

**PRACTICAL ACTIVITIES**

**Subject: 26521. ECOLOGY**

Undergraduate Studies

in Biology and Marine Sciences.

Ecology Department

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**AUTORSHIP:**

**DATE: PLACE:**

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| Team Nº | Members names | Observations |
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**PRACTICAL ACTIVITY 1:** CHARACTERIZATION OF A MEDITERRANEAN FOREST ECOSYSTEM.

*FIELD NOTEBOOK*

**Objective:**

Evaluate the effect of environmental factors variations on the ecological characteristics of an ecosystem.

For this purpose, we will study the south exposed (S) and north exposed hillsides (N) of a hill near the *Maigmó massif* (check the aerial photograph at the end of the guide). It is intended to obtain data needed to answer the following questions:

¿How affects the orientation of the hillside to:

- Biological and spatial structure of a pine forest,

- Foliar morphology of the dominant species,

- Pines growth,

- The floristic composition of the community?

For this task and with prior knowledge given in the lectures you should set out the study hypothesis\*. These hypothesis or others, that may arise when we are analysing the field data, should be responded with the collected data.

This practical activity will consist of:

- A FIELD session, where it will be taken data from physical environment and different parameters of vegetation.

This session has half an hour in the laboratory to weight fresh samples and introduce them into the fridge or oven.

- A LABORATORY session where samples will be handled organized or filled tables and will obtain or calculated additional data. Once all the necessary data are available, they will be introduced in a common database before the end of the practical activity.

- A COMPUTER session where from the common database will attempt to obtain relations between environmental variables and biological parameters to corroborate the hypothesis.

To evaluate if the horizontal and vertical structure of vegetation or other functional variables differ between the two hillsides, it will be done all the next steps to both, south exposed (S) and north exposed hillsides (N)

*\* Each team can take all additional data if appropriate to demonstrate or reject the different hypothesis but must provide the necessary material.*

**TASKS IN THE FIELD (Summary)**

**Both** south exposed (S) and north exposed hillsides (N) **, will follow the next outline:**

0.- Vegetation transect line of 15 meters in length.

1.- Horizontal and vertical structure of shrub and herbaceous species. It will be taken on the transect line.

1.1.- Data collection of the shrub horizontal structure.

1.2.- Data collection of the shrub vertical structure.

2.- Horizontal forest pine structure and growth of pine shoots which will be determined by a total of five pines per hillside.

3.- Soil temperature measurements.

4.- Collect soil samples for gravimetric moisture determination in the laboratory

5.- Sampling of plant material for laboratory determinations related to the amount of water content and foliar characteristics.

**You must complete the tables whose numbers are given in this text.**

**Note:** Consider that for the same number of table, there is one for the south exposed (S, refers to the south exposed slope) and one for north exposed (N)

**METHODOLOGY**

**0.- Vegetation transect line of 15 meters in length**.**.** Methodology:

Every team should:

- Be located in a place of the hillside, which is not influence by the “edge effect” whose produce in it the interaction with the adjacent ecosystems.

- Respect a safe distance between teams (at least 3-4 meters).

- Select randomly a point for the beginning of the 15m transect

- Hold the beginning of the tape (15 meters or more) to the soil or a shrub.

- Extend the tape respecting the level curve to the end point.

This transect will be used to study the horizontal and vertical structure of the vegetation (following step).

**NOTE 1. Mark the transect following the** level curve**.**

**NOTA 2. The tape itself marks the line at which it will be sampled the height and coverage of the understory.**

**1.- Horizontal and vertical structure of herbaceous and shrub layer along the transect.**

**1.1. Linear plant cover (horizontal structure).**

On the transect line (tape) will be made the method of the linear plant cover.

For this purpose it will be imaginary extended to the soil (over the plants) the line created by the tape. In the **Table 1** (1-S for the south exposed, or 1N for the north exposed slopes), it will be written down the centimetres occupied by each species along the 1500 cm (15 m). The **Table A** is an example of how it should be taken the data of the linear plant cover and how to do the first calculations if from the beginning of the transect (cm 0), to the (cm 15) there is bare soil+ litter, from the (cm 15) to the (cm 82) there is kermes oak, from (cm 82) to (cm 92) there is boulders, from (cm 92) to (cm 120) there is rosemary, etc…. In addition to the different herbaceous vegetable and shrubs species majority, it will be consider other types of coverage such as: bare soil, lichens, stones, herbaceous species, bare soil + litter, etc) that will allow to analyse with more detail the type of plant cover and structure of the ecosystem, both vegetation and edaphic types.

In order to give an accurate description of the vegetation, you must obviously indicate the plant cover by species and for that, it has to be distinguished each one of the species that composed the ecosystem. To have some help with the species it will be provided some photographs printed of some of them. Also, in the Table B it will be provided a list of the majority species in the area. If you find different species with a considerable presence whose name is unknown, you can ask the teacher or write a code to identified it (check the example in the interval from 250 to 305 of the table A) and take a small sample to identify in the laboratory.

**1.2. The vertical structure of the scrub.**

It will be estimated with the height data of the herbaceous and shrub layer. You should measure the height in the point where the tape marks the beginning of each meter. The data will be written down in the TABLE 1 (1-S y 1-SS).

**2.- Horizontal structure of the forest pine and pine sucker growth**

**2.1.** For the study of the tree stratum (formed almost exclusively of Aleppo pine, *Pinus halepensis*), each team will have to select five pines and they SHOULD ESTIMATE the surface in which they are living. In this way, we will have a calculation of the density of the pines per surface. For that, you will measure the length of the sides of an imaginary square in which these five pines are included. THIS MEANS EACH TEAM WILL SAMPLE THE tree DENSITY ON ITS OWN sampled SURFACE.

In each of the pines it will be measured: a) the perimeter at 130 cm to the soil (P130)\*, for the calculation of DBH-diameter at breast height; b) Also it will be obtained the cover of the crown (horizontal projection) by measuring the diameter (∅) of the crown projection on its longest side and the perpendicular side. These data will also recorded in **Table 2 (2-S and 2-N)**.

From these measures, you should calculate in the laboratory: density of trees (nº trees/hectare); canopy cover (square meterscovered by leaves/hectare) and basal area (trunks surface/hectare). The data will be recorded in **TABLE 3 (3-S and 3-N).**

* ***Caution: if the pine to be measured is lower than 130 cm to the soil you should measure the perimeter at 50cm from the soil and write it down.***

**2.2.** To estimate the pine shoot growth, you should measure with a ruler (without cutting the twigs) the length of the twig growth of the last year with a precision of 0,1cm. You should take 5 measurements per pine\*\* (25 total data) and the data will be written down in the **TABLE 4 (4-S and 4-N)**

***\*\* Note: If the pines are too high it can be taken another smaller individual for this measure.***

**3.- Soil temperature .**

It will be measured using the digital thermometers for the field**\*\*\***. You should take the temperature at 1 cm of depth, in 6 points in open areas and 6 points located under the pines or shrubs. They will be written down in the **TABLE 5 (5-S y 5-N).**

***\*\*\* Caution: To measure the temperature not force the thermometer when goes into the ground. Choose a place where you can insert it comfortably.***

**4.-** **Collecting soil samples for gravimetric moisture determination in the laboratory.**

It will be taken **composited samples\*\*** of approximately 200 gr of soil in open areas and in areas under plant cover in each hillside (north and south). The samples will be taken between 1 and 5 cm depth after remove the litter. Then, you will keep the samples into plastic bags until you arrive to the laboratory. Take into account you have to zip them well to avoid the loss of moisture and then code them properly: Team/cover type (open or under vegetation) S or N (depending of the hillside where it is).

IN TOTAL YOU WILL HAVE 4 **composited** SAMPLES (two PER HILLSIDE), that it will be transported to the laboratory.

The table 6 refers to these data.

**\*\*** Composited samples means you should mix the soil from different points (take 5 points per each sample). To obtain a homogeneous and correct sample, you can take a soil sample in 5 different points from the same depth and in a similar amount, put them together, mix them and put in a bag about 200gr. This sample will be transported to the laboratory. Keep the excess *“in situ”* .

**5.-** **Sampling of plant material for laboratory determinations.**

***The teacher will assign two shrub species to each team*** to taking samples that it will be analysed in the laboratory.

**5.1.** Select 4-5 individuals of each assigned species.

**5.2.** Take three terminal twigs about 10 cm length of each. Introduce the three twigs in the same plastic bag and code*: Team/ Team’s code/ T (twigs)/ species X or and (depends on which it is)/ S or N (depending on the hillside)\*\*\*\*\*.* These samples will be kept in the FRIDGE when they arrive to the laboratory and they will be used to determine the specific leaf weight (SLW) and the ratio leaves weigh to stem weigh in the next session.

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**5.3.** Leaves sampling.

From the same species selected in points 5.1. and 5.2., each team will sampling a total 30 leaves (approximately) of each species. The samples will be introduce in different plastic bag, so you will have one bag per sample and it will be coded as: *Date/team’s code/ L (leaves)/ species X or and (depends on which it is)/ S or SS (depending on the hillside)\*\*\*\*\*.*

**NOTE: When you**  arrive from the field, you have to weight the samples and put them into the OVEN. In the next laboratory session, you will have the dry weight and the ratio between the dry weight and fresh weight.

The Table

***\*\*\*\*\* Each team should keep all the collected samples until they arrive to the laboratory (with special caution those taken in points 5.1 and 5.2).***

**WARNINGS:**

**1 You should distribute** the tasks**.** Not recommended that the 4 people do the same.

**2. Label (code)** the samples correctly **(VERY IMPORTANT).** Not to mix them either in the field or in the laboratory.

**3 Avoid** moisture loss in the samples for a correct data determination. It is recommended to do the sampling of the soil and twigs at the end of doing the other tasks in each hillside.

**4 Control** all you personal and materials belongings all the time.

**5 Material devolution.** You will have a list of the borrowed materials to return at the end of the activity.

**The basic data will be obtained for each hillside are, at least:**

|  |  |
| --- | --- |
| Hillside slope | Percentage of water in plants. |
| Orientation | Specific leaf weight (SLW) |
| Shrubland cover, % | Soil temperature and moisture. |
| Cover of different shrub species, % | Density of the pines |
| % bare soil, stony, … | Tree/canopy cover |
| Shrub height average and by every species | Basal area. |

**This amount of data will allow us to differentiate between both hillsides in case that there were differences.**



Workplace Maigmó petrol station and restaurant.

**PRÁCTICAL ACTIVITY 1:** CHARACTERIZATION OF A FOREST MEDITERRANEAN ECOSYSTEM.

*TABLES FOR DATA COLLECTION*

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| --- | --- | --- | --- | --- |
| **Table 1**- South slopes | | | | |
| initial  cmi | final  cmf | Dist. interval  (cm) | Plant species or surface | Height (cm) shrub vegetation at: |
|  |  |  |  | 1 m = |
|  |  |  |  | 2 m = |
|  |  |  |  | 3 m = |
|  |  |  |  | 4 m = |
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|  |  |  |  | Shrub height average in cm:  (mean and standard deviation of the 15 height data) |
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cmi is the distance to the zero point of the transect where it begins to cover the species indicated.

cmf is the distance to the zero point where the coverage of that species ends.

**Calculation results examples from the table above.**

From the data in the upper table, a summary must be done indicating the centimetres occupied by each species. You can see how to do it in the following tables. Table A is the example for the activity, the table A-BIS would be the one to fill. Your data has to be put in the table 1BIS of this booklet template (both suntrap and shady spot)

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| **Table A.** Data example for taking data of horizontal structure of scrub in the transect | | | | |
| Measurement interval  (from the beginning) | | Interval distance |  | Plant species or type of surface |
| cm initial  cmi | cm final cmf | cm |  |  |
| 0 | 15 | 15 |  | Bare soil+ Litter (fallen leaves, dead leaves) |
| 15 | 82 | 67 |  | *Quercus coccifera* |
| 82 | 92 | 10 |  | Rock/stones |
| 92 | 120 | 28 |  | *Rosmarinus officinalis* |
| 120 | 154 | 34 |  | Quercus coccifera |
| 154 | 202 | 48 |  | Rosemary |
| 202 | 230 | 28 |  | Bare soil+ Litter |
| 230 | 250 | 20 |  | *Thymus vulgaris* |
| 250 | 305 | 55 |  | Unidentified species (blue flowers, seems Compositae) |
| 305 | 335 | 30 |  | *Brachypodium cf retusum* |
| 335 |  | ……. | etc to 1500 cm | |

|  |  |  |  |
| --- | --- | --- | --- |
| **Tabla A-BIS.**  Grouped values by species coverage (or type of substrate) | | | |
| Results | Interval distance | TOTAL for each species | Plant species or type of surface |
| cm | cm |  |
| 55 | 55 | Unidentified species (blue flowers, seems Compositae) |
| 30 | 30 | *Brachypodium sp* |
| 67+34 | 101 | *Quercus coccifera* |
| 10 | 10 | Rock/stones |
| 28+48 | 76 | *Rosmarinus officinalis* |
| 15+28 | 43 | Bare soil+ Litter |
| 20 | 20 | *Thymus vulgaris* |
| ……. | etc con el resto de especies y espacio | |

DATA OBTEINED: The most abundant species is *Quercus coccifera*. It Represents the ….% of the scrub in the suntrap/shady spot studied. The next in abundance is the Rosemary and blue flower (must be classified) and a percentage of …..is bare soil.

Caution: there are no percentages because is an example that does not included the 1500 cm but it has to be calculated.

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| **Table 1-BIS** South slopes.- Grouped values of species hedges (or type of substrate) . | | | | |
| Total  cm | Species |  | Total  cm | Species |
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|  | ALSO INDICATE THE % SURFACE. |  |  |

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| **Table 2**- South slopes | | | | | | |
| Sampled surface (m2) = | | | | | | |
|  | Trunk  Perimeter  measured | Trunk  Diameter  calculated | Pine canopy cover measured:  ∅ in each projection (Proj). | | Basal area. (surface occupied by the trunk) | Pine canopy cover |
| Pine | P130  (cm) | ∅ 130  (cm) | ∅ en Proj. A  (m) | ∅ en Proj. B  (m) | (cm2). | (m2) |
| nº 1 |  |  |  |  |  |  |
| nº 2 |  |  |  |  |  |  |
| nº 3 |  |  |  |  |  |  |
| nº 4 |  |  |  |  |  |  |
| nº 5 |  |  |  |  |  |  |
| ALL  Average and deviation | ------------- |  |  |  | ∑ | ∑ |

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| **Table 3-** South slopes | | | | |
| Variable | | Data | Calculations from the sampled surface and each variable | Result |
| 1 | Sampled surface (m2) |  |  |  |
| 2 | Number of pines |  | Density = number ft (feet)/hectare  variable 2 |  |
| 3 | Σ of the canopy cover of the 5 pines measured (m2) |  | Canopy cover (m2/hectare)  variable 3 |  |
| 4 | Σ Area of the trunk of the 5 pines |  | Basal area (m2/hectare) taking the 4 variable |  |

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| **Table 4-** South slopes  Growth of the pine’s twigs of the last year ( in cm and with millimetre precision) | | | | | | | |
|  | | Pine nº | | | | | |
| 1 | 2 | | 3 | 4 | 5 |
| Twigs nº | 1 |  |  | |  |  |  |
| 2 |  |  | |  |  |  |
| 3 |  |  | |  |  |  |
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| 5 |  |  | |  |  |  |
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| Average growth (cm) and standard deviation | | | |  | | | |

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| **Table 5**- South slopes  Temperature of the soil at 1 cm of depth (ºC) | | | | | | | | |
|  | Measure nº | | | | | |  | |
| 1 | 2 | 3 | 4 | 5 | 6 |
| Sampling area |  | | | | | | Average | Standard  deviation |
| Bare soil |  |  |  |  |  |  |  |  |
| Under vegetation cover |  |  |  |  |  |  |  |  |

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| **Table 1**-North slopes | | | | |
| inicial  cmi | final  cmf | Interval  distance  (cm) | Plant species or type surface | Shrub vegetation height at (cm): |
|  |  |  |  | 1 m = |
|  |  |  |  | 2 m = |
|  |  |  |  | 3 m = |
|  |  |  |  | 4 m = |
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|  |  |  |  | Shrub height average in cm:  (mean and standard deviation of the 15 height data) |
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cmi is the distance to the zero point of the transect where begins to cover the species indicated.

cmf is the distance to the zero point of the transect where the species coverage ends.

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| **Table 1-BIS**-North slopes.- Grouped values of species coverage ( or type of substrate) | | | | |
| Total  cm | Species |  | Total  cm | Species |
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|  | ALSO INDICATE THE % SURFACE. |  |  |

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| **Table 2**- North slopes | | | | | | |
| Sampled surface (m2) = | | | | | | |
|  | Trunk  Perimeter  measured | Trunk  Diameter  calculated | Pine canopy cover measured:  ∅ in each projection (Proj). | | Basal area. (surface occupied by the trunk) | Pine canopy cover |
| Pine | P130  (cm) | ∅ 130  (cm) | ∅ en Proj. A  (m) | ∅ en Proj. B  (m) | (cm2). | (m2) |
| nº 1 |  |  |  |  |  |  |
| nº 2 |  |  |  |  |  |  |
| nº 3 |  |  |  |  |  |  |
| nº 4 |  |  |  |  |  |  |
| nº 5 |  |  |  |  |  |  |
| ALL  Average and deviation | ------------- |  |  |  | ∑ | ∑ |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table 3**- North exposed | | | | |
| Variable | | Data | Calculations from the sampled surface and each variable | Result |
| 1 | Sampled surface (m2) |  |  |  |
| 2 | Number of pines | Density = number ft (feet)/hectare  variable 2 | Density = number ft (feet)/hectare  variable 2 |  |
| 3 | Σ of the canopy cover of the 5 pines measured (m2) | Canopy cover (m2/hectare)  variable 3 | Canopy cover (m2/hectare)  variable 3 |  |
| 4 | Σ Area of the trunk of the 5 pines | Basal area (m2/hectare) taking the 4 variable | Basal area (m2/hectare) taking the 4 variable |  |

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| **Table 4**- North slopes  Growth of the pine’s twigs of the last year ( in cm and with millimetre precision) | | | | | | | |
|  | | Pine nº | | | | | |
| 1 | 2 | | 3 | 4 | 5 |
| Twigs nº | 1 |  |  | |  |  |  |
| 2 |  |  | |  |  |  |
| 3 |  |  | |  |  |  |
| 4 |  |  | |  |  |  |
| 5 |  |  | |  |  |  |
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| Average growth (cm) and standard deviation. | | | |  | | | |

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| **Table 5**- North slopes  Temperature of the soil at 1 cm of depth (ºC) | | | | | | | | |
|  | Measure nº | | | | | |  | |
| 1 | 2 | 3 | 4 | 5 | 6 |
| Sampling area |  | | | | | | Average | Standard  deviation |
| Bare soil |  |  |  |  |  |  |  |  |
| Under vegetation cover |  |  |  |  |  |  |  |  |

**SOIL TABLES (gravimetric moisture)**

**Table 6**. Soil Moisture. Estimate the gravimetric moisture. Values, mean and standard deviation.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Position | Pot number | PR (g) | PA (g) | PD (g) | % Soil moisture |
| South | Canopy cover |  |  |  |  |  |
| North | Canopy cover |  |  |  |  |  |
| South | Bare area |  |  |  |  |  |
| North | Bare  area |  |  |  |  |  |

**(PR)** Weight of the empty pot (in grams)

**(PA)** Pot weight + fresh soil (in grams)

**(PD)** Pot weight + dry soilafter oven at 105ºC (in grams)

**% moisture** = calculated as : ((PA-PD)/ (PD-PR))\*100

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| --- | --- | --- | --- |
| **Table 7**. Ratio Dry weight / fresh weight in shrub leaves | | | |
|  | **FW** (mg) | **DW**(mg) | **DW/FW** |
| ss\* 1= South exposed |  |  |  |
| ss\* 1= North exposed |  |  |  |
| ss\* 2= South exposed |  |  |  |
| ss\* 2= North exposed |  |  |  |

ss\*. Sampled species.

**FW** Fresh weight 30 leaves (mg); **DW** Dry weight 30 leaves (mg)

**Table 8**. Ratio *leaves fresh weight / stem fresh weight* in shrubs.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Team Date | | | | | | |
|  |  | FW leaves(mg) | FW stem (mg) | FW leaves / FW stem | Mean | Standard deviation |
| ss 1=  …………  SOUTH  EXPOSED | Branch1 |  |  |  |  |  |
| Branch2 |  |  |  |
| Branch3 |  |  |  |
| ss 1=  NORTH EXPOSED | Branch1 |  |  |  |  |  |
| Branch2 |  |  |  |
| Branch3 |  |  |  |
| ss 2=  …………  SOUTH  EXPOSED | Branch1 |  |  |  |  |  |
| Branch2 |  |  |  |
| Branch3 |  |  |  |
| ss 2=  …………  NORTH EXPOSED | Branch1 |  |  |  |  |  |
| Branch2 |  |  |  |
| Branch3 |  |  |  |

**Table 9**. Specific foliar weight and specific foliar area foliar in shrub leaves

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Team Date | | | | | |
| SPECIES AND HILLSIDE | Fresh weight  6 leaves (mg) | Dry weight  6 leaves (mg) | Surface  6 leaves (cm2) | SLW  (mg/ cm 2 ) | SLA  (cm2 / mg) |
| ss1 South exposed |  |  |  |  |  |
| ss1 North exposed |  |  |  |  |  |
| ss2 South exposed |  |  |  |  |  |
| ss2 North exposed |  |  |  |  |  |

**Table 10**. Dimension of the leaves in the shrub species in two slope aspects.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Team Date | | | | | | | | | |
|  |  | 1 | 2 | 3 | 4 | 5 | 6 | Mean | Standard deviation |
| SOUTH EXPOSED  Ss 1 | Le.(mm) |  |  |  |  |  |  |  |  |
| Width(mm) |  |  |  |  |  |  |  |  |
| Surf.(mm2) |  |  |  |  |  |  |  |  |
| NORTH EXPOSED  Species:xxx | Le. (mm) |  |  |  |  |  |  |  |  |
| Width(mm) |  |  |  |  |  |  |  |  |
| Surf.(mm2) |  |  |  |  |  |  |  |  |
| SOUTH EXPOSED  Species:yyy | Le. (mm) |  |  |  |  |  |  |  |  |
| Width(mm) |  |  |  |  |  |  |  |  |
| Surf.(mm2) |  |  |  |  |  |  |  |  |
| NORTH EXPOSED  Species:yyy | Le.(mm) |  |  |  |  |  |  |  |  |
| Width(mm) |  |  |  |  |  |  |  |  |
| Surf.(mm2) |  |  |  |  |  |  |  |  |

**PRÁCTICAL ACTIVITY 1:** CHARACTERIZATION OF A MEDITERRANEAN FOREST ECOSYSTEM.

*LABORATORY: FIELD DATA AND SAMPLES TREATMENT.*

**A.-SOILS CHARACTERIZATION. Gravimetric soil moisture content.**

**Introduction**

Water and soil atmosphere are filling the gaps between the soil particles and components of the solid phase. The gaps that can contain water or air are called SOIL POROSITY. The gaps or pinholes do not have the same size but for each type of soil there is a distribution of types of pinholes size according to the bedrock type, organic matter and other physical factors. For example, in clay soils predominate fine porosity (small pinholes), thick porosity in sandy soils, medium porosity in silty soils and in the sandy loam soils a balanced proportion of the three types. Different pinholes sizes will have different water holding capacity and this is important to understand the water dynamics in the soil. Large pinhole size will have a low water holding capacity and that is because the forces of capillarity that maintain the adhesion forces between water and pinholes walls are weak. Therefore, water easily flows through them. This low retention force allows the roots to acquire water with low energy cost (i.e low suction force) but in return water can drain by gravity to deeper horizons where roots cannot reach it. In the small pinholes, capillary forces that retain water in the pinholes are very important. Here, water is strongly retained in the pinhole and its extraction by the root requires a high energy cost. In return, the water cannot escape easily by gravity. Thus, it is available water but at higher cost. The intermediate size pinholes have intermediate water holding characteristics. The soils more suitable for plan water availability (i.e. water absorption) will be soils with a good heterogeneity in the pinholes size. That is, equilibrate proportion of the different types of size.

Soil water is moving constantly and retained with more or less force depending on the pinholes size. To a certain point, water content in a specific volume of soil will depend on the water taken by the roots, atmosphere evaporation, drain and entries by irrigation or rainfall. Therefore, this amount can be extremely variable over time and space. But if we wish to know the soil water status in a certain moment for a specific ecosystem we can measure simultaneously at different points the amount of moisture, evaluate the existing variability, compare different environments and analyze the different causes of these differences.

The water content in a soil sample in a specific point can be obtained by different methods. Some of them consist on measure *in situ* the moisture using specific sensors. In others such as **gravimetric soil moisture method** you should take soil samples in the field, weight them after and dry them in the oven to know their water content by weight difference. To get an idea to the spatial variation of the gravimetric moisture within an ecosystem, you should take several soil samples simultaneously in different microenvironments. For example, in our case we will take samples in south slopes and north slopes, and within them in areas situated under the pines cover or others located in the open spaces.

**¿How is calculated the gravimetric moisture?**

The soil samples are taken to the laboratory. Once they are in the lab, the sample is sieved with the 2mm sieve to eliminate the thick fraction (stones and gravels), which would introduce errors in the measurements due to the heterogeneity. This sample, once is sieved, will be weighted in fresh (FW) and then put into the oven at 105ºC until constant weight (24h-48h approximately) with the purpose to produce the evaporation of all the water in the sample. After that time the sample is weighted again to obtain the soil dry weight (DW). The moisture content of the sieved sample will be the difference between FW-DW. But we have to express this amount in relative terms to be comparable with other samples. In the case of gravimetric moisture we should express as water content per dry weight unit of soil. Multiplying by 100, will get the soil moisture or soil water content (%).

That is, % soil water content = [(FW-DW)/ DW ] \*100

**\*\*LABORATORY PROCEDURE AFTER FIELD ARRIVAL (To do the same day as field activity).**

1.- Sieve every soil samples (4 samples) through the 2mm sieve separately on a filter paper. Throw the thick fraction to the bin (fraction >2mm what remains in the sieve).

2.- Take a numbered aluminium pot (write down the pot number in **Table 6**)

3.- Weight the empty pot and write it down (PR) in **Table 6.**

4.- Without tare weight the pot, put the sieved sample until about the half of the pot approximately. Write down the weight (pot+ fresh soil)=(PA) in the **Table 6**

5.- Introduce the pot in the oven (105º C, 24h)

6.- Repeat these steps for all the soil samples from both south slopes and north slopes samples.

**\*\*LABORATORY PROCEDURE IN THE NEXT SESSION.**

7.- Monday or Tuesday when the activity at the laboratory, take out the samples from the oven and weight them. Write down the weight (pot weight+ dry soil)= (PD) in **Table 1.** Make the appropriate calculations to obtain the gravimetric soil moisture.

The weight of the pots should be expressed in grams with 2 decimal accuracy (000.00 g)

**B.- PLANT MATERIAL CHARACTERITATION.**

Calculations between dry weight/fresh weight, leaves weight/stem weight, specific leaf weight (SLW), specific leaf area (SLA) and foliar dimensions in shrubs species. Intraspecific comparison and between sought slopes plants and north slopes plants.

**B1.- Water content (Ratio dry weight/ fresh weight)**

In terrestrial ecology when we speak about leaves production, woody biomass, N content or chlorophyll of foliar material, we always use the dry weight of the tissue. This is because the water content in tissues is highly variable and could never have an adequate comparison. So, if we want to compare the chlorophyll content in leaves of two different species or even in two leaf types within the same species, we always have to refer the quantities (chlorophyll, in this case), to a dry leaf weight unit. Also when we analyse SLW –specific leaf weight- (which represent the ratio between weight and leaf surface), in this case the weight is dry weight not fresh weight.

The ratio between dry and fresh weight in leaves is an indicator of hydration conditions presented by a specific tissue. Under conditions of water stress and high evaporation fresh leaves have lower water content in their tissues. In fact the ratio [(fresh weight-dry weight) / dry weight] in the leaves of these species is used as a fuel flammability index and it is used to evaluate the susceptibility of a type of vegetation to suffer a wildfire.

On the other hand the level of hydration compared between species also represents some functional characteristics of the species. In general, several studies in drylands classify plant species within two main strategies: isohydric and anisohydric species. Both strategies are related to the maintenance of foliar hydration and with the gas exchange dynamics and water potential. Therefore it is considered that isohydric species maintain higher and constant water content throughout the year than anisohydric species whose would show higher fluctuations in their water content.

Although we are in the Mediterranean winter (time of the year with high water availability in the soils and lower evapotranspiration rates), it can be expected differences in moisture content between types of leaves within the same species according to their slope aspect (south or north conditions) because of environmental conditions vary between both orientations, although there are more subtle differences in winter than in spring or summer.

Due to the fact that leaves collected in the field are living leaves with a specific water content, for the laboratory calculations we need to remove the water. For this purpose, a sample of thirty leaves taken in the field will be weighted in fresh and will be introduced in the oven at about 80 º until constant weight. Weighing the leaves before placing into the oven (fresh weight) and after (dry weight without water) we will be able to know not only the amount of water they had at the time of sample collection, but the ratio of dry weight / fresh weight, and thus leaf moisture or leaf water content.

**\*\**LABORATORY PROCEDURE AFTER FIELD ARRIVAL.***

1.- Weight an empty plastic bag like the ones used for leaf collection at the activity and write down its weight.

2.- Weight the leaves introduced in its bag (fresh weight) and write it down in **Table 2.**

3.- Introduce in the oven (around 24h at 60ºC) to obtain its dry weight. Remember they should be labelled correctly: Team number/ team name/ sample (sp XXX suntrap, ss XXX shady spot, sp YYY suntrap, ss YYY shady spot).

**With the twigs samples that are in plastic bags:**

1.- Put them into the FRIDGE to conserve to the next session.

***\*\*LABORATORY PROCEDURE IN THE NEXT SESSION.***

4.-In the next laboratory activity, weight again each sample leaves, write down the dry weight and the ratio between dry weight/ fresh weight in the **Table 2.**

**B2.- Ratio leaves weight/stem weight in scrub twigs.**

The ratio between leaves weight and stem weight is an easy and fast measurement that allows to identify production variations or photosynthetic biomass per unidad de soporte conductor (leadframe). We will evaluate this coefficient in collected twigs in types from the same species that are in suntrap and shady spot.

**\*\*LABORATORY PROCEDURE**

1. Take out the twigs samples of each species and orientations kept in the FRIDGE. You should have three twigs per species and hillside. For each of them without confusing you should:
2. Separate all the stem leaves for each twigs.
3. Weight in fresh and separately the leaves and stems of the twigs 1, 2 and 3.
4. Write down the values in the **Table 3**.
5. Ones you have written all the weights, KEEP LEAVES FROM EACH SPECIES AND ORIENTATION FOR THE NEXT STEP. The rest can be pulled.
6. NOTE: One sample would represent three twigs of the species xxx in south slope, another sample would represent three twigs of the species xxx in the north slope etc.

**B3.- Specific leaf weight (SLW) and specific leaf area (SLA)**

The SLW is a variable that relates leaf dry weight with the surface of that tissue. In this way it indicates how much leaf biomass are invested by unit of leaf surface. In flat, wide and low thickness leaves such as many deciduous species (walnut, beech, chestnut, etc) the SLW are low, whereas the species that present thick, small, narrow leaves and with thick cuticles SLW is high. Threfore,sclerophyllous species, common in Mediterranean vegetation (oaks, pines, gorse, rosemary, etc.) have high SLW. For these reasons, SLW is considered a variable to indicate the level of sclerophylly in leaves. Sclerophylly is an adaptive response of many Mediterranean species to water scarcity, but also to high light conditions. In Mediterranean environments characterized by a strong evaporative demand, plants need to save water and not to lose it too easily. Since water is lost through the surfaces, what matters is to reduce it in relation to the weight in order to reduce water losses. This is achieved with foliar morphologies that develop small, strong leaves and sometimes transformed into spines, sometimes rolled up on themselves (as Stipa tenacissima) and in which the ratio weight / surface is high (high SLW).

The specific leaf area (SLA) expressed in cm2 / mg is the inverse of the SLW, that means SFA = 1 / SLW. Inversely to the SLW, higher SLA involve higher transpiration area per leaf tissue weight unit and therefore higher risk for the leaf to lose water.

In this activity, we will calculate the SLW and SLA of two representative shrub species of Mediterranean ecosystem in the Maigmó area, and we will compare these rates taking leaves of the same species in south and north slopes. Although most of the species we are going to study will have sclerophyllous leaves, you will be able to see differences between them. However, in Maigmó area. there are some shrubs with softer and wider leaves (like for instance the *Anthyllis cytisoides* and the *Cistus albidus* where we would expect lower values of SLW).

To calculate the SLW are different methodologies. All of them based on the collection of a representative sample of the leaves of specific species to **which the area should be measured (just for one side of the leaves) and the sample is dried and calculated its dry weight**.

The ratio dry weight/surface generally expressed in mg/cm2 units is the **specific leaf weight (SFW)** and its inverse surface/dry weight cm2/mg is what is called **specific leaf area (SFA).**

The surface of the leaves samples is always measured in fresh leaves. Three methods are the most commonly used. Let suppose a ten leaves sample, methods are:

1. Trace on graph paper each sample leaf, cut their silhouettes and count the mm2 or cm2 within them. It is an accurate method but very laborious.
2. Trace on vegetal paper each sample leaf, cut their silhouettes and weight them together. Later, cut a square or rectangle of the same vegetal paper and known surface. For example a 2 by 2 cm square and weight it. As shown below, we calculate the total surface of the 10 silhouette leaves (X) which also equals to the total area of the 10 real leaves.

*4 cm2 (pattern surface)--------------------pattern weight*

*X cm2 (10 silhouette surfaces)--------------10 silhouette weight*

In this example the pattern is 4 cm2. But you have to put the exact pattern surface that you do in case is different to the value. This method is convenient, fast and accurate as the previous one.

1. Leaves scanner. You should scan the leaves in a high resolution (300 ppt) and the resulting image is saved as a bitmap. Then, with an appropriate software you will measure the leaves surface either individually or in combination. The software we have allows to see the exact surface of the elements the program is measuring, allows to adjust the colour spectrum and indicate the surface and the error in the measurement of each leaves as well as the one caused by putting the data to a excel sheet.

***In this activity we will use the c) method for the shrub leaves***

**\*\*LABORATORY PROCEDURE.**

**SURFACE AREA OF LEAVES (c method)**

1. To see the proportion between leaves height/ stem weight in the twigs. Take six leaves of each shrub species and orientation (in total 4 samples of 6 leaves each one). Be careful not to mix them. Weight in fresh each sample and write down the values (mg) in **Table 4.**
2. Place over acetate the samples leaves one by one, labelled appropriate (ss XXX south, ss XXX north, ss YYY south, ss YYY north) each sample should be in a row and the leaves should not touch each other. The **greener part of the leaf should be putted down**. You should have four leaves rows, each of them corresponding to a sample. Finally, place over acetate a reference square of 2x2 cm.

You should reference each row with the species/orientation they belong in your notebook.

1. Cover carefully the acetate where the samples and the reference square are taking care the leaves do not bend if they move.
2. Fix the two acetates with a paperclip and bring them to the scanner.
3. Scan and save them with the team name and BMP extension.
4. Apply the programme *MIDE*, it gives the individual area, length and width of each leaf. Do the addition of the leaves areas to know the total area of each sample in each shrub species per orientation (en mm2). Write down these values (cm2) in **Table 4**.
5. Once you have the total and individual surface of each leaves samples, you have to calculate the dry weight. For that, you will use the leaves ratio DW/FW calculated in **Table 2.** Put the values in **Table 4**. Then, calculate the SLW in (mg /cm2) and the SLA in (cm2 /mg). Write the down in **Table 4.**

**4. Leaf size**

Note down the length and width of the 6 leaves of each sample given by the program *MIDE*. So, you can obtain the foliar dimensions average in the two shrub species both in south and north slopes. Put the values in **Table 5.**

**5. Tables preparation and discussion.**

Complete the tables information and laboratory to later discussion. Moreover, you should put the final data in an excel template to calculate means and standard errors, and to make comparisons of the main characteristics.

**NOTEBOOK**

ACTIVITY OBJECTIVES (LABORATORY)

**Laboratory**

Concept of specific leaf weight as an index of sclerophylly. Ecological meaning.

Procedure and foliar areas calculations.

Fresh weight vs Dry weight in ecology.

Soil water content. Spatial variation, measurement and ecological meaning.

Mean values and standard deviations of the different variables analysed.

Get familiar with the material and laboratory equipment.

**PRACTICAL ACTIVITY 2:** CHARACTERIZATION OF AQUATIC AND LITTORAL ECOSYSTEMS.

*INTRODUCTION*

The objective of these activities is to demonstrate if the environmental factors variations have ecological responses.

The study area is to 40 kilometres south of Alicante. In this area we will study some ecology characteristics of aquatic and coastal ecosystems. The activity will be started in the FIELD (observation and sampling) to be continued in the LABORATORY (water analysis, get results and data collection) and continues with the data processing in the COMPUTER session. All the results will be evaluated in the REPORT that each team should provide.

The field work in this activity is divided in two parts:

1. In the morning, there will be a walking tour along the last 3500 m of the Segura river.

In this activity, you should observe, quantify some variables and take water samples to be analysed in the next laboratory session. With the analysis of samples, you must look for differences in the ecosystem in relation to water salinity.

1. After lunch, we will work in the coastal pine forest located on the dunes. There, vegetation transects 600m long will be traced perpendicular to the sea line. In them, some variables will be recorded according to plan of work in this practical activity. These variables will serve to look for differences in dune vegetation due to the marine spray

You should take notes about observations in the field that can be useful to study and understand the ecological environment and to prepare the final report.

**GENERAL WARNING FOR THE ACTIVITY.**

**1. To distribute** the tasks. It is not operating four people doing the same task.

**2. Label** correctly the samples (**VERY IMPORTANT)**. Do not confuse either in the field or laboratory

**3. Avoid** sample loss.

4. **Carry some compass**; It is not necessary to be very accurate (some phones has this function, the GPS also serves, etc)

**5. Wear appropriate clothing and footwear.**

**6. Bring food and drink.** The practice will start at 8:30h and we will return at 19.00h.

**7. Returning material.** You will have a list of the material received to return at the end of the activity.

**PRACTICAL ACTIVITY 2.1:** CHARACTERIZATION OF AQUATIC ECOSYSTEMS**.**

*ANALYSIS OF ECOSYSTEM CHANGE DUE TO WATER SALINITY OF THE RIVER.*

The rapid regression in extension and environmental quality that have been suffered all the wetlands around the world in this century has also occurred in Spain. It is estimated the 60% of Spanish wetlands have disappeared in the last 40 years.

Wetlands are considered ecosystems with high biological interest and they are subjected to different rules and regulations either national, international and locals (see example: Directive 79/409 / EEC 2 April on the Special Protection Areas Bird whose application in the *Comunidad Valenciana* referes to the existence of 7 ZEPA zones located in wetlands areas, the Directive 92/43 / EEC May 1992 relative to the natural habitats conservation).The wetlands called “Dunas de Guardamar” in the Comunidad Valenciana’s law is listed as LIC (“Lugar de Interés Comunitario”).

Segura River wetland’s mouth will be the area to study in this practical activity. It is a dynamic ecosystem of great potential, altered by the large amount of nutrients provided by the farmland drainage, which has led to a significant increase in abundance and diversity of fish species.

**ITINENARY AND SAMPLING POINTS**.

Segura River rises in *Pontonés*, province of Jaén, and its 325 km is mostly used for irrigation. This water returns to the river downstream but charged with ions, which results in a high conductivity at the end of the river.

In less than three Km during the practical activity, water conductivity changes will be even more pronounced in connection with seawater. It can be seen channels that draw water from the river (called *“azudes” or* *diversion dam*) and others that incorporate water that has been used irrigation practices (called “*azarbes”*). This allows to find in this short distance very different aquatic environments, mainly due to the type of dissolved salts in water and it determine the different species presence, both animal and plants.

Four stops will be made along the itinerary. In every stop you should follow the same protocol for the observations, taking measurements and samplings.

The natural trail (the “old” one) and the artificial (the “new” one) in the last Km of the river as well as the route and four stops locations are presented in figure 1.

|  |  |
| --- | --- |
| Figure 1. Mouth Segura River. | |
| The “old” riverbed is highlighted with the “new” one. | Location of the four points in this activity. |
|  |  |

The first stop is next to the diversion dam of San Antonio, which is the last dam on Segura River. From here, water is derived to the orchards. Conductivity values are around 2.000 and 5.000 μS/cm on the upstream, but depending on rains. Riparian vegetation has changes in response of the river clean up and a recent riverbank restoration where it have been planted tree species, herbaceous perennials, hydrophytes and hemicrypthophytes.

The second stop will be about 500 meters downstream from the weir. Water conductivity is usually much higher if the river water does not run over the weir, since the sea water entry can alter the chloride concentration. In this zone it can be seen the river restoration results.

The third stop is up to the beginning of the old channel. Here water is incorporated from *azarbe* “La Villa”. The lack of fresh water to the new channel due to the water infiltration through the soil and the presence of a high seawater percentage, determine higher water conductivities. These conditions severely effect the vegetation and fauna development.

The fourth stop is 1 Km downstream (and around 900 meters from the mouth) where the scarcity of vegetation can be seen.

**FAUNA AND FLORA OF THE AREA.**

**Vegetation**

During the itinerary we can find marsh areas with herbaceous dominance such as reed (*Phragmites australis*) or cane (*Arundo donax*) and large tendency to flooding conditions. You will find areas with saline pasture with rushes (*Juncus maritimus),* halophytic schubs with presence of *Sarcocornia sp, Salicornia sp, Suaeda sp, Limonium sp* and *Arthrocnemum macrostachyum,* or Mediterranean saline steppes which have a set of perennials as *Frankenia pulverulenta, Hordeum marinum, Hymenolobus procumbens, Lygeum spartum, Senecio auricula, Sphenopus divaricatus, Suaeda spicata,* and several species of *genus Limonium*. It can also be found Tarayal (Tamarix sp community) scattered in the area. In abandoned agricultural areas there are halo-nitrophyllous shrubs classified as halo-nitrophyllous bushes (Pegano-Salsoletea).

**Fauna**

Most abundant fauna in the area include several species of fish (eel, carp, mullet), amphibians (common toad, natterjack toad, common frog), reptiles (lizards, eyed-lizard and snakes) and mammals (hedgehog, squirrel, dormouse, rabbit or hare), but they are difficult to see due to human disturbances. However, it will be easy to wacth many water births. To assist in the identification of most of the species you can check the photographic annex.

**WORK METHODOLOGY**

In each of the stops you will take the same measures with the aim to establish differences or similarities between the different zones where, presumably, the salinity of the water is different and determine different habitats.

For the fauna, the annotations will not be just in each point due their mobility. This quantification will allow us to observe possible changes in the presence of one or the other species, especially those related to water conditions. You should write down each species observed before arrive to each point.

Once you are in each sampling point, the tasks you have to make are:

1.-Water sampling:

Each team will label one 300ml bottle (found in the activity material) with the group name, date and sampling place (P-1 in the farthest point to the sea, P2, P3 to P4 nearest point to the sea). You should fill the bottle with river water. If there are any difficulties, the teacher will fill a bigger bottle. Then, in the laboratory session, you should measure the pH, conductivity, Chlorides and Alkalinity (which basically indicate the bicarbonate concentration of the sample).

2.- Vegetation presence and quantification: ­­

You will identify vegetation species from different environments. Many of them are difficult to identify and you will have a photographic booklet with the most abundant fauna and flora.

You should extend a parallel tape along 20m to the river. In the space between the tape and the river you should write down all the present vegetation species. On the same surface it will be determined the species coverage listed in **Table 1.** This will be made accordingto the Braun-Blanquet abundance-dominance scale indicated in the following table.

|  |  |
| --- | --- |
| Braun-Blanquet abundance-dominance scale | |
| Índex | Meaning |
| r | One individual, negligible plant cover |
| + | Some individuals, low plant cover |
| 1 | Lower cover than 5% |
| 2 | Cover of 5 to 25% |
| 3 | Cover of 25 to 50% |
| 4 | Cover of 50 to 75% |
| 5 | Cover equal or more than 75% |

3.- Leaves sampling to measure its size.

You should collect *Atriplex halimus* leaves in two parts of the plant. This allows us to study the leaf size variations along the different stops. Methodology:

* Select 3 large types of *Atriplex halimus*, which are close to the water.
* From the top of the plant and sun exposed parts, you have to take five leaves of each type (fifteen leaves in total) and keep them together in a bag labelled with the team name, the point and plant zone.
* From the bottom of the plant, it will be sampled and kept the same number of leaves.
* CAUTION: When sampling, you can appreciate that leaves coming out from the stem are bigger than those developed in the lateral buds. You should sample avoid those from the stem and select leaves in similar positions.
* In the laboratory, you should write down the length and width of each leaf sampled in the top of the plant in Table 2. Measures of the leaves from the lower part of the plant will be written down in Table 3.

4.- Compilation of different animal species observed on the routes. It will be counted the individuals observed and **Table 4** and **5** will be fulfilled.

**FIELD SAMPLING TABLES**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Table 1**.-Abundance quantification (according to Braun-Blanquet abundance-dominance scale)  of the most common vegetation species along the river in the four sampling points (P1, P2, P3 y P4). P1 is taken as the farthest point to the sea. | | | | | |
| **Photo nº** | **Species** | **P 1** | **P2** | **P3** | **P4** |
|  | *Sarcocornia fruticosa* |  |  |  |  |
|  | *Scirpus maritimus* |  |  |  |  |
| 1 | *Urtica urens* |  |  |  |  |
| 2 | *Cynara scolymus* |  |  |  |  |
| 3 | *Suaeda vera* |  |  |  |  |
| 4 | *Salsola vermiculata* |  |  |  |  |
| 5 | *Chenopodium album* |  |  |  |  |
| 6 | *Hordeum murinum* |  |  |  |  |
| 7 | *Atriplex postrata* |  |  |  |  |
| 8 | *Salsola soda* |  |  |  |  |
| 9 |  |  |  |  |  |
| 10 |  |  |  |  |  |
| 11 |  |  |  |  |  |
| 12 |  |  |  |  |  |
| 13 | *Mesembryanthemum cristallinum* |  |  |  |  |
| 14 |  |  |  |  |  |
| 15 |  |  |  |  |  |
| 16 |  |  |  |  |  |
| 17 |  |  |  |  |  |
| 18 |  |  |  |  |  |
| 19 |  |  |  |  |  |
| 20 | *Ditrichia viscosa (Inula)* |  |  |  |  |
| 21 |  |  |  |  |  |
| 22 |  |  |  |  |  |
| 23 |  |  |  |  |  |
| 24 |  |  |  |  |  |
| 25 |  |  |  |  |  |
| 26 |  |  |  |  |  |
| 27 |  |  |  |  |  |
| 28 | *Limonium sp. 28* |  |  |  |  |
| 29 |  |  |  |  |  |
| 30 |  |  |  |  |  |
| 31 | *Tamarix sp (tamarit) 31* |  |  |  |  |
| 32 |  |  |  |  |  |
| 33 |  |  |  |  |  |
| 34 | *Salix purpurea 34* |  |  |  |  |
| 35 |  |  |  |  |  |
| 36 | *Atriplex halimus 36* |  |  |  |  |
| 37 | *Juncus maritimus 37* |  |  |  |  |
| 38 | *Juncus acutus 38* |  |  |  |  |
| 39 | *Phragmites australis (carrizo) 39* |  |  |  |  |
| 40 | *Arundo donax (caña) 40* |  |  |  |  |
| 41 | *Typha sp 41* |  |  |  |  |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 2**.- Leaves dimensions of *Atriplex halimus* in the top plant (mm) in the four points. | | | | | | | | |
|  | **P1** | | **P2** | | **P3** | | **P4** | |
|  | **Top** | | **Top** | | **Top** | | **Top** | |
|  | **Length** | **Width** | **Length** | **Width** | **Length** | **Width** | **Length** | **Width** |
| 1 |  |  |  |  |  |  |  |  |
| 2 |  |  |  |  |  |  |  |  |
| 3 |  |  |  |  |  |  |  |  |
| 4 |  |  |  |  |  |  |  |  |
| 5 |  |  |  |  |  |  |  |  |
| 6 |  |  |  |  |  |  |  |  |
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| 13 |  |  |  |  |  |  |  |  |
| 14 |  |  |  |  |  |  |  |  |
| 15 |  |  |  |  |  |  |  |  |
| Mean\* |  |  |  |  |  |  |  |  |
| σ\*\* |  |  |  |  |  |  |  |  |
| \* Average. In EXCEL corresponds to the *PROMEDIO* or AVERAGE function.  \*\* Standard deviation. DESVEST in EXCEL. | | | | | | | | |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 3**.- Leaves dimensions of *Atriplex halimus* in the low part of the plant (mm) in the four points. | | | | | | | | |
|  | **P1** | | **P2** | | **P3** | | **P4** | |
|  | **Low** | | **Low** | | **Low** | | **Low** | |
|  | **Length** | **Width** | **Length** | **Width** | **Length** | **Width** | **Length** | **Width** |
| 1 |  |  |  |  |  |  |  |  |
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| 15 |  |  |  |  |  |  |  |  |
| Mean\* |  |  |  |  |  |  |  |  |
| σ\*\* |  |  |  |  |  |  |  |  |
| \* Average. In EXCEL corresponds to the *PROMEDIO* or AVERAGE function.  \*\* Standard deviation. DESVEST in EXCEL. | | | | | | | | |

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| --- | --- | --- | --- | --- | --- |
| **Table 4**. -Number of different individuals in each of the most common **fish** **species, amphibians, reptiles and mammals**, along the points. You can check the photographic booklet to identify them. | | | | | |
| **Photo nº** | **Species** | **P 1** | **Between P1 and P2** | **Between P2 and P3** | **Between P3 and P4** |
|  |  |  |  |  |  |
|  | **FISHES** |  |  |  |  |
| 1 | *Anguilla anguilla* (anguila) |  |  |  |  |
| 2 | *Cyprinus carpio* (carpa) |  |  |  |  |
| 3 | *Mugil cephalus* (pardete) |  |  |  |  |
| 4 | *Chelon labrossus* (mujol, lisa) |  |  |  |  |
|  |  |  |  |  |  |
|  | **AMPHIBIANS** |  |  |  |  |
| 1 | *Bufo bufo (*sapo común*)* |  |  |  |  |
| 2 | *Bufo calamita (*sapo corredor*)* |  |  |  |  |
| 3 | *Rana perezi (*rana común*)* |  |  |  |  |
|  |  |  |  |  |  |
|  | **REPTILES** |  |  |  |  |
|  |  |  |  |  |  |
| 1 | Acanthodactylus erythrurus (L. coliroja*)* |  |  |  |  |
| 2 | *Lacerta lepida* (lagarto ocelado) |  |  |  |  |
| 3 | *Podarcis hispanica* (L. ibérica) |  |  |  |  |
| 4 | *Psammodromus algirus* (L. colilarga) |  |  |  |  |
| 5 | *Elaphe scalaris* (culebra de escalera) |  |  |  |  |
| 6 | *Natrix natrix (*culebra de agua) |  |  |  |  |
|  |  |  |  |  |  |
|  | **MAMMALS** |  |  |  |  |
| 1 | *Erinaceus europaeus* (erizo común) |  |  |  |  |
| 2 | *Sciurus vulgaris* (ardilla) |  |  |  |  |
| 3 | *Eliomys quercinus* (lirón careto) |  |  |  |  |
| 4 | *Oryctolagus cunículus* (conejo) |  |  |  |  |
| 5 | *Lepus granatensis* (liebre) |  |  |  |  |
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|  | **OTHERS** |  |  |  |  |
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| **Table 5**.- Number of different individuals in each of the most common **birds species**, along the points. You can check the photographic booklet to identify them. | | | | | |
| **Photo nº** | **Species** | **P 1** | **Between P1 and P2** | **Between P2 and P3** | **Between P3 and P4** |
|  |  |  |  |  |  |
| 1 | *Tadorna tadorna* (tarro blanco) |  |  |  |  |
| 2 | *Anser anser* ( ansar común) |  |  |  |  |
| 3 | *Anas platyrhynchos* (pato azulón) |  |  |  |  |
| 4 | *Tachibaptus ruficolis* (zampullín chico) |  |  |  |  |
| 5 | *Milvus migrans* (milano negro) |  |  |  |  |
| 6 | *Falco tinnunculus* (cernícalo) |  |  |  |  |
| 7 | *Porphyrio porphyrio* ( calamón común) |  |  |  |  |
| 8 | *Fulica cristata* (focha moruna) |  |  |  |  |
| 9 | *Fulica atra* ( focha común) |  |  |  |  |
| 10 | *Himantopus himantopus* (cigueñuela) |  |  |  |  |
| 11 | *Recurvirostra avosetta* (avoceta) |  |  |  |  |
| 12 | *Gallinula chloropus* (gallineta) |  |  |  |  |
| 13 | *Larus cachinnans* ( gaviota patiamarilla) |  |  |  |  |
| 14 | *Phalacrocorax diomedei* (cormoran moñudo) |  |  |  |  |
| 15 | *Phalacrocorax carbo* (cormorán grande) |  |  |  |  |
| 16 | Calonectris diomedea (pardela cenicienta) |  |  |  |  |
| 17 | *Larus ridibundus (gaviota reidora)* |  |  |  |  |
| 18 | *Larus genei* (gaviota picofina) |  |  |  |  |
| 19 | *Erithacus rubecula* (petirrojo) |  |  |  |  |
| 20 | *Parus major* (carbonero común) |  |  |  |  |
| 21 | *Sterna sandvicensis* ( charrán patinegro) |  |  |  |  |
| 22 | *Parus cristatus* (herrerillo capuchino) |  |  |  |  |
| 23 | *Cisticola juncidis* ( buitron) |  |  |  |  |
| 24 | *Upupa epops* (abubilla) |  |  |  |  |
| 25 | *Turdus merula* (mirlo común) |  |  |  |  |
| 26 | *Acrocephalus* sp (carricerín) |  |  |  |  |
| 27 | *Gallinago gallinago* (agachadiza) |  |  |  |  |
| 28 | *Sturnus sp (e*stornino) |  |  |  |  |
| 29 | *Passer domesticus* (gorrión) |  |  |  |  |
| 30 | *Carduelis carduelis* (jilguero) |  |  |  |  |
| 31 | *Motacilla alba* (lavandera blanca) |  |  |  |  |
| 32 | *Serinus serinus* (verdecillo/ gafarró) |  |  |  |  |
| 33 | *Carduelis chloris* (verderón/ verderol) |  |  |  |  |
| 34 | *Alcedo atthis* (martín pescador) |  |  |  |  |
| 35 | *Chaladrius alexandrinus* (chorlitejo) |  |  |  |  |
| 36 | *Tringa ochropus* (andarríos grande) |  |  |  |  |
| 37 | *Bubulcus ibis* ( garcilla bueyera) |  |  |  |  |
| 38 | *Egretta alba* (garceta grande) |  |  |  |  |
| 39 | *Ardea cinérea* (garza real) |  |  |  |  |
| 40 | *Egretta garzetta* (garceta común) |  |  |  |  |
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**PRACTICAL ACTIVITY 2.2:** CHARACTERIZATION OF LITTORAL ECOSYSTEMS**.**

*MARINE INFLUENCE ON DUNE VEGETATION*

The second part of this activity will be to work in the littoral zone. There you should look for differences in dune vegetation that respond to the effect of the sea and marine spray on it.

In *Guardamar* there are coastal dune systems (i.e. ridge of dunes) due to the sediment movement produced by the river, wind and sea weaves. The last two phenomena also have a significant effect on the dune vegetation.

Dune succession causes an alteration of exposed areas to sea breeze (Windward\*) and others located in the opposite side (Leeward\*\*\*) where vegetation won’t be so influenced (Figure 1). Therefore, sea influence is evident not just in the specific composition of the dunes but the morphological characteristics of species are also influenced by the effect of marine spray.

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| **Figure 1-Dune fences (ridge of dunes) outline system:**  - Windward\*: Part where the wind comes. Therefore, part of the dune facing the sea.  - Crest\*\*: Top of the dune.  - Leeward\*\*\*: The opposite part of the windward. Related to the part of the dune where does not receive the wind from the sea. |

*Because in these systems is very easy to become disoriented and to follow the transect as straight as possible it is convenient to carry a compass (or other corresponding application) and walk E to W direction.*

**FIELD WORK**

Each team will work on the same field transect along the dune system. This transect will run perpendicular to the sea. Depending on the place we are, the transect will have between 500-1100m long. You should write down characteristics and measures per each dune in **identical tables** (founded in this guide). IT IS VERY IMPORTANT TO PLACE (and write it down) THE DUNE POSITION REGARDING THE SEA.

Each team will sample always in the same dune position.

**Methodology and variables to register:**

On the dune:

* Dune location regarding to the sea.
* Compare the height of the dunes between each other.
* Sampling location in the dune (W, C or L)
* Windward (W) part of the dune facing the sea,
* Crest (C), top of the dune
* Leeward (L) part of the dune that is protected from the wind of the sea.

In six pines of this area, Chosen by team members randomly:

* Pine number
* Pine perimeter, measured at 130 cm to the soil (in cm).
* Height class (h). We will be distinguish between:
* h1 if the height is less than 1 meter.
* h2 if the height is between 1 and 2 meters.
* h3 if the height is between 2 and 4 meters.
* h4 if the height is over 4 meters.
* Trunk inclination. It will be distinguished only three types,
* I1 For pines whose trunks are vertical or almost vertical.
* I2 For pines with intermediate inclination trunks.
* I3 For pines with very inclined trunks. (Angles smaller than 30º to the soil)
* Pine canopy affectation. Most of the pines in the pine forest have a lot of dry branches in the lower part, but you do not have to measure that. You have to measure the canopy defoliation in green leaves. You must see if the pines have loss their leaves in the top side. It will be considered:
* YES, if there are more than 50% defoliated or damaged cup.
* NO, if there are less than 50% defoliated or damaged cup.

**OBTAINING DATA MEANS**

**.** First, you should convert the perimeters into diameters.

Perimeter

Pines mean diameter = 2 π r; Ø= 2r. Then, calculate the mean and standard deviation.

For the following variables: **pines height, pines inclination and canopy damage,** which don´t have a numeric value only class type, you should indicate in the table which is the most common type in the plot.

**DATA, RESULTS TREATMENT.**

To achieve the objective of this activity, that means, whether or not exist differences in dune vegetation due the marine effect, it will be made graphs or correlations between variables in the computer session. You will have access to a bigger database with lots of data to find relationships between variables.

Each team must present the results in a report, which will be evaluated.

|  |  |  |  |  |  |  |  |  |  |  |  |
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| **TABLE dune-1**. *Pinus halepensis* structure in a perpendicular transect to the sea. Dunes North of the Segura River mouth. | | | | | | | | | | | |
| TEAM | Dune situation regarding the sea | | Height of the dune compared to the others | Sampling location in the dune  (W,C or L) | | Pine  (nº) | | PERIMETER at 130 cm to the soil (cm) | h | I | Canopy  damage |
|  |  | |  |  | | 1 | |  |  |  |  |
| 2 | |  |  |  |  |
| 3 | |  |  |  |  |
| 4 | |  |  |  |  |
| 5 | |  |  |  |  |
| 6 | |  |  |  |  |
| **Dune characteristics:**  Size, continuity…  Total height, (height meters approximately):  Relative height to neighbours plants.  Vegetation, type y density:  Psammophyte plants presence:  Animal presence signs  …..  Location drawing | | | | | | | | | | | |
| **Fence (ridge) situation:** will be numbered in ascending order from East (E) to West (W).  **Pine:** will be numbered without any established order.  **P-130** = Perimeter (P) at 130 cm to the soil.  **h** = Pine height. 4 classes will be distinguished as indicated\*  **I** = Trunks inclination. According to the following codes\*\*  **Dune situation:** indicateif the dune it is in Windward (W), Crest (C) or leeward (L)\*\*\*  **Cup damage:** Indicate YES or NO, depending if it has more or less the 50% of the cup defoliated or damage. | | | | | | | | | | | |
| **\* Height types**  h1 Height <1 meter.  h2 Height between 1 and 2 m.  h3 Height between 2 and 4m.  h4 Height > 4 m. | | **\*\* In: Trunks inclination.**  Deviation level from the vertical:  I-1 almost vertical  I-2 Around 45º  I-3 Is separated from the soil less than 30º | | | **1**  **3**  **2** | | **Dune situation \*\*\***  W Sea  E  Dune situation can be located roughly in this sketch. | | | | |

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| **TABLE dune-2.** *Pinus halepensis* structure in a perpendicular transect to the sea. Dunes North of the Segura mouth**.** | | | | | | | | | | | |
| TEAM | Dune situation regarding the sea | | Height of the dune compared to the others | Sampling location in the dune  (W,C or L) | | Pine  (nº) | | PERIMETER at 130 cm to the soil (cm) | h | I | Canopy damage |
|  |  | |  |  | | 1 | |  |  |  |  |
| 2 | |  |  |  |  |
| 3 | |  |  |  |  |
| 4 | |  |  |  |  |
| 5 | |  |  |  |  |
| 6 | |  |  |  |  |
| **Dune characteristics:**  Size, continuity…  Total height, (height meters approximately):  Relative height to neighbours plants.  Vegetation, type y density:  Psammophyte plants presence:  Animal presence signs  …..  Location drawing | | | | | | | | | | | |
| **Fence (ridge) situation:** will be numbered in ascending order from East (E) to West (W).  **Pine:** will be numbered without any established order.  **P-130** = Perimeter (P) at 130 cm to the soil.  **h** = Pine height. 4 classes will be distinguished as indicated\*  **I** = Trunks inclination. According to the following codes\*\*  **Dune situation:** indicateif the dune it is in Windward (W), Crest (C) or leeward (L)\*\*\*  **Cup damage:** Indicate YES or NO, depending if it has more or less the 50% of the cup defoliated or damage | | | | | | | | | | | |
| **\* Height types**  h1 Height <1 meter.  h2 Height between 1 and 2 m.  h3 Height between 2 and 4m.  h4 Height > 4 m. | | **\*\* In: Trunks inclination.**  Deviation level from the vertical:  I-1 almost vertical  I-2 Around 45º  I-3 Is separated from the soil less than 30º | | | **1**  **3**  **2** | | **Dune situation \*\*\***  W Sea  E  Dune situation can be located roughly in this sketch. | | | | |

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| **TABLE dune-3**. *Pinus halepensis* structure in a perpendicular transect to the sea. Dunes North of the Segura mouth. | | | | | | | | | | | |
| TEAM | Dune situation regarding the sea | | Height of the dune compared to the others | Sampling location in the dune  (W,C or L) | | Pine  (nº) | | PERIMETER at 130 cm to the soil (cm) | h | I | Canopy damage |
|  |  | |  |  | | 1 | |  |  |  |  |
| 2 | |  |  |  |  |
| 3 | |  |  |  |  |
| 4 | |  |  |  |  |
| 5 | |  |  |  |  |
| 6 | |  |  |  |  |
| **Dune characteristics:**  Size, continuity…  Total height, (height meters approximately):  Relative height to neighbours plants.  Vegetation, type y density:  Psammophyte plants presence:  Animal presence signs  …..  Location drawing | | | | | | | | | | | |
| **Fence (ridge) situation:** will be numbered in ascending order from East (E) to West (W).  **Pine:** will be numbered without any established order.  **P-130** = Perimeter (P) at 130 cm to the soil.  **h** = Pine height. 4 classes will be distinguished as indicated\*  **I** = Trunks inclination. According to the following codes\*\*  **Dune situation:** indicateif the dune it is in Windward (W), Crest (C) or leeward (L)\*\*\*  **Cup damage:** Indicate YES or NO, depending if it has more or less the 50% of the cup defoliated or damage | | | | | | | | | | | |
| **\* Height types**  h1 Height <1 meter.  h2 Height between 1 and 2 m.  h3 Height between 2 and 4m.  h4 Height > 4 m. | | **\*\* In: Trunks inclination.**  Deviation level from the vertical:  I-1 almost vertical  I-2 Around 45º  I-3 Is separated from the soil less than 30º | | | **1**  **3**  **2** | | **Dune situation \*\*\***  W Sea  E  Dune situation can be located roughly in this sketch. | | | | |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **TABLE dune-4**. *Pinus halepensis* structure in a perpendicular transect to the sea. Dunes North of the Segura mouth. | | | | | | | | | | | |
| TEAM | Dune situation regarding the sea | | Height of the dune compared to the others | Sampling location in the dune  (W,C or L) | | Pine  (nº) | | PERIMETER at 130 cm to the soil (cm) | h | I | Canopy damage |
|  |  | |  |  | | 1 | |  |  |  |  |
| 2 | |  |  |  |  |
| 3 | |  |  |  |  |
| 4 | |  |  |  |  |
| 5 | |  |  |  |  |
| 6 | |  |  |  |  |
| **Dune characteristics:**  Size, continuity…  Total height, (height meters approximately):  Relative height to neighbours plants.  Vegetation, type y density:  Psammophyte plants presence:  Animal presence signs  …..  Location drawing | | | | | | | | | | | |
| **Fence (ridge) situation:** will be numbered in ascending order from East (E) to West (W).  **Pine:** will be numbered without any established order.  **P-130** = Perimeter (P) at 130 cm to the soil.  **h** = Pine height. 4 classes will be distinguished as indicated\*  **I** = Trunks inclination. According to the following codes\*\*  **Dune situation:** indicateif the dune it is in Windward (W), Crest (C) or leeward (L)\*\*\*  **Cup damage:** Indicate YES or NO, depending if it has more or less the 50% of the cup defoliated or damage | | | | | | | | | | | |
| **\* Height types**  h1 Height <1 meter.  h2 Height between 1 and 2 m.  h3 Height between 2 and 4m.  h4 Height > 4 m. | | **\*\* In: Trunks inclination.**  Deviation level from the vertical:  I-1 almost vertical  I-2 Around 45º  I-3 Is separated from the soil less than 30º | | | **1**  **3**  **2** | | **Dune situation \*\*\***  W Sea  E  Dune situation can be located roughly in this sketch. | | | | |

**PRACTICAL ACTIVITY 3:** ESTIMATION SECONDARY PRODUCTION AND ECOLOGICAL EFFICIENCY

*SILKWORMS AS A MODEL*

**OBJECTIVES**

* To estimate secondary production of an herbivorous.
* Estimating Ecological Efficiencies: Assimilation, Production, Growth
* Relate the above variables with the size of the animal

**STUDY SPECIES**

Butterfly or silkworm (*Bombyx mori*) is a moth of the family Bombycidae, domesticated in Asia around 3000 BC for silk production. The caterpillars feed on mulberry leaves while the imago is not fed. The caterpillar passes through five larval stages and four moults, with increases in its weight by over 1000 times. Larval development lasts about 25-28 days (Fig 1).



Figure 1.Change in weight of silkworm larvaes. The vertical dashed lines mark the moults. (From: Tong et al., 2011. Bulletin of Entomological Research. 101: 613-622).

**MATERIAL REQUIRED**

- Animals: silkworm (*Bombyx mori*).

- Mulberry leaves.

- Small containers.

- Balance.

- Oven.

- Graph paper

- Petri dishes

**PROCEDURE**

During the laboratory work, all data will be written down on table 1.

Beginning of the experiment:

Each worm must be in a single, clean compartment with enough mulberry leaves to feed “ad libitum”.

Each worm must have been weighed at the beginning of the experiment and their fresh weight, date and time must be recorded in the compartment of the worm.

Mulberry leaves of each worm should be weighted and their fresh weight, date and time must be recorded in the compartment of the worm.

Each group of students will have 3 petri dishes with its corresponding worm.

Three leaf samples of similar shape to those used for the worms should be putted in similar compartments, but without worms. Fresh weight of these leaves should also be registered at the beginning of each experiment.

These leaf samples will be common to all students within the same group of practice and it will serve to assess the loss of water from leaves during the whole experiment.

End of the experiment (each group of students):

1. *Silkworms:*

Every group should weight individually each of your worms. It will also measure the length of the body putting the worm on a ruler or graph paper (you can push the worm gently with the tip of a pen to take a position approximately straight).

You should count the number of droppings produced each worm and their fresh weight for each worm separately.

In the binocular magnifier, you have to measure the length and diameter of a random sample of three worm droppings individually.

Once done the above, all droppings from each worm will be introduced in the oven for dry weight.

After weighting every warm, they will be killed in the freezer and then introduced into the oven to calculate dry weight.

1. *Mulberry leaves*

Fresh weight and intermediate fresh weight of the leaves should be corrected due to the loss by evaporation: leaf litter of each worm will be weighted separately. These leaf blades have lost weight due to consumption by worms but also by evaporation of water.

You should also weight the leaves samples not consumed by worms which were maintained in the experiment as a control for estimating the loss of water. Then:

Loss of weight due to evaporation= (Initial leaves weight - Final leaf weight)/ Initial leaves weight

For leaves consumed by worm:

Fresh weight of leaves without the loss of water can be calculated as

Initial fresh weight –(Final fresh weight/(1- Loss of weight due to evaporation))

Dry weight: After weighting the fresh samples, portions of leaves not consumed by worms will be introduced in the oven to calculate its dry weight.

Also, control leaf samples (i.e. leaves used to compute loss of water) should be weighed to calculate its dry weight. These three leaves samples are common for all groups.

**CALCULATIONS**

Calculations:

Data should be introduced in the spreadsheet (model provided by teacher), identifying each group and students team. Excel file has to be sent to the teacher once data has been introduced. Calculations should be done in both, dry and fresh weight.

Parameters to compute:

They are:

1. *Ingested food (I)*

I=Dry weight of leaves=Initial weight-Final weight

Fresh weight calculation should be corrected for water loss according to the above formula

1. *Secondary Production (P)*

P=Worm growth= Final weight- initial weight

1. *Assimilation (A)*

A=I-weight of droppings

1. *Respiration (R)*

R = A – P

1. *Assimilation Efficiency= A/I*
2. *Production Efficiency = P/A*
3. *Ecological Growth Efficiency =P/I*

**REPORT OF PRACTICES**

**Question:** A researcher put under a series of containers collectors in a Mulberry forest. These containers collect the droppings of the caterpillars eating the leaves. Do you think you could use that information to estimate the amount of worms living in the trees and their sizes? Could you calculate the amount of leaves that caterpillar population is consuming per day?

To answer these questions you should calculate the average of droppings per hour in your samples (Num. Droppings / Time) and explore graphically the relationships between final size of worms (final length) and the diameter (Diam Excre Med) and longitude (Lon Excre Med ) of worm dropping. Also you can explore the consumption of leaves dry weight / hour (Consumption (I) PS / hour).

Calculate the mean for efficiencies: A / R, A / P and P / I

To perform the calculations, you should use all data collection from your group. It will be necessary to complete the different fields in the Excel datasheet provided by the teacher and send it again to upload the complete dataset in the Campus Virtual.

On the next page there is a table to record the information in the laboratory. Below you can see some examples of ecological efficiencies.

|  |  |  |  |
| --- | --- | --- | --- |
| Production efficiency (P/Ax100) of several groups of animals | | | |
|  | **Group** | **P/A (%)** |  |
|  | Mice | 4,10 |  |
|  | Little Mole | 2,63 |  |
|  | Poultry | 1,26 |  |
|  | Fish | 9,74 |  |
|  | Social insects | 8,31 |  |
|  | Orthoptera | 41,67 |  |
|  | Hemiptera | 41,90 |  |
|  | Other insects | 41,23 |  |
|  | Mollusks | 21,59 |  |
|  | Crustaceans | 24,96 |  |
|  | Non-insect invertebrates |  |  |
|  | Herbivores | 18,81 |  |
|  | Carnivores | 25,05 |  |
| Data from Humphreys, 1979. | | | |

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | | | | | | | | |
|  | Assimilation efficiencies and production for homeothermic and poikilothermic (%) | | | | | | | | | |  |
|  | Efficiency | All homeotherms | | Arthropods herbivorous | | Herbivorous sucking sap | | Lepidoptera | All poikilothermic | |  |
|  | Assimilation | |  | |  | |  |  | |  |  |
|  | A/I | | 77,5±6,4 | | 37,7±3,5 | | 48,9±4,5 | 46,2±4,0 | | 41,9±2,3 |  |
|  | Production | |  | |  | |  |  | |  |  |
|  | P/I | | 2,0±0,46 | | 16,6±1,2 | | 13,5±1,8 | 22,8±1,4 | | 17,7±1,0 |  |
|  | P/A | | 2,46±0,46 | | 45,0±1,9 | | 29,2±4,8 | 50,0±3,9 | | 44,6±2,1 |  |
|  |  | |  | |  | |  |  | |  |  |
|  | | | | | | | | | | | |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table 1.**  Quantified variables for the three types of *Bombyx mori* of the experiment | | | | |
| Group’s Name: | Group components | | | |
|  |  | | | |
| **Control leaves weight (en mg)** | **Sheet 1** | **Sheet 2** | **Sheet 3** | Average  value |
| Initial fresh weight |  |  |  |  |
| Final fresh weight |  |  |  |  |
| Final DRY weight (after oven) |  |  |  |  |
|  |  |  |  |  |
| **Worms variables and their food** | **Sample 1** | **Sample 2** | **Sample 3** |  |
| **Worms weight (en mg)** |  |  |  |  |
| Initial worm fresh weight |  |  |  |  |
| Final worm fresh weight |  |  |  |  |
| Final worm DRY weight |  |  |  |  |
| **Experiment time** |  |  |  |  |
| Date and hour experiments starts |  |  |  |  |
| Date and hour experiments ends |  |  |  |  |
| Total time for the experiment (hours and minutes) |  |  |  |  |
| **Worm length (mm)** |  |  |  |  |
| Final worm length |  |  |  |  |
| **Excrements** |  |  |  |  |
| Total number |  |  |  |  |
| Excrement fresh weight (mg) |  |  |  |  |
| Excrement dry weight (mg) |  |  |  |  |
| Excrement diameter 1 |  |  |  |  |
| Excrement length 1 |  |  |  |  |
| Excrement diameter 2 |  |  |  |  |
| Excrement length 2 |  |  |  |  |
| Excrement diameter 3 |  |  |  |  |
| Excrement length 3 |  |  |  |  |
| **Food weight of each worm (mg)** |  |  |  |  |
| Initial fresh weight mulberry leaf |  |  |  |  |
| Final fresh weight mulberry leaf\* (FFW) |  |  |  |  |
| Final fresh weight CORRECTED \*\* |  |  |  |  |
| Final DRY weight mulberry leaf |  |  |  |  |
|  |  |  |  |  |
| \* Refers to the leaf rest was offered to the worm. | | | | |
| \*\* For this data, you should add the water weight that the leaf has loss by evaporation to the previous (above) data (FFW). The CORRECTED value considers the water loss percentage that has been calculated in the control leaves. | | | | |