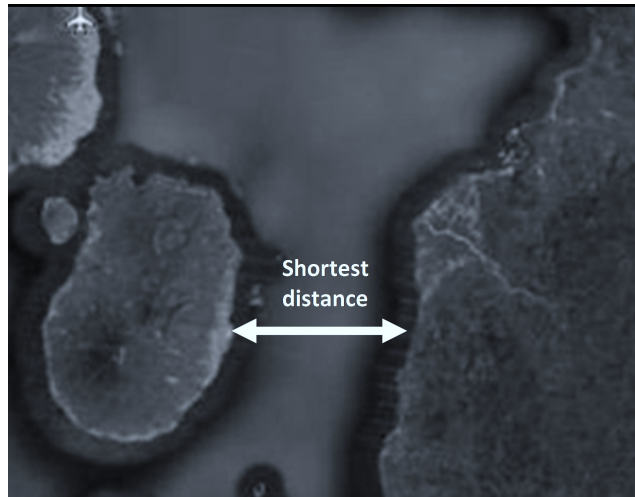


Figure 1. Measurement of shortest distance between islands.

Question 1.1. (5 points) For each island, determine its distance from the mainland and record your measurements in the **Answer Sheet**.

Question 1.2. (10 points) Fill in the appropriate islands whose rates of colonization and extinction have been depicted in the graph in the **Answer Sheet**, using the island codes provided below:

Code	Island
A.	Ternate
B.	Tidore
C.	Mare
D.	Moti
E.	Makian

Question 1.3. (2 points) Which two islands listed have the largest number of species in equilibrium?

- A. Ternate
- B. Tidore
- C. Mare
- D. Moti
- E. Makian

Task 2. Primary Succession After Volcanic Eruption (52 points)

Introduction

The Krakatau islands are a small volcanic group located in the strait of Sunda which separates the large islands of Java and Sumatra (see aerial photograph coded C). In 1883, Mount Krakatau produced one of the most catastrophic volcanic eruptions in human history and a new volcanic island, Anak Krakatau (Child of Krakatau) emerged in 1927 and is still active today.

Part A : Succession and Plant Community Structure

Anak Krakatau has now become a habitat for a variety of flora and fauna. Table 1 presents part of the data collected on plant species richness and abundance on Anak Krakatau in 1934, 1949, 1963, 1979 and 1991. Using data from Table 1, compare the plant community structure on Anak Krakatau across these years, using diversity and similarity indices.

Table 1. List and abundance of plant species on Anak Krakatau.

No	Life Form*	Plant Species	Habitat**	Plant Abundance (number of individuals per unit area)				
				1934	1949	1963	1979	1991
1	T	<i>Calophyllum inophyllum</i>	I	0	0	1	3	35
2	H	<i>Canavalia rosea</i>	C	1	0	3	7	37
3	T	<i>Casuarina equisetifolia</i>	C	0	0	2	8	59
4	T	<i>Cocos nucifera</i>	C	0	0	2	11	69
5	S	<i>Eupatorium odoratum</i>	C	0	0	3	17	121
6	T	<i>Ficus septica</i>	I	0	0	0	5	16
7	T	<i>Hibiscus tiliaceus</i>	C	2	0	8	26	56
8	H	<i>Imperata cylindrica</i>	C	0	0	15	94	256
9	H	<i>Ipomoea pes-caprae</i>	C	18	15	37	80	212
10	H	<i>Nephrolepis hirsutula</i>	C	15	12	30	58	278
11	T	<i>Nypa fruticans</i>	C	0	0	2	11	87
12	T	<i>Pandanus tectorius</i>	C	2	0	8	28	101
13	T/S	<i>Pongamia pinnata</i>	C	2	0	0	0	65
14	H	<i>Saccharum spontaneum</i>	I	0	0	5	19	189
15	T	<i>Samanea saman</i>	I	0	0	2	3	45
16	S	<i>Scaevola taccada</i>	C	0	0	2	18	37
17	H	<i>Spathoglottis plicata</i>	I	0	0	0	0	28
18	T	<i>Tamarindus indica</i>	I	0	0	0	2	21
19	T	<i>Terminalia catappa</i>	I	0	0	3	4	121
20	H	<i>Vigna marina</i>	C	2	3	4	5	46
		DIVERSITY INDEX (H')		??	0.94	2.17	2.33	2.70

*T=tree, H=herb, S=shrub; **I=inland, C=coast

- The Shannon-Wiener diversity index (H') as in Table 1 is calculated using the formula below.

$$H' = - \sum_{i=1}^n (p_i \ln p_i)$$

where : H' = diversity index
 p_i = the proportion of the i^{th} species
 $\ln p_i$ = the natural logarithm of p_i
 n = the number of species in the community

Question 2.1. (3 points). The diversity index for vegetation on Anak Krakatau in 1934 was:

- A. 0.70
- B. 0.83
- C. 0.95
- D. 1.40
- E. 1.73

- The similarity between two communities is often expressed using indices, such as the following Sørensen similarity index:

$$\beta = \frac{2c}{S_1 + S_2}$$

where : β = similarity index
 c = number of species common to both communities
 S_1 = number of species in community 1
 S_2 = number of species in community 2
 β values range from 0 (no species overlap) to 1 (complete overlap)

Question 2.2. (5 points) Calculate the similarity between pairs of observation years of Anak Krakatau vegetation using the Sørensen index. Record your results in the table in the **Answer Sheet**.

Question 2.3. (2 points) Using data from Question 2.2, the vegetation on Anak Krakatau was most different between observations conducted in the years:

- A. 1934 and 1949
- B. 1949 and 1963
- C. 1963 and 1979
- D. 1979 and 1991
- E. 1949 and 1991

Question 2.4. (4 points) Indicate, using the data above, whether the following statements are true or false.

- A. Plant species richness at a particular observation year is always higher than the previous observation year.
- B. Vegetation in 1979 shows lower species evenness compared to 1963 (evenness is defined as H' divided by the natural logarithm of the number of species).
- C. On Anak Krakatau, the first pioneer plant species were dispersed by sea.
- D. On this island, the first pioneer plant species were woody plants.

Question 2.5. (6 points) Using the data from Table 1, plot the number of species colonizing the coastal and inland areas of Anak Krakatau against time (in years) in the graph provided in the **Answer Sheet**.

Question 2.6 (2 points) Based on your graph, state whether the following statements are true or false.

- A. The coastal colonization of Anak Krakatau stabilized after 45 years.
- B. The inland colonization of the island occurred 15-30 years after that of the coast.

Part B: Dispersal Biology of *Ficus*

The size of a plant's fruit and seed is related to its ability to disperse and colonize new sites. Three different species of fig (*Ficus*) are found on Anak Krakatau, i.e., *Ficus hispida*, *Ficus septica* and *Ficus variegata* (Table 2).

Table 2. Description of fruits of *Ficus* species found on Anak Krakatau

Species	Fruit Description
<i>Ficus hispida</i>	having the form of a pear, greenish yellow, diameter 24-35 mm.
<i>Ficus septica</i>	globular, light green, longitudinally ribbed with many pole warts, diameter 15-25 mm.
<i>Ficus variegata</i>	globular, red with white lines and blotches, or yellowish green, diameter 20-25 mm

- On your table, you will find a bag containing a mixture of 30 *Ficus* “fruits” (*they are not actual fruits, but are acrylic chips that are representative of the actual form and size of the original fruits*). Each chip has a serial number printed on it.
- Observe the morphology and color of the fruits and separate them according to species.
- Using the Vernier caliper provided, measure the diameter of each fruit, i.e., the widest part of the fruit, as in the example below. Record your measurements in millimeters (Precision: two places after the decimal point).

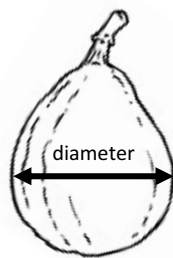


Figure 2. Example of fruit diameter measurement at the widest part.

Question 2.7. (15 points) Enter your measurements of all fruits into the table in the **Answer Sheet**. Be sure to record your data in the appropriate species column and according to the serial number printed on each fruit. After all fruits have been measured, calculate the average diameter and standard deviation for each species.

Question 2.8. (8 points) Using your data from Question 2.7, draw a bar graph, to illustrate the size comparison among fruits of the three species. You may use the color pencils provided.

Fig fruits are highly variable and are consumed by almost all taxa of terrestrial vertebrates, such as birds and bats. Birds have a relatively narrow gape, lack teeth, have tetrachromatic vision and a poor sense of smell. Bats have teeth, eat fruit piecemeal, are mostly nocturnal, and have a keen sense of smell. Birds usually prefer seeds/fruits with a size of around 11.3 mm, while bats can eat fruits greater than 50 mm in diameter.

Question 2.9. (4 points) Indicate whether the following statements are true or false.

- A. Bats will choose fruit colors such as green, yellow, pale, orange or brown, i.e., colors that do not contrast with its surroundings.
- B. Birds eat *Ficus benjamina*, another species with red fruits that are 9.8 mm in diameter.
- C. Based on fruit size, *Ficus hispida* is more likely to rely on bats for its dispersal than *Ficus septica*.
- D. Birds will eat *Ficus variegata*.

Using a magnifying glass, observe the sample of *Ficus* fruit on your table. This 'fruit' is actually a unique structure called syconium, which has many flowers on its inner surface. The syconium is pollinated by specialized fig wasps, which enter and pollinate the flowers as they lay eggs in the ovules. The syconium is essential for wasp larvae development and their eventual mating.

Question 2.10. (3 points) Based on the above information and observation, indicate if each of the following statements is true or false.

- A. The *Ficus* syconium contains both male and female flowers.
- B. The interaction between *Ficus* and fig wasps is not mutualistic.
- C. The relationship between these two species is an example of coevolution.

Task 3. Speciation in Songbirds (31 points)

Introduction

Songbirds communicate to other animals using clear sound or vocal signals, which are important cues for both intra- and interspecific recognition.

An ornithologist has been studying several songbirds in the tropical forests of Indonesia and uses song variations to investigate bird speciation. He analyses all songs using spectrograms (sonograms) and oscillograms, which are produced by an instrument called a sound spectrograph (sonograph). A spectrogram, which is a plot of sound frequency (pitch) on the vertical axis against time on the horizontal axis, reveals (1) the frequency range and (2) the duration of the song. The oscillogram plot is the variation in amplitude (loudness) against time.

Bird sounds typically consist of: **call** (short and simple) and **song** (long and complex). In addition to a repertoire ("collection") of song types, birds also produce pure tonal or whistled sounds that can be modulated/varied in both frequency and amplitude. A typical spectrogram and oscillogram, with explanation is given below:

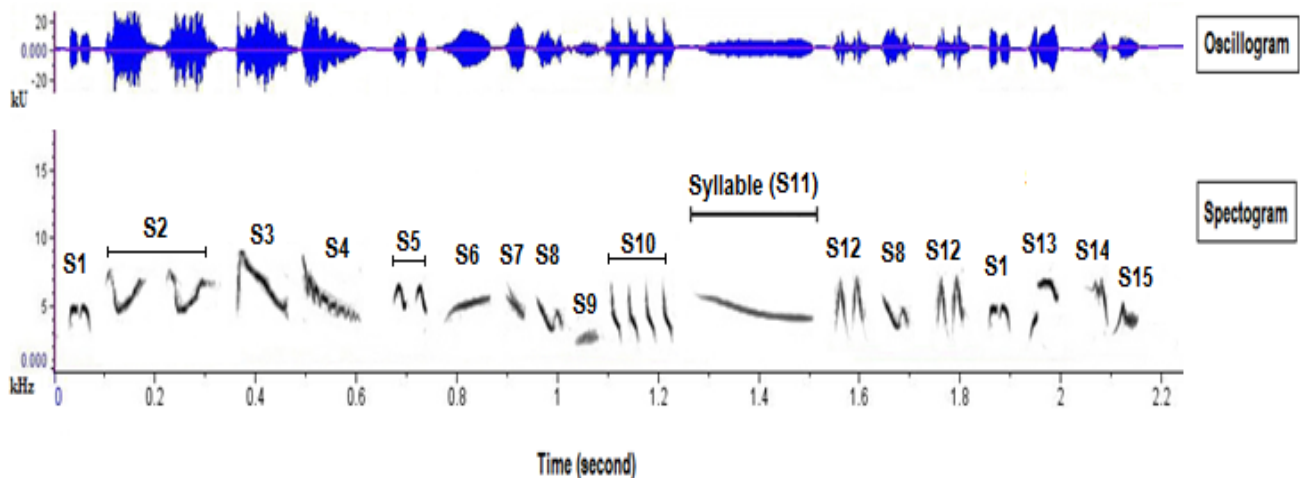


Figure 3. Spectrogram and oscillogram of different syllables from a songbird's repertoire.

- Syllable (S): One or more continuous traces, consisting of one, two or three elements, which appear together in a sequence
- Syllables can be distinguished according to the syllable's frequency range
- Syllable Repertoire (SR): number of different syllables in a song (Fig. 3, $\sum SR = 15$)

You have been provided with the spectrograms of nine individual songbirds. Your task is to analyze all the spectrograms using the terminologies described above, and identify different groups of songbirds. You have also been given an MP3 player and ear phone to listen to the recorded songbirds (see Appendix B if needed).

Question 3.1. (1 points) Which of the syllable types below are unmodulated frequency traces?



- A. 1
- B. 2
- C. 3
- D. 4
- E. 5

Question 3.2. (18 points) Count the number of different syllables for each song from the nine spectrograms provided and enter them into the table in the **Answer Sheet**. Different syllables in a song can be more clearly distinguished by comparing between the oscillogram and the spectrogram.

Question 3.3. (3 points) Assign the individual songbirds to three putative species groups A, B and C, based on the closeness of their syllable repertoires as well as their characteristic spectrograms.

Question 3.4. (8 points) Compare the syllable repertoire of the two species groups that have the highest and lowest numbers of syllables, identified in Question 3.3, using the two-sample t-test with 95% confidence level (Appendix A). Tabulate the results of your analysis in the **Answer Sheet**.

Question 3.5. (1 points) Assuming that difference in syllable repertoires implies different species, indicate which of the following statements are correct.

- A. The two groups belong to the same species.
- B. The two groups belong to two different species.

APPENDIX A: Student t-test

Statistical analysis by Student t-test can be used to compare between two groups. The calculated value is interpreted according to the t-table value at the significance level (i.e., if the calculated value \geq table value, the null hypothesis is rejected and it can be concluded that the difference is greater than what would be expected by chance; or if the calculated value $<$ table value, the null hypothesis is accepted and it can be concluded that the observed difference could have been the result of chance).

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

\bar{X}_1 = mean of sample 1

\bar{X}_2 = mean of sample 2

n_1 = number of subjects in sample 1

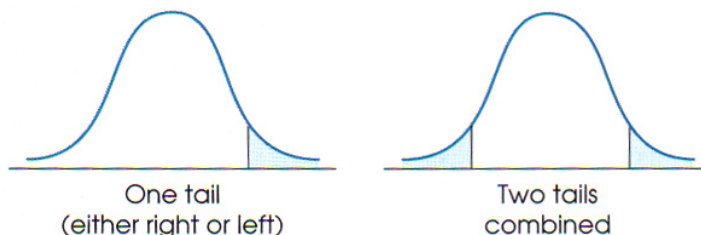
n_2 = number of subjects in sample 2

S_1^2 = variance of sample 1 = $\frac{\sum (x_1 - \bar{X}_1)^2}{n_1}$

S_2^2 = variance of sample 2 = $\frac{\sum (x_2 - \bar{X}_2)^2}{n_2}$

Statistical Tables Table t - distribution

Table entries are values of t corresponding to proportions in one tail or in two tails combined



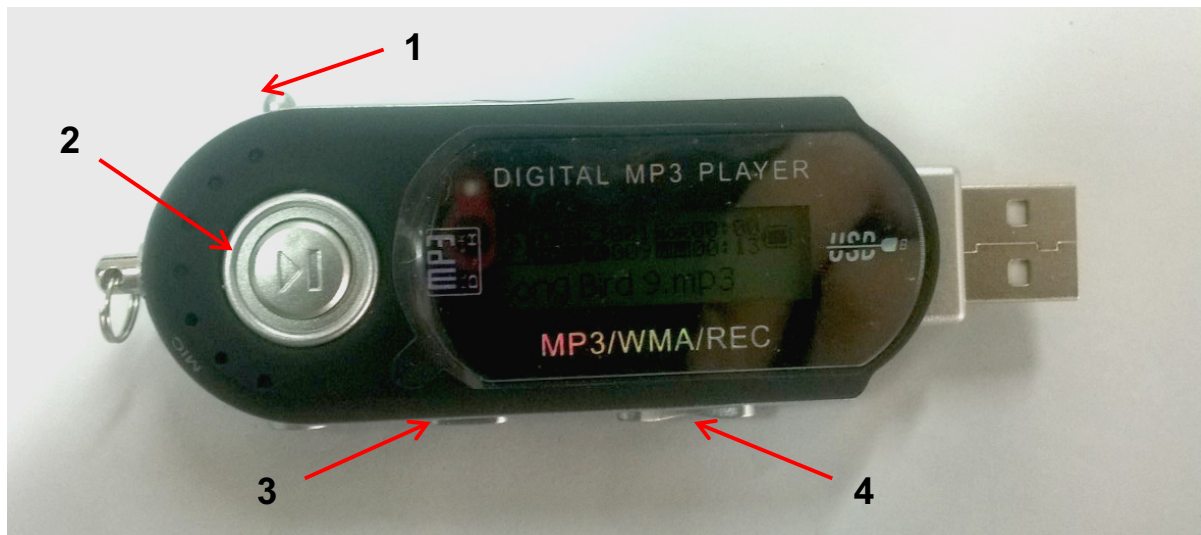
t Table

cum. prob	<i>t</i> _{.50}	<i>t</i> _{.75}	<i>t</i> _{.80}	<i>t</i> _{.85}	<i>t</i> _{.90}	<i>t</i> _{.95}	<i>t</i> _{.975}	<i>t</i> _{.99}	<i>t</i> _{.995}	<i>t</i> _{.999}	<i>t</i> _{.9995}
one-tail	0.50	0.25	0.20	0.15	0.10	0.05	0.025	0.01	0.005	0.001	0.0005
two-tails	1.00	0.50	0.40	0.30	0.20	0.10	0.05	0.02	0.01	0.002	0.001
df											
1	0.000	1.000	1.376	1.963	3.078	6.314	12.71	31.82	63.66	318.31	636.62
2	0.000	0.816	1.061	1.386	1.886	2.920	4.303	6.965	9.925	22.327	31.599
3	0.000	0.765	0.978	1.250	1.638	2.353	3.182	4.541	5.841	10.215	12.924
4	0.000	0.741	0.941	1.190	1.533	2.132	2.776	3.747	4.604	7.173	8.610
5	0.000	0.727	0.920	1.156	1.476	2.015	2.571	3.365	4.032	5.893	6.869
6	0.000	0.718	0.906	1.134	1.440	1.943	2.447	3.143	3.707	5.208	5.959
7	0.000	0.711	0.896	1.119	1.415	1.895	2.365	2.998	3.499	4.785	5.408
8	0.000	0.706	0.889	1.108	1.397	1.860	2.306	2.896	3.355	4.501	5.041
9	0.000	0.703	0.883	1.100	1.383	1.833	2.262	2.821	3.250	4.297	4.781
10	0.000	0.700	0.879	1.093	1.372	1.812	2.228	2.764	3.169	4.144	4.587
11	0.000	0.697	0.876	1.088	1.363	1.796	2.201	2.718	3.106	4.025	4.437
12	0.000	0.695	0.873	1.083	1.356	1.782	2.179	2.681	3.055	3.930	4.318
13	0.000	0.694	0.870	1.079	1.350	1.771	2.160	2.650	3.012	3.852	4.221
14	0.000	0.692	0.868	1.076	1.345	1.761	2.145	2.624	2.977	3.787	4.140
15	0.000	0.691	0.866	1.074	1.341	1.753	2.131	2.602	2.947	3.733	4.073
16	0.000	0.690	0.865	1.071	1.337	1.746	2.120	2.583	2.921	3.686	4.015
17	0.000	0.689	0.863	1.069	1.333	1.740	2.110	2.567	2.898	3.646	3.965
18	0.000	0.688	0.862	1.067	1.330	1.734	2.101	2.552	2.878	3.610	3.922
19	0.000	0.688	0.861	1.066	1.328	1.729	2.093	2.539	2.861	3.579	3.883
20	0.000	0.687	0.860	1.064	1.325	1.725	2.086	2.528	2.845	3.552	3.850
21	0.000	0.686	0.859	1.063	1.323	1.721	2.080	2.518	2.831	3.527	3.819
22	0.000	0.686	0.858	1.061	1.321	1.717	2.074	2.508	2.819	3.505	3.792
23	0.000	0.685	0.858	1.060	1.319	1.714	2.069	2.500	2.807	3.485	3.768
24	0.000	0.685	0.857	1.059	1.318	1.711	2.064	2.492	2.797	3.467	3.745
25	0.000	0.684	0.856	1.058	1.316	1.708	2.060	2.485	2.787	3.450	3.725
26	0.000	0.684	0.856	1.058	1.315	1.706	2.056	2.479	2.779	3.435	3.707
27	0.000	0.684	0.855	1.057	1.314	1.703	2.052	2.473	2.771	3.421	3.690
28	0.000	0.683	0.855	1.056	1.313	1.701	2.048	2.467	2.763	3.408	3.674
29	0.000	0.683	0.854	1.055	1.311	1.699	2.045	2.462	2.756	3.396	3.659
30	0.000	0.683	0.854	1.055	1.310	1.697	2.042	2.457	2.750	3.385	3.646
40	0.000	0.681	0.851	1.050	1.303	1.684	2.021	2.423	2.704	3.307	3.551
60	0.000	0.679	0.848	1.045	1.296	1.671	2.000	2.390	2.660	3.232	3.460
80	0.000	0.678	0.846	1.043	1.292	1.664	1.990	2.374	2.639	3.195	3.416
100	0.000	0.677	0.845	1.042	1.290	1.660	1.984	2.364	2.626	3.174	3.390
1000	0.000	0.675	0.842	1.037	1.282	1.646	1.962	2.330	2.581	3.098	3.300
Z	0.000	0.674	0.842	1.036	1.282	1.645	1.960	2.326	2.576	3.090	3.291
	0%	50%	60%	70%	80%	90%	95%	98%	99%	99.8%	99.9%
	Confidence Level										

APPENDIX B: MP3 Player User Guide

On your MP3 player, you will find 4 buttons which are: mode button, play button, hold button and volume button (see Figure A).

- The mode button (1) can be used to go forward to the next song (by turning the button right) or backward to the previous song (by turning the button left). This mode button can also be used as an OK button to choose the selection in the root menu by pressing down.
- The play button (2) is used to play a song or to pause a song. This button is also used to turn the MP3 player on/off.
- The hold button (3) is used to freeze this MP3 player so no button will be responsive as long as the hold button is on the left position.
- The volume button (4) is used to raise the volume (the + button) or to reduce the volume (the – button)



To play a song, first turn on your MP3 player (by pressing the play button more than 3 seconds), then select music (the musical note icon, spelled MSC) from the main menu (Figure B) then press the mode button on the MP3 player's upper left corner. Here you will find your songbird's song, with each song title numbered according to the respective sonogram. Push the play button to play the song. Turn the mode button right for going forward to the next song, or turn left for going backward to the previous song. You can also hold the button right to fast-forward the song you are listening to and hold left for reversing backward. On the screen upper right corner, you can see the time and duration of the song.



Figure B. MP3 Main Menu