INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

IBO CHALLENGE II

32nd INTERNATIONAL BIOLOGY OLYMPIAD

Lisbon, PORTUGAL - July 19th, 2021

THEORETICAL-PRACTICAL TEST

(Time available: 180 minutes)

- This test consists of eight parts
- 1 point each correct answer
- Maximum score: 170 points
- Only answers marked in the ANSWER SHEET will be validated

GENERAL INSTRUCTIONS

1. Your exam is composed of eight parts, some of them with multiple sections to accommodate different subjects.
2. Some questions involve calculations, and the students are allowed to use basic calculators.
3. Your exam is composed of four types of questions:
   - Multiple choice questions
   - True / False questions
   - Matching items questions
   - Fill in the spaces questions
4. The students are not supposed to use words to answer the questions, only codes of letters placed in the appropriate locations.
   - Multiple choice questions should be answered by selecting the correct answer on the Answer Sheet.
   - True / false questions should be answered by selecting either a “T” for true or an “F” for false, on the Answer Sheet.
   - Matching items questions and fill in the spaces questions should be answered on the Answer Sheet by selecting the appropriate letter in the key provided in each case.

ENJOY AND HAVE FUN!!!

Introduction and context

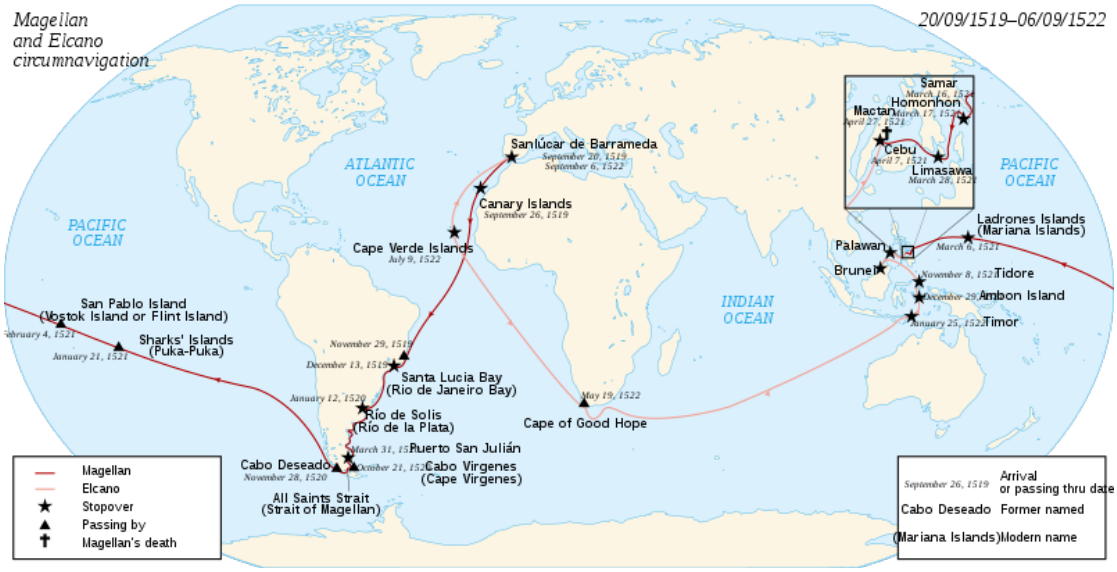
The IBO Challenge II – IBO 2021 is associated with the commemorations of the 500th anniversary (in 2019) of the first voyage around the Globe, initiated by Fernão de Magalhães.

Fernão de Magalhães (Ferdinand Magellan), in a 16th/17th century anonymous portrait.

Fernão de Magalhães (1480-1521), also known as Fernando de Magallanes (Spanish) or Ferdinand Magellan (English) was a Portuguese-born experienced sailor who planned, organized and led a naval expedition to the East Indies by following a western route. That voyage was financed and done on behalf of the Spanish king Charles I, the future Emperor of the Holy Roman Empire and father of the Spanish King Philip II (who would become Philip I of Portugal).

The purpose of the journey was to reach the “Spice Islands”, the Moluccas, located in Southeast Asia in the transition between the Indian and the Pacific Oceans, and to open a new trade route that would not collide with the rights of the Portuguese to control the East route through the Atlantic and the Indian Oceans. In fact, as a result of the Tordesillas Treaty the Moluccas belonged to the Spanish part of the world, but as the eastern route was dominated by the Portuguese, the Spanish could only reach the Moluccas by sailing west.

An armada of five ships commanded by Fernão de Magalhães left Sanlúcar de Barrameda, in the Gulf of Cadis, Spain, on 20 September 1519 and travelled Southwest through the Atlantic towards the eastern coast of South America down to Patagonia. Once there, the fleet searched for a passage to the Pacific Ocean, a passage that even today bears Magellan's name. Reaching the Pacific Ocean, Magellan sailed in the direction of the Philippine Islands where, on 27 April 1521, during a battle in the Island of Mactan, he was killed by the natives.



Source: https://upload.wikimedia.org/wikipedia/commons/thumb/a/ab/Magellan_Elcano_Circumnavigation-en.svg/800px-Magellan_Elcano_Circumnavigation-en.svg.png

The journey continued and, after reaching the Moluccas, in November 1521, and having laden the ships with spices, Juan Sebastián Elcano, in command of the Victoria, the only surviving ship, returned to Spain on 8 of September 1522 through the Indian Ocean and up the Atlantic coast of Africa, completing what turned out to be the first circumnavigation of the Earth.

This **IBO Challenge II** test is based on the **journey of Fernão de Magalhães** since the departure from the Gulf of Cadis to the Philippines. Along the way, the students will have to answer some questions and solve some problems that are posed in association to locations that represent some selected stopovers of the Magellan's armada along its journey. Each stopover corresponds to one of the eight parts that compose the present test. **ALL ABOARD!**

Part 1
The departure – The Gulf of Cadis I

Subject: Cell Biology and Biotechnology

Theme: Plant adaptations to biotic and abiotic factors

Time proposed: 20 min

Introduction

Plants interact with the environment using several mechanisms, either against biotic and abiotic detrimental factors, and/or by attracting pollinators and animals for seed dispersal. In Mediterranean climates, plants reveal several adaptations that allow them to succeed and thrive in these dry and hot habitats. Certain characteristics will provide protection for excessive radiation and UV light.

Section I - Observe the micrographs A and B in **Figure 1,** below.

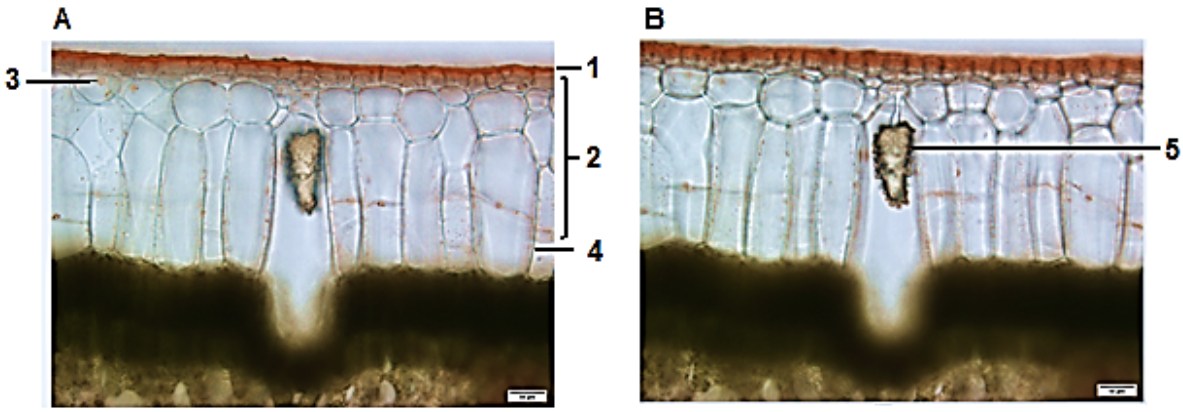


Figure 1 - Two focusing planes of a transversal leaf section observed under light microscopy. The section was stained with Sudan red IV, a fat-soluble dye that stained in red the general lipids. Scale bar represents 50 μm.

1. Use the key below (**options A to J**) to complete the legend of **Figure 1** (**numbers 1 to 5**). Notice that the number of options in the key exceeds the number of correct answers. **[5 points, 1 point each correct answer]**

KEY	
A	Stratified columnar epithelium
B	Plant epidermis
C	Cell membrane
D	Cell wall
E	Intercellular space
F	Chloroplast
G	Cuticle
H	Nucleus
I	Cell Wall modification
J	Vacuole with crystal content

2. In Figure 1A the structure marked 1 represents [**choose the correct option**]. **[1 point]**

a) Secondary cellulosic cell wall

b) Lignified secondary cell wall

c) Suberized secondary cell wall

d) Cutinized cell wall

3. Concerning the cells represented in Figure 1A and 1B, classify as true (T) or false (F) each of the sentences below **[4 points, 1 point each correct answer]**

a) The top layer has a protective function, isolating the cells from the environment

b) All the cells in the transparent layer have no intercellular spaces

c) All the cells in the transparent layer have the same function

d) Cell lengths vary between 25 µm and 300 µm

4. The video associated to this question shows the changes that occur when a microscope slide with leaf sections similar to those presented above is irrigated with a HCl solution that replaces the water of the mounting medium. The event is accelerated 3x to shorten the duration of the time reaction. [video]

a) Based on the changes that occurred, what would be the composition (chemical nature) of the structure that "disappears"? [**choose the correct option**] **[1 point]**

a) Calcium oxalate
b) Calcium carbonate
c) Silica aggregates
d) Cellulose fibers
e) Lignin deposition
f) Lipids
g) Proteins

b) What is the cell location of the aforementioned structure? [**choose the correct option**] **[1 point]**

a) Vacuole
b) Cell wall
c) Cytoplasm
d) Cell membrane

Section II - Observe the micrographs A and B in **Figure 2,** below.

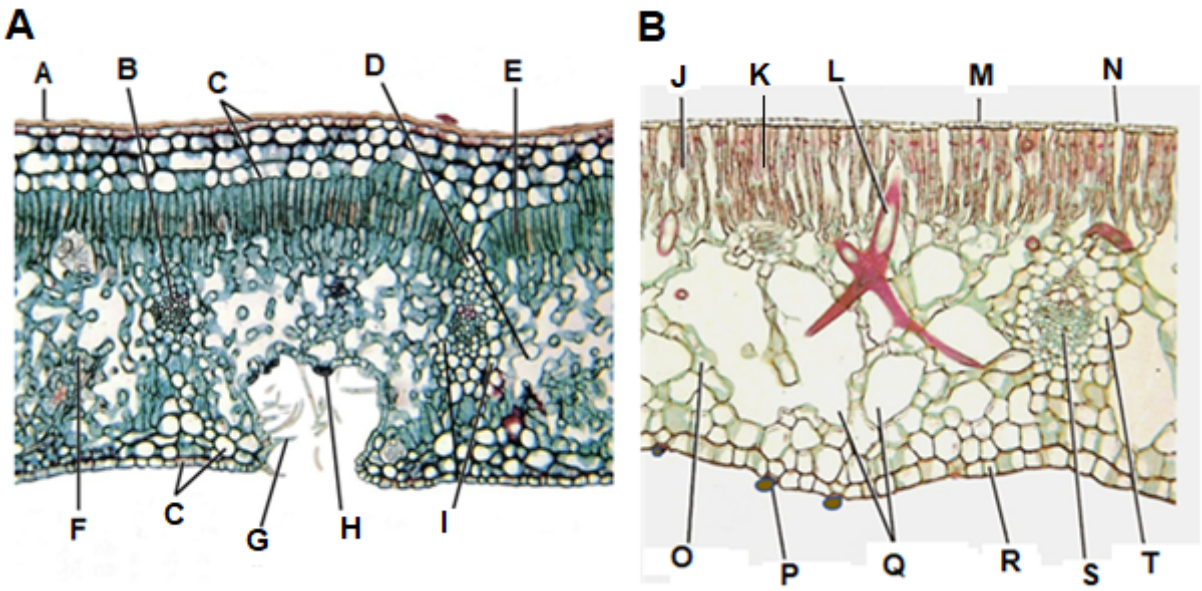


Figure 2 - Transverse sections of leaves from two distinct species.

5. Use the key below (**options 1 to 20**) to choose in the optional term that would best designate each of the labelled structures and complete the figure captions Figure 2 (**letters A to T**).Note: Not all terms are necessarily used and some might be used more than once. [**20 points, 1 point each correct answer]**

KEY	
1	Stomata
2	Glandular trichome (glandular hair)
3	Ab axial epidermis
4	Palisade parenchyma
5	Branched sclereid (Astrosclereid)
6	Bundle sheath
7	Spongy parenchyma
8	Cuticle
9	Lacunae
10	Sclerenchyma
11	Multiple epidermis
12	Chlorenchyma
13	Intercellular space
14	Metaxylem
15	Covering trichome (non-glandular trichome)
16	Crystal
17	Vascular bundle
18	Adaxial epidermis
19	Cambium
20	Resin duct

6. Still considering **Figure 2**, classify each of the following statements as true (T) or false (F).

a) Concerning image A [4 points, 1 point each correct answer]

a) The palisade cells are perpendicularly elongated to the leaf surface

b) The mesophyll pattern organization is characteristic of an isobilateral leaf

c) The vascular bundles are double open and collateral

d) The leaf exhibits anatomical features that can be interpreted as adaptations to habitats with low water availability and high light intensity

b) Concerning image B [4 points, 1 point each correct answer]

a) The mesophyll pattern organization is characteristic of a dorsiventral leaf

b) The sclerenchyma is poorly developed

c) In what concerns the stomata position, the leaf is epistomatic

d) The leaf anatomy suggests that the species is a hydrophyte with submerged leaves

Section III - The effects of environmental factors on leaf morphology and anatomy have produced a wide variety of adaptations in different taxa. Adaptations enable plants to survive in adverse conditions. Observe carefully **Figure 3**, below.

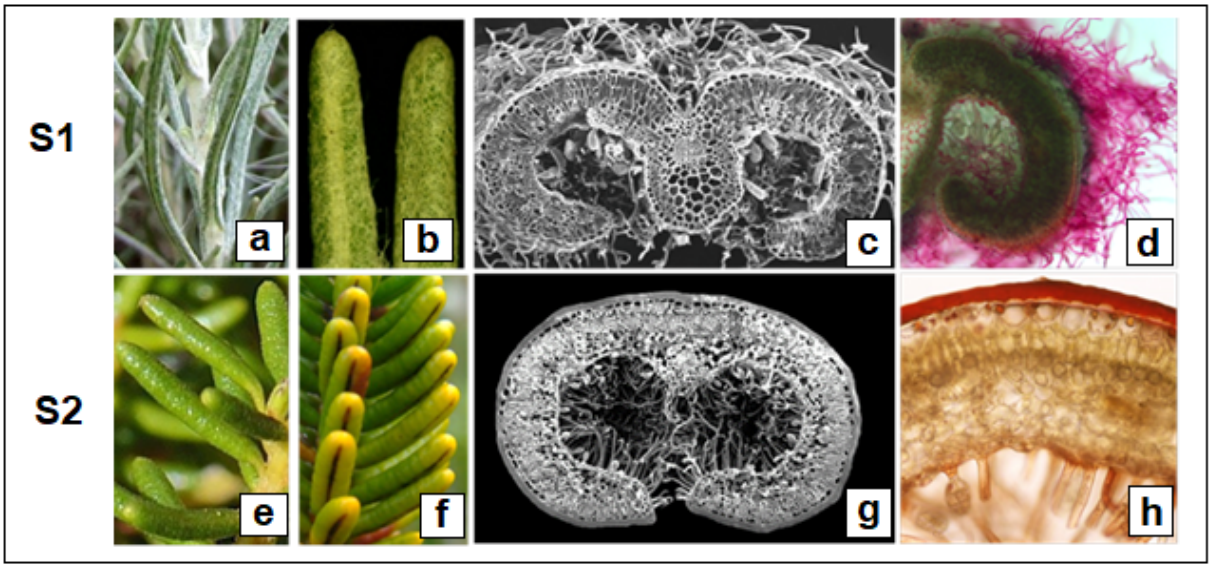


Figure 3 - Features of the leaves of two distinct species S1 and S2. **a, b** and **e, f** are macrographs of the adaxial and abaxial leaf surface; **c** and **g** are SEM micrographs of leaf cross sections; **d** and **h** are light micrographs of leaf cross sections stained with Ruthenium Red (d) and Sudan IV (h) to identify cell compounds through histochemical techniques. Ruthenium Red stained pectins, highly hydrophilic compounds well known by their ability to form gels with water, with a pink color; and Sudan IV-stained red general lipids.

7. Considering **Figure 3, classify each of the statements in the following items as true (T) or false (F).**

a) Concerning the images of both species **[4 points, 1 point each correct answer]**

a) The leaves exhibit a mesophytic structure characteristic of species that grow in temperate zones with average or optimal water availability

b) The leaves show features that are often associated with water plants (hydrophytes)

c) The leaves present features that are characteristic of plants adapted to dry or seasonally dry habitats

d) The leaves possess a typical structure of plants living on forest floors

b) Which leaf features help species to deal with the environment stress factors in the respective habitats? [4 points, 1 point each correct answer]

a) Leaves with a cylindrical outline and prominent enrolled margins

b) Leaves with flattened blades and thin cuticles

c) Stomata confined to hair-lined grooves

d) A high leaf surface/volume ratio

c) The hairs covering the leaves of S1 may have the following functions: [4 points, 1 point each correct answer]

a) Increase light absorption

b) Regulate leaf internal temperature

c) Increase the diffusion of gases across the leaf and air interface

d) Reduce predation by insects and herbivores

d) According with the results of the histochemical tests performed (images d and h) [4 points, 1 point each correct answer]

a) The grey-whitish appearance of S1 leaves is due to the non-glandular trichomes (hairs) that covering the leaf surface absorb the visible light

b) The smooth, long, and flexuous non-glandular trichomes of S1 leaves can entrap and retain dew and fog water

c) The glossy appearance of S2 leaves is due to the thick hydrophobic cuticular layer that reflects the light as a mirror

d) The cutinized upper epidermal cells of S2 leaves reduce the evapotranspiration water loss

Part 2
The departure – The Gulf of Cadis II

Subject: Ecology

Theme: The passage of fire over the soil

Time proposed: 30 min

Introduction

The Mediterranean basin is a human made ecosystem traditionally shaped by agriculture and fire. Fire affects ecosystems in many and complex ways, but several scientists have highlighted the need to assess the fire impact on soil biodiversity and functionality as a driving force of post-fire land degradation independently from that of the removal of vegetation, soil roughness and other fire-induced changes. Many wildfire events leave behind a mosaic of burned and unburned areas that can be used to study the fire effects on soil.

Section I - To assess the effect of fire on soil properties, 10 soil samples were randomly collected from each plot and immediately mixed to obtain a representative and homogeneous sample of each of the 5 burned and 5 unburned plots. Samples (± 1 kg each) were collected at 0 -20 cm depth, after removing the litter layer. Samples were packed in open plastic bags, kept at 4°C, and sieved through a 2 mm sieve to remove the coarse fragments (stones, roots, etc.). Afterwards, each sample was divided into 3 aliquots.

- The first aliquot was air-dried to constant weight for determination of pH, electrical conductivity (EC), cation exchange capacity (CEC), and total organic carbon (C_{org}) content.
- The second aliquot was stored at 4 $^{\circ}$C for the assessment of water content (to express microbial variables to dry weight), total microbial biomass carbon (C_{mic}), and fungal mycelium (FM) content, and microbial respiration.
- The third aliquot was stored at -20 $^{\circ}$C for DNA extraction and protein analysis.

Table 1 - Results obtained for several parameters in unburned and burned soils

Variable	Soil before fire mean (± sd)	Soil 2 weeks after fire mean (± sd)
pH	6.0 (± 0.3)	6.8 (± 0.2)
EC (dS m^{-1})	0.30 (± 0.06)	0.50 (± 0.06)
CEC (cmol kg^{-1} dw)	38.0 (± 3.3)	26.1 (± 9.6)
Corg (g kg^{-1} dw)	98.0 (± 10.2)	86.0 (± 2.7)
Cmic (mg g^{-1} dw)	1.2 (± 0.8)	1.9 (± 1.2)
FM (mg g^{-1} dw)	1.1 (± 0.3)	2.9 (± 0.3)
Respiration (mg CO_2-C g^{-1} dw d^{-1})	0.15 (± 0.01)	0.15 (± 0.03)

dw = dry weight - sd = standard deviation

1. Why is it necessary to determine the soil water content of each sample? **Classify each of the following statements as true (T) or false (F). [4 points, 1 point each correct answer]**

   a) To express the results in terms of soil dry weight (dw) avoiding the water weight which varies among samples
   b) Because it is impossible to determine some parameters in wet soil
   c) To evaluate the percentage of living organisms that died with fire
   d) To ass ess if the fire was very intense

2. After the fire, why does the electrical conductivity of the soil increase? **Classify each of the following statements as true (T) or false (F). [4 points, 1 point each correct answer]**

   a) Due to increased content of ions in the soil solution
   b) Due to decreased content of ions in the soil solution
   c) Due to the decrease in the soil cation exchange capacity
   d) Due to the dead of soil organisms including microbes

3. Which hypotheses are supported by the results presented in the table? **Classify each of the following statements as true (T) or false (F). [4 points, 1 point each correct answer]**

   a) The microbial community of the soil may increase after a fire

   b) The soil respiration and soil biotic activity increase after the fire

   c) The fire contributes to the loss of organic matter in the soil

   d) The fire is a cause of soil acidification

4. The observed increment in fungal mycelium after fire… **Classify each of the following statements as true (T) or false (F). [4 points, 1 point each correct answer]**

   a) Cannot justify the observed decrease in soil organic carbon after fire

   b) May result from the proliferation of certain fungal species able to grow fast when nutrients are available

   c) Contributes to an increase in soil electrical conductivity

   d) May represent a substantial contribution for the observed increment in the soil microbial carbon

5. What does the soil organic carbon include? **Classify each of the following statements as true (T) or false (F). [4 points, 1 point each correct answer]**

   a) The soil organic matter, as well as the living biomass

   b) The bacterial biomass, but not the fungal one

   c) All the carbon in the soil

   d) The carbon fraction that can be used by plants

Section II - People often realize that after a fire, water lines change their path across fields. Several factors contribute to these changes. One of them is soil hydrophobicity, which can be understood as the soil's water repellency. This phenomenon is associated with the covering of soil particles by hydrophobic organic substances, and there are currently numerous reports of water repellency in areas with burnt soils. Fungi such as *Fusarium graminarum* or *Penicillium nigricans* can release hydrophobic organic substances. The hydrophobicity of the mycelium is determined by low molecular weight

proteins that are unique to fungi and are excreted in monomora/monomers that acquire their final conformation by self-assembly – the hydrophobins.

In the following experiment, soil hydrophobicity was determined using the angle between the water drop and the soil surface, as represented in the **Figure1**.

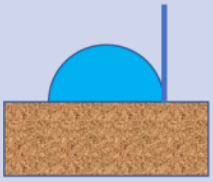
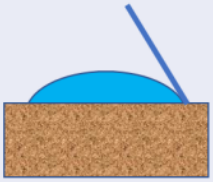
	1	2	3
A		Yes	≥ 90°
B		No	0° ≤ x ≤ 90°

Figure 1 - Schematic representation of the angle formed between the water drop and the soil surface. 1 - Schematic representation of the water drop on the soil surface; 2 - Soil water repellent; 3 - Contact between soil and water.

6. Based on figure 1, answer the following questions **by filling the space** with the letter corresponding to the correct situation (A or B). **[5 points, 1 point each correct answer]**

    a) Which soil is more hydrophobic?

    b) Which soil favours run off?

    c) Which soil favours water infiltration?

    d) In which soil do you expect to find a higher hydrophobin concentration?

    e) Which soil may belong to a burned stand?

Section III - After a fire soil water infiltration decreased by 50% and the fungal mycelium increased by 30%.

7. Based on the statement above, **classify each of the following conclusions as true (T) or false (F). [5 points, 1 point each correct answer]**

a) the proportion of *Fusarium graminarum* or *Penicillium nigricans* increased among the soil filamentous community and they are producing more hydrophobin than before fire

b) There is an increased production of hydrophobin, which contributes to an enhancement of water run off

c) There is an increased production of hydrophobin, which contributes to an enhancement of water infiltration

d) Fungal mycelium is occupying the free space left by the decrease in water infiltration

e) The decrease in water infiltration increases the run off and the risk of soil erosion

Part 3
The departure – The Gulf of Cadis III

Subject: Animal Physiology

Theme: Microplastics in aquatic insect larvae (20 min)

Time proposed: 20 min

Introduction

Five hundred years ago Ferdinand Magellan, commanding a fleet of five vessels, headed south through the Atlantic Ocean to Patagonia. Despite a series of storms and mutinies, they made it through the Strait of Magellan into a "peaceful sea" (Pacific Ocean).

If Ferdinand Magellan left today the likelihood of finding a sea of plastic particles would be enormous. In early times, microplastic contamination was associated to the sea, but nowadays we know that its origin is terrestrial and that our rivers are one of the main vehicles of these particles for our oceans…

… Thus, in this stopover, you will have the opportunity to know what microplastics cause in the larva of an insect that lives in contact with the contaminated sediments of a river in the Iberian Peninsula. It is intended that you understand the physiological responses of these organisms when they are forced to ingest inert particles due to microplastic contamination of the sediments they live in.

Context

The non-biting midge larvae *Chironomus riparius* is one of the most abundant macroinvertebrate species in freshwater benthic ecosystems. Its lifecycle presents an adult aerial period of 2-4 days for reproduction and a period for development of 14-16 days, where larvae go through 4 metamorphic stages (from L1 to L4) until reaching a pupal stage and emerge as adults. This lifecycle is represented in figure 1.

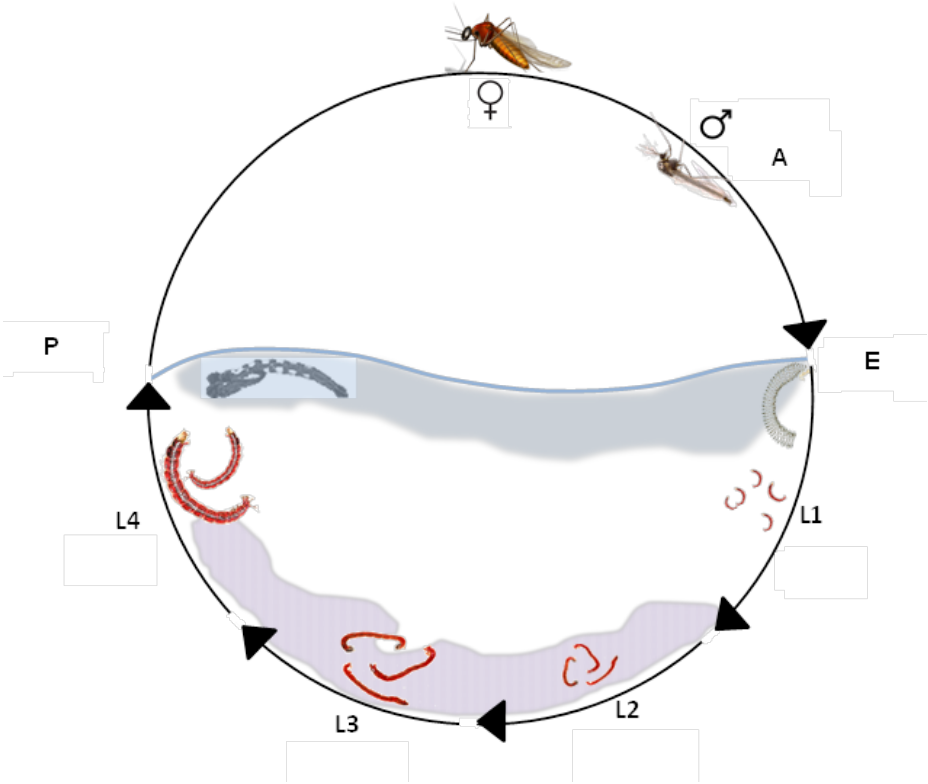


Figure 1 - Life cycle of *Chironomus riparius*. A, adult; E, egg rope; L1-L4, 1st to 4th instar larvae; P, pupa.

1. One characteristic of the life cycle of *Chironomus riparius* that allows scientists to use this species in freshwater studies concerning the effects of contaminants is: [**choose the correct option**] **[1 point]**

   a) a relatively short period developing in sediments

   b) a relatively long period of its aerial phase

   c) a relatively long period developing in sediments

   d) a relatively long-life cycle

Context

Inhabiting the uppermost layers of sediment, the larvae of *Chironomus riparius* act as deposit-feeders, feeding on sediments and particulate organic matter (detritus). Their feeding behavior is mostly non-selective determined by bioavailability. Typically, first instar (L1) larvae of *Chironomus riparius* ingest sediment particles up to 20 µm as part

of their regular feeding activity. Conversely, final instars (3rd–4th instar; L3-L4, figure 2) can ingest particles up to almost 200 µm.

Figure 2 – Schematic representation of L4 larvae of *Chironomus riparius* in freshwater sediments. (This figure was kindly provided by the project comPET).

2. Due to the feeding behaviour of L4 larvae of *Chironomus riparius*, it can be hypothesized that: [**choose the correct option**] **[1 point]**

a) The mouth apparatus of L1 enables the larvae to ingest particles up to 200 µm
b) The mouth apparatus of L4 enables the larvae to ingest particles up to 200 µm
c) L1 larvae can be a good model to determine the ingestion of particles of the size range 32-63 µm
d) L4 larvae is not a good model to determine the ingestion of particles of the size range 32-63 µm

Context

Continuous release and long-term deposition of microplastics (MPs) are the main causes of their presence and persistence in freshwater sediments, especially near highly industrialized or densely populated areas where they can reach levels up to 9 g kg^{-1} of sediment (Hurley *et al.*, 2018). Therefore, riverine sediments might be major sinks of MPs imposing a potential threat to freshwater benthic invertebrates.

Figure 3 – Photographs of L4 larvae of *Chironomus riparius* exposed 48 h to sediments containing polyethylene microplastics (PE-MPs) of the size range 32-63 µm (left photograph) and sediments without microplastics (right photograph). (The photographs were kindly provided by the project comPET).

3. The observation and comparison of larvae on both photographs is a good method to: [**choose the correct option**] **[1 point]**

a) quantify the number of PE-MPs of the size range 32–63 µm inside the gut of L1 larvae

b) quantify the number of PE-MPs of the size range 32–63 µm inside the gut of L4 larvae

c) show that L1 larvae ingested PE-MPs of the size range 32–63 µm

d) show that L4 larvae ingested PE-MPs of the size range 32–63 µm

Context

Three replicates (of 15 organisms each) per condition were used to estimate the number of MPs inside the gut of the larvae of *Chironomus riparius* exposed for 48 hours to concentrations of 0.00, 1.25, 5.00, and 20.00 g kg^{-1} dry sediment of PE-MPs of the 32-63 µm size class. Table 1 presents the results of such experiment.

Table 1 - Number of polyethylene microplastics (PE-MPs) of size-class 32-63 µm inside the gut of L4 of *Chironomus riparius* after 48 h of exposure to different concentrations of PE-MPs in the sediment.

Concentration of PE-MPs in the sediment (g Kg^{-1} sediment)	Number of ingested PE-MPs ± standard error of the mean (number/organism)
0.00	0,00 ± 00.0
1.25	525 ± 51.1
5.00	2047 ± 54.1
20.00	2389 ± 31.0

4. ~~The proportion of the number of PE-MPs inside the gut of L4 observed between the different concentrations of PE-MPs in sediment is: [**choose the correct option**] **[1 point]**~~

~~a) always the same (5/1.25 = 20/5 =2 X 20/1.25), and higher than 0~~

~~b) two times higher for 20/5 than 5/1.25~~

~~c) Almost the same when comparing 5/1.25 with 20/1.25~~

~~d) always 1~~

5. The observed proportions between the number of PE-MPs inside the gut of L4 as a function of its concentration in sediments might mean that: [**choose the correct option**] **[1 point]**

a) The volume of the gut of L4 is not finite

b) The expected number of PE-MPs of L4 for the highest concentration in sediments could be lower

c) The number of ingested PE-MPs is near its maximum value

d) None of the above

6. The number of PE-MPs inside the gut of L4 was 0 for the control condition. This is because: [**choose the correct option**] **[1 point]**

   a) L4 did not ingested PE-MPs despite their presence in sediments

   b) L4 ingested PE-MPs in control sediments, but the egestion was faster than ingestion

   c) L4 did not ingested PE-MPs since sediments of control do not have them

   d) none of the above

Context

Seven replicates (of 15 organisms each) per condition were used to estimate oxygen consumption rate of larvae of *Chironomus riparius* exposed for 48 hours to concentrations of 0.00, 1.25, 5.00, and 20.00 g kg^{-1} dry sediment of PE-MPs of the 32-63 µm size class. Briefly, samples were homogenised for 30 seconds at 4 °C. From each homogenized sample, 300 µL were collected, mixed with a detergent, centrifuged (1000 g X 10 min, 4ºC), and the resulting supernatant used for the analysis of the electron transport system (ETS) activity. The oxygen consumption was estimated by following the increased absorbance at 490 nm throughout a 3 min period and expressed as KJ $hour^{-1}$ mg^{-1} organism. The results of such experiment are presented in figure 4.

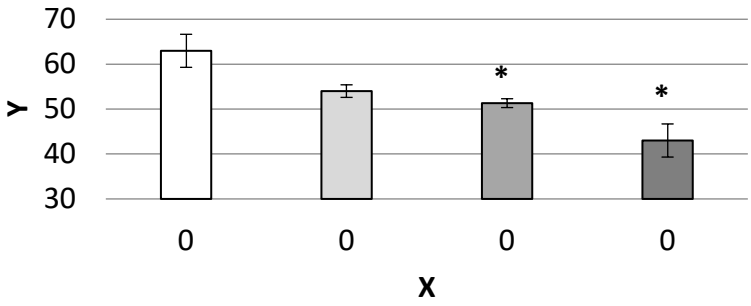


Figure 4 – Oxygen consumption rate (mJ h^{-1} mg^{-1} organism) in isolated samples of larvae of *Chironomus riparius* exposed for 48 hours to concentrations of 0.00, 1.25, 5.00, and 20.00 g kg^{-1} dry sediment of PE-MPs of the size class 32-63 µm. Axes: **X** - concentration of polyethylene microplastics (g PE-MPs/Kg sediment); **Y** - oxygen consumption rate (kJ/h/mg organism). Values are presented as mean ± standard error of the mean (n=7). *denotes a significant ($p < 0.05$) difference when compared with the control (0) treatment after one-way ANOVA followed by the post-hoc Dunnett's tests.

7. The oxygen consumption rate (Figure 4) of larvae of *Chironomus riparius*: [**choose the correct option**] **[1 point]**

   a) Was not significantly affected by the ingestion of PE-MPs
   b) Was significantly increased due to ingestion of PE-MPs
   c) Was significantly decreased due to ingestion of PE-MPs
   d) Is not shown in figure 4

8. The results obtained with larvae of *Chironomus riparius* indicate that: [**choose the correct option**] [**1 point]**

   a) The ingested PE-MPs are inert and consequently aerobic energy production might be compromised due to lack of nutrients
   b) A decreased ETS activity enables larvae to ingest less PE-MPs due to an excess of nutrients
   c) All the above options are correct
   d) All the above options are incorrect

9. The electron transport system occurs inside: [**chose the correct option**] [**1 point**]

   a) Lysosomes
   b) The nuclei
   c) The Golgi apparatus
   d) The mitochondria

10. What stage of aerobic respiration does the protocol focus on to determine oxygen consumption? [**choose the correct option**] **[1 point]**

    a) Oxidative phosphorylation
    b) Krebs cycle
    c) Glycolysis
    d) Gluconeogenesis

Part 4

The first stopover – The Canary Islands

Subject: Ecology

Theme: Cory's shearwaters: mighty travellers of the Atlantic seas

Time proposed: 20 min

Introduction

Although poorly known, Cory's shearwaters, *Calonectris borealis* (Cory, 1881), are the most emblematic seabirds in the Portuguese coastal and oceanic waters. They nest exclusively on islands, occurring in the archipelagos of the Azores, Madeira, and also in Berlengas, off the Portuguese coast.

Cory's shearwaters are formidable oceanic migrants. They are present in their breeding areas from mid-February to mid-October. They nest in burrows and rock crevices where they lay just one egg, and are unable to replace it in the same year, in case of loss. In late October, the vast majority of birds departs to the southern hemisphere, heading towards Uruguay and Brazil, and then crossing to South Africa, where they spend most of the non-breeding season in the rich waters of the Benguela current. In February, they return to the colonies for the next breeding season.

According to the International Union for Conservation of Nature (IUCN), the conservation status of Cory's shearwaters is "Least Concern", due to the relatively large populations, some of which are actually growing.

The largest known colony of Cory's shearwaters is located in the Selvagens Islands, ca. 300 km south of the island of Madeira, Portugal. In this island, with about 270 ha, there was a breeding population of shearwater that some researchers speculated to have reached 130,000 breeding pairs! However, the continued exploitation of birds for human consumption, and especially two slaughter episodes of adult birds, in 1978 and 1979 (coinciding with the creation of a Nature Reserve) decimated the population, drastically reducing the number of breeding pairs. From 1980 onwards, a biological station was set on the island, ensuring a permanent presence of nature wardens, which has allowed the recovery of the shearwater population. Since then, several studies have been undertaken on the demography and migratory ecology of this species.

Context

In 1980, the population of Cory's shearwaters was estimated at only 7000 breeding pairs (Mougin and Stahl 1982). In 2005, the population was estimated again suggesting the presence of 29,540 breeding pairs (Granadeiro *et al*. 2016).

1. Assuming that the rate of population increase has remained constant throughout the entire period; determine the annual growth rate of the population. **Perform the necessary calculations, starting from the function**:

$$N_{(t+1)} = R \times N_{(t)} \quad \Leftrightarrow \quad N_{(t)} = R^t \times N_0$$

Where:
N = Number of individuals
R = Growth rate of the population
t = time

and **choose the correct option**. **[1 point]**

a) 5.93% per year
b) 3.22% per year
c) 4.22% per year
d) 3.05% per year

2. Considering that the population growth rate has remained unchanged since 2005, and assuming a hypothetical growth rate of 4.6% per year, determine how many years it will take this colony to reach 130 000 breeding pairs. Perform the necessary calculations using the function above and **choose the correct option**. **[1 point]**

a) around 26 years (25.7 years)
b) around 33 years (32.9 years)
c) around 48 years (48.2 years)
d) around 51 years (51.4 years)

Context

During the breeding period, Cory's shearwaters feed in a vast area around the Selvagens, including the pelagic waters around the island, the seamounts and also the African coast, i.e., in very different oceanographic domains. Figure 1 portraits 3 real shearwater tracks collected when birds were foraging during the chick-rearing period, as well as the variation of temperature and bathymetric conditions during each trip.

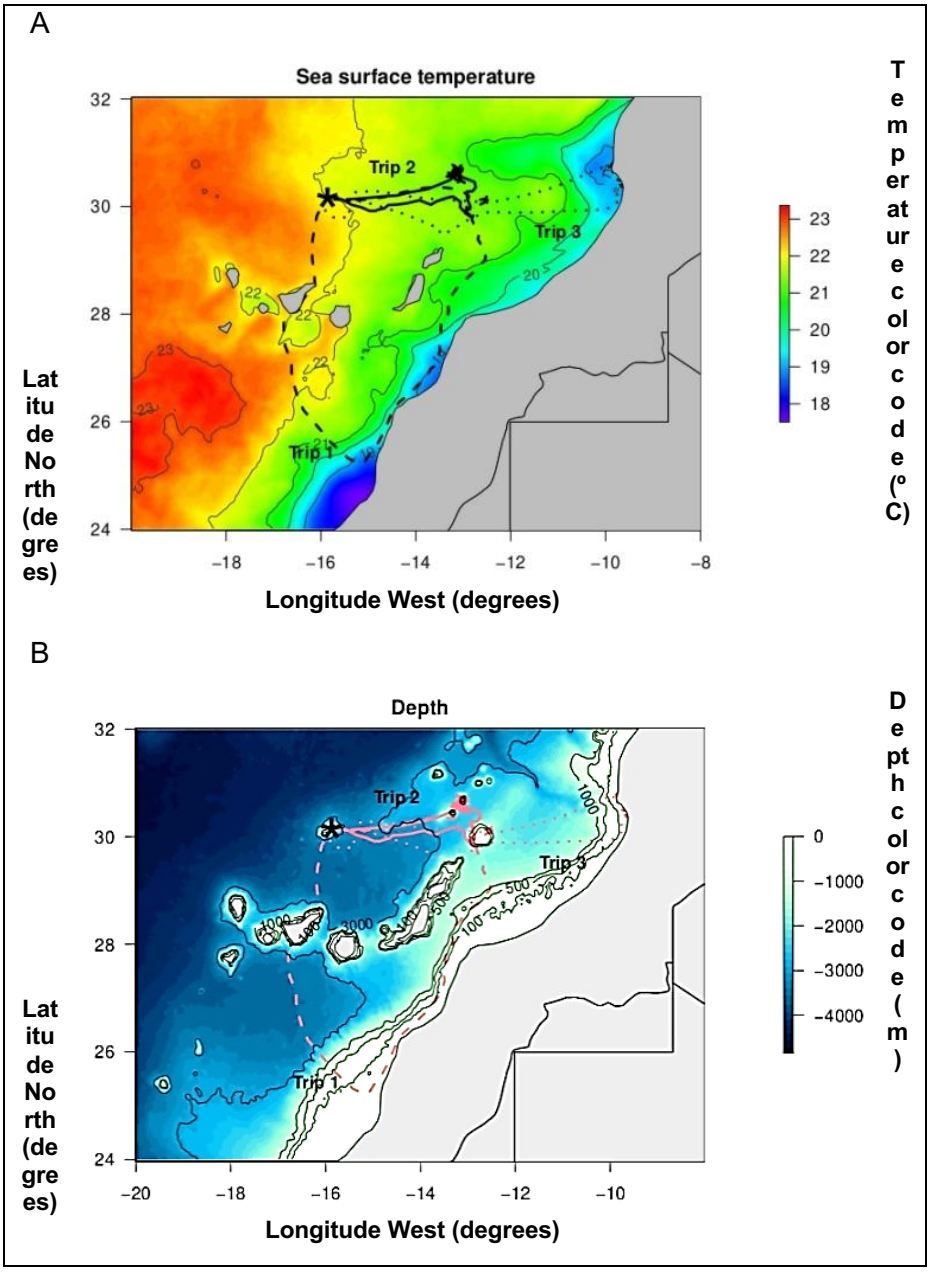


Figure 1 – Three real shearwater tracks obtained (with GPS loggers) in Selvagem Grande (the island is marked by a black asterisk), plotted over maps of sea surface temperature (A) and of depth (B), showing the variation in temperature and bathymetry experienced by shearwaters during the course of each trip. Trip 1 - dashed line, trip 2 - solid line, trip 3 - dotted line.

3. **Figure 2** shows three profiles of temperature and depth variation related with the trips displayed in **Figure 1**. Associate each of the trips (1, 2 and 3) to the respective profile of variation in temperature and depth conditions shown in figure 2 (A, B and C). [**Fill the spaces with the letters A, B or C, as appropriate**] **[3 points, 1 point each correct answer]**

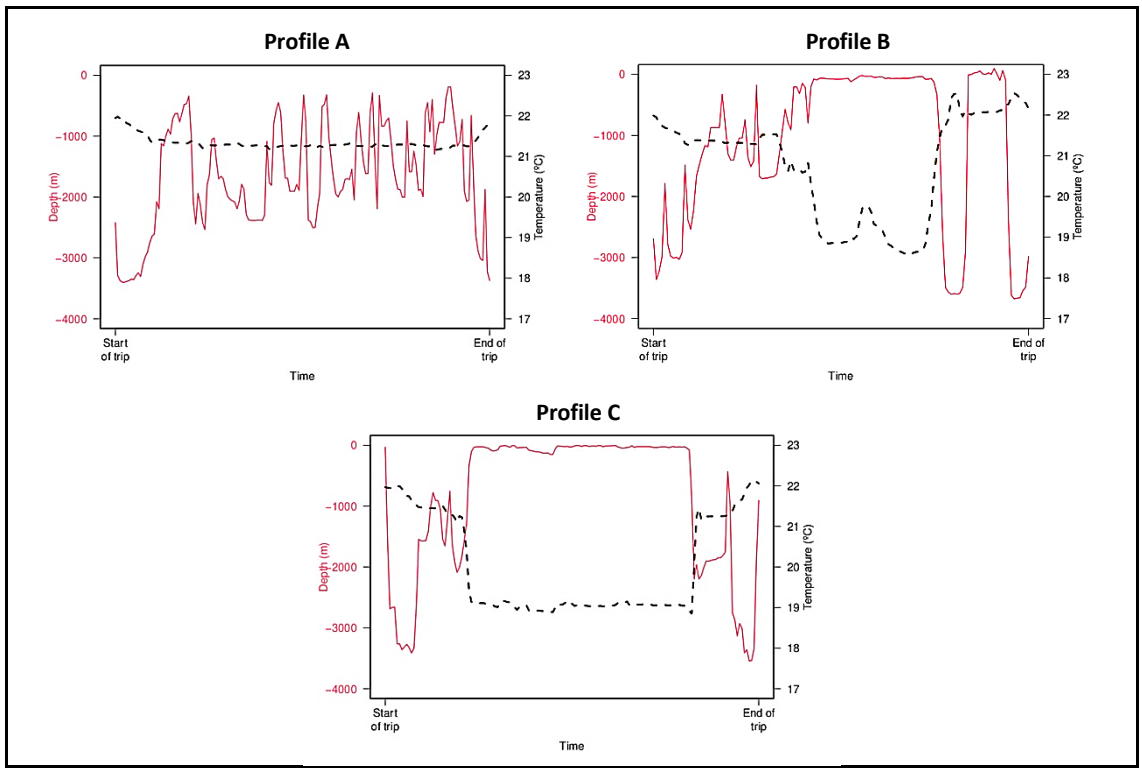


Figure 2 – Profiles of variation in temperature and bathymetry experienced by shearwaters along each of the three trips represented in figure 1.

Context

During the breeding period, Cory's shearwaters feed in different areas, and therefore prey on distinct marine communities. Table 1 shows the results of diet studies of birds foraging in three areas.

Table 1 - Prey consumption by Cory's Shearwaters in three different oceanic domains (the figures in the table represent number of prey)

Prey species	Coastal zones	Seamounts	Pelagic areas
Sardina pilchardus	280	70	21
Scomber colias	28	140	28
Macroramphosus scolopax	20	140	70
Engraulis encrassicolus	20	84	42
Trachurus sp.	16	210	161
Diretmus argenteus	12		28
Naucrates ductor	12		350
Ommastrephes bartramii	8		
Histioteuthis arcturi	4		

4. Using the Shannon-Wien er index (H') rank the studied areas by ascending order of diversity. [**choose the correct option**] **[1 point]**

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

Where:
pi = relative proportion of each prey species

a) Seamounts < African Coast < Pelagic areas
b) African Coast < Seamounts < Pelagic areas
c) African coast < Pelagic areas < Seamounts
d) Pelagic areas < African coast < Seamounts

5. ~~The Cory's shearwater colony in Selvagem Grande depends to a large extent on the Canary Current Large Marine Ecosystem region, where one of the most important upwelling systems in the Atlantic Ocean is located. Complete the following statements concerning this oceanographic phenomenon. [**choose one option from the appropriate key in each case to fill the blanks in each of the sentences**] **[7 points, 1 point each correct answer]**~~

~~a) Coastal upwelling is an oceanographic process in which _____ water, _____ nutrients _____ deeper areas to the surface water.~~

a)	Key				
	1		2		3
A	warm	A	rich in	A	rise from
B	cold	B	poor in	B	descend to
C	cleaner	C	contaminated with	C	dilutes into
D	saltier	D	with rare		

~~b) In this region, the upwelling is more intense when the winds blow from _____, which causes the surface water to move _____ the coast.~~

b)	Key		
	1		2
A	North	A	toward
B	South	B	away from
C	East	C	along (to the North)
D	West	D	along (to the South)

c) Across the globe, coastal upwelling occurs mainly in areas close to the _____ coasts of the continents.

c)	Key
	1
A	North
B	South
C	East
D	West

d) The availability of nutrients in ocean water is mostly essential for the growth of _____.

d)	Key
	1
A	phytoplankton
B	zooplankton
C	fish and squids
D	marine mammals

Context

Cory's shearwaters are trans-equatorial migrants, heading to the southern hemisphere in mid-October and only returning to the breeding grounds in mid-February. A group of researchers tried to understand whether the date of departure from the colony influenced their migratory strategies, and particularly the decision to carry out (or not) temporary stops during the migration, commonly known as stopovers. To address this issue, researchers deployed tracking devices (geolocators) to 97 individuals in September, before they left the colony to migrate. The data obtained in the following breeding season allowed for the construction of Figure 3.

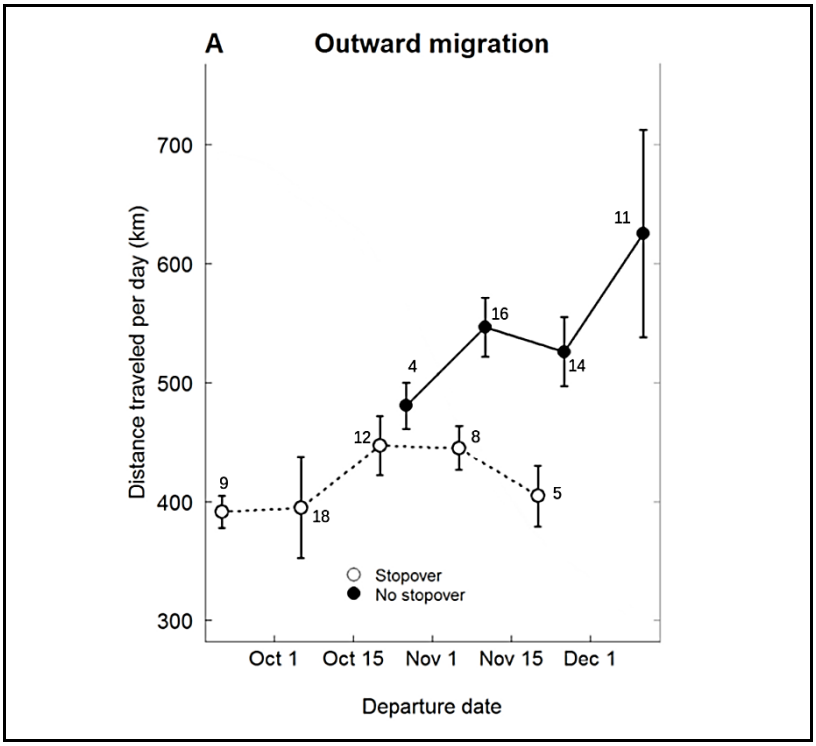


Figure 3 – Relation between the date of departure of Cory's shearwaters for their migration and the mean distance travelled per day (± standard deviation) in birds that made stopovers (open symbols) and in birds that did not (solid symbols); the numbers next to each dot represent the number of individuals tracked in each situation.

6. Based on the interpretation of the Figure, **classify each of the following statements as true (T) or false (F)**. **[4 points, 1 point each correct answer]**

   a) The decision to make migratory stopovers does not depend on the date of departure from the colony

   b) When birds choose to stopover, it is because they can travel faster

   c) The distance travelled per day increases throughout the migratory period, regardless of stopovers

   d) The probability of carrying out a stopover tends to decrease with the increase in the departure date

Part 5
The second stopover – Rio de Janeiro, Brazil

Subject: Cellular Biology and Biotechnology

Theme: Natural products in plants

Time proposed: 20 min

Introduction

There is growing interest in the use of herbal medicinal products (HMP). Among the most sought-after HMP are those containing *Hypericum perforatum* L., prescribed, essentially, for the relief of moderate depressive states and of anxiety. These products are available on the market in various forms, such as, for example, capsules, tablets, alcoholic extracts, plant for infusions and food supplements. Antidepressant activity has been attributed to hypericin and hyperforin. These compounds, however, do not exist in all *Hypericum* species. In *H. perforatum*, hypericin, with a characteristic dark red color, occurs in black glands present in the leaves and petals.

Section I - To evaluate the presence of hypericin in two sachets of HMP, marketed for infusions, and in capsules, marketed as food supplements, the morphological and chemical characteristics were evaluated. For this, the material was observed under an optical microscope, and extracted with methanol. In this way it was possible to differentiate which HMP were from *H. perforatum*, or from another species, frequent in Portugal.

The images in **Figure 1** show, randomly, phases of the process.

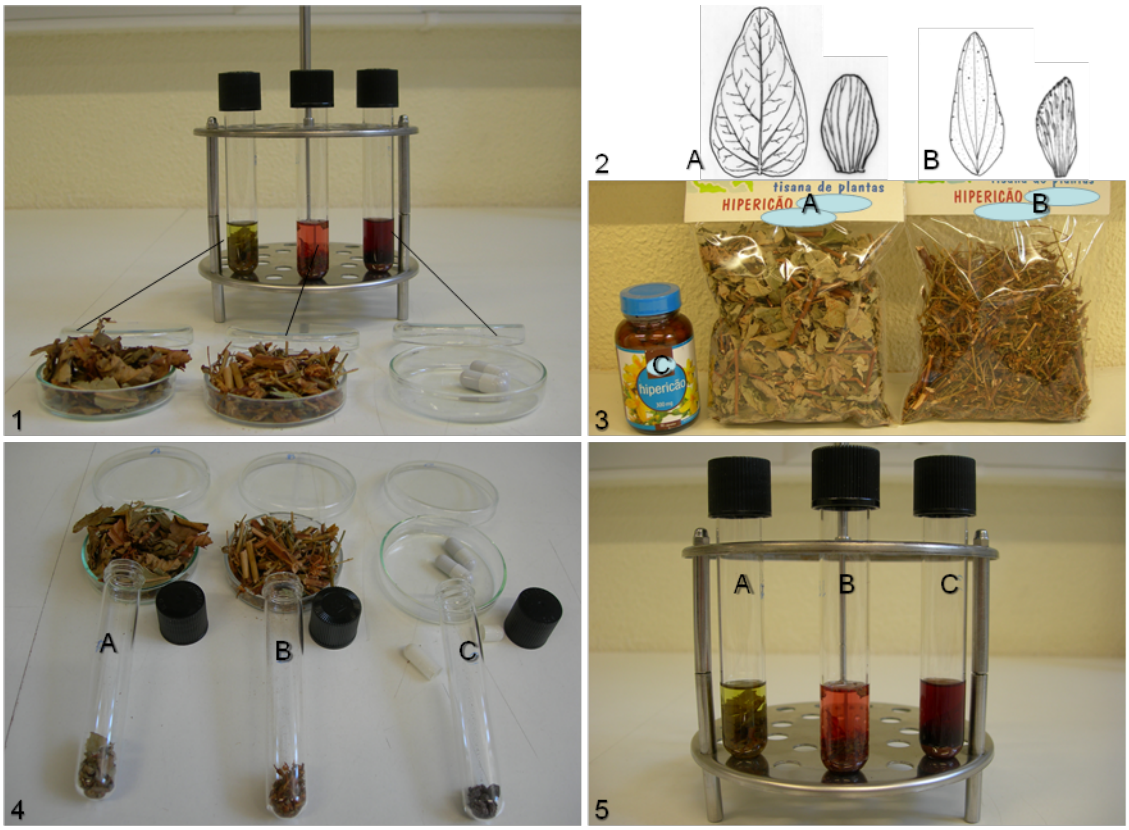


Figure 1 – Different phases of the determination of the presence of hypericin and of the extraction process, based on the method of methanol extraction.

1. To present correctly and sequentially the observations of the morphological and chemical characteristics of the samples, the ordering of the figures should be: [**choose the correct option**] **[1 point]**

a) 2, 4, 3, 1, 5

b) 3, 2, 4, 1, 5

c) 1, 2, 3, 4, 5

d) 4, 2, 3, 1, 5

2. **Classify as true (T) or false (F) each of the sentences below**. **[6 points, 1 point each correct answer]**

   a) Image 5 shows the maceration of the plant material in methanol

   b) Image 2.B represents schematically the observation of sample 4.C under the microscope

   c) Image 2.A schematically represents leaves and petals from sample A, which has no black glands

   d) Chlorophyll is the dominant pigment in sample C

   e) Based on the observed reaction, we can conclude that sample A is from *Hypericum perforatum*

   f) Only two samples show the presence of hypericin

Section II - Allelopathy is a natural phenomenon of biological interaction. Mediated by substances (allelochemicals) produced by an organism, the interaction can affect the surrounding community positively (to be beneficial) or negatively (to be harmful). Many allelochemicals produced by a plant affect germination, growth, survival, and reproduction of other plants. Some aquatic plants (e.g. *Nymphaea odorata*) release allelopathic substances that suppress the growth of duckweed *(Lemna minor*). Man often uses this type of allelochemicals to obtain formulations that act as natural herbicides. For this purpose, it is necessary to test the allelopathic potential of plant extracts, such as, for example, essential oils.

In the present experiment, Figure 2, the allelopathic effect of *Eucalyptus globulus* (eucalyptus) essential oils was tested on *Lemna minor* (duckweed). The multiwell plate A served as a control (without the addition of essential oil). In each well of the multiwell plate B, 100 µl of essential oil were added. The allelochemical effect of the essential oil was evaluated, among others, by determining leaf chlorosis (a condition characterized by a decrease in the content, or absence of chlorophyll, visible by the pale green or yellowish aspect).

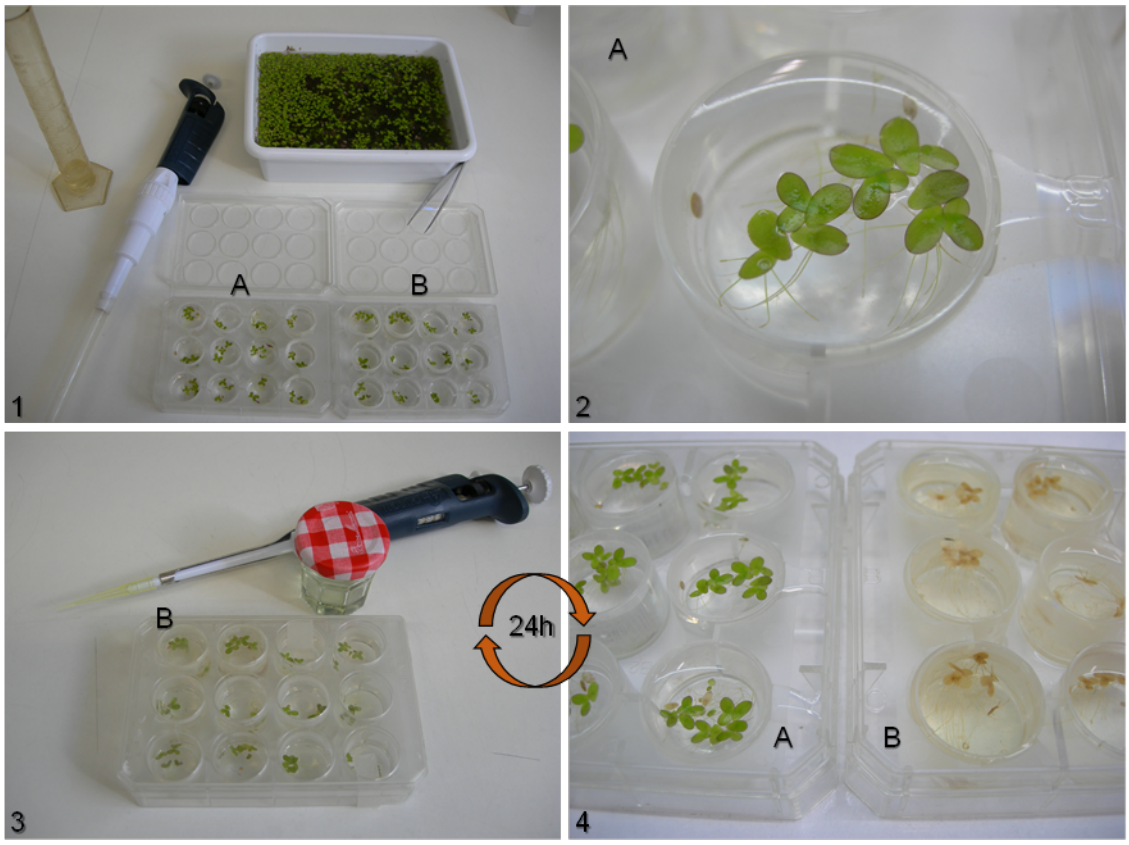


Figure 2 – Different steps of an experiment performed to test the allelopathic effect of *Eucalyptus globulus* essential oils on *Lemna minor*.

3. Classify as true (T) or false (F) each of the sentences below. [6 points, 1 point each correct answer]

a) An allelopathic potential of 100% means that 2 out of 4 leaves of *Lemna minor* did not show leaf chlorosis

b) The whole experiment could be carried out in the same multiwell plate

c) Leaf vigor was assessed by the absence of leaf chlorosis

d) The experiment should be carried out under conditions of natural light and humidity, like the natural growth conditions of *Lemna minor*

e) Based on the observed reaction, we can conclude that the essential oil of *Eucalyptus globulus* has no allelopathic effect, under these conditions

f) The physiological state of *Lemna minor* can condition the result of the experiment

Part 6

The third stopover – The Strait of Magellan

Subject: Ecology

Theme: Demography of penguin populations

Time proposed: 20 min

Introduction

The Magellanic penguin (*Spheniscus magellanicus* Forster, 1781) is an aquatic bird, native to the southern region of the South American continent, particularly to the coasts of Patagonia, Argentina, and the Falkland/Malvinas Islands, in the Atlantic Ocean, and of Chile, in the Pacific Ocean, where the species forms densely populated breeding colonies. After the breeding season the Magellanic penguins migrate North in search for food, in both oceans, reaching the coasts of Brazil, in the Atlantic, or of Peru, in the Pacific.

The common name of the species is associated to that of Ferdinand Magellan (Fernão de Magalhães), as its existence was noted for the first time in 1520, during the circumnavigation voyage lead by the Portuguese sailor while passing by the Tierra del Fuego.

According to the *International Union for Conservation of Nature* (IUCN), the Magellan penguins' populations are estimated as composed by 1.3 million reproducing couples and the species is classified as "*Least Concern*", although following a decreasing tendency (globally) during the last decades. This decrease is essentially due to the reduction in prey abundance, caused by fishing, to climatic changes and to pollution.

The adults may attain 70 cm in height, and weight up to 4-6 kg, the females being a little less robust than males.

The Magellan penguins are monogamous and reproduce between September and February. They build nests on the floor or in small burrows or lairs, normally protected by low vegetation and, in the most populated colonies, which may have up to 200 000 individuals, the nests may be as close as 1.2-2.5 m. The female lays up to four eggs per year, in two broods of two eggs, laid with an interval of 4 days. The eggs are incubated for about 40 days. The mortality is rather high, both *in ovo*, as in the juvenile phase,

before leaving the nest, and especially during the first migration. From then on, the mortality stabilizes, and the adults may live up to an age of 10-20 years.

Note: the experience upon which the exercise is built, is fictitious, as well as the figures that were obtained from it. Nevertheless, all the elements produced for the purpose of this test, are realistic and possible.

Context

Since several decades, the population dynamics of two Magellan penguins' breeding colonies, in two natural reserves dedicated to the conservation of the species – Cape Vígenes, Argentina and Isla Magdalena, Chile – have been regularly monitored.

Recently, a study on the abundance and population structure of these two colonies, which involved a mark-recapture experiment, was conducted.

One week before the beginning of the experiment, in each of the reserves, a 3.0 ha (hectares) study area was selected and three funnel shaped rails were placed by the beach, just as represented in **Figure 1.**

Such devices are intended to guide and concentrate the penguins, making it easier to count them and mark them in their return from the sea towards the respective nests.

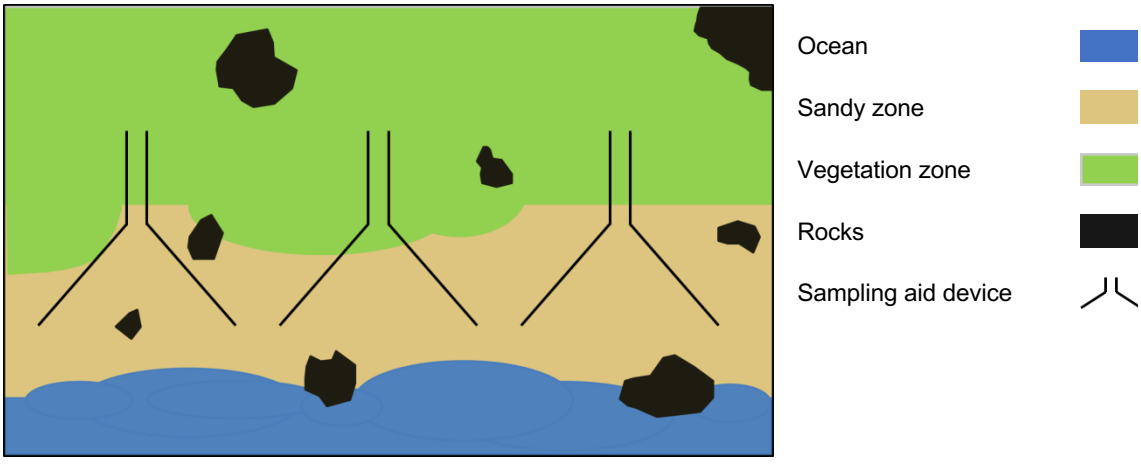


Figure 1 – View of the sampling aid device installed in each of the studied reserves in order to sample the Magellanic penguins (the image is merely illustrative, as the various components it portrays are not represented in the same scale).

In the first session of the experiment an observer, positioned in the narrow extreme of each rail device, counted and marked all penguins that passed by him in their way from the sea to the nest. The marks were small circular spots, made with a harmless colour paint spray on the external face of the left wing of each animal.

In the second session of the experiment, the same observer counted again all penguins that march in their way from the sea to the nest and, among them, all those which were marked in the previous session.

The conditions of the study were those displayed in **Table 1**.

Table 1 – General conditions of the experiment performed on the population dynamics of the reproductive colonies of the Magellan penguin, *Spheniscus magellanicus*, living at Cape Vírgenes and Isla Magdalena

	Cape Vírgenes (Argentina)	Isla Magdalena (Chile)
Study area	3 ha	3 ha
Sessions	2 days	2 days
Sampling effort	5 hours	5 hours

The results of the experiment, related to the two 3 ha areas under study, are presented in **Table 2**.

Table 2 – Results obtained from the experiment performed on the population dynamics of the reproductive colonies of the Magellan penguin, *Spheniscus magellanicus*, living at Cape Vírgenes and Isla Magdalena

		Cape Vírgenes (Argentina)	Isla Magdalena (Chile)
1st Session	Counted	1 068 inds.	1 077 inds.
	Previously Marked	0 inds.	0 inds.
2nd Session	Counted	1 239 inds.	1 245 inds.
	Previously Marked	36 inds.	34 inds.

Other information on both populations has been collected for some years and part of it, namely that related with reproduction and mortality, is reproduced in **Table 3**, while the population structure is presented in **Figure 2.**

Table 3 – Information relative to sex ratio, number of eggs per female and mortality rates estimated for the populations of the Magellan penguin, *Spheniscus magellanicus*, living at Cape Vírgenes and Isla Magdalena.

		Cape Vírgenes (Argentina)	Isla Magdalena (Chile)
Sex ratio	M/F F/M	1,0145 0,9857	0,9231 1,0833
Number of eggs per female		4	4
Mortality rate	*In ovo* Juvenile 1st migration	53% 65% 71%	53% 59% 65%

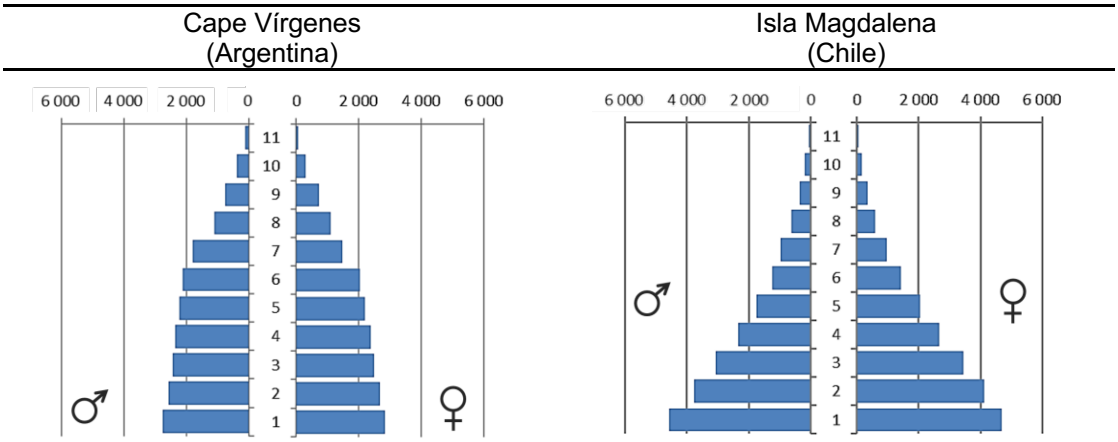


Figure 2 – Population structure of the populations of the Magellan penguin, *Spheniscus magellanicus*, living at Cape Vírgenes and Isla Magdalena. Vertical axis represents age in years, and horizontal axis represents number of individuals.

Based on the data provided, help the researchers in the characterization of the studied populations by finding the solution for the problems below, assuming that in both colonies the juveniles have not left the nest yet.

1. Estimate the number of adult individuals that composes the population of Cape Vírgenes [**choose the correct option**] **[1 point].**

	Cape Vírgenes (Argentina)	~~Isla Magdalena (Chile)~~
a)	44 604 inds	~~Estimated number of~~
b)	38 448 inds	~~adult individuals~~
c)	36 757 inds	~~39 437 inds~~
d)	35 206 inds	

2. The estimate made in the question above was based on a model with the following assumptions [**classify as true (T) or false (F) each of the sentences below**] **[8 points, 1 point each correct answer]**

   a) The marks and the marking do not affect the survival/behaviour of the animals
   b) The marked animals are easier to detect than the unmarked
   c) The marked animals must have a recuperation period
   d) The probability of capture of marked and unmarked animals is similar
   e) The marked animals mix randomly within the population
   f) The marked animals are more abundant than the unmarked ones
   g) The marks are not lost between the two sessions of the experiment
   h) All marked animals go to the nests after being marked

3. Estimate the average adult individuals' density in the population of Isla Magdalena [**choose the correct option**]. **[1 point]**

	~~Cape Vírgenes (Argentina)~~	Isla Magdalena (Chile)
a)	~~Estimated adult~~	1.41 inds/m^2
b)	~~Individuals' density~~	1.31 inds/m^2
c)	~~1.23 inds/m^2~~	1.28 inds/m^2
d)		1.22 inds/m^2

4. Estimate the number of adult males and females in the population of Isla Magdalena [**choose the correct option**] **[1 point].**

	~~Cape Vírgenes (Argentina)~~		Isla Magdalena (Chile)	
	~~Males~~	~~Females~~	Males	Females
a)	~~Estimated number of~~		18 930 inds	20 507 inds
b)	~~males~~	~~females~~	18 202 inds	21 361 inds
c)	~~18 511 inds~~	~~18 246 inds~~	21 361 inds	18 202 inds
d)			19 628 inds	20 894 inds

5. Estimate the average density of nests in the population of Cape Vírgenes [**choose the correct option**] **[1 point]**.

	Cape Vírgenes (Argentina)	~~Isla Magdalena (Chile)~~
a)	0.63 nests/m^2	~~Estimated average density~~
b)	1.63 nests/m^2	~~of nests~~
c)	0.82 nests/m^2	~~0.63 nests/m^2~~
d)	0.61 nests/m^2	

6. How many new individuals (recruits) can be estimated to enter the population of Cape Vírgenes in the following year [**choose the correct option**]. **[1 point]**.

	Cape Vírgenes (Argentina)	~~Isla Magdalena (Chile)~~
a)	3 5 83 inds	~~Estimated new individuals~~
b)	4 463 inds	~~in the next year~~
c)	3 482 inds	~~5 107 inds~~
d)	4 8 63 inds	

7. Taking into consideration the graphs of population structure in each reserve, characterize the growth of each of the populations [**choose the correct option**]. **[1 point]**.

	Cape Vírgenes (Argentina)	Isla Magdalena (Chile)
a)	Moderate growth	Stable
b)	Slow growth	Fast growth
c)	Stable	Moderate growth
d)	Fast growth	Slow growth

Part 7
The fourth stopover – Ladrones Islands (Marianas)

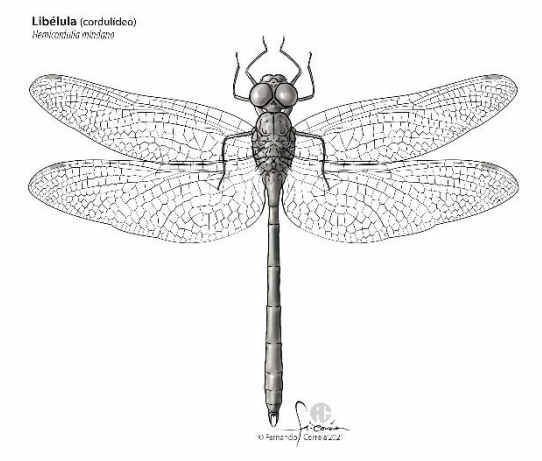
Subject: Ecology and Anatomy

Theme: Insect anatomy and ecology

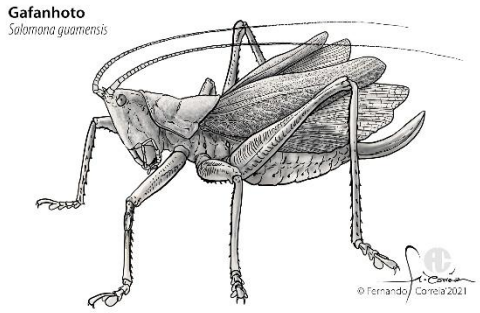
Time proposed: 20 min

Introduction

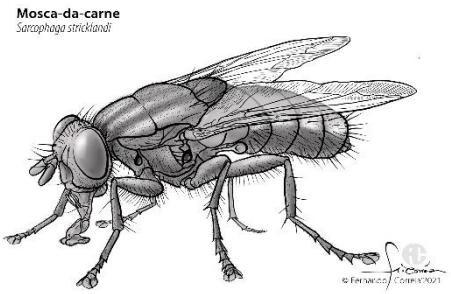
The Marianas were the first islands Magellan encountered after crossing the Pacific from the southern tip of South America. They set in an area of about 1000 km^2 in the Pacific Ocean and the flora and fauna biodiversity is relatively valuable. The community of insects, although neglected, has been studied and several autochthonous species were found. Among these, there are four endemic species (**Figure 1**) with morphological adaptations to different habitats and diverse behavior.



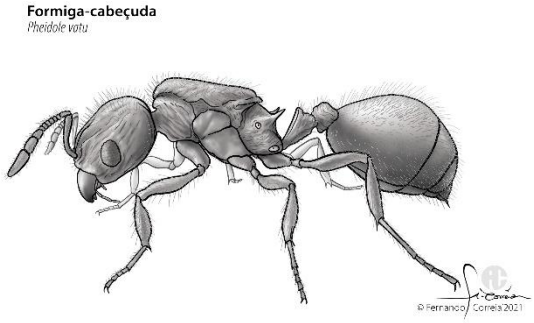
A - *Hemicordulia mindana* Needham & Gyger, 1937. [Odonata, Ani soptera]



B - *Salomona guamensis* Hebard, 1922. [Orthoptera, Ensifera]



C - *Sarcophaga stricklandi* Hall & Bohart, 1948. [Diptera, Sarcophagidae]



D - *Pheidole vatu* Mann, 1921. [Hymenoptera, Formicidae]

Figure 1 - Four Marianas Islands' endemic insect species.

Section I. Based on the characteristics of each of the insects present in **Figure 1,** answer the following questions by **selecting the correct option**.

1. The modified hind wings of *Sarcophaga (= Bezziela) stricklandi* are: **[1 point]**

a) Elytra

b) Hamuli

c) Haltere

d) Tegmina

2. Another name for the walking legs of *Pheidole vatu* is: **[1 point]**

a) Cursorial

b) Ambulatory

c) Fossorial

d) Saltatory

3. *Salomona guamensis* can detect sounds through an auditory sense structure called: **[1 point]**

a) Antenna

b) Sensilla

c) Tympanum

d) Bristle

4. *Hemicordulia mindana* uses a characteristic mouthpart to eat: **[1 point]**

a) Sucking mouthpart

b) Chewing mouthpart

c) Siphoning mouthpart

d) Sponging mouthpart

5. The forewings are attached to which body segment? **[1 point]**

a) First abdominal

b) Prothorax

c) Metathorax

d) Mesothorax

6. Insects can create vibrations that are transmitted through a substrate… **[1 point]**

   a) And send very specific intraspecific messages, e.g. courtship songs
   b) And used for efficient intraspecific communication over short distances
   c) And represent a secure means of intraspecific communication
   d) All of the above

7. The chemical trail produced by *Pheidole vatu* would be classified as a(n)… **[1 point]**

   a) Kairomone
   b) Pheromone
   c) Allomone
   d) None of the above

8. *Sarcophaga (= Bezziela) stricklandi* larvae (maggots) move away from a bright source of light. This is an example of a… **[1 point]**

   a) Taxis
   b) Kinesis
   c) Reflex
   d) Transverse orientation

9. Like in most insects, *Salomona guamensis* has the sense of smell located in… **[1 point]**

   a) The tarsi
   b) The maxillary palps
   c) The antennae
   d) The frons

10. *Hemicordulia mindana* mechanoreceptors would not be receptive to… **[1 point]**

   a) Body movement
   b) Wind speed
   c) Sound vibration

d) Water vapor

Section II. Match species with characteristics. Use the key to indicate the characteristics of each of the insect species in the right column. [**Notice that each element of the key may be used once, more than once, or not at all**] **[4 points, 1 point each correct answer]**

KEY		Species characteristics
A	Apterous	*Hemicordulia mindana*
B	Halteres	
C	Hamuli	
D	Jumping legs	*Pheidole vatu*
E	Sponge-like mouthpart	
F	Furcula	
G	Small hair-like antenna	*Salomona guamensis*
H	Elytra	
I	Tegmina	
J	Wasp-waist	*Sarcophaga stricklandi*
K	Two pairs of wings	
L	Geniculated antenna	

Section III. Match each behaviour listed in the right column with its typical function listed in the left column [**Notice that each element of the key may be used once, more than once, or not at all**] **[5 points, 1 point each correct answer]**

KEY		Behaviours	
A	Light flash in firefly	Courtship	
B	Waggle dance in honey-bee	Dispersal	
C	Stridulation in cricket	Nestmate recognition	
D	Trophallaxis in ants	Location of food	

E	Hissing in cockroach	Alarm	

Part 8

The end of the journey – The Island of Mactan, Philippines

Subject: Microbiology

Theme: Bioremediation

Time proposed: 20 min

Introduction

Currently, diverse human activities without respect for the environment make pollution a serious problem. The Earth can only continue to be our home if we adopt sustainable living strategies. **Bioremediation** uses the metabolic potential of microorganisms to reduce pollution in contaminated environments. There are several bioremediation strategies: *in situ*, handling pollution where it exists; *ex situ*, collecting contaminated material and treating it at specific sites; by **biostimulation** of microorganisms indigenous to an environment by adding nutrients or improving environmental conditions to promote the development of that microbiome; by **bioaugmentation**, addition of cultures of exogenous microorganisms to promote degradation of pollutants.

Section I – An experiment about the purification of mine drainage fluids

The problem: To study the role of sulphate-reducing bacteria in the bioremediation of acidic mine water.

The goal of the experiment: To remove metals and sulphate from polluted waters through the use of sulphate-reducing bacteria, reducing those pollutants to levels that allow the waters to be used for irrigation.

The experiment: A bioremediation system, at laboratory scale, was developed using acidic water from an abandoned copper mine.

The results:

I. Chemical composition, sulphate concentration and chemical parameters of the original acidic water were analysed (**Table 1**).

II. Chemical composition, sulphate concentration and chemical parameters of the water after the biologic treatment were analysed (**Table 1**).

III. Electrophoretic profiles of bacterial communities based on the 16S rRNA gene (**Figure 1**).

Table 1 - Chemical composition, sulphate concentration and chemical parameters of the water collected in the mine before and after biological treatment

Chemical composition	Concentration ($mg\ L^{-1}$)	
	Before treatment	After treatment
As	0.10	< 0.03
Cd	0.12	< 0.06
Co	0.99	< 0.01
Cr	0.08	0.06
Cu	28	< 0.01
Fe	70	0.09
Hg	< 0.01	< 0.01
K	0.30	3.70
Mn	7.10	3.70
Mo	< 0.01	< 0.01
Ni	0.26	< 0.02
Pb	< 0.04	< 0.04
Sb	< 0.1	< 0.1
Se	< 0.007	< 0.007
Sn	< 0.1	< 0.1
Sr	0.14	0.47
Zn	13	< 0.007
SO_4^{2-}	1800	303
Chemical parameters (Eh = Reduction Potential)		
Eh (mV)	401	-376
pH	2.8	6.5

1. Based on the information provided relative to the experiment described above, **classify the statements (a to d) using the key (A to C) [4 points, 1 point each correct answer]**

Key	
A	The statement is supported by the data
B	The statement is contradicted by the data
C	The statement is unrelated to the data

a) Before the sulphate-reducing bacteria's activity the redox potential was lower than after bioremediation

b) After the treatment, the water´s acidity decreased

c) An efficient removal of the three most abundant metals in the acidic mine water has occurred during the treatment

d) The water obtained after the treatment is not suitable for irrigation

2. Figure 1 shows the electrophoretic profiles of bacterial communities, based on the 16S rRNA gene, present in four bioremediation systems. **Select the key option (A, B or C) that correctly evaluates the whole set of statements** regarding the band that corresponds to sulphate-reducing bacteria. **[1 point]**

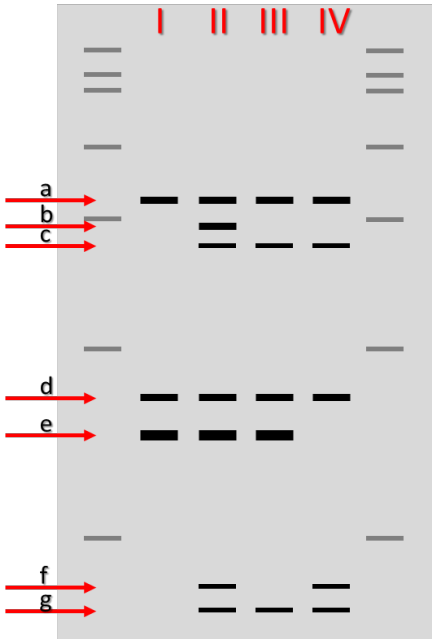


Figure 1 - Electrophoretic profiles of bacterial communities, based on the 16S rRNA gene, present in four bioremediation systems. Only one system contains sulphate-reducing bacteria. Each band (**a** to **g**) corresponds to a different group of bacteria.

Key	
A	a) is true, b) is true, but c) is false
B	a) is false, b) is false, and c) is false
C	a) is false, b) is false, but c) is true

a) The band that corresponds to sulphate-reducing bacteria is **e** because it is more intense

b) The band that corresponds to sulphate-reducing bacteria is **g** because it exists in a profile that does not have **f**

c) The band that corresponds to sulphate-reducing bacteria is **a** or **d** because it exists in all the profiles

3. Only one of the bacterial community profiles shown in Figure 1 corresponds to the bioremediation system under analysis in Question 1 . Which is the profile? [**choose the correct option**] **[1.0 points]**

a) Profile I

b) Profile II

c) Profile III

d) Profile IV

Section II – An experiment about the purification of food and feed industrial effluents rich in oily products

The problem: Food and feed industries produce oily effluents, with high fat, oil and grease (FOG) content.

The goal of the experiment: Select bacteria that exist naturally in polluted sites and evaluate them to improve bioaugmentation strategies.

The experimental steps:

1. Bacterial isolation from activated sludge of residues treatment stations.
2. DNA extraction from the isolated bacteria.
3. Molecular identification of the isolates.
4. Evaluation of FOG-biodegradation potential of the isolates.

The results:

I. In total, 196 isolates were screened for biodegradation potential with pollutants as sole carbon sources for growth.

II. Best biodegradation results, in 7 days assay of FOG content removal, were 37.9% for oleic acid and 19.1% for triolein by an *Aeromonas* sp. isolate and a *Staphylococcus cohnii* isolate, respectively.

4. Based on the experiment described above, **classify as true (T) or false (F) each of the following sentences [4 points, 1 point each correct answer]**

a) The work described aims to evaluate the biostimulation process in the presence of FOG

b) A biostimulation process will be more successful if point 3. of the experiment is previously applied

c) Biostimulation can only be carried out if the described in points 2. and 3 of the experiment are carried out

d) The use of bacteria for bioremediation can be a sustainable strategy against pollution

Section III – Bioremediation of oily sludge-contaminated soil

5. Consider that you need to create a bioaugmentation system for oily sludge-contaminated soils and you have at your disposal the elements that are presented in **Table 2** . **Select the option that corresponds to an appropriate bioaugmentation system for oily sludge-contaminated soils [3 points, 1 point each correct answer]**

Table 2 – Elements available to build a bioaugmentation system for oily sludge-contaminated soils

1. oily sludge-contaminated soil	
2. lipolytic bacteria naturally present in the soil	
3. bacteria with enhanced lipolytic activity after some selective growth in lab	
4. nutrients used by lipolytic bacteria	
5. surfactant that breaks down oils into smaller molecules	

a) 1 + 2 + 4

b) 1 + 2 + 3

c) 1 + 2 + 3 + 4

6. Which strategy, among those presented below leads to an effective bioremediation system for fats and oils? **[choose the correct option]** **[1 point]**

a) *In situ* promotion of the growth of indigenous lipolytic bacteria

b) Addition of a surfactant to a soil containing lipolytic bacteria

c) *In situ* addition of bacteria with high lipolytic activity

d) Confirmation that there is no antagonism between the soil microbiome and the added bacteria

e) All the previous strategies

CONGRATULATIONS!

YOU MADE IT TO THE END!