# Practical evaluation of the relationship between community biodiversity and ecosystem productivity using a simple plant model

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## Abstract

This educational activity aims to test the relationship between the biodiversity of plant communities and their productivity. In two laboratory sessions, students assemble duckweed communities at different levels of species richness and determine their productivity. The data collected is interpreted by applying a biodiversity partitioning metric to assess the roles of complementarity and selection in driving the observed relationship between diversity and productivity. This activity also offers the possibility to measure species morphological traits which can be used to quantify the functional diversity of communities, investigate its effect on community productivity, and monitor phenotypic plasticity as a possible mechanism. This activity includes a theoretical aspect investigating the relationship between biodiversity and

ecosystem functioning, and a practical aspect involving the cultivation of experimental plant communities and the measurement of quantitative traits.

## Learning objectives

The primary objective of this hands-on activity is to demonstrate the link between community biodiversity and ecosystem functioning, in this case primary productivity. In the laboratory, students assemble a series of aquatic plant communities, in monocultures and polycultures of two and three species, and, after a period of growth, measure their productivity as biomass production. Students will learn about experimental design and replication in addition to culturing experimental plant communities. With the collected data, students will explore the question of how plant diversity affects ecosystem productivity. This relationship can be further analysed by partitioning the overall biodiversity effect into components representing species complementarity and a selection effects. Through a lab report, students will learn how to test the main hypothesis, and use theory to speculate on mechanisms responsible for the observed results. At the instructor's discretion, the study may be extended to explore possible mechanisms. For example, simple plant traits can be measured allowing students to quantify functional diversity and phenotypic plasticity. In addition to giving students an opportunity to collect and analyse quantitative data, trait measurements will allow students to ask whether functional and taxonomic measures of diversity equally affect productivity, whether growth in community as opposed to monoculture causes the plastic modification of species' traits, and whether this phenotypic plasticity plays a role in driving the observed biodiversity effects. Another possibility is to run the

experiment under different environmental conditions (by modifying nutrient availability, light intensity, or temperature) to test the stress-gradient hypothesis which states that diversity effects should be more important under stressful conditions (Maestre et al., 2009). Lastly, the experiment could be run over a longer duration to determine if species interactions and the strength and direction of diversity effects vary through time.

## Overview, timeframe, and list of materials

The experiment takes place during two three-hour laboratory sessions, which are spaced two weeks apart to allow for the growth of the plant communities. Of course the experiment can run longer with multiple productivity measurements to investigate how diversity effects develop through time (Urgoiti et al., 2022 ; Couture, 2022), but as the measurements are destructive, more replicate communities would need to be established. Duckweed (*Lemnaceae* spp.) and other floating aquatic macrophytes are suggested as a model system because of their rapid reproductive rate, small size, and ease of culture (Laird and Barks 2018, Jewell and Bell, 2022). Prior to the first session, the instructor must prepare the culture medium (recipe in Appendix 1), obtain the plants, and ensure their growth and reproduction. Although the lab work isn't done under sterile conditions, it is best to begin with axenic plants and culture media to avoid algal growth which could impact the community dynamics. This would be especially important if the duration of the experiment is increased beyond the suggested two weeks to look at how diversity effects change over time, Many species can be purchased in axenic culture online (e.g., *Canadian Phycological Culture Centre* in Canada, *Rutger's Duckweed Stock Cooperative* in USA), or field samples can

be sterilized (or at least cleaned of most algae) using 10% bleach (Jewell et al. 2023a). Preparation for the first laboratory session is likely to require the greatest investment on the part of the instructor. However, the time required may vary depending on how quickly the biological material is received and the number of students. The instructor must also ensure that the students have received the theoretical background necessary to understand the project (during a class on community ecology for example).

The first three-hour laboratory session is entirely devoted to assembling the various plant communities. The simplest experimental design, (and the one described here), involves three species grown in monoculture, in all 2-species mixtures, and in a full 3-species community (total of seven cultures). Students are broken up into small groups (suggested group size of two), each responsible for a number of experimental communities. Data from all groups is then pooled by the instructor for analysis. To avoid confounding group and treatment, it is best to assign one replicate of all seven treatments to each group, or if this is not feasible, to randomly assign communities to groups. Issues of replication and confounding factors can be discussed and included in the lesson if desired.

The second three-hour session, two weeks later, involves separating and counting individuals of each species and weighing the culture biomass. After individuals are sorted by species, the entire population is gently blotted with absorbent paper to remove excess water and then weighed to obtain a measure of species wet mass. Trait measurements could also be taken during this session to address bonus questions if desired. After the second lab session, students are responsible for

analysing the pooled data and writing a lab report (suggested two weeks). The student handout describing the lab instructions is included as Appendix 2.

This list enumerates the materials needed per group of two students, responsible for one replicate of each of all seven treatments.

Laboratory Session 1:

- 1.5 L of modified Hoagland's culture medium
- Graduated cylinder (to fill Erlenmeyer flasks)
- 7 250 mL Erlenmeyer flasks*
- 2 bacterial inoculation loops
- 3 containers each containing one species
- Labeling tape
- Marker

*alternative containers like mason jars or plastic cups may be used instead of Erlenmeyer flasks

Laboratory session 2:

- the 7 Erlenmeyer flasks from part 1
- 3 beakers
- 2 bacterial inoculation loops

- 2 counters
- 1 basin
- a balance (precision to 1mg)
- weighing trays
- absorbent paper to blot plants dry before weighing
- strainer (0.5 mm pores)
- optional - camera (cell phone) and ImageJ software (free) to measure plant traits (Appendix 3)

**Procedures and general directions for the instructor**

Biodiversity and ecosystem functioning

Although most of the learning activity takes place in two laboratory sessions, the integration of the theoretical foundation on which it is based is imperative to the students' understanding. The theory concerns the relationship between biodiversity and ecosystem functioning, or BEF, which has been increasingly studied in recent decades (Cardinale et al. 2013, Gonzalez et al. 2020). It is generally recognized that biodiversity promotes community productivity (Cardinale et al. 2012, Urgoiti et al. 2022). Two phenomena are at the basis of this relationship: selection and complementarity effects. The selection effect describes how communities consisting of a greater number of species are more likely to contain a productive species (Loreau, 1998; Mulder et al., 2001). The complementarity effect in resource use (or niche partitioning) describes the phenomenon of how differences in species' resource use, including spatial and temporal variation

in uptake, can result in greater overall community resource use, a reduction in intraspecific competition, and therefore greater community productivity (Gross et al., 2007; Cardinale, 2013). Together, the effect of selection and complementarity, i.e. the net biodiversity effect, can lead to the overyielding of communities, where community productivity is higher than the weighted productivity of monocultures of the constituent species (Loreau and Hector, 2001; Tobner et al., 2014). Overyielding can be estimated using the Relative Yield Total (RYT) equation (Eq. 1), which compares the yield of a community to the yield of monocultures of species in that community. In this case, yield represents productivity in terms of biomass.

**Eq. 1**

$$\mathrm{RYT} = \frac{Yield\ of\ species\ 1\ in\ polyculture}{Yield\ of\ species\ 1\ in\ monoculture} + \frac{Yield\ of\ species\ 2\ in\ polyculture}{Yield\ of\ species\ 2\ in\ monoculture} + \cdots (number\ of\ species\ in\ the\ polycutlure)$$

The net biodiversity effect (NE) can be partitioned into components representing the selection effect (SE) and complementarity effect (CE) using a covariance decomposition, based on the Price equation (Loreau and Hector, 2001) (Eq. 2). This decomposition can be easily applied to the class data to quantify net diversity, selection, and complementarity effects and uncover the mechanisms responsible for the observed diversity-productivity relationship.

**Eq. 2**

$$NE = SE + CE$$

OR

$$\Delta Y = Y_O - Y_E = \Sigma_i RY_{Oi} M_i - \Sigma_i RY_{Ei} M_i = \Sigma_i \Delta RY_i M_i$$

$$= N\overline{\Delta RY}\,\bar{M} + N\mathrm{cov}(\Delta RY, M)$$

Where, for a given mixture,

- $M_i$ = the yield of species i in monoculture;
- $Y_{Oi}$ = the observed yield of species i in the mixture;
- $Y_O = \Sigma_i Y_{Oi}$ = the total observed yield of the mixture;
- $RY_{Ei}$ = the expected relative yield of species i in the mixture, which is simply its proportion seeded or planted;
- $RY_{Oi} = Y_{Oi} / M_i$ = the observed relative yield of species i in the mixture;
- $Y_{Ei} = RY_{Ei} M_i$ = the expected yield of species i in mixture as the product of its initial relative yield in mixture and its yield in monoculture;
- $Y_E = \Sigma_i Y_{Ei}$ = the total expected yield of the mixture;
- $\Delta Y = Y_O - Y_E$ = the deviation from the total expected yield of the mixture;
- $\Delta RY_i = RY_{Oi} - RY_{Ei}$ = the deviation from the expected relative yield of species i in the mixture
- N = the number of species in the mixture.

In this equation, NE is represented by ΔY, CE is represented by $N\overline{\Delta RY}\,\bar{M}$ and SE is represented by Ncov(ΔRY, M). An Excel file (see Appendix) can be provided to students to facilitate the application of the equation. Since this statistical procedure is complicated to understand and interpret (Bourrat et al. 2023), depending on the class level, it may be desired to instead focus only on calculating relative yields (Eq. 1) and then discuss the possible explanations (e.g., complementarity and selection) at a conceptual level.

Functional diversity and phenotypic plasticity (extensions)

In addition to quantifying species richness and biomass, students can measure simple morphological traits in order to quantify functional diversity and phenotypic plasticity. A theoretical foundation is also useful for student understanding. Functional diversity is a measure of diversity that is linked to the niche complementarity hypothesis since species' resource use can be partially captured by their traits, such that communities made up of functionally similar species may be more redundant in their resource use (Loreau et al., 2001; Paquette and Messier, 2011). Functional redundancy occurs when different species overlap in the niche positions (for example in their resource use) and leads to a saturation in the diversity-functioning relationship, where adding more species results in a functionally equivalent community (Tobner et al., 2014). For this reason, functional diversity is thought to have a more linear relationship with ecosystem functioning compared to species richness (Tobner et al., 2014).

Functional diversity indices are calculated from measurements of species functional traits (Cantarel et al., 2013) which link species to the ecological impacts they have in the ecosystem.

Functional traits can be physiological, morphological, or phenological, so long as they impact fitness through effects on survival, growth, or reproduction (Violle et al., 2007). Simple traits like frond area and total root length are easily measured on floating aquatic plants, are highly plastic, and are known to be related to resource acquisition including competition for nutrients and space (Jewell et al. 2023a; Jewell and Bell 2023). These traits can be measured by students by imaging, and subsequently used to calculate functional diversity using *functional dispersion*, an index which integrates species functional similarity and their relative abundances in a community (Laliberté and Legendre, 2010). This index is calculated for each community using the FD Package in R. A script can be provided to students (see Appendix 4).

Although calculations of functional diversity rely on species expressing more or less consistent values of the given traits, intraspecific variation may arise for a number of reasons. Firstly, intraspecific genetic diversity may result in substantial trait variation within species. If cultures are purchased from banks, they will be clonal since reproduction in these species is almost exclusively asexual. However, if cultures originate from field samples, genetic diversity and intra-specific variation may be discussed (Jewell et al. 2023b). Secondly, a genotype may express different phenotypes in different environments, known as phenotypic plasticity (Bradshaw, 1965). Plasticity allows species to respond to their abiotic and biotic environment within the lifetime of an individual (Miner et al., 2005). It is therefore possible that the mean trait values of species in monocultures differ from the mean values obtained within polycultures, which would indicate a plastic response to intraspecific competition.

Strengths of duckweed as a model plant system

This learning activity is carried out using communities of floating aquatic plants in the family *Lemnaceae*, *aka*. duckweed (Appenroth et al., 2013; Laird and Narks, 2018). These plants are aquatic monocotyledons that have been described as the smallest and simplest Angiosperms (Les et al., 2002). Duckweed is distinguished primarily by its single-frond morphology to which there may be between zero and more than a dozen unbranching roots. (Landolt, 1998; Laird and Narks, 2018). Their small and simplified morphology, combined with vegetative asexual reproduction results in rapid growth and short generation times making them ideal models for research and teaching (Ziegler et al., 2015; Laird and Barks, 2018). Their small size makes feasible the establishment and manipulation of many replicate communities consisting of thousands of individuals, while their short generation time enables multi-generational experiments to be carried out over just a few weeks (Laird and Barks, 2018). The two weeks of growth prescribed here are sufficient to observe considerable biomass production in optimal environmental conditions (~3 doublings), allowing for both selection and complementarity effects to occur. Other species of floating aquatic macrophytes that are functionally similar to duckweeds and naturally coexist with them can also be used, such as some aquatic liverworts (see example below).

Although all species of duckweed share a similar basic morphology and life history, they differ in a number of ecologically important characteristics. These include variation in frond size, the quantity and length of roots, position in the water column (floating or partially submerged), competitive ability, longevity, and reproductive rate (Lemon et al., 2001, Jewell et al. 2003b).

These functional differences result in differences between inter- and intraspecific competition, and therefore the possibility of biodiversity effects such as resource use complementarity.

Three naturally cooccurring species are recommended to facilitate manipulation. The first species, *Lemna minor*, is the common duckweed whose morphology consists of a single oval frond (2-5mm in diameter, weighing ~1mg) to which a single root is attached. This species is mainly characterized by a rapid growth rate and is the superior competitor in most environments (Jewell et al., 2023b). The second species is *Spirodela polyrhiza, "*greater duckweed*,"* and consists of a frond of larger diameter than *L. minor* (5 to 10 mm in diameter and weighing 4 mg on average) to which between 2 and 12 roots are attached. This species is easily distinguished from *L. minor* by its larger fronds and purple coloured ventral surface. Growth rate is often slower than that of *L. minor* (Lemon et al., 2001). The third recommended species is *Ricciocarpus natans*, an aquatic liverwort. Like duckweed, *R. natans* floats on the surface of the water and reproduces almost exclusively by asexual vegetative budding. The morphology of *R. natans* consists of a heart-shaped cordiform thallus (5 to 15 mm in diameter and weighing 15 mg on average) whose ventral face is covered with rhizomes. Other species of duckweed or liverworts can also be used depending on the supply possibilities. For example, *Lemna trisculca*, "star duckweed", which forms branching chains of fronds, is a functionally unique species that may exhibit complementarity in the use of space. This species is partially submerged, floating just under the surface of the water, and therefore its inclusion in a mixture should decrease the strong competition for space in high nutrient growth environments, and indeed produced strong diversity effects in Couture *et al.,* (2022). However, some species should be avoided for teaching purposes, specifically species in the genera *Wolffia* or *Wolfiella* are not recommended because of

their small size which makes manipulation and measurement tedious (although for the same reasons they could be expected to show strong complementarity with other duckweed species).

For the instructor, the first laboratory session consists of distributing the materials and supervising the inoculation of the plants into their culture media. Seven cultures are assembled per group. These are three monocultures (one per species), three polycultures of two species (all possible combinations) and one polyculture including all three species. This is considered a minimum and is feasible in the time frame suggested. However, in larger or more advanced classes, additional species, communities, and richness levels could be used and the protocol easily adapted accordingly. Each culture should contain 150 mL of culture medium and 150 mg of biological material in total. At the end of the session, the instructor must ensure that the cultures are clearly identified and closed to avoid contamination. The instructor should then place the cultures in a growth chamber for two weeks under the following conditions: 200 µmol light/$m^2$/s, Light:Dark 16:8 and 25 ° C.

The second laboratory session is allocated for data collection. For each culture, the measurements include the total biomass, the biomass per species and the number of individuals of each species. The role of the instructor is to supervise the manipulations and compile the student data into a database, which can be given to the students at the end of the session. This database will allow the application of the RYT and partition equations which the students will use as the principal analyses to write their reports.

If the instructor wishes to extend the experiment to include questions related to functional diversity or phenotypic plasticity, students will measure traits at the individual level which are averaged for each species in each flask. Suggested traits are total root length (root number x root length), frond area, frond mass and specific frond area (frond area / frond mass). Traits should be measured on a subset of each population (suggested 10 individuals per species per flask). Trait measurements can be taken by photographing plants pressed against a white background with a standard ruler mark and analyzed later using ImageJ (Appendix 3). Since plants are too light to be weighed individually, measurements of wet mass are estimated by dividing the total biomass of a species by the number of individuals of that species. Students will then be able to calculate the mean specific leaf area by dividing the mean frond area by the mean individual mass. These results can be added to the database initially provided by the instructor at the end of the second lab session. Students will then be able to calculate the functional dispersion index and compare individual trait values obtained in polycultures to those obtained in monocultures.

Expected results

It is expected that the more diverse cultures will have a higher total biomass produced. The more diverse cultures are then expected to show an overyielding (RYT greater than 1) and a net effect of biodiversity. It is also expected that the mean trait values of the species will vary with the presence of other species in the cultures.

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**Appendix 1 -** Recipe for modified Hoagland's E Medium.

| | |
|---|---|
| **$MgSO_4$** | 12.300 mg/L |
| **$Ca(NO_3)$ x 4 $H_2O$** | 27.140 mg/L |
| **$KH_2PO_4$** | 4.3530 mg/L |
| **$KNO_4$** | 12.625 mg/L |
| **$H_3BO_3$** | 71.50 µg/L |
| **$MnCl_2$ x 4$H_2O$** | 45.50 µg/L |
| **$ZnSO_4$ x 7 $H_2O$** | 5.500 µg/L |
| **$NaMoO_4$ x 2 $H_2O$** | 2.250 µg/L |
| **$CuSO_4$ x 5 $H_2O$** | 3.500 µg/L |
| **$FeCl_3$ x 6 $H_2O$** | 0.484 mg/L |
| **EDTA** | 1.500 mg/L |

*The pH is set to 5.8 before autoclaving the media.*

**Appendix 2 – Student handout**

*Biodiversity – Ecosystem functioning in duckweed communities*

## Question

What is the relationship between the biodiversity in community and its productivity?

## Summary

The primary objective of this activity is to illustrate the relationship between plant diversity and community productivity. In this lab you will assemble a series of experimental duckweed communities, manipulating species richness. Each of the three species will be grown in monoculture, in every possible two-species combination as well as the full three-species community. All communities will be inoculated with the same total biomass. Mixed-species communities are inoculated with equal biomass of each species where biomass is measured as number of individual fronds multiplied by the species' average frond mass.

This exercise is completed over two laboratory sessions. In the first part you will assemble the experimental communities which will develop in growth chambers for two weeks. In the second part you will measure primary productivity as production of new biomass for each species of each community. This will be done by first sorting the communities into their constituent species and then counting the number of fronds for each species. Finally, to assess phenotypic plasticity in terms of average frond mass, the total biomass for each species for each community is weighed.

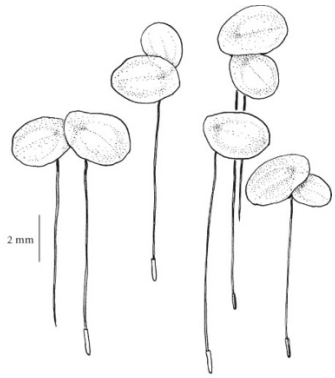
## Introduction

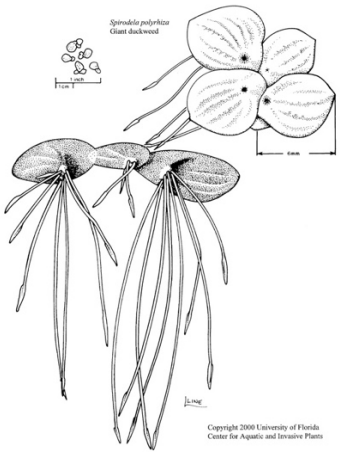
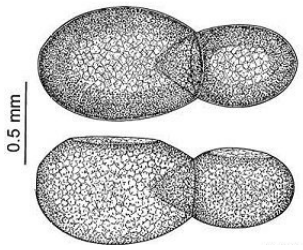
Duckweed (*Lemnaceae*) is a family of small, morphologically reduced floating aquatic monocots. Consisting of five genera and 37 species, they are widespread, growing on every continent except Antarctica. Although reproduction is almost always by asexual and vegetative, certain environmental conditions may lead to the production of flowers and sexual reproduction making them the smallest known flowering plants (Angiosperms). Rapid growth often leads to the formation of clonal mats covering still mesotrophic and eutrophic ponds.

Their reduced morphology consists of a single floating frond or thallus and in the case of the genus *Lemna*, a single root, *Spirodela* several roots, or *Wolffia* and *Wolffiella*, no roots.

The last couple decades have seen a rapid growth in duckweed research and application. Two species in particular, *Lemna minor* and *Spirodela polyrhiza* have become model systems in ecotoxicology and are being developed for applications including agricultural and aquaculture animal and fish feed, wastewater remediation and biofuel production. They also serve as a useful model for ecological experiments.

Although the common ducked (*L. minor*) sometimes grows in dense monocultures covering the entire surface of ponds, it is often found in diverse communities, coexisting with other species of duckweed and other floating plants like liverworts. Liverworts are a group of primitive non-vascular seedless plants that reproduce using spores and often resemble mosses, to which they are closely related. Although most species of liverworts are terrestrial, some have reverted to an aquatic life, and some, like *Ricciocarpus spp*. May have both terrestrial and aquatic forms. Although they possess a sexual phase, like duckweed, the vast majority of their reproduction is asexual and vegetative.

| **Lemna Minor** | **Common name:**<br>Minor duckweed<br><br>**Description:**<br>Morphology consists of a single frond and single root. The ventral surface is green. Frond diameter between 2-5mm. Daughter and grand-daughter fronds often remain attached to the grandmother to produce clusters of 3-8 individuals. | |
|---|---|---|
|  | |  |

| | | |
|---|---|---|
| **Spirodela polyrhiza**<br>  | **Common name**:<br>Major duckweed<br><br>**Description:**<br>Morphology consists of a single frond, each with several (between 2-12) roots. The ventral surface is purplish. Daughter and grand-daughter fronds often remain attached to the grandmother to produce clusters of 3-8 individuals. The largest frond diameter of the 3 species, on average 5-10mm. |  |
| **Wolffia Columbiana**<br>  | **Common name:**<br>Watermeal<br><br>**Description:**<br>Morphology consists of a single frond with no roots. Fronds measure only ~1 mm, significantly smaller than other species. Mother fronds will produce a single daughter frond and then divide before the daughter frond can produce their own offspring resulting in 2 attached fronds. |  |

## ***PART 1***

Develop your predictions:

1. How might community biomass change as a function of the number of species in the community? Describe both graphically and in writing.
2. What mechanisms might influence productivity in a multi-species community?

**Materials** (per group of 2 students):

- 1.5L of 10% Hoagland's growth media
- graduated cylinders to dispense media into flasks
- 7 250mL Erlenmeyer flasks
- 2 bacterial loops
- 3 beakers full of each of the 3 species
- labelling tape
- marker

**Methods:**

Clonal populations of each species have been propagated in the lab under sterile conditions. Given that populations originate from a single individual, intraspecific diversity is negligible, originating only form mutation. Fresh nutrient-rich growth media has been prepared in advance in which to grow the experimental communities.

- In a group of 2, acquire all necessary materials.
- Fill all (7) Erlenmeyer flasks with 150 mL of growth media
- Label the flasks as follows:
- Species richness, Species codes, Group number
  For example,

  1, Lm, 3 indicates *Lemna minor* in monoculture, belonging to group 3

  3, Lm-Sp-Wc, 3 indicates the full 3-speces community, belonging to group 3

- Next, you will inoculate your flasks with the corresponding duckweed species to generate the desired communities. Each flask should start with a total of 150mg of biomass. Using the bacterial loop, hook fronds one at a time, taking care not to break off roots.

| Species name | Species Code | Average frond mass |
|---|---|---|
| *Lemna minor* | Lm | 1mg |
| *Spirodela polyrhiza* | Sp | 4mg |
| *Ricciocarpus natans* | Rn | 15mg |

Calculate the number of fronds for each species to be added to each flask.

Monoculture

Lm: _____

Sp: _____

Rn:_____

2 species communities

Lm: _____

Sp: _____

Rn:_____

3 species community

Lm: _____

Sp: _____

Rn:_____

**A note on frond counting.

Since data will be pooled across groups, it is essential that there is consistency between groups when it comes to frond counting. The simplest standardized protocol is to count all daughter and grand-daughter fronds as individuals, even when still attached. This means that frond count should include all budding fronds visible to the naked eye. For *Ricciocarpus natans*, count each lobe as an individual.

Cultures are then transferred to controlled growth chambers for two weeks at the following conditions: 200umol light /m2/s, light-dark cycle of 16/8, 25$^{o}$C.

## PART 2

**Materials** (per group of 2):

- the 7 flasks from Lab 1
- 3 beakers
- 3 bacterial loops
- 2 counters
- 1 large tub
- 1 balance
- 1 strainer
- weighing trays
- paper towel
- camera (phone)

**Methods:**

For each flask:

- Empty the contents into the tub.
- Sort the community by species, isolating each species into its own beaker. Use your clickers to count the number of individuals as you go.
- Record frond number on your data sheet.
- After species have been sorted, counted and recorded for a community, measure the wet mass of each species in the community.
- Strain one species, empty the biomass onto paper towel, blot dry by pressing plants between two sheets (like pressing leaves), then empty contents into a weighing tray.
- Record the total mass for each species for each community on your data sheet.

## Data sheet

| Species | Species richness | Other species in the community | Number of fronds | Total wet-mass (mg) |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |

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|---|---|---|---|---|
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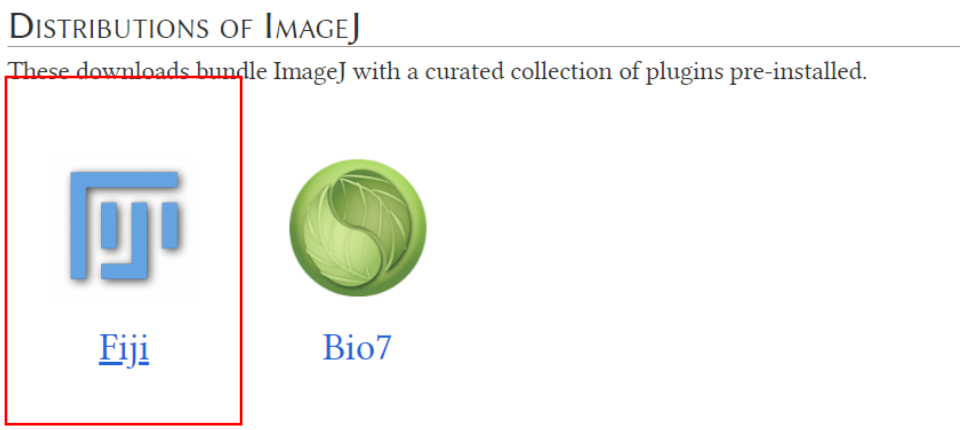
**Appendix 3 -** Protocol for trait measurements using ImageJ

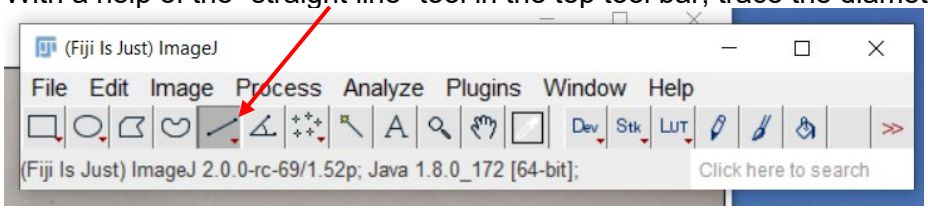
This document describes the protocol to measure frond area, and can be extended to use other traits such as root length. Before beginning, note that the use of an external mouse instead of a track pad will greatly facilitate measurements.

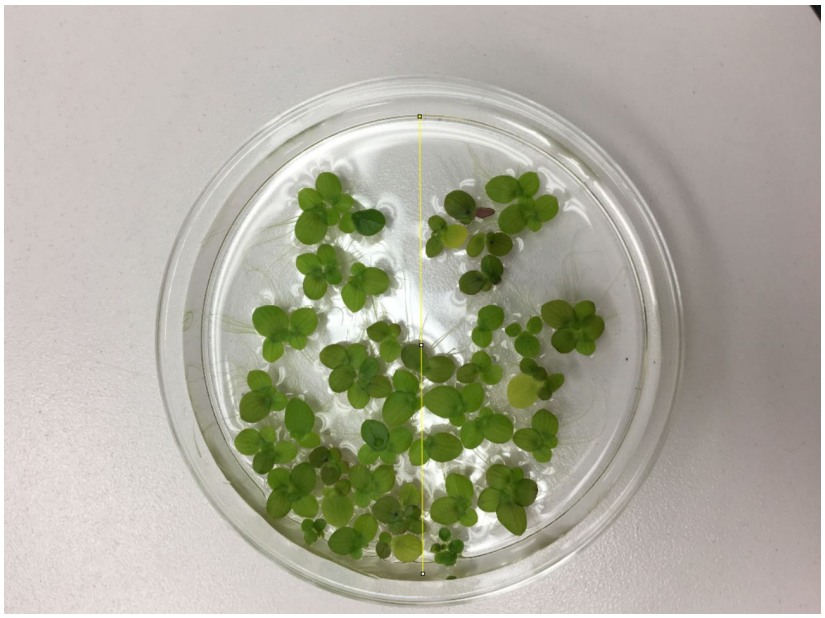
- Download the program Image J (imageJ.net)



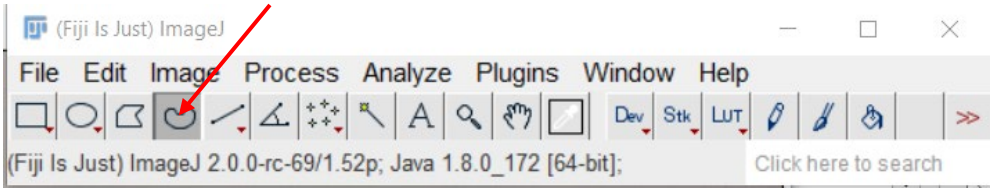

- Open one image at a time in ImageJ
  File -> Open -> Choose image

- With a help of the “straight line” tool in the top tool bar, trace the diameter of the beaker





- Analyse -> Set scale. Use the known measure of the beaker's diameter to calibrate the "Known distance". Don't forget to use the correct units,.
- Choose the "freehand line tool"




- In each image, measure the surface area of 10 randomly selected fronds
- Start by carefully tracing the perimeter of the frond


- Select "Analyse -> Measure" to obtain the surface area

## Appendix 4 – R Code for Functional Dispersion

```
##set working directory
setwd("")
#import the the files containing trait measurements and biomass
Traits <-read.csv("Traits.csv", header = TRUE, sep = ";", row.name=1)
M.biomass <-read.csv("M.biomasse.csv", header = TRUE, sep = ";",
row.name=1)
#convert the biomass file into a matrix
M.biomasse<-as.matrix(M.biomass)
#calculate functional dispersion
library(FD)
d=dist(Traits)
#calcul de la dispersion fonctionnelle
DF <- fdisp(d, M.biomasse)
DF$FDis
```