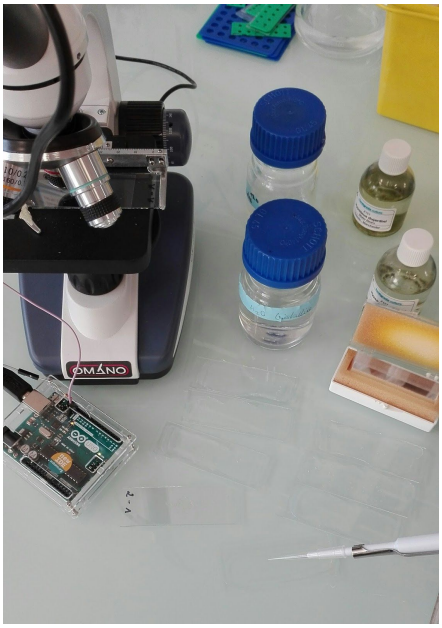
Labnotebook
Tardigrades and LDR

By Léonie Ledrein, Nina Varchavsky and Nikola Zarevski



The goal of our intensive week project was to see how a biologic sensor is responding to light stimuli and to compare this response with an electronic sensor. For that, we decided to work with Tardigrades, more specially with *Hypsibius dujardini* because this specie is known to have eyes, also called “ocelli”, and we already had them in the laboratory. On the electronic side, we chose a Light-Dependent-Resistance which has an analogic variation of its inner resistance depending on the ambient light’s intensity.

Biologic sensor Protocol

19/01/17

First, we started to try out the microscope in order to see *H. dujardini* with a camera. For that we had to make special microscope slides because Tardigrades cannot move well under a glass surface. We asked Nicolas Sénécaut, an alumni of the

bachelor program, to share his protocol with us since he had to work with Tardigrades in seemingly conditions.

To realize this protocol, you will need :

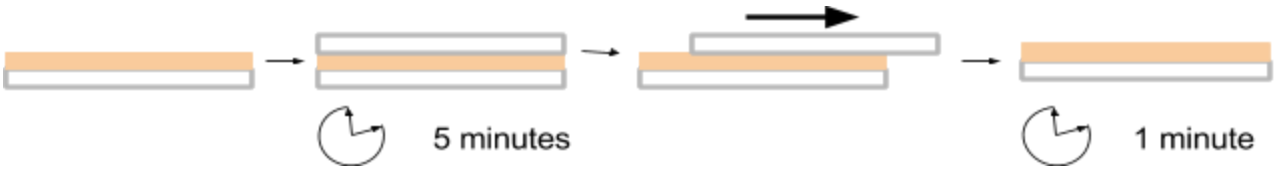
- A microscope
- A camera
- ToupView Software (or another software allowing to record via a camera)
- A “black box”
- A green LED
- An Arduino
- A 300Ω resistance
- Microscope slides
- Agarose
- Cristaline® water
- *Hypsibius dujardini*

The special microscope slides are covered with a layer (1mL) of an agar solution applied thanks to a pipette.
The agar solution recipe is :

- 0.75 mg of agarose
- 80 mL of Cristaline® water

Tip : Since the agar solution tends to solidify at room temperature, warming it up with a microwave to liquify might be necessary (don’t forget to loosely open the lid allowing pressure to come out).
Once 1mL of agar is deposed on the slide, another slide is placed on the top to smoothen the surface of the agar. After 5 minutes, the upper slide can be removed. 1 minute waiting is necessary to let the agar dry completely.

Agar slides manipulation

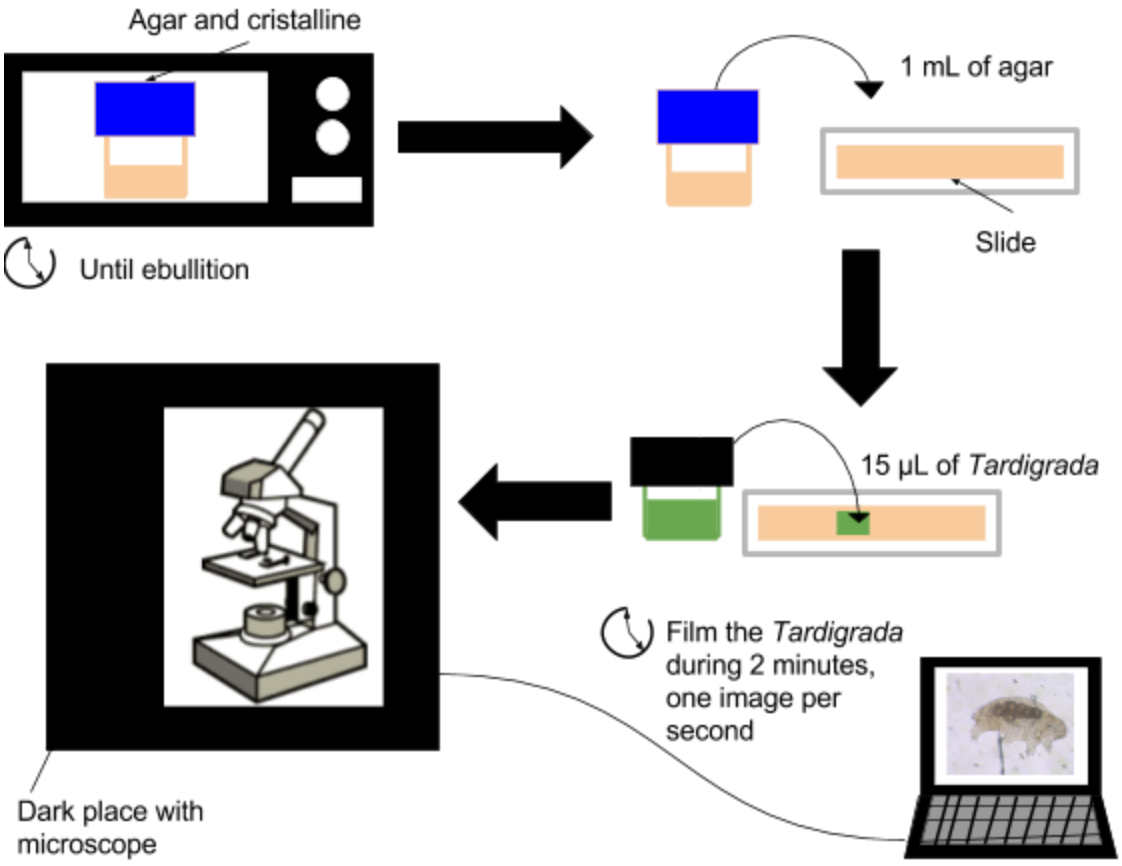


Tip :You can leave the two slides together if you want to conserve them for later use.

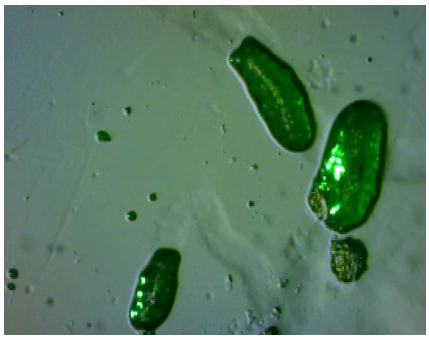
After that , 15 microliters of tardigrade's liquid culture can be deposit on the agar slide. At first, we were putting a coverslip to flatten the drop of tardigrade's liquid culture in order to have a better focus and limit water evaporation.
But master students made the point that it may compress the tardigrades, killing them or at least slowing their movements.

Now, the tardigrades can be observable under a microscope (we were at x40 magnification). A camera was used to record in live everything under the microscope using the software **ToupView** (under Mac and Windows only).

Method for the preparation and observation



The “black box” that we used to reduce the ambient light and work in seemingly dark conditions was a black plastic bag that we put over the microscope.



Then an green LED was light up on one side of the microscope slide and the camera recorded for two minutes (~300 images at the end of the capture, which means a frame rate of ~2.5 images per second). The different intensities tested were given through an Arduino and had the arbitrary values of 0, 1, 10, 100 and 255. 0 being our negative control with no exposition to green light and 255 being our positive control with a maximum light exposition.

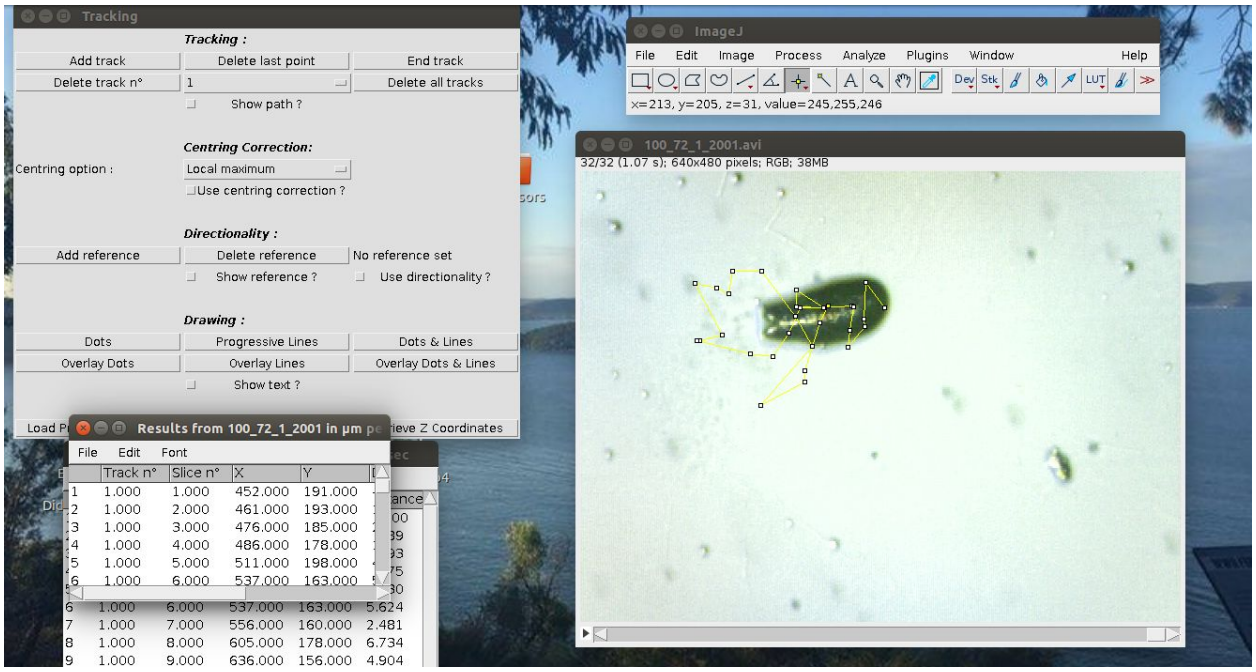
We wanted to see how does *H. dujardini* respond to different intensity of light.

Data were gathered for one Tardigrade per slide, with 10 slide, for each light intensity. Ending up with 50 video files to analyze.

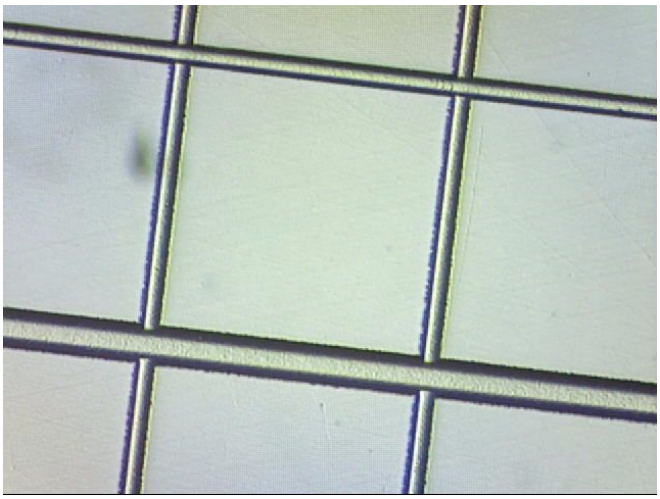
Data for intensities 0, 1 and 10 were collected on Friday (20/01/17) and for intensities 100 and 255 three days later on Monday (23/01/17) due to a lack of time.

Data Analysis

We used a specific plugin in ImageJ named Manual Tracking to track one back foot and the head of *H. dujardini* for each video.



Before all, we used a special picture (see to the right) to calibrate the software by changing pixel unit into a millimeter unit. One side of the square measures 0,33mm.



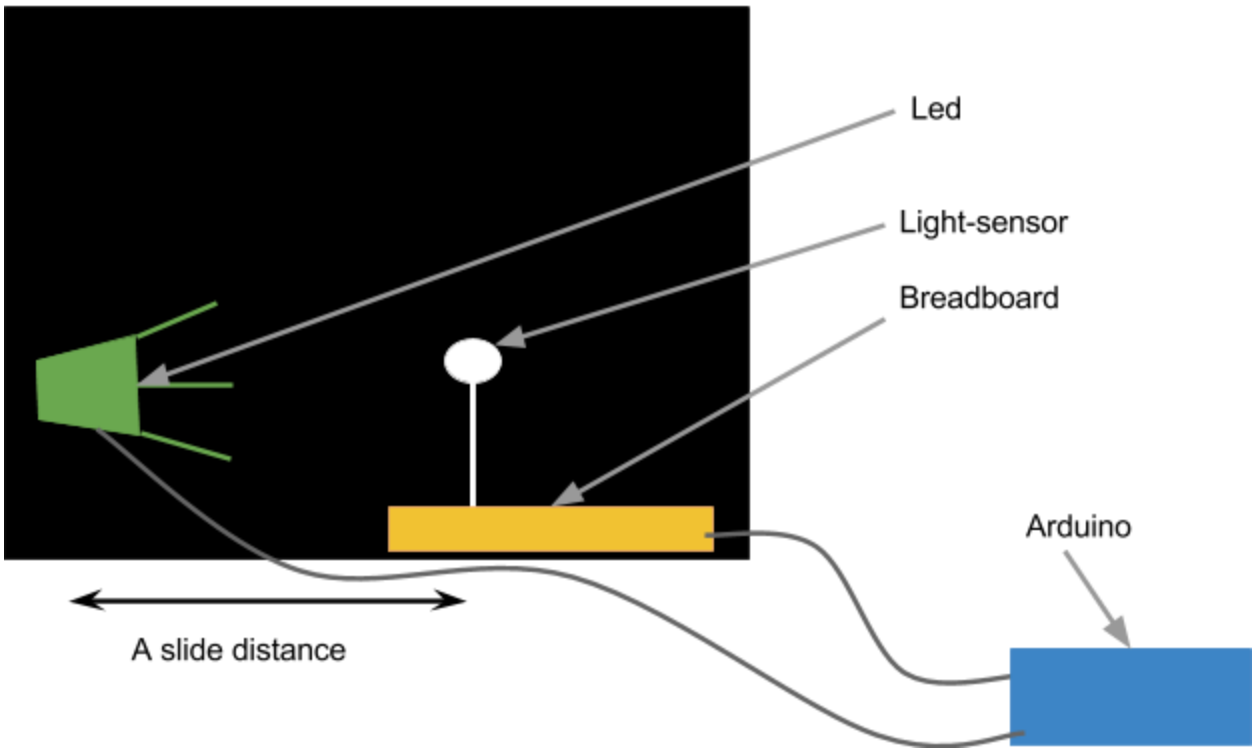
We recorded each movement on a csv format, in which we have the position x and y, the number of the picture, the distance between one point and the previous one. By a parsing script, we extracted the distance with the previous point and we have done an average speed of the back leg and of the head of each tardigrades. Then, we have done an average between the speed of the back leg and of the head to plot a graph that depict the average speed in $mm.s^{-1}$ in function of the intensity.

Electronic sensor Protocol

In parallel, we made the experiment concerning our electronic sensor.
For that you will need :

- An Arduino
- A Light-Dependent-Resistance (LDR)
- A 10kΩ Resistance
- A green LED

Final electric-sensor experiment



The all circuit was placed under a black bag to dim the light intensity.
We had 10 different LDR of the same kind and we registered their resistance for 1 minute for 5 different values of intensity (0, 1 , 10, 100 or 255).

To gather those data, we used both Arduino and Python programming to collect the data and then store them in .csv files in order to facilitate data manipulation, plotting and analysis.
You can find our Arduino and Python codes on our GitHub account.
Here is how our .csv file looks like :

Time	Light-value
100	0281
200	0303
300	1259
400	1319
500	2267
600	2322

21/01/17

Today we did a replicate of the electronic-sensor experiment. This replicate would test, if the place where we practiced the experiment can impact our results because of a different ambient light intensity.
Yesterday, we did it in Léonie's house at 11pm while today we did it in Cochin's classroom at 7pm.
However, we noticed that some sensors had troubles measuring the intensity.
Indeed, they gave impossible values (which means superior to 1024). Thus, our sensors are not resistant enough to be reused twice. This point seems to indicate that the bio-sensor model and the electric-sensor model have the same disadvantage: it is difficult to keep them working to replicate the experiment another time.

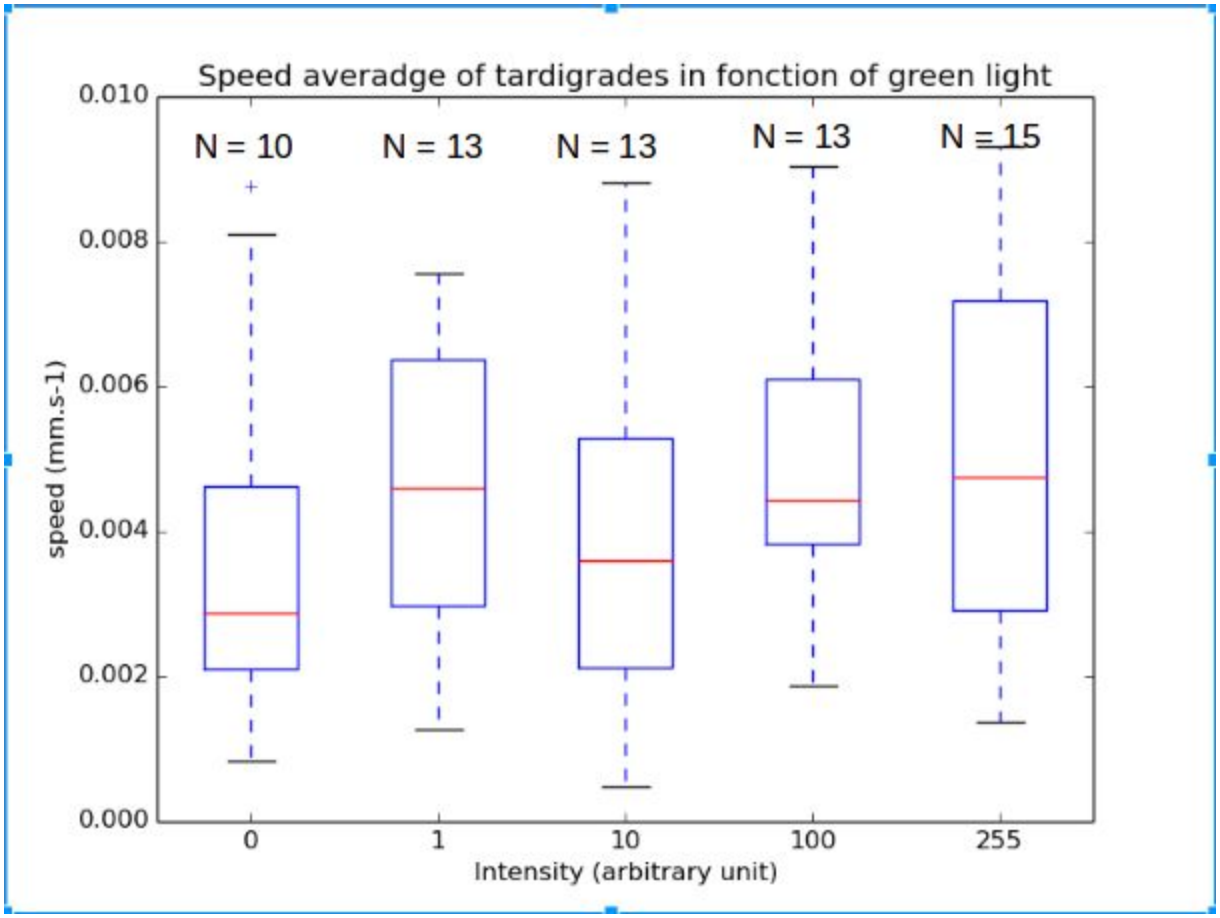
23/04/16

Last day of the project, we finished all remaining tests to do :

- We took the video and the measures of the tardigrades as we have done before but the the intensity 100 and 255. Nikola took the footages and Nina analysed them with ImageJ.
- Léonie took a new set of data from the electronic sensor. Indeed, we realised that the data previously extracted from the LDR by Léonie and Nikola haven't been done with the same program, so we chose the easiest one to parse and allowed us to make another replicate and use other datas than those who failed (ie, resistance over 1024). Finally we kept the set of Nikola (21/01/16) and the new set of Léonie (23/01/16).

In the end, we obtained the following results :

For the Biological sensor:



The number on top of the boxes indicates the number of individuals measured for each intensity. We can observe that there is no tendencies of phototaxis or photokinesis with the increase of light intensity. We probably should do more repetitions and replicates and also improve our protocol to control settings such as the humidity or the ambient light. Elements that may have an impact on *H. dujardini* 's mobility.

For the Electronic sensor :

The number on top of the boxes indicates the number of individuals measured for each intensity. We can see that the higher the intensity is, the higher the resistance of the LDR will be, meaning that it will sense an increase of intensity. Moreover, speaking about the variability, we can observe an increase of the variance with the increase of intensity. In comparison, the variance of the biological sensor is much higher and quite constant with the intensity. This can be explained by the inter-individual variability (biological noise).

Conclusion

Our data were inconclusive due to a lack of repetitions concerning the observation of the response of tardigrades. But we can observe a real difference in precision for low intensity light. On another hand, there is a lot of improvements to make to our protocol. Have a better control on the light intensity, on the humidity level on the slide, both having an impact on tardigrades mobility.

If you are interested and want to know more about our project, you can check our presentation, GitHub or Blogpost !