Laboratory Notebook

Sarah Talon Sampieri, Tanguy Chotel, François Sacquin - Biosensors Week 1 - Light Sensors

All the design plan illustrated is available here:
https://docs.google.com/presentation/d/1OPqVPsu4H2yyu3Pjg0fByXw8JIA9_LCRb3b70cnSuwA/edit?usp=sharing
please make sure to take a look to it, it can be helpful to visualize each step of the experience.

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Just like in a real Lab Notebook, we will not erase any content and will write down every manipulations, interrogations and errors.

Day V : Biological and Arduino sensors Test

Today we begin our experiments. We will test our biological sensors, Daphnia, in order to study their reaction to intensity of blue light.

Material

- x3 500mL beakers
- One Arduino and led light (blue)
- Smartphone for filming
- Tape to fix the arduino
- One resistor (220 Ohm)
- Rulers
- Cardboard to create a dark room

Protocol

- Put all the daphnias in an aquarium previously prepared.
- Prepare the water needed to grow daphnias in it (let clore evaporates)
    - Mineral Water and microalgae
- The all experience will be done in a dark room (DIY dark room = cardboard dark room), to avoid other lights to interfere with our experience.

- Place a beaker full of water on a horizontal, non-reflective surface. Put a moderate amount of Daphnias inside (moderate meaning not too much too avoid overlapping and insure they can all stay at the same level). N=+20
- Write on the beaker every 1 cm
- Place a camera on the side filming the beaker.
- Place a blue light above the beaker vertically at 0 intensity connected to an arduino and a computer to program the good intensity (0 intensity = 4 lux).
- Very slowly change the intensity of the light up. (30 intensity at every step)
- Stop at regular intervals to observe the Daphnias (30 seconds floors) and turn off the light after the 30 second floor to reset the Daphnia.
- Go until you reach the 240 intensity (227 lux).
- Repeat the experience for the Replicates (2). And do the 4 Repetitions of the experience (with the same population of daphnia).
- Analyze the video.
- Preparation of the beakers: around 20 daphnias are put inside (probably a little bit more than 20); while the arduino is fixed to the first beaker, the second beaker is being prepared with water and ~ 20 daphnias. Also the third beaker of water is prepared.

<u>Part I: Biological Sensor</u>

Preparation of the experience:

- Buy daphnias but make sure to take them alive: for this, they have to be put in **spring water**, in a **constant room temperature**, away from direct sunlight, nourished by algae or yeast extract, and in a enough big space.

For the repetitions, we will use the same 3 beakers previously prepared with the same populations.

Order of the **manipulations**:

- **Negative Control**: The first sample beaker with is used as a negative control: the experience is done without blue light, for the eight intensities.

- Started with the experiences for our **3 samples**: a part the first population, we have other 2 beakers with **2** different populations: those are our **replicates**. To have more

data and can make an accurate data analysis, we also have **2 repetitions** of the experiment for each beaker, which makes 9 different analysis at each time: the analysis consists in putting blue light up of each beaker and test the 8 different intensities for each beaker.

- For **each analysis**, wi record a video of 30s for each intensity. Between each record, we wait 30s to reset the conditions: the population is in dark for 30s, to let them 'readapt' to normal start conditions, and avoid getting them used to blue light stimuli.

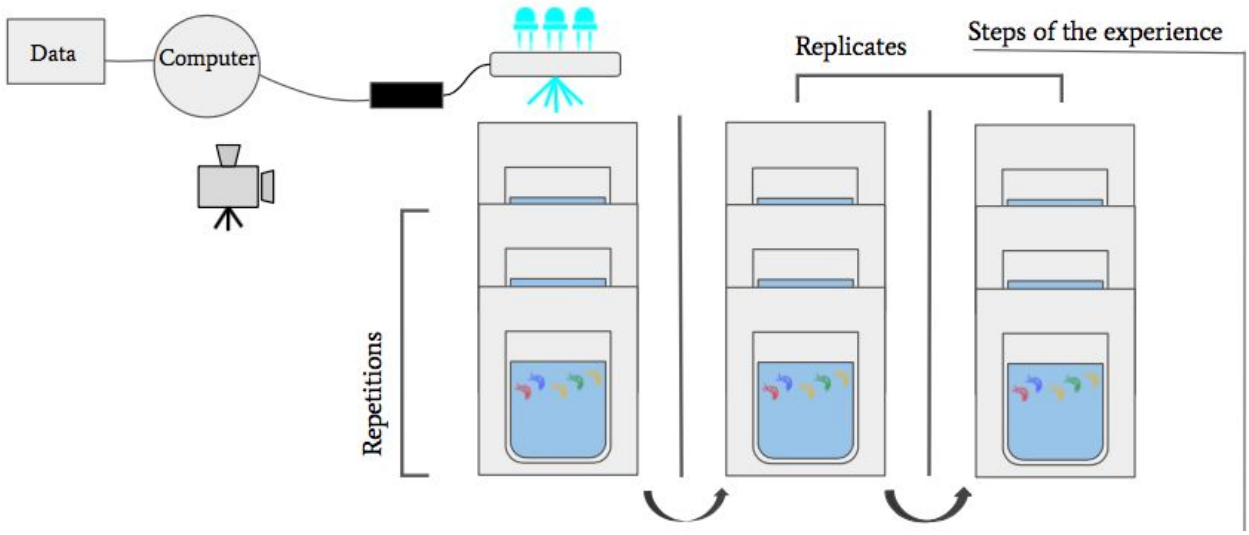
30s of 30 nm → Dark for 30s → 30s for 60 nm → Dark for 30s → 30s of 30 nm → Dark for 30s → 30s for 120 nm → Dark for 30s → 30s for 150 nm → Dark for 30s → 30s for 180 nm → Dark for 30 s → 30s of 210 nm → Dark for 30s → 30s of 240 nm.

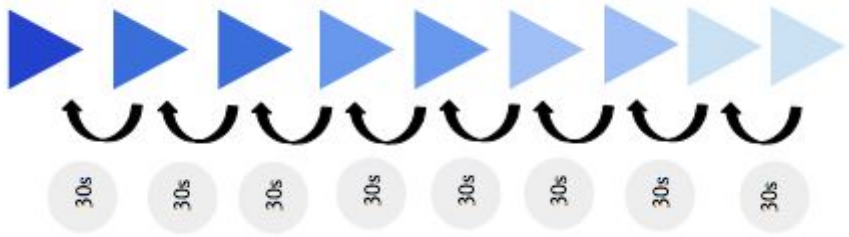
- At the end of each video which is recorded by an iphone, the video are saved on **drive box**, that is shared by all team members.

- **Positive control:** for the positive control, we took an the 3rd beaker as our population sample and we exposed it to a constant intensity of light of **240 nm,** 8 times. A repetition has been done for the positive control.

- At the end of the experience, we put again our daphnia in the aquarium.

Set up of the experience: 3 different populations, 2 replicates for each population. Each experience is recorded by a camera. The light comes from an arduino, regulated by computer.





Order of intensities: 30 nm - 60 nm - 90 nm -120 nm - 150 nm - 180 nm - 210 nm - 240 nm: between each variation of intensity, wait **30s** to reset.

- We succeeded in doing all our analysis for each replicate and its repetitions. These are our comments on our experiments:

Comments/errors:

- not sure that the 30 seconds between each intensity (30 seconds of dark) are precise, it is sometimes longer. So in reality, this parameter has not been kept constant along all the experience.
- The change on intensity of arduino (téléversement) is not instantaneous, it takes a little bit of time.
- The number of videos taken for the controls is 7 instead of 8, due to a mistake in the preparations. Therefore the mean made when analysing data will have more standard deviation.
- The videos we took were not easily usable, since sometimes the focus was not always made on the beakers but sometimes on the labcoat serving as a floor for the experiment.
- The white labcoat used as the floor reflected the light, therefore a lot of light was projected on the floor.
- For positive and negative controls: we succeeded in doing one repetition for the positive control, but no repetition for the negative control. Also, we used the first and third population for these tests, and we should have done a positive and negative control for each beaker, as each beaker have a different population. We should have also do a positive and a negative controls before and after all our experiments.
- To be even more rigorous, we should have made positive and negative controls with each population, before and after the experiment, but also once with another beaker with another population, to confirm our first positive and negative control with our populations.

Schéma: 1 negative control → First simple: 8 intensities, 2 repetitions → Second sample: 8 intensities, 2 repetitions, Third sample: 8 intensities, 2 repetitions → 2 positive controls (did in two different times, one was did in the morning and one in the afternoon of the same day: the first at the first lab session at **10h** and the second at the second lab session at **16h**).

Part II: Electronic Sensor (Friday 20 and saturday 21)

Material:

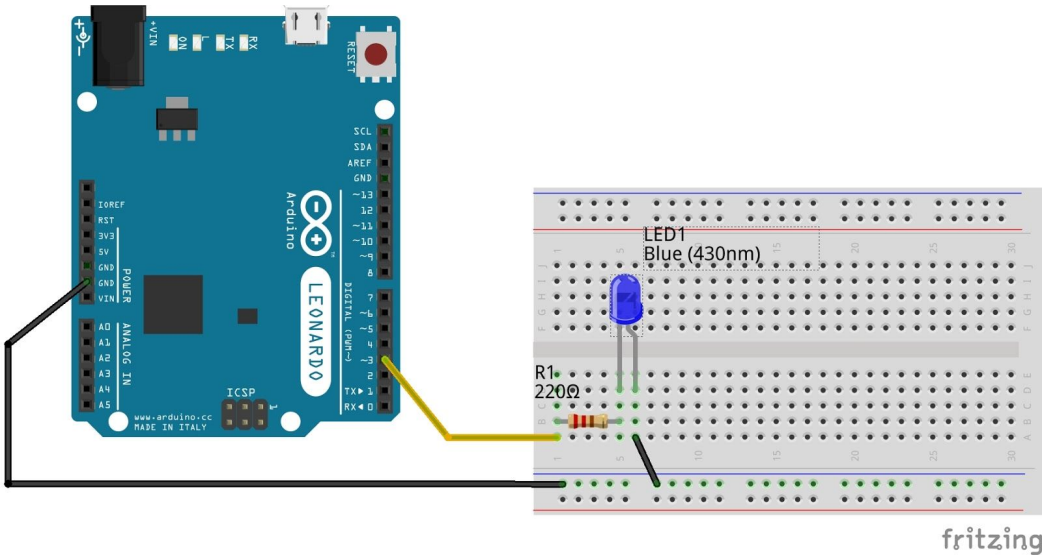
- Two arduino
- Tape to fix the arduino
- Two LDR (photo resistors)
- One blue LED (know intensity)
- 2 resistors (9,1k Ohm and 220 Ohm))
- One lentil box
- One cardboard dark room

Protocol:

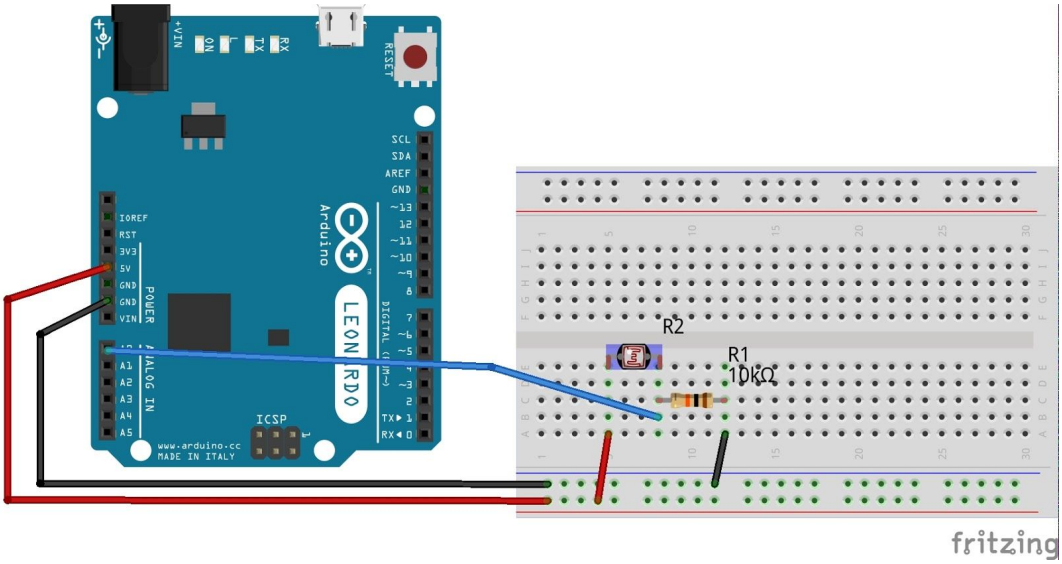
- Put the Arduino under the cardboard at the same height as the water level of the Daphnia (200mL)
- Place a blue light above the beaker, on the lentil box to attain the same height as the beaker vertically at 0 intensity connected to an arduino and a computer to program the good intensity (0 intensity = 4 lux).
- Very slowly change the intensity of the light up. (30 intensity at every step)
- “Reset” the sensor with 30s of dark between each measure
- The data will be gathered directly thanks to this code (**Check the github**)
- Repeat the experience for the replicate and 2 repetitions
- Controls are the same, for 0 intensity and 240 intensity

Preparation of Arduinos

Here is the schematic for the LED arduino setup :



And the schematic for the LDR arduino setup



The two codes can be found here on the github of the project
Experiment Setup:

In order to analyse the photoreceptor (LDR), we tried to create a setup that was very similar to the setup used for the biological sensor experiments.

Therefore we used a lentil box to simulate the height of arduino put on the beaker top (as you can see on the picture).

This setup was then put under the cardboard dark room, just like for the biological sensor.

Measures:

FIrst LDR :

- Negative control
- 3 sets of measures by increasing intensity 30 by 30, with 30s pause in the dark.
- Positive control

Second LDR:

- Negative control
- 3 sets of measures by increasing inteNsity 30 by 30, with 30s pause in the dark.
- Positive control

⇒ In conclusion, we did two LDR measures, containing 3 sets of measures with an increasing intensity, one positive control and one negative control.

Comments/error:

- The led was not directly lighting the sensor, therefore the intensity received by the sensor might not be its maximum capacity.
- We used two different LDR. However each LDR has a different calibration, which means that a different resistor should be used for each sensor. We chose to use the same resistor (9,1k Ohm) not to change too much parameters, which is why our two different sets of measures are so different.

Schematic

Day 6,7,8: Data Analysis

Part I: Biological Sensor

For the Data analysis part, we decide to evaluate as many parameters as possible to see how daphnia react to the variation of blue light intensity. What we would like to analyze:

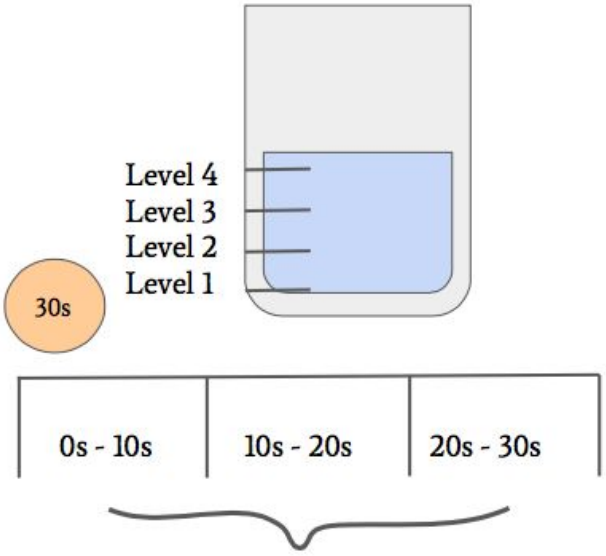
- The reaction time of daphnia to the variation in intensity of blue light
- The rapidity through which they move (move tracker with ImageJ)
- The direction of their phototaxis
- Possible interactions between daphnia

In fact, as we did have an enormous amount of data (**1152 data**, with positive and negative control, repetitions and replicates).

This is **how** we managed to to do our data analysis:

For each experience, one video has been recorded. One video has been made for each intensity (30s of video). We had 8 videos for each replicate and for each repetitions, which makes **72** videos for sample replicate and repetitions and **24** videos for positive and negative control.

We analyzed the videos by looking at how daphnia would distribute every 10s of each video. To do this, we marked 4 levels in the beaker, and at each time we stopped the video we counted out how many daphnia were present at each level of the beaker.



Errors/remarks:

- Maybe there were too little quantity of water in the beaker, as during data analysis, it seemed the had not too much space to move. Maybe having more space would have helped to see how they would move, not “obliged” by space constraints.
- The video have been finally charged on google drive: the quality was very low, pixels were really big and it has been very difficult to identify daphnias in the beaker.
- In the population, there were big daphnia as well as little ones: littles ones were hard to visualize during the analysis of video.
- It seems daphnia tend to be more dynamic and homogeneously distributed in the beaker in the negative control.
- There were reflects of light in beakers in a lot of videos, so the light intensity was not really good regulated.

- A lot of daphnia were overlapping: this was hard then to count daphnia at each instant of the video.
- Half of the data analysis have been made with imageJ, half have been made by eye. Even more, two different persons have been done the analysis: this doesn't make the scientific analysis rigorous because the measure tools are not the same.

Part II: Electronic Sensor

Conversion Intensity to Lux

First, in order to use an unity that was comparable we had to convert the intensity delivered by the led, into Lux, a light intensity unity. (**Check the github for the "functionLux")**

- We used an Iphone 6 and the App Luxcamera to measure the lux given by the intensity of the LED light. Therefore we were able to plot those values and find a function that would convert LED intensity into Lux.
- Using this function we converted all the values that we got from the LDRs, in order to compare two lux intensities.

Then, we plot the values comparing the emission and reception intensity of the led and sensors.

Data analysis

We collected the data directly from the arduino code, and therefore already had a .csv file (**check github**) filled with all the data.

Using this data, we plotted the intensity received by the LDR in the function of the intensity of LED, both in lux.