Lab Notebook

Lemna Balloons

Louise Dagher, Corentin Mathé--Deletang, Simon Fradet

Question: Is *Lemna minor* a good tool against eutrophication?

Scientific problem: Studying the effect of nitrogen uptake efficiency on competitive outcome between *Lemna minor* and *Chlamydomonas reinhardtii*.

Objective: Study the proportion of the algae individuals when they are in competition with Lemna for nitrates.

- **Variable:** Concentration of nitrate in freshwater
- **Constants:** Light, temperature, pH, amount of Lemna and algae, water

MATERIAL

- 27 x 100mL glass bottle
- JBL NO3 test
- *Chlamydomonas reinhardtii* cultures
- *Lemna minor* 318
- Grid slide to count algae under microscope
- Scale with 1 µg precision
- Thermometer
- Luxmeter (smartphone application)
- pH test (pH paper)
- Graduated cylinder
- Potassium nitrate
- Potassium phosphate mono hydrate
- Magnesium sulfate

06/02/17

We prepared 27 solutions of 50 mL, corresponding to our 3 nitrate concentrations, 1 test and two control per concentration, and three replicates per test and controls.

Preparation of potassium, magnesium and nitrate solutions

In order to set the *Lemna minor* environment with nitrates as for *Lemna minor*, we decided to use tap water and to add some fundamental minerals for organisms such as potassium, phosphate, sulfate and magnesium.

By this, we prepared two preliminary solutions :

- A solution of magnesium sulfate composed of 100 mg of $MgSO_4$ powder and 100 mL of tap water : so, by adding 1 mL in our 27 solutions, we would obtain a concentration of 20 $mg.L^{-1}$
- A solution of potassium phosphate monohydrate composed of 100 mg of K_2HPO_4 powder and 100 mL of tap water : so, by adding 1 mL in our 27 solutions, we would obtain a concentration of 20 $mg.L^{-1}$

Two nitrate solutions were also prepared in order to compose the base of the environment.
As we wanted to be able to track nitrate concentration at the beginning and the end of the experiment in our beakers, it was necessary not to exceed maximum threshold value of our NO_3 test which is 240 $mg.L^{-1}$.

Since we wanted to cultivate our algae and lentils in 50 mL, that corresponded to a mass of 12 mg of nitrate (240 * 0.05 = 12). However, we have potassium nitrate powder : in this form, nitrate composes 60 % of the mass of the powder. So, to add a net amount of 12 mg of nitrate, we would have to add 20 mg of potassium nitrate : indeed, 20 * 0.6 = 12.

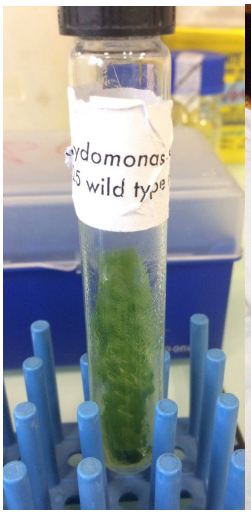
With that in mind, we decided to test another concentration of nitrate, 120 mg/L^{-1}, which correspond to a net mass of potassium nitrate of 10mg to add in 50 mL.

By that, we decided to prepare :

- A solution of nitrate with a concentration of 10 g.L-1 composed of 100 mg of KNO_3 powder diluted in 10 mL of tap water (SOLUTION 1) : so we just have to pour 1 mL in our desired solutions (9 solutions for this mass of nitrate) to have our 10 mg.

- A solution of nitrate with a concentration of 20 g.L-1 composed of 200 mg of KNO3 powder diluted in 10 mL of tap water (SOLUTION 2) : so we just have to pour 1 mL in our desired solutions (9 solutions for this mass of nitrate) to have our 20 mg

Preparation of algae solution



We took algae from an initial culture on agar, and release that in 3 mL of tap water.

To determine the algae concentration in the solution, we decided to count the number of organisms using grid slides under the microscope.

We found that the concentration was approximately $5.5 * 10^{9}$ algae.L^{-1}.

Since we had to add the same amount of algae in all the solutions except for the nine controls where only would grow, we had to divide these 3 mL by 18. To have some leftovers, we decided on an amount of 150 µL, which corresponded roughly to 825 000 algae. So, we had an initial concentration of algae in our 18 solutions of $1.65*10^{7}$ algae.L^{-1}.

counting

We counted the number of in the lab.
We found out 318 individuals, that's why we choose to put 8 of them per beaker in order to have a general sample of 216 individuals. Other individuals will be used to be dried and weighed at the beginning of the experiment to be compared with characteristics of experiment individuals at the end of the project.

Here is the final experiment design :

Nitrate concentration (added)	Experiment	Control 1 (*Chlamydomonas reinhardtii* only)	Control 2 (*Lemna minor* only)
0mg.L-1 (0mM)	8 Lemna minor 150µl of algae solution 48mL of tap water 1mL of MgSO4 solution 1mL of K2HPO4 solution 3 REPLICATES	150µl of algae solution 48mL of tap water 1mL of MgSO4 solution 1mL of K2HPO4 solution 3 REPLICATES	8 Lemna minor 48mL of tap water 1mL of MgSO4 solution 1mL of K2HPO4 solution 3 REPLICATES
120mg.L-1 (2mM)	8 Lemna minor 150µl of algae solution 1mL of solution 1 47mL of tap water 1mL of MgSO4 solution 1mL of K2HPO4 solution 3 REPLICATES	150µl of algae solution 1mL of solution 1 47mL of tap water 1mL of MgSO4 solution 1mL of K2HPO4 solution 3 REPLICATES	8 Lemna minor 1mL of solution 1 47mL of tap water 1mL of MgSO4 solution 1mL of K2HPO4 solution 3 REPLICATES
240mg.L-1 (4mM)	8 Lemna minor 150µl of algae solution 1mL of solution 2 47mL of tap water 1mL of MgSO4 solution 1mL of K2HPO4 solution 3 REPLICATES	150µl of algae solution 1mL of solution 2 47mL of tap water 1mL of MgSO4 solution 1mL of K2HPO4 solution 3 REPLICATES	8 Lemna minor 1mL of solution 2 47mL of tap water 1mL of MgSO4 solution 1mL of K2HPO4 solution 3 REPLICATES

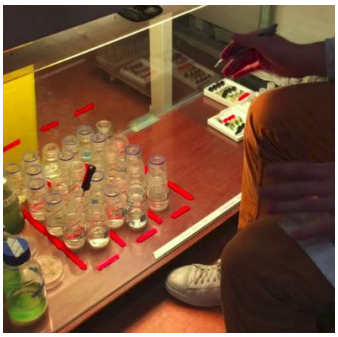
Nitrate test of the tap water:

To test the amount of nitrate in the tap water we used JBL NO3 test.
According to it, our water seems to have a concentration between 10 and 15 mg/L-1 of nitrates.

100mL beakers were placed in the greenhouse of the lab following the experiment design.

To pay attention about environment conditions we will measure continuously pH, light, temperature values but also concentration in the different beakers.

07/02/17



Measurements of temperature of all the beakers with a thermometer and light intensity using a luxmeter.

Temperatures were very different between the front and the back of the greenhouse. Since we didn't want to set the temperature as a supplementary variable in the experiment, we decided to change the positioning of the beakers as below :

Nitrate concentration (added)	0mg.L-1 (0mM)	120mg.L-1 (2mM)	240mg.L-1 (4mM)
	E1 R1 C1 R1 C2 R1	E2 R1 C1 R1 C2 R1	E3 R1 C1 R1 C2 R1
	E1 R2 C1 R2 C2 R2	E2 R2 C1 R2 C2 R2	E3 R2 C1 R2 C2 R2
	E1 R3 C1 R3 C2 R3	E2 R3 C1 R3 C2 R3	E3 R3 C1 R3 C2 R3

E(#concentration) R(#replicate)

C(#control) R(#replicate)

Experiment

3 concentrations :

0 mM → #1

2 mM → #2

4 mM → #3

Control 1 (*Chlamydomonas reinhardtii* only)

Control 2 (*Lemna minor* only)

Drying leftover . 11h:

We put them in a 60 °C oven for a day.

pH measurements at 14h30 :

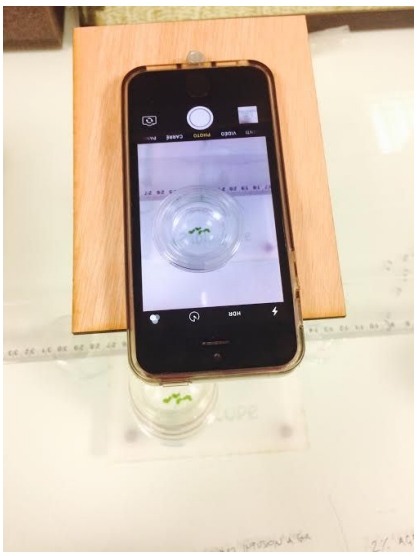
27 samples of pH paper were disposed on a clean surface. One by one, each of the solutions were tested, pipetting 20 µL of solutions and putting it on a new piece of paper. Colors were taken 10 seconds after the liquid and paper came into contact.

Algae counting at 15h30:

One by one, each solution was resuspended using a 1 mL pipette, and then 6.6 µL of each solution was put in individual frame of grid slides for counting. Sadly, due to the relatively low generation time of and the fact that we couldn't place them in a shaking incubator for concerns, they hadn't grown enough to be countable on this setup.

8/02/17

9h20-9h40 : Taking pictures of the surface of the lentils. Same protocol as the 7/02/2017.



9h20- 10h : taking temperatures : Thermometer inserted into each pot, the water was shaken thanks to the thermometer rod, temperature was registered after 5 seconds stabilization.

9h40-10h30 : Weighing the dry leftovers of . They were recuperated from the oven, deposited on a paper towel with a tweezer and weighed one by one on a 0.1 mg precision scale. We had 46 values.

<u>14h : pH measurements</u>

<u>15h-19h30 : Counting algae</u>

9/02/17

<u>9h - 13h30 :</u>

Temperature sampling, taking pictures of the leaves and the roots, putting the lentils in 60 °C oven for 1 day, each batch of lentils from one solution was put in a separate beaker for drying.

Nitrate test for all beaker solutions and also for a solution with a given concentration of 240mg.L-1 of nitrates.

Analysis of absorption of the solutions using the spectrophotometer TEKAN (blue light exposure 450 nm) so that we will be able to do a graph with absorption and corresponding nitrate concentration.