Lab notebook

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Brainstorming

16/01/2017

For our experiment, we want to see the reaction time variation of Daphnia[1] individuals when exposed to different color-alternating frequency[2].
We assume that the smaller the color-alternating frequency, the smaller the reaction-time will be.
We will choose blue and red light because Daphnia undergo a positive phototaxis[3] when the light is between 420 nm and 600 nm wavelengths and an negative phototaxis between 260 nm and 380 nm wavelengths[4]. Nevertheless, we have also seen during the Biosensors introduction that Daphnia seem to go up with the blue light, and "fall" with the red light … Thus, another goal of the experiment will be to determine which of these statements is correct.

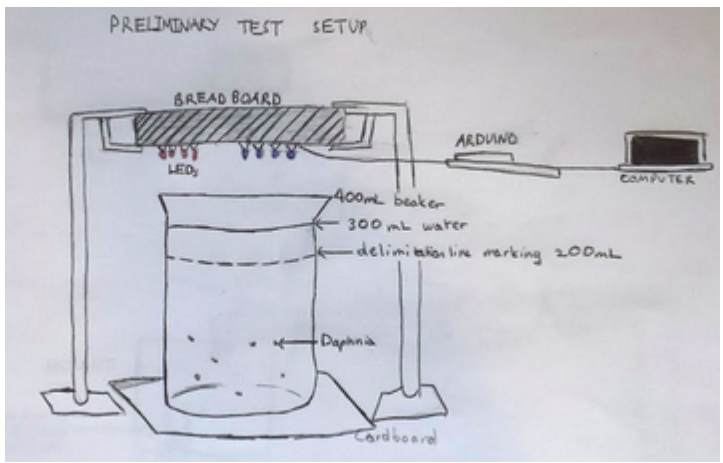
First experiment

19/01/2017

What is the reaction time variation of a sensor when exposed to increasing color-alternating frequency?

MATERIAL

- ❏ Daphnia
- ❏ Aquarium
- ❏ 400mL beaker
- ❏ 100mL beaker
- ❏ Blue and red LED
- ❏ RGB Sensor
- ❏ 2 Arduino LEONARDO
- ❏ Chronometer
- ❏ 25 mL pipet
- ❏ Pro Pipet
- ❏ Scotch
- ❏ Paper



[1] Ebert, Dieter. *Introduction to Daphnia Biology*. National Center for Biotechnology Information (US), 2005. https://www.ncbi.nlm.nih.gov/books/NBK2042/.
[2] Cellier, S., M. Rehaïlia, J.-L. Berthon, et B. Buisson. « Le rôle de l'œil, dans les rythmes migratoires de Daphnia magna et Daphnia longispina (Cladocères) ». *Annales de Limnologie - International Journal of Limnology* 34, nº 2 (1 juin 1998): 159‑64. doi:10.1051/limn/1998015.
[3] KN, Zhang L. and Baer. « The influence of feeding, photoperiod and selected solvents on the reproductive strategies of the water flea, Daphnia magna. - PubMed - NCBI ». Consulté le 20 janvier 2017. https://www.ncbi.nlm.nih.gov/pubmed/15092821.
[4] Storz, U. C., et R. J. Paul. « Phototaxis in Water Fleas (Daphnia Magna) Is Differently Influenced by Visible and UV Light ». *Journal of Comparative Physiology A* 183, nº 6 (1 décembre 1998): 709‑17. doi:10.1007/s003590050293.

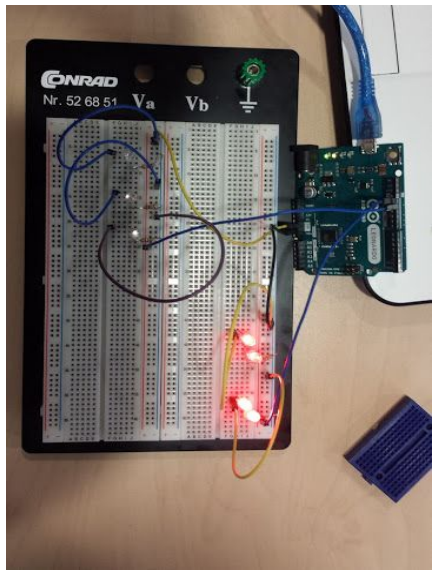
For our first experiment, we designed a structure using an Arduino Leonardo, two breadboards, 3 blue LEDs for one, and 3 red LEDs. The code was designed for the red and blue LEDs to light alternately according to a given time[5][6].

Sequential_blinking | Arduino 1.6.12

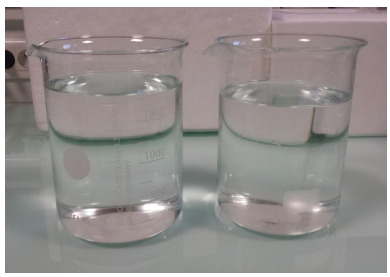
Fichier Édition Croquis Outils Aide

Sequential_blinking §

```
/* A simple program to sequentially turn on and turn off 2 set of LEDs */

int LED1 = 12;
int LED2 = 13;
int LED3 = 2;
int LED4 = 3;

void setup() {
   pinMode(LED1, OUTPUT);
   pinMode(LED2, OUTPUT);
   pinMode(LED3, OUTPUT);
   pinMode(LED4, OUTPUT);
}


void loop() {
  digitalWrite(LED1, HIGH);    // turn on LED1
  digitalWrite(LED2, HIGH);    // turn on LED2
  delay(1000);                  // wait for x ms
  digitalWrite(LED1, LOW);     // turn off LED1
  digitalWrite(LED2, LOW);     // turn off LED2

  digitalWrite(LED3, HIGH);    // turn on LED3
  digitalWrite(LED4, HIGH);    // turn on LED4
  delay(1000);                  // wait for x ms
  digitalWrite(LED3, LOW);     // turn off LED3
  digitalWrite(LED4, LOW);     // turn off LED4
}
```



Preparation of the experiment



Chlorine is toxic for Daphnia[7]. Thus, to get rid of it, we prepared two beakers with tap water and we left them in the laboratory overnight to let chlorine evaporate.

Daphnia-package(~300 individuals) shopping at Truffaut(pet shop) and immersion of individuals in the aquarium.

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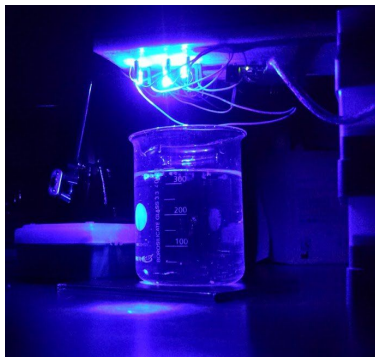
[5] « Check out the course “Tableaux et jeux de lumière avec plusieurs LED” on OpenClassrooms ». *OpenClassrooms*. Consulté le 20 janvier 2017. https://openclassrooms.com/courses/programmez-vos-premiers-montages-avec-arduino/jeux-de-lumiere-et-tableaux-avec-plusieurs-led.

[6] « Sequential_blinking.ino ». *Dropbox*. Consulté le 20 janvier 2017. https://www.dropbox.com/s/bivwdnehp7ln1iq/Sequential_blinking.ino?dl=0.

[7] « Éliminer le chlore de l’eau du robinet gratuitement ». *consoGlobe*, 7 mai 2016. http://www.consoglobe.com/eliminer-chlore-eau-robinet-gratuitement-2895-cg.

Beginning of the experiment at 10h

Most of the Daphnia bought at Truffaut on 01/18/17 are dead. Our sample size is composed of one adult and 15 juveniles (including two dead individuals and two others that don't seem to move).



We put our Arduino installation with our two sets of LEDs, in the dark room above the 400ml beaker containing Daphnia. We drew a delimitation line on the 200ml mark, the beaker being filled with 350mL of water. Our goal is to observe and test the impact of the light wavelength on Daphnia behaviour.

We hope to see our population go up in our beaker while exposed to blue light and go down while exposed to red light.

To observe their reaction time, we measure the time between the light shift from blue to red and the moment when the last Daphnia of the population (hopefully entirely on the top of the beaker) is under the delimitation line.

We realised the experiment with an alternating-lighting frequency of three minutes (that is to say three minutes of blue light, and then three minutes of red light).

Observations

- First session of three minutes:
  - Blue-light session : Very few individuals above the delimitation line, only one or two individuals at different moments.
  - Red-light session : The number of Daphnia above the line during the whole experiment is 3 or 4 depending on the moment.
- Second session of three minutes:
  - Blue-light session : juvenile Daphnia move far more visibly than larger individuals, making observation difficult. Their movement seems to move in no clear direction overall. We therefore decide to only study adult Daphnia.

Results

Obtained results are very different to our expectations.

We didn't observe a "group" behaviour like we had hoped. It's possible that the juvenile population hadn't acquired daily or adult behaviours in their environment yet.

Because of this, we rapidly stopped the experiment and didn't measure parameters. We concluded our experiment had to be redesigned.

2nd session of experiment

We came back to the laboratory to retry the first experiment, but with a new population of Daphnia composed of more adults.

We take 20 adult Daphnia (+/- 1)

-Under white light, three individuals stay at the bottom of the beaker and the rest stay at the surface. Two individuals swim in seemingly random vertical direction between the bottom and the delimitation line.
- We turn on the red light during three minutes, and during all this time we don't observe any change in behavior.
- We observe the Daphnias on the head of the beaker begin to regroup them under the blue light, directly underneath the LEDs.
The Daphnias at the bottom of the beakers don't have this behaviour when the light is on the surface.
-During the alternations of colors, we moved the beaker to be directly under the LED. The phenomenon of concentration under the red light is like what we had observes before, regardless of light color.
-We turn off the light during 8 minutes to observe their behavior. The phenomenon of concentration has not appeared.
During all of our experiments we observed only horizontal migration. Daphnia are only attracted to light that shines at the same depth as the one they are swimming at..
-We illuminate the beaker with the white light of a phone and the daphnia concentrate in one point directly under the light. We place the light on the sides of the beaker and move along the surface of the water. We notice that the Daphnias located on the surface of the water all come close to the light. We try this again at the bottom of the beaker and notice that the individuals at the bottom are now attracted to the light.

We decide to change our protocol.
We will evaluate the migration speed of Daphnias from one side of the beaker to the other side. To do that, we place two lights on opposite sides of a Petri dish, and we turn on and off the light alternately, and measure the time it takes for the Daphnia to go in a certain area close to the light.

We replace the beaker with a 13.7 cm diameter petri dish. We trace areas on a piece of paper placed under the container. When 5 individuals arrived in the darkest area, the migration is considered to be complete. Only the movement of adults in the population (20 Daphnia) is taken into account.

After performing the experiment once, we realize that the area that the Daphnias are attracted to when a light is on is larger than the one we had decided on. We extend the area and repeat the experiment. At this point we begin collecting data that we will use. We have 5 repetitions for every color-alternating frequency. We had planned on replicating the experiment 3 times, but time constraints only allowed us to do this once.

Second experiment

20/01/2017 What is the reaction time variation of Daphnia depending on changes in light stimulus location ?

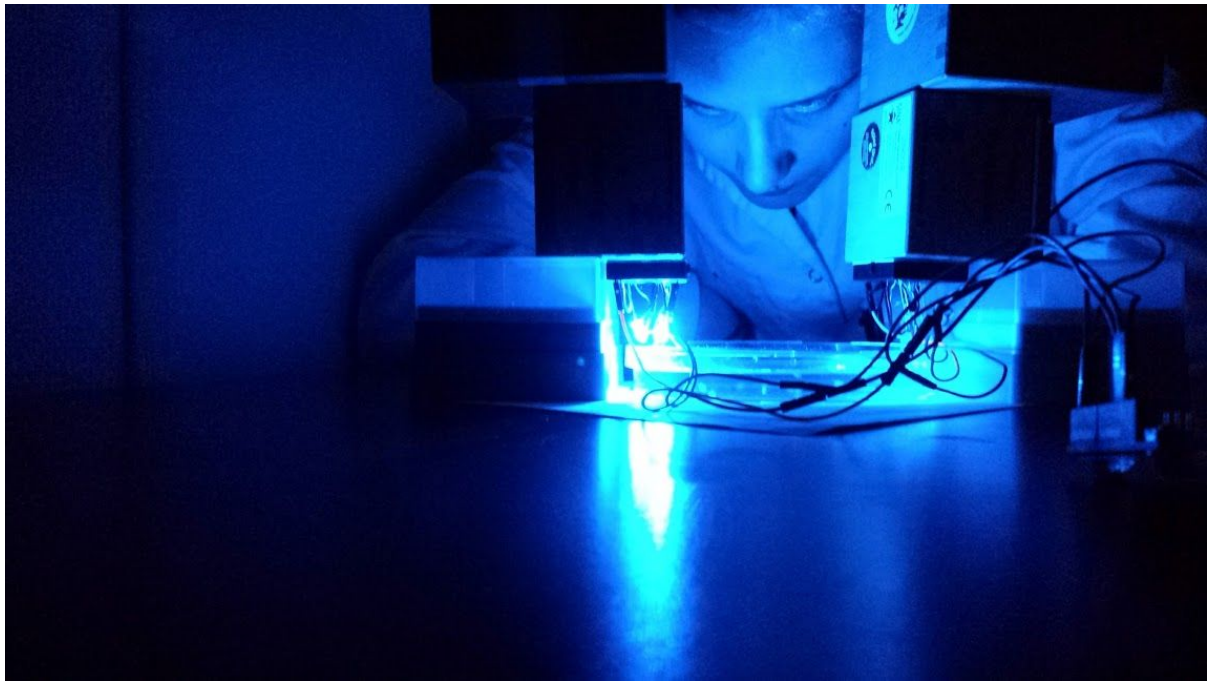
Protocol:

MATERIAL

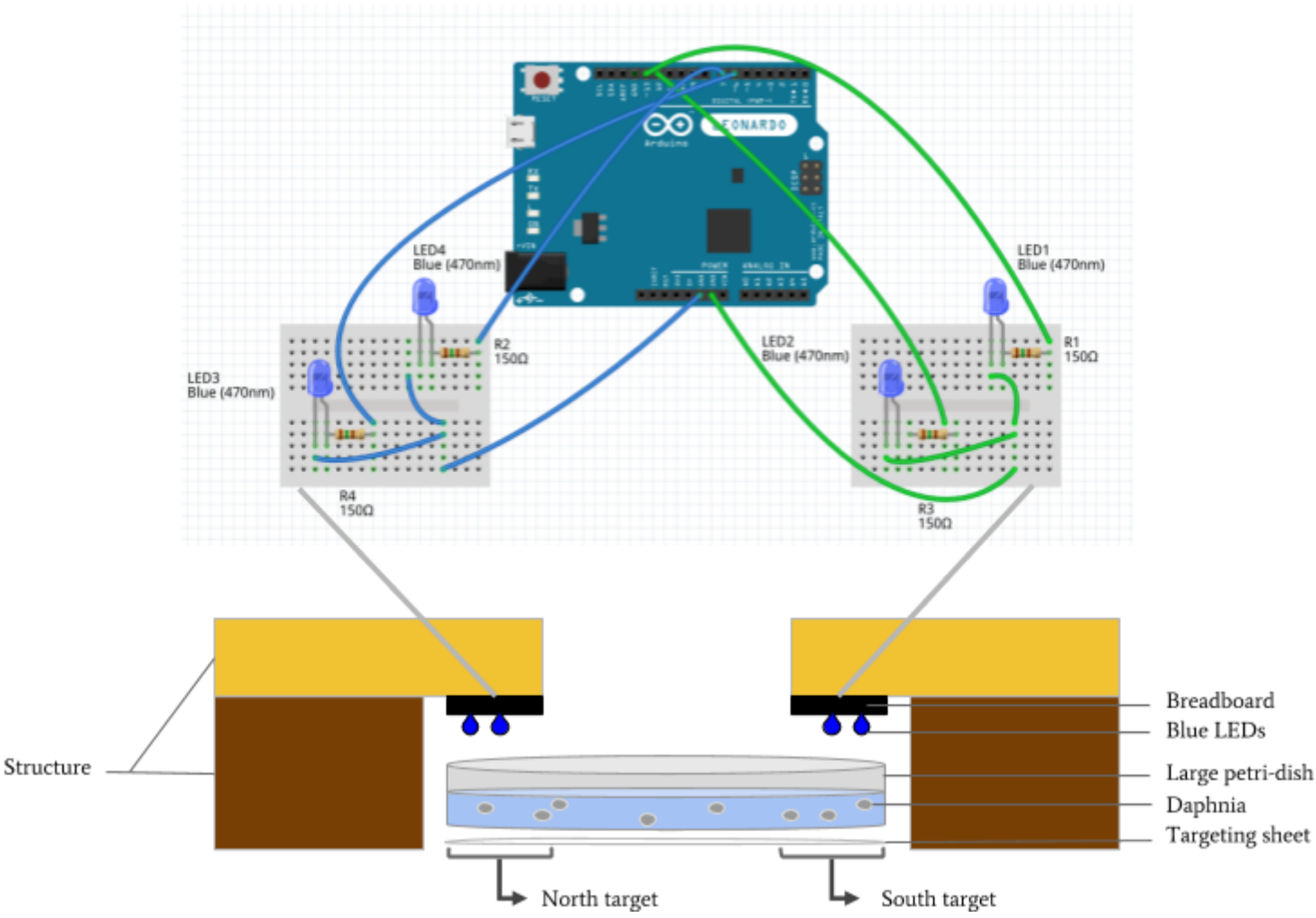
- Daphnia (~ 20 organisms per experiment)
- Aquarium
- 2 x 400mL beakers
- 100mL beaker
- Large petri-dish, diameter: 13.7 cm
- Blue and red LEDs
- RGB Sensor
- 2 Arduino LEONARDO[8]
- Chronometer
- 25 mL pipet
- Pro Pipet
- Scotch
- Paper

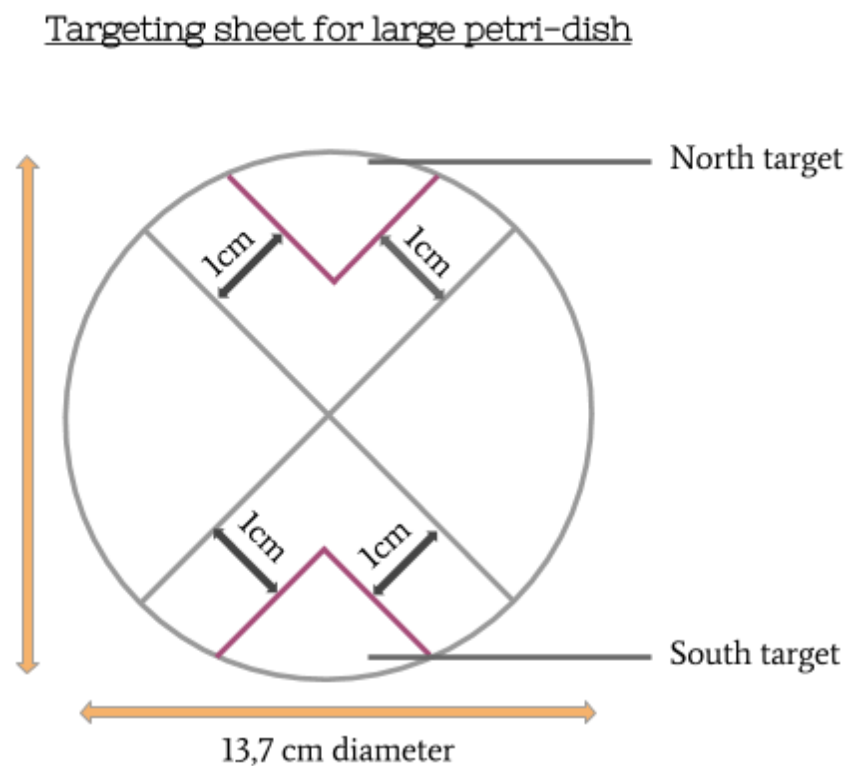
- Take water from the aquarium to organize Daphnia environment
- Remove twenty daphnias from the aquarium using a propipet
- Put the two sets of lights on two opposite sides of the plate
- Set light switch frequency.
- Measure the time it takes for 5 daphnia to be located in the target area.
- Let 5 light switches pass and record migration time for each one.
- Turn off light for one minute.
- Frequencies of alternating-lighting : 1min, 3min, 3min20s, 4min, 4min20s

[8] « Anatomy of a Mini Breadboard - Pimoroni Yarr-niversity ». Consulté le 20 janvier 2017. https://learn.pimoroni.com/tutorial/170pt-projects/anatomy-of-a-mini-breadboard.

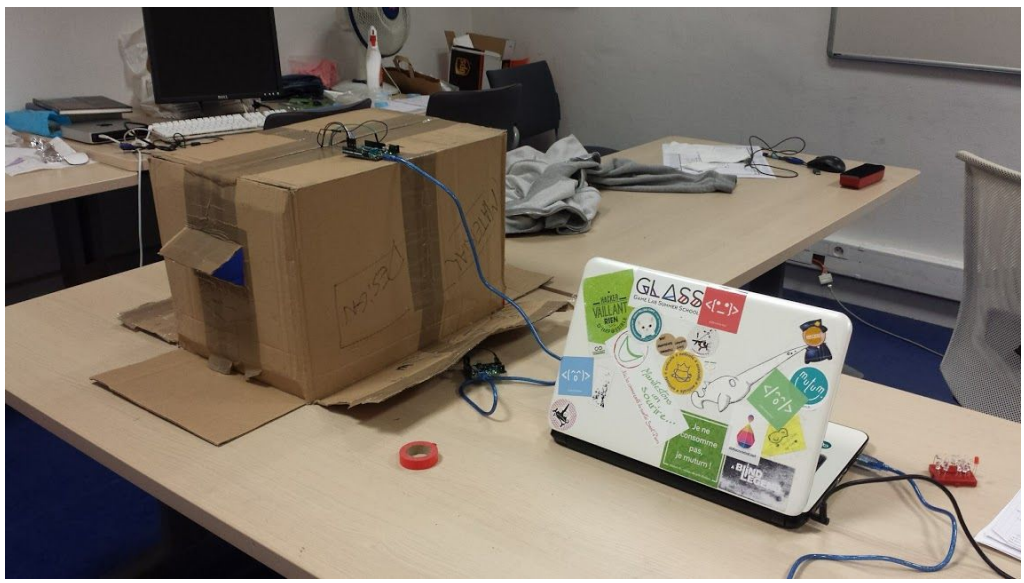


Experiment Setup



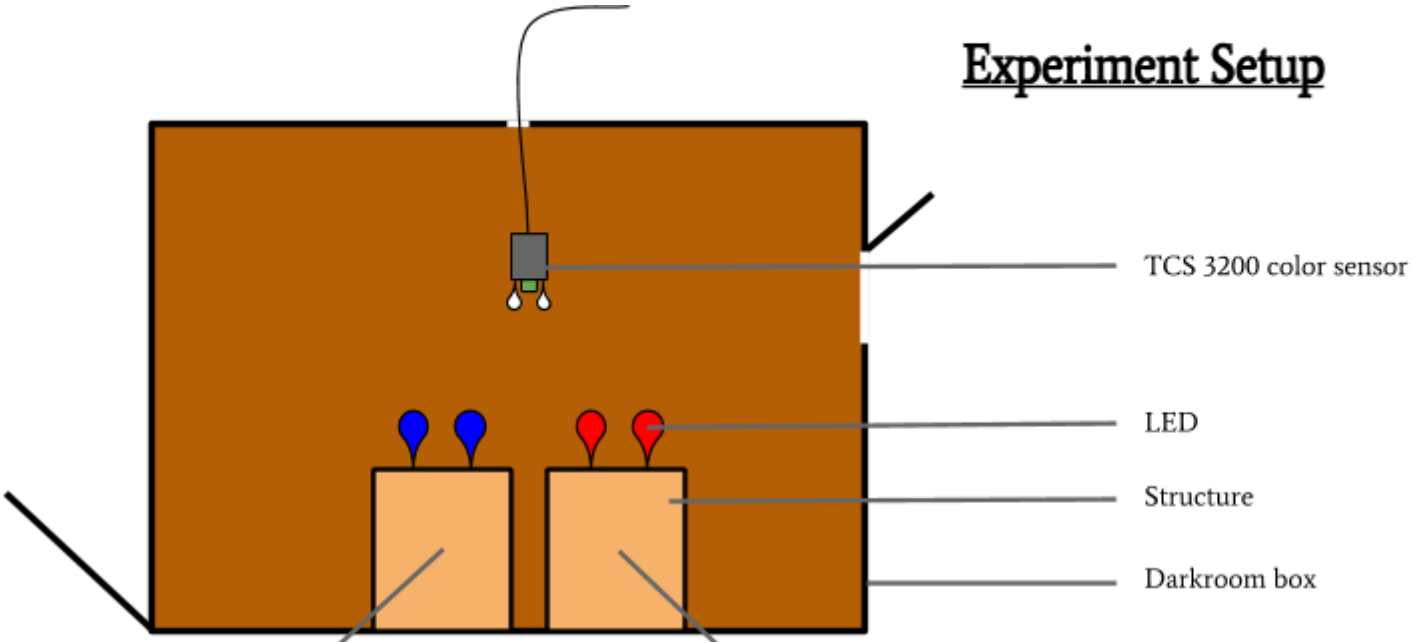


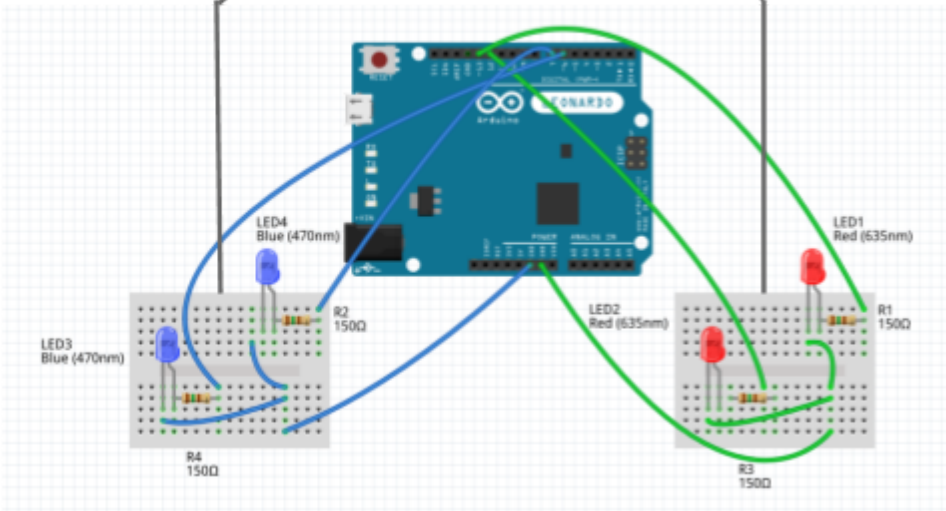
- Put the sensor in a darkroom or in a box. Attach the sensor to the top of the box.
- Put the two set of LED (blue and red one) below the sensor.
- Collect measurements of the sensor before each alternating of light. (We kept the same frequencies of alternating-lighting for the Daphnia and the sensor).
- As for the lab session, repeat the experiment 5 times for each alternating-lighting frequency.

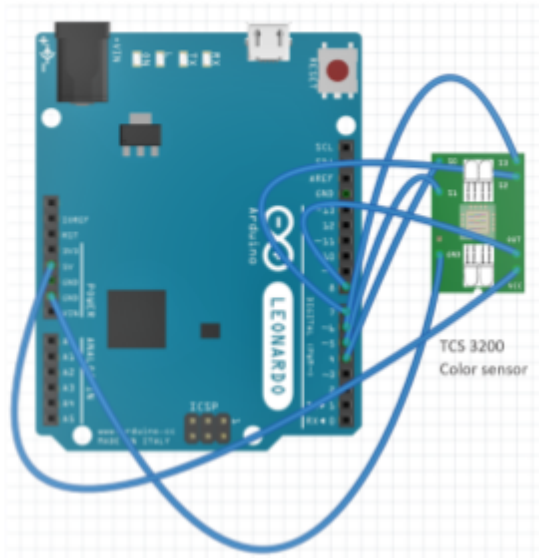


[9] « Arduino Color Sensing Tutorial - TCS230 TCS3200 Color Sensor - HowToMechatronics ». Consulté le 23 janvier 2017. http://howtomechatronics.com/tutorials/arduino/arduino-color-sensing-tutorial-tcs230-tcs3200-color-sensor/.

Experiment Setup







During the experiment:

We did this experiment twice, with two set of 20 Daphnia at 10h00 am to 2pm.

We perform 6 sessions, with different times that determine the time during which a LED is lighting: 4:20min, 4min, 3:20min, 3min, 2min, 1:30m. After each of the different sessions we left the daphnia in the dark for 1 minute.

We take all measurements by counting the time between the lighting of the LED and the time taken by 5 Daphnias to come into a gray triangle under the plate below the LED.

For each session we take 5 measurements.
We performed this experiment twice but changed the area that we took into account for migration as our initial area was deemed to be too small to obtain results. We also increased the time for which each LED set was lit, as our initial chosen times were too short for any complete migration to take place.
We observe during the experiment that daphnia come more slowly or don't move. We can think their tired, after all the move, or they have an habituation to the light.

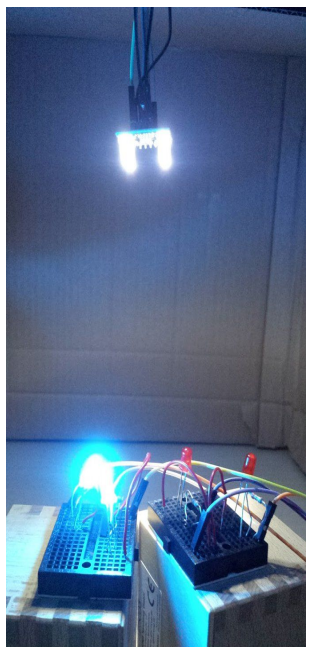
RGB §

```
#define S0 4
#define S1 5
#define S2 6
#define S3 7
#define sensorOut 8
int frequency = 0;
void setup() {
  pinMode(S0, OUTPUT);
  pinMode(S1, OUTPUT);
  pinMode(S2, OUTPUT);
  pinMode(S3, OUTPUT);
  pinMode(sensorOut, INPUT);

  // Setting frequency-scaling to 20%
  digitalWrite(S0,HIGH);
  digitalWrite(S1,LOW);

  Serial.begin(9600);
}
void loop() {
  // Setting red filtered photodiodes to be read
  digitalWrite(S2,LOW);
  digitalWrite(S3,LOW);

  // Reading the output frequency
  frequency = pulseIn(sensorOut, LOW);

  // Printing the value on the serial monitor
  Serial.print("R= ");//printing name
  Serial.print(frequency);//printing RED color frequer
  Serial.print("  ");
  delay(100);
```

RGB §

```
  // Reading the output frequency
  frequency = pulseIn(sensorOut, LOW);

  // Printing the value on the serial monitor
  Serial.print("R= ");//printing name
  Serial.print(frequency);//printing RED color frequency
  Serial.print("  ");
  delay(100);

  // Setting Green filtered photodiodes to be read
  digitalWrite(S2,HIGH);
  digitalWrite(S3,HIGH);

  // Reading the output frequency
  frequency = pulseIn(sensorOut, LOW);

  // Printing the value on the serial monitor
  Serial.print("G= ");//printing name
  Serial.print(frequency);//printing RED color frequency
  Serial.print("  ");
  delay(100);

  // Setting Blue filtered photodiodes to be read
  digitalWrite(S2,LOW);
  digitalWrite(S3,HIGH);

  // Reading the output frequency
  frequency = pulseIn(sensorOut, LOW);

  // Printing the value on the serial monitor
  Serial.print("B= ");//printing name
  Serial.print(frequency);//printing RED color frequency
  Serial.println("  ");
  delay(1000);
}
```

During the afternoon we didn't have access to the laboratory, so we did the experiment with the sensor in a room with a low quantity of light, and we put the sensor and the installation with the LED in a box. We pass the sensor through the top of the box and place the box above the twos sets of LEDs. The Arduino code that we use is just above.

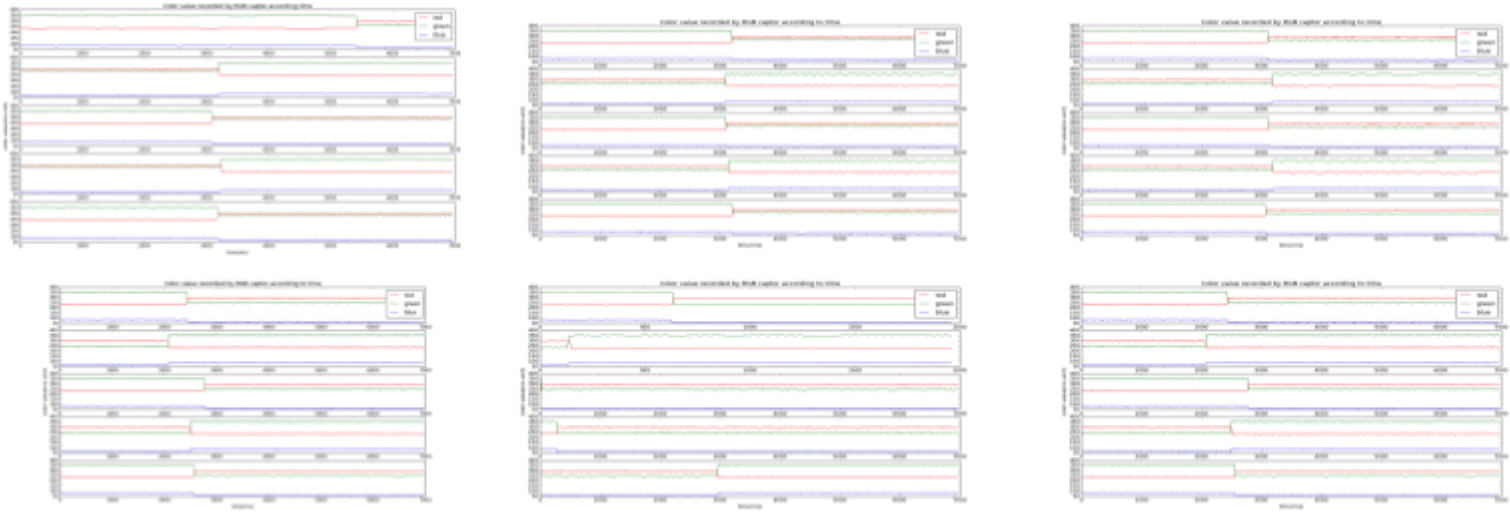


We take 5 measurements for all the different intervals of lighting (same intervals as previously).
10 seconds before the changing of light, we activate a python code that records 700 measurements taken by the sensor in a CSV file.

The CSV file is then opened and the 'R=', 'G=', and 'B=' are removed so that only numbers are left.
The data is then parsed and plotted using this python code.

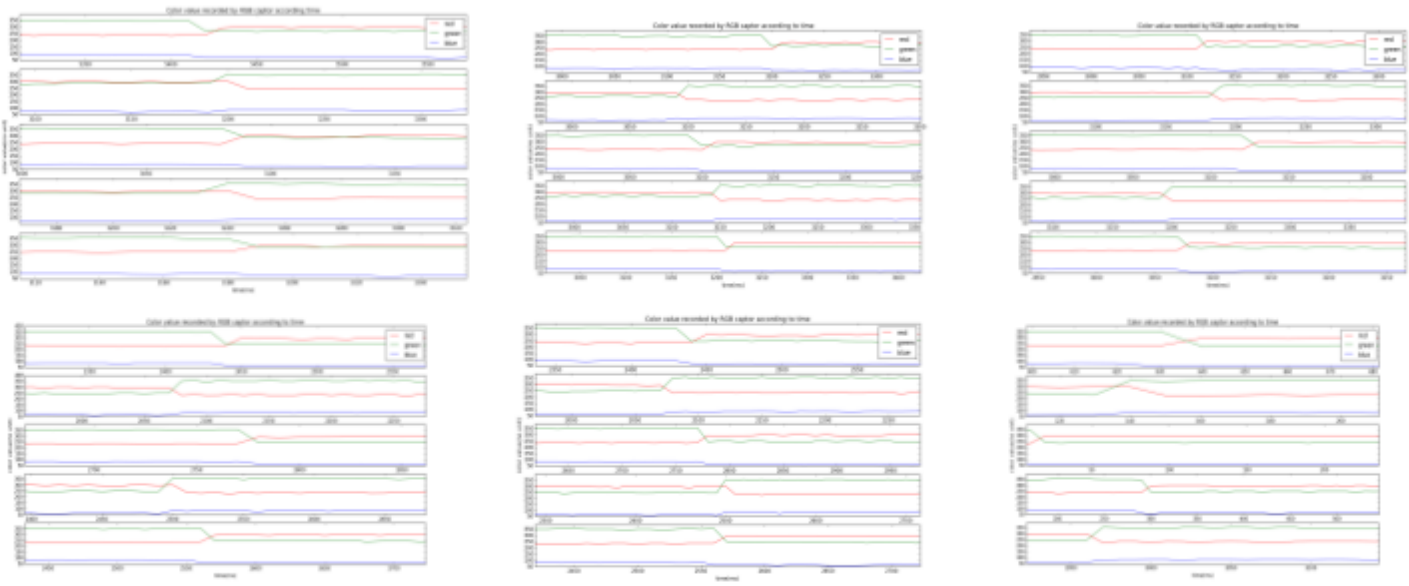
```
import matplotlib.pyplot as plt #for creating graphs

frq = 10 #frequency of measurements taken by the sensor in milliseconds

file = open('data.csv', 'r') #retrieve recorded data

grosseliste = [] #this list will stock each RGB value for each measurement
time = [] #this list will contain the times at which measurements were taken, starting with the first measurement at 10ms

mesure1 = [] #these lists seaparate the values recorded for each repetition
mesure2 = []
mesure3 = []
mesure4 = []
mesure5 = []

listeR1 = [] #these lists separate the color values for each repetition
listeG1 = []
listeB1 = []

listeR2 = []
listeG2 = []
listeB2 = []

listeR3 = []
listeG3 = []
listeB3 = []

listeR4 = []
listeG4 = []
listeB4 = []

listeR5 = []
listeG5 = []
listeB5 = []

for aline in file.readlines():
    miniliste = aline.split('  ')   #read lines and remove unwanted characters
    del miniliste[-1]
    grosseliste.append(miniliste) #add each measurement to a large list
for i in range(0, 699):           #split this list into one list for each repetition
    mesure1.append(grosseliste[i])
for i in range(700, 1399):
    mesure2.append(grosseliste[i])
for i in range(1400, 2099):
    mesure3.append(grosseliste[i])
for i in range(2100, 2799):
    mesure4.append(grosseliste[i])
for i in range(2800, 3499):
    mesure5.append(grosseliste[i])

del mesure1[0]    #removes every first and last value of the new lists because they are always not properly formatted.
del mesure1[-1]
del mesure2[0]
del mesure2[-1]
del mesure3[0]
del mesure3[-1]
del mesure4[0]
del mesure4[-1]
del mesure5[0]
del mesure5[-1]

for i in range(697):  #adds time values to time list
    time.append(i * frq)

for i in range(len(mesure1)):     #separates the red, green and blue values into separate lists for every repetition
    listeR1.append(mesure1[i][0])
    listeG1.append(mesure1[i][1])
    listeB1.append(mesure1[i][2])
for i in range(len(mesure2)):
    listeR2.append(mesure2[i][0])
    listeG2.append(mesure2[i][1])
    listeB2.append(mesure2[i][2])
for i in range(len(mesure3)):
    listeR3.append(mesure3[i][0])
    listeG3.append(mesure3[i][1])
    listeB3.append(mesure3[i][2])
for i in range(len(mesure4)):
    listeR4.append(mesure4[i][0])
    listeG4.append(mesure4[i][1])
    listeB4.append(mesure4[i][2])
for i in range(len(mesure5)):
    listeR5.append(mesure5[i][0])
    listeG5.append(mesure5[i][1])
    listeB5.append(mesure5[i][2])

plt.subplot(511)                                  #creating plots of R, G and B values according to time.
plt.plot(time, listeR1, color = 'r', label ='red')
plt.plot(time, listeG1, color = 'g', label = 'green')
plt.plot(time, listeB1, color = 'b', label = 'blue')
plt.legend()
plt.title('Color value recorded by RGB captor according to time')
plt.subplot(512)
plt.plot(time, listeR2, color = 'r', label ='red')
plt.plot(time, listeG2, color = 'g', label = 'green')
plt.plot(time, listeB2, color = 'b', label = 'blue')
plt.subplot(513)
plt.plot(time, listeR3, color = 'r', label ='red')
plt.plot(time, listeG3, color = 'g', label = 'green')
plt.plot(time, listeB3, color = 'b', label = 'blue')
plt.ylabel('color value(no unit)')
plt.subplot(514)
plt.plot(time, listeR4, color = 'r', label ='red')
plt.plot(time, listeG4, color = 'g', label = 'green')
plt.plot(time, listeB4, color = 'b', label = 'blue')
plt.subplot(515)
plt.plot(time, listeR5, color = 'r', label ='red')
plt.plot(time, listeG5, color = 'g', label = 'green')
plt.plot(time, listeB5, color = 'b', label = 'blue')
plt.xlabel('time(ms)')
plt.show()

file.close()
```

These are the plots obtained:



Graphs of RGB values according to time for light change intervalls of 1min30s, 2min, 3min, 3min20s, 4min, 4min20s respectively(moving clockwise from top left)

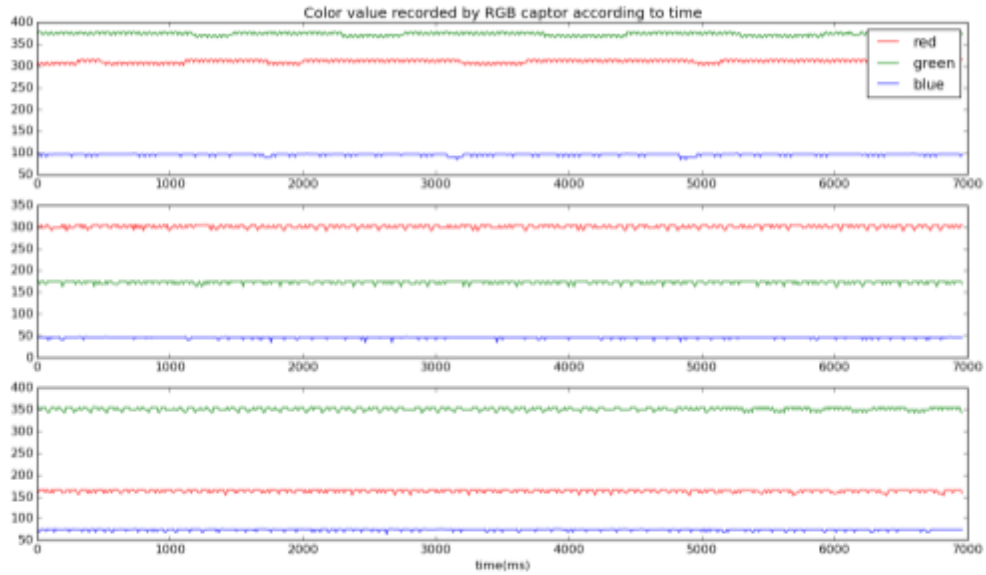
Each graph showed what seemed to be constant values, except for one huge change, which represents the change in LED color. We then used the zoom tool in matplotlib to see the change closer and evaluate how much time it took for the sensor to adapt to it and return to constant value.



Zooms taken on graphs to evaluate electronic sensor reaction time

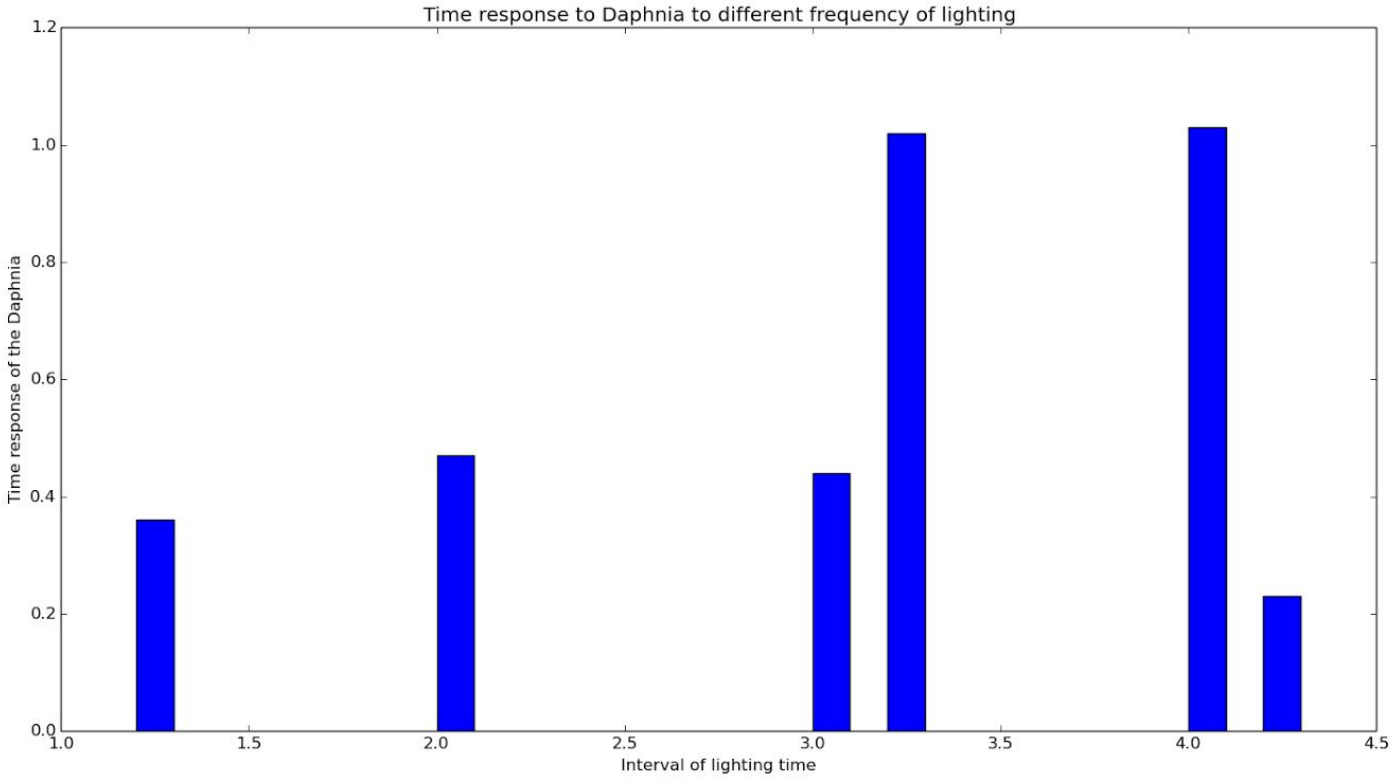
We then printed out the zoomed versions of the graphs and used a ruler to calculate the reaction time as precisely as possible according to proportionality rules.

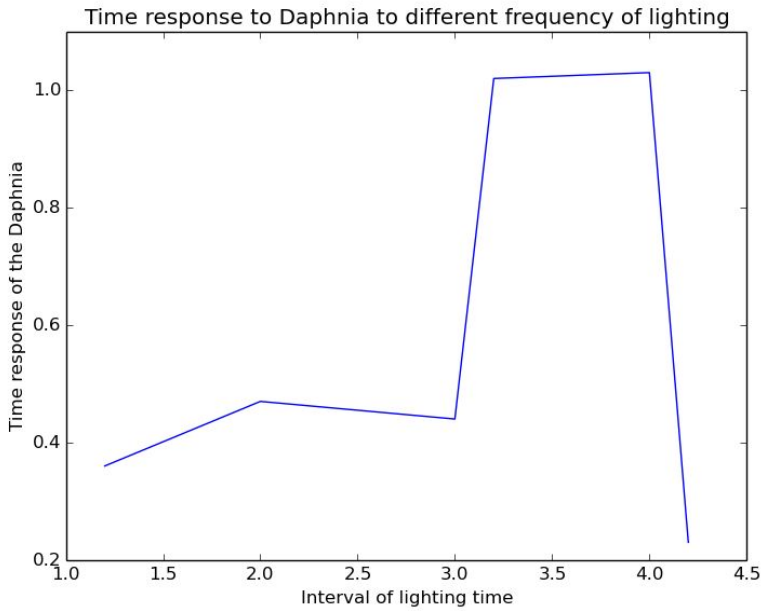
Once we had values for reaction time, we calculated the average reaction time for a given frequency of change and plotted them. We then evaluated how much the values varied from one value to another. The graphs showed no clear pattern or correlation to comment on. No conclusions were drawn at this point. We still wanted to know whether or not the differences we had observed were significant or not. We performed a Student's statistical test on each value to obtain the associated p-value. All were under 5% and therefore we concluded that changes were not significant and all values could be considered statistically identical. We concluded that the reaction time of the sensor was a constant between 10 and 25 milliseconds. This conclusion is coherent with our hypothesis.

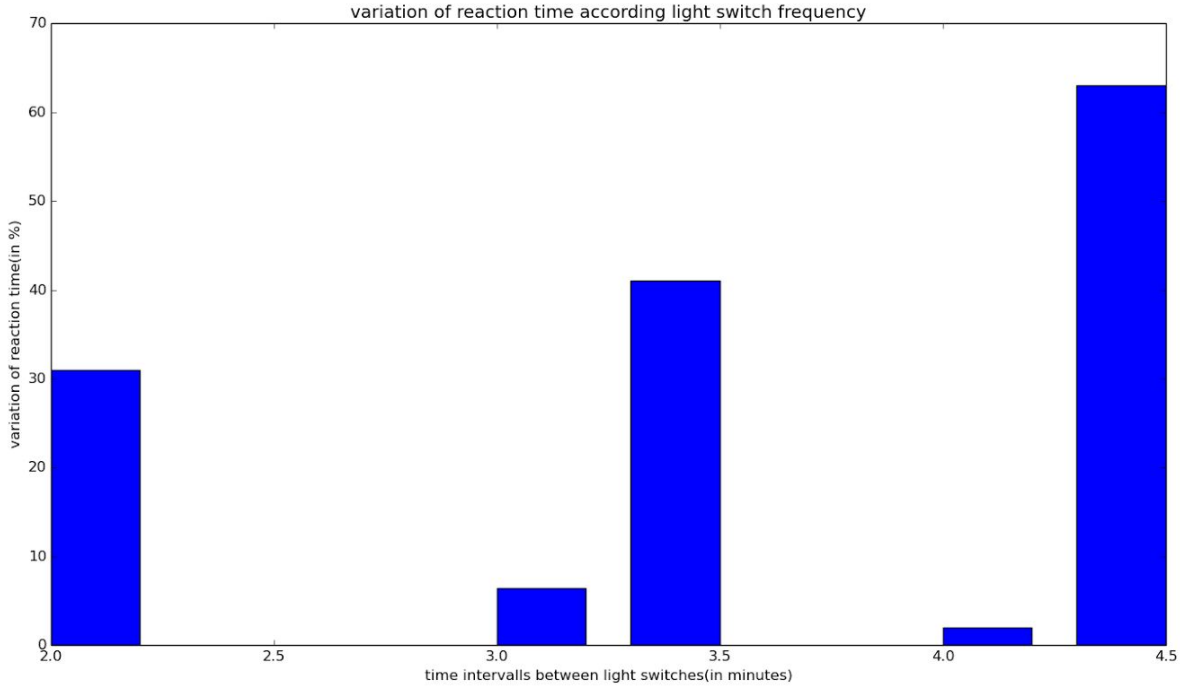


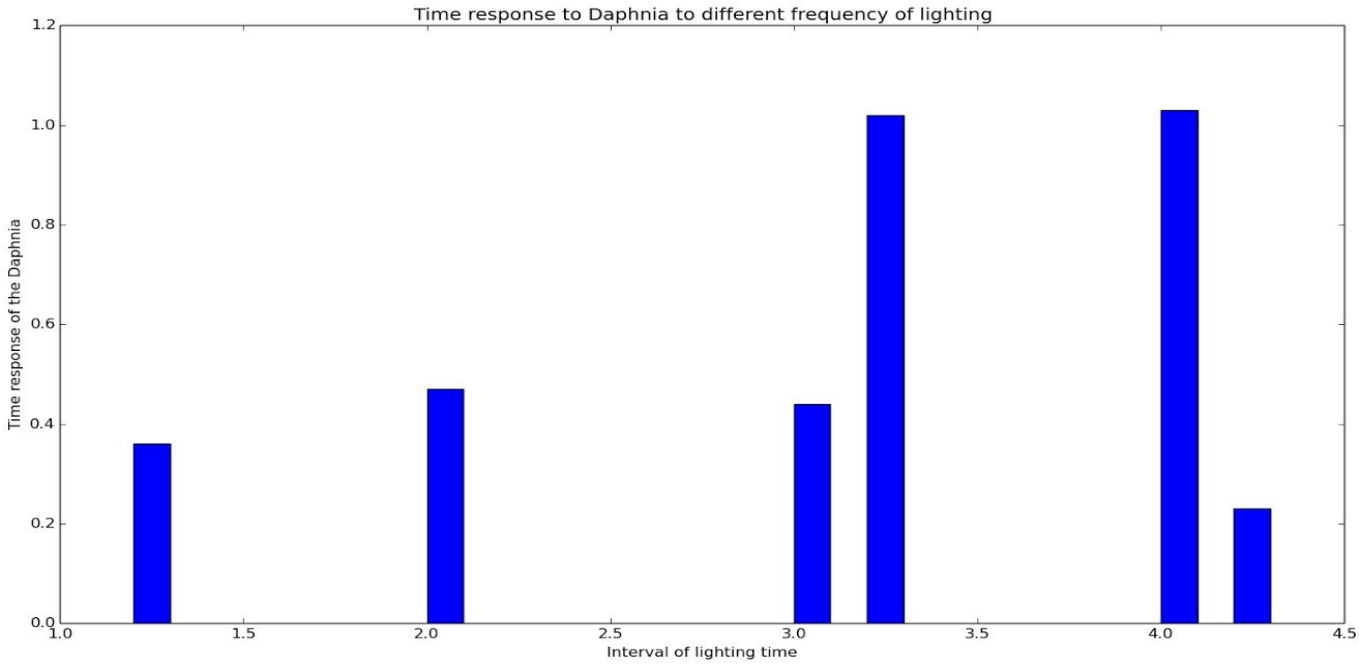
Graphs of controls performed with the RGB sensor. Top: No light, Middle: Only Blue Light, Bottom: OnRed light.

We then created a graph of migration times of Daphnia populations as a function of light change frequency and again noticed no clear trend. It seemed our method of evaluating migration time lacked precision and accuracy. In addition, repeating the experiment on one population of Daphnia may have tired them or made them unreactive to the light regardless of their capacity to react. More replicas would be needed to confirm, but due to time constraints, this will not be possible. The percentage of variation between each value also revealed no clear trends(graphs can be seen below). Students test yielded p-values that were consistently above 5% meaning that we could not consider reaction time to be constant, but we could not determine how the frequency of light change affected this reaction time.


Time response to Daphnia to different frequency of lighting
Time response of the Daphnia
Interval of lighting time
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5


Time response to Daphnia to different frequency of lighting
Time response of the Daphnia
Interval of lighting time
1.0
0.8
0.6
0.4
0.2
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5


variation of reaction time according light switch frequency
variation of reaction time(in %)
time intervalls between light switches(in minutes)
0
10
20
30
40
50
60
70
2.0
2.5
3.0
3.5
4.0
4.5


Time response to Daphnia to different frequency of lighting
Time response of the Daphnia
Interval of lighting time
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5