AN INNOVATIVE LIGHT SOURCE

INVESTIGATING THE BIOLUMINESCENT ACTIVITY IN THE *PYROCYSTIS FUSIFORMIS* ALGA

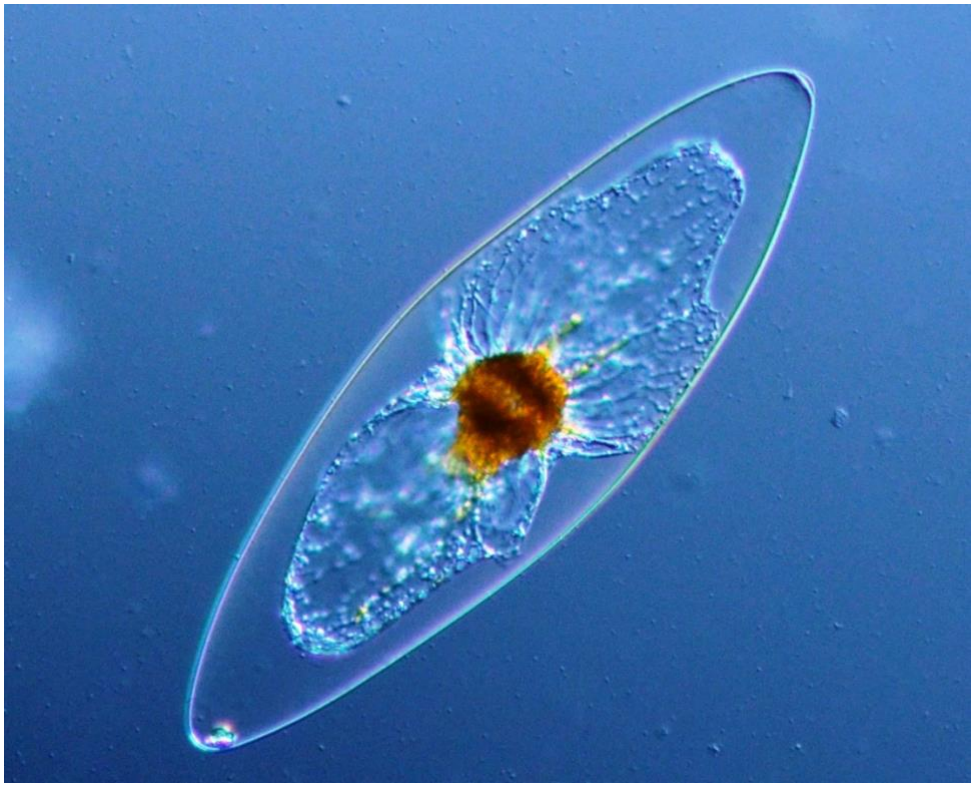

Image 1: Single Pyrocystis fusiformis *alga*[1]

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[1] https://i2.wp.com/exploringtheinvisible.com/wp-content/uploads/2013/11/dsc_0302.jpg (*Pyrocystis fusiformis,* Magnification 1'000x, DIC Microscopy) last accessed 20.11.20

Abstract

Climate change questions our energy consumption, and high expectations are in the field of sustainable light sources. In search of such sources with high outputs and minimal environmental costs, researchers have found the phenomenon of bioluminescence in organisms like the *Pyrocystis fusiformis* alga. This study investigated whether these algae present a persisting light source in terms of light intensity, performance duration and recovery ability. A self-designed measuring device involving light-sensitive photodiodes was used to analyse the stated parameters in six standardised algae samples over the course of two weeks.

On average, an algae sample emitted light with a maximum intensity of 0.15 milliwatt at which structural changes in peripheral objects became visible. Their emission of detectable light lasted for around 22 seconds. Further, the study showed that the algae possess a fast recovery ability as they regained 30-40% of their initial light intensity during a recovery period of 40 minutes.

Currently, the *Pyrocystis fusiformis* algae cannot be seen as an alternative light source because the measured parameters were too small to replace common lighting techniques. However, the study revealed the potential of *Pyrocystis fusiformis,* which encourages future research and innovation in this area.

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Introduction

Investigating natural processes has enabled us to increase our knowledge about our surrounding, which in return has often been the key to progressive inventions. As we now face increasing global population and climate change, the need for such inventions to maintain global sustainability is unprecedented. One area with much ongoing research involves energy-efficient light sources. The growing demand for the latter has forced researchers to investigate completely new concepts of light sources, such as the natural phenomenon of "*Bioluminescence"*.

Bioluminescence is the loanword defining the natural production and emission of light by a living organism[2]. It is a type of chemiluminescence, meaning that the emitted light is produced through a series of chemical reactions. The probably most famous example of bioluminescence is the Lampyridae, better known as the firefly. However, it is found that this ability to produce light is not only found among certain insects, but also among marine organisms, such as bacteria, jellyfish and algae. This study analyses the group of planktonic bioluminescent algae called *Dinoflagellates*[3], more specific the protozoon species known as *Pyrocystis fusiformis*. This species is known for its nocturnal bioluminescent behaviour when actively stimulated by a physical impact such as the abrupt change in water movement. As external influence triggers the glowing of the algae, it allows one to control the bioluminescent behaviour via the purposeful application of physical force in the form of hydrodynamical stress (Maldonado and Latz, 2007). Hence, the investigation focuses on their capability of acting as an energy-efficient light source that may present a sustainable alternative to commercial ones. The topic of this study is expressed in the following structured research question.

Could the bioluminescent activity of a standardised *Pyrocystis fusiformis* algae population present an energy-efficient and sustainable light source in terms of light intensity, performance duration and recovery ability?

[2]Refer to: https://www.oxfordlearnersdictionaries.com/definition/english/bioluminescence last accessed 26.8.20

[3]Algae obtained from: https://pyrofarms.com/products/pyrodinos200ml last accessed 30.9.20

Scientific Background

The marine *Pyrocystis fusiformis* algae are a protozoan species. As the name suggests, their cell has a fusiform shape[4]. A single cell may reach a length of up to 1000µm and a width of around 150µm (Widder, 2002). As *Pyrocystis fusiformis* prefer temperatures from 17°C to 26°C, they are predominant in warm water bodies such as the South Pacific Ocean[5]. A single alga has an average life expectancy of six months, and researchers have found that if environmental conditions are suitable, its replication cycle may therefore span a short period of only seven days (Elbrächter and Drebes, 1978). Elbrächter and his colleague found that they reproduce asexually by forming zoospores in the parent's cell. During a process similar to cell division, the zoospores from the parent cell result in independent algae offspring.

Regarding their bioluminescent behaviour, research has shown that *Pyrocystis fusiformis* live according to a so-called circadian rhythm (Christianson, 1976). While sunlight is present, the algae photosynthesise as a way of metabolism by converting the solar radiation into a form of chemical energy. During the night they emit bioluminescent blue light with the help of the obtained energy from photosynthesis. Prior research has shown that a single *Pyrocystis fusiformis* alga can flash for around 500ms (Widder and Case, 1981) and that less than 20% of the emitted light generates thermal radiation[6]. The thermal aspect gives it the suitable technical term "*cold light"*. As known from physics, this cold blue light has high energy due to its short wavelength ranging from 460-560nm (Eckstein, 1991) and as a result, travels the furthest among the spectrum of the visible light. With this idea in mind, researchers are currently investigating the purpose of the bioluminescent activity in *Pyrocystis fusiformis*. Theories concerning the purpose include the self-protective mechanism, such as sending warning signals via the spontaneous emission of blue light caused by a change in the water movement. If environmental conditions are suitable and a high density of bioluminescent *Dinoflagellates* is present on a shallow beach, one may observe these spontaneous emissions caused by the breaking of waves. This sensation of a collective light emission by thousands of algae is called "*Sea Glowing*".

[4] Refer to Image 1+ further Information at: https://en.wikipedia.org/wiki/Pyrocystis_fusiformis last accessed 12.12.20
[5] Refer to: https://eol.org/pages/896863 last accessed 12.12.20
[6] Refer to: https://www.nationalgeographic.org/encyclopedia/bioluminescence/ last accessed 25.9.20

The bioluminescent process behind the "*Sea Glowing*" is a subcategory of chemiluminescence, meaning that the emitted light is produced in a series of chemical reactions. There exist many species capable of emitting light in the process of chemiluminescence. However, scientists distinguish between two main groups; bacteriogenic and autogenic bioluminescent organisms. Bacteriogenic means that the emitted light is not produced by the organism himself, but rather by so-called symbiotic bacteria that live, as their name indicates, in symbiosis with the organism. The firefly, as well as the anglerfish, are classified as bacteriogenic organisms. The autogenic species, on the other hand, produce the emitted light themselves without the need of symbiotic bacteria. As *Pyrocystis fusiformis* belong to the group of autogenic bioluminescent species, this study will focus on their type of light production. Although the chemical processes underlying their bioluminescence are not yet fully understood, researchers have managed to identify the central pieces to this puzzle.

Pyrocystis fusiformis are classified as autotrophic due to their ability to perform the process of photosynthesis. As previously stated, they live according to a circadian rhythm that is split into a daytime part that involves photosynthesis and a nocturnal part that includes the bioluminescent activity. The chemical concept underlying the process of the latter involves exergonic oxidation, meaning that energy is released (here in the form of visible blue light) in a reaction where electrons are transferred. The following three formulas illustrate the basic underlying reaction present in most of the known bioluminescent *Dinoflagellates* (Lee, 2015).

$$Dinoflagellate\ Luciferin + O_2 \xrightarrow{Luciferase} Presumed\ Intermediate\ Product$$

$$Presumed\ Intermediate\ Product \rightarrow Oxyluciferin\ (excited\ state) + H_2O$$

$$Oxyluciferin\ (excited\ state) \rightarrow Oxyluciferin + hv(light)$$

The molecule *Luciferin* is a natural compound known for its contribution in the bioluminescent processes in various organisms; hence it's Latin name "*lucifer*"- to bring light. The stoichiometric aspect of the molecule may vary among species resulting in subcategories such as the *Dinoflagellate Luciferin.* In the investigated algae, the enzyme *Luciferase* catalyses a

reaction between *Luciferin* and O_2, resulting in an intermediate product that researchers presume to exist (Fajardo et al., 2020). In the next step, this intermediate product releases an H_2O molecule and oxidises to *Oxyluciferin (excited state).* However, this *Oxyluciferin* is excited, meaning that an electron is located in a higher orbit than usual and therefore has surplus energy. As this state is highly unstable, the electron returns to its previous location while releasing energy in the form of light; here denoted as *hv (light).* As a result, the *Oxyluciferin (excited state)* becomes *Oxyluciferin.* The understanding of these chemical reactions can be enhanced with Figure 1 showing the change in the structural formula of the *Dinoflagellate Luciferin*.

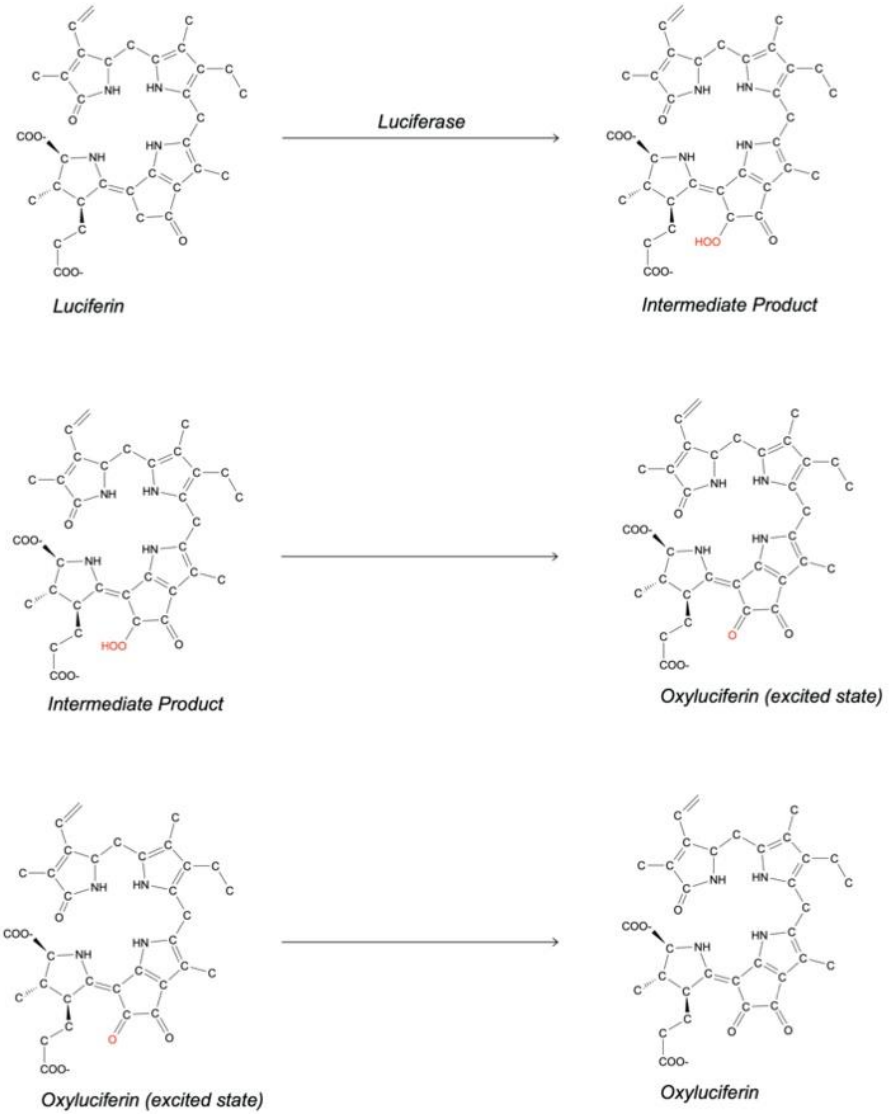


Figure 1: Structural changes in the chemical compound "Dinoflagellate Luciferin" during the exergonic oxidation[7]

[7] Figure 1 was created with the application "MoleculeSketch"

Figure 1 shows the molecular structure of the investigated type of *Luciferin*. Due to its four five-membered carbon rings with a nitrogen atom, known as a "*Pyrrole*"[8], the whole complex is called a "*Tetrapyrrole*". The atoms marked in red visualise which molecules are added and released from the compound during the reaction of bioluminescence. First, *Luciferase* catalyses the reaction leading to the formation of the intermediate product by adding an oxygen molecule. Then a water molecule splits from this intermediate product resulting in the excited *Oxyluciferin*. By emitting energy in the form of visible light, the excited *Oxyluciferin* now becomes *Oxyluciferin* in its ground state. With the help of ATP, the *Oxyluciferin* can be reverted into *Luciferin*, and the reaction of bioluminescence can be repeated. Knowing the underlying reactions of the bioluminescent behaviour in *Pyrocystis fusiformis*, one may now look at how the influence of an external force such as hydrodynamic pressure triggers these reactions to happen.

The interaction of *Luciferin* and *Luciferase* takes place in a dense vesicle within the cytoplasm called "*Scintillon*" (Fogel and Hastings, 1972). It has an average diameter of 0.5-0.9µm, and it was shown that they are most likely to be located in the periphery of the cell (Seo and Lawrence, 2000). Under no influence of external force, this intracellular structure contains an inactive form of *Luciferase* and a molecular complex consisting of *Dinoflagellate Luciferin* and a *Luciferin Binding Protein (LBP)*[9]. The *LBP* prevents the *Luciferin* from oxidising in a non-luminescent way that may harm the cell. If the *Pyrocystis fusiformis* cell membrane is stimulated through shear stress such as the movement of the surrounding water, a cascade of events is triggered that results in an ultimate drop of pH[10] from 8 to around 6 within the scintillon. This drop in pH activates the *Luciferase* and releases the *Luciferin* from the bond with the *LBP.* Figure 2 shows the mechanisms behind this pH drop.

[8] Refer to: http://www.chem.uzh.ch/robinson/lectures/AC_BII/Kap6/kap6.html last accessed 12.12.20
[9] Refer to Eckstein, 1991
[10] Refer to Fogel and Hastings, 1972

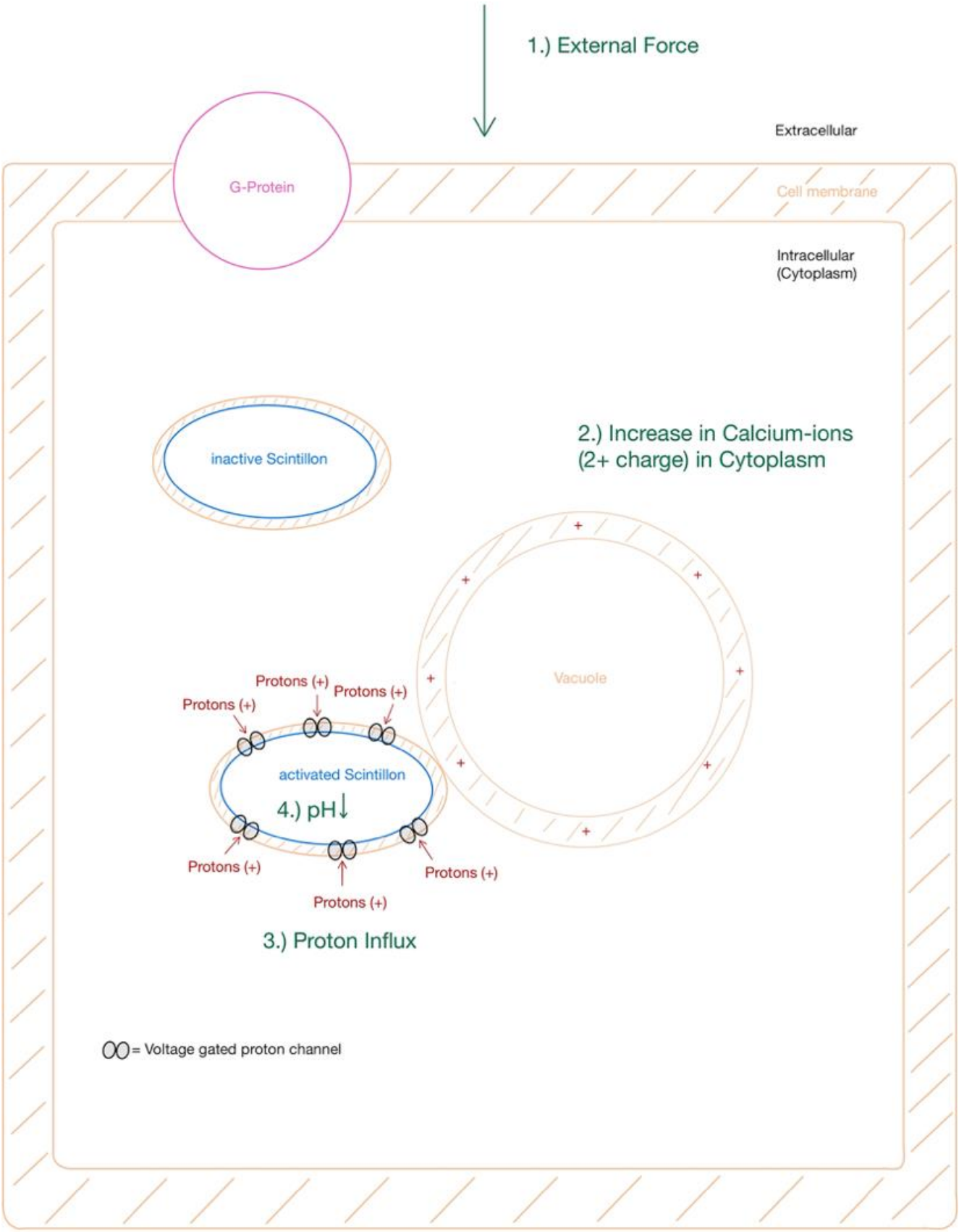


Figure 2: Sequence of events behind the pH drop in an activated Scintillon (Valiadi and Iglesias-Rodriguez, 2013)

In Figure 2, the four steps written in green explain the events resulting in the acidification of the scintillon. At first, the cell membrane is agitated under the influence of an external force. Intracellular storages start releasing Ca^{2+} ions which results in an increased concentration of the latter (Dassow and Latz, 2002). Researchers have found that so-called "*Guanine Nucleotide-Binding Proteins*", short G-Proteins are involved (Chen et al., 2007) in the transition from 1.) to the resulting 2.). This family of proteins is known for their function of transmitting external signals to the interior of the cell. The G-Protein causes the translation of the external signal into a chemical one. This chemical signal is the formation of an action potential on the membrane of some vacuoles (illustrated as + signs in Figure 1), and it is the consequence of the previous increase in cytosolic Ca^{2+} ion concentration. A scintillon may now be acidified if its membrane comes into physical contact with the charged vacuole membrane. This is the case for the so-called "*activated Scintillon*"[11]. As it stands in physical contact with the vacuole, an electric current occurs. This current leads to the activation of the discovered "*Voltage-Gated Proton Channels*" (Rodgriguez et al. 2017 & Smith et al., 2011) on the scintillon membrane. To equalise the electrical charge, the proton channels open to promote a proton influx in the scintillons interior. As the value of the pH depends on the concentration of protons in a solution, the proton influx leads to a drop in the scintillons interior pH. With the drop in pH, the explained chemical reactions can take place, and the bioluminescent behaviour becomes visible. The following image shows the bioluminescent activity of the investigated *Pyrocystis fusiformis*.

[11] Refer to Figure 1

Image 2: Bioluminescent activity of Pyrocystis fusiformis *(photo taken during measuring series)*

Hypothesis

On the basis of the previously explained scientific background it is hypothesised that the involved standardised *Pyrocystis fusiformis* algae population presents an energy-efficient and sustainable light source in terms of light intensity, performance duration and recovery ability.

Methodology

The goal of this study is to answer the stated research question by confirming the stated hypothesis. As it focuses on the light emission of the *Dinoflagellates*, an accurate measuring device used to quantify the luminosity of the latter was needed. For this purpose, the sensitivity of various instruments such as a so-called light meter were investigated. Although those methods to measure light are said to be accurate, they were not satisfactory regarding the goal of this study. Hence, a new measuring device was designed to guarantee the required accuracy.

Measuring Device

The designed measuring device enabled the identification of the relative change in light intensity over a definite time period. The following materials were used for this instrument.

- Photodiodes[12]
- Arduino Uno open-source microcontroller board[13]
- Jump wire & regular wire
- Breadboard
- Cardboard

The idea behind the measuring instrument lies in the ability of photodiodes to convert radiation into electrical voltage if light hits their integrated sensors. This permits one to identify the light intensity of a source relative to the resulting voltage. Hence, photodiodes were used as the basic building block of the instrument. Using a breadboard enabled the usage of multiple photodiodes simultaneously. They were placed on the breadboard so that two diodes were parallel connected and 27 of such diode pairs were serial connected (see Image 3). In this setup, the photodiodes cover a maximised area, making the absorption of a higher amount of light possible and therefore increase the accuracy of the measurement.

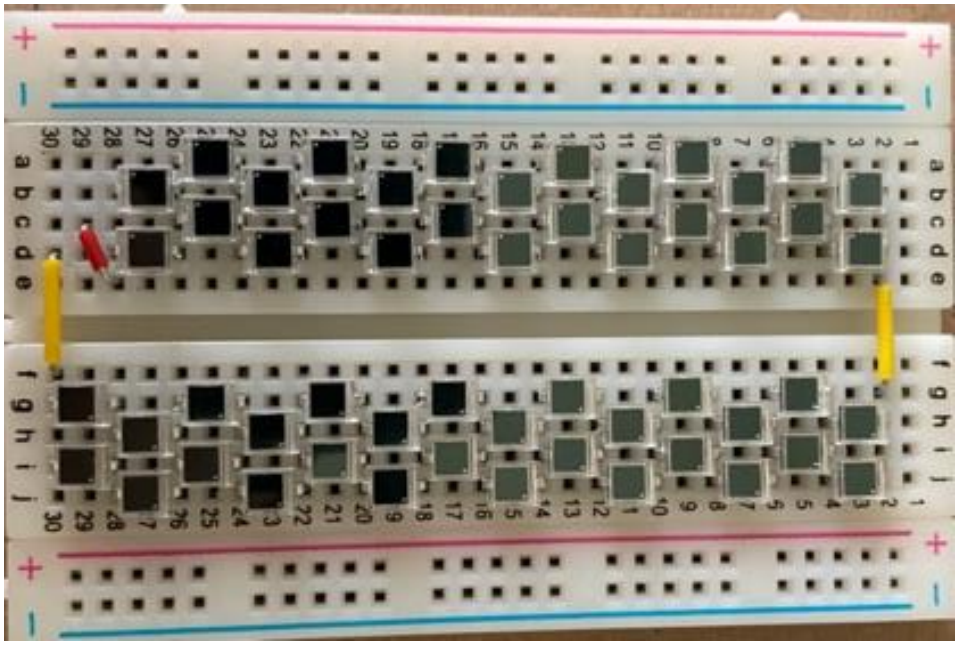


Image 3: Arrangement of photodiodes on a breadboard

[12] For more information about used photodiode: https://www.reichelt.com/silizium-pin-fotodiode-50-a-420-1120nm-bpw-34-oso-p217043.html?search=photodiode last accessed 12.12.20

[13] Refer to: https://store.arduino.cc/arduino-uno-rev3 last accessed 12.12.20

If radiation hits the breadboard, the photodiodes produce an electric current. The voltage can be accurately defined by measuring the voltage difference between a hole in the 29th and one in the 30th row, such as a29 and a30. According to the governing laws about circuits, the total voltage in a serial circuit is the sum of the voltage in each component. For a parallel circuit, the total voltage is equal to the average out of the generated voltage. Hence the total voltage in this setup is equal to the sum of the average voltage in each diode pair. With this knowledge, four such complexes were built and each one was glued onto a separate piece of cardboard. The cardboards were put together in a perpendicular arrangement and the breadboards were parallel connected (see Image 4). If a light source is now placed in this instrument, the sensors absorb light from all four sides, nearly spanning 360°. Further, the generated voltage is the average out of many voltages which increases the accuracy as it equalises potential errors within single sensors.

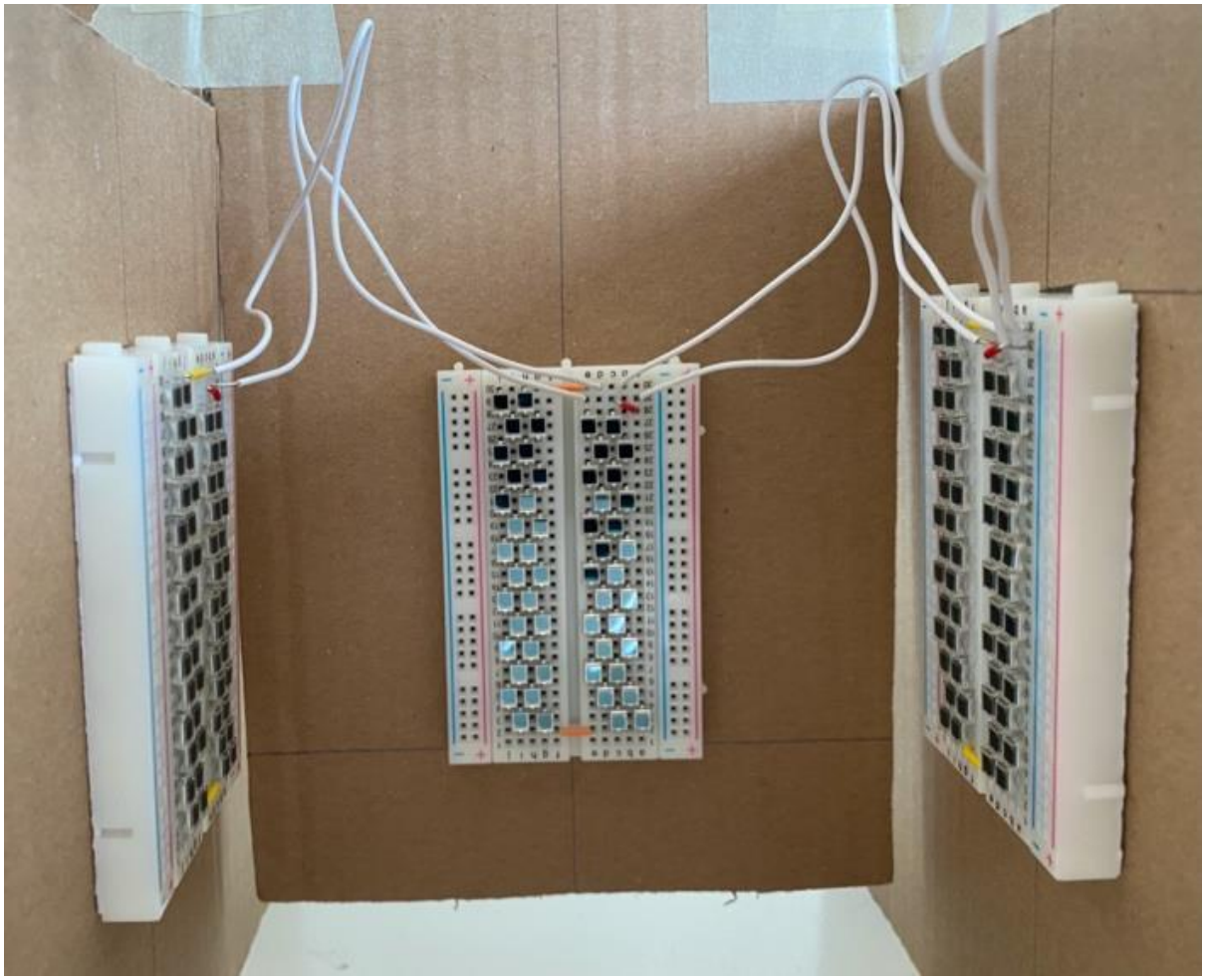


Image 4: Interior of the measuring device from the perspective of one breadboard

Image 4 shows the finished measuring device. However, to record data, a tool was needed that enabled the accurate transfer of obtained data into a file. For this purpose, the so-called Arduino Uno[14] was used. It is a programmable microcontroller that has the feature of measuring voltage differences. To facilitate an efficient process, an SD-Card system was attached, which enabled the direct transfer of obtained data into a file on the card. Further, a programme was written that instructed the microcontroller on how to record and display the data points[15]. Image 5 shows how this programmed microcontroller is integrated into the measuring system.

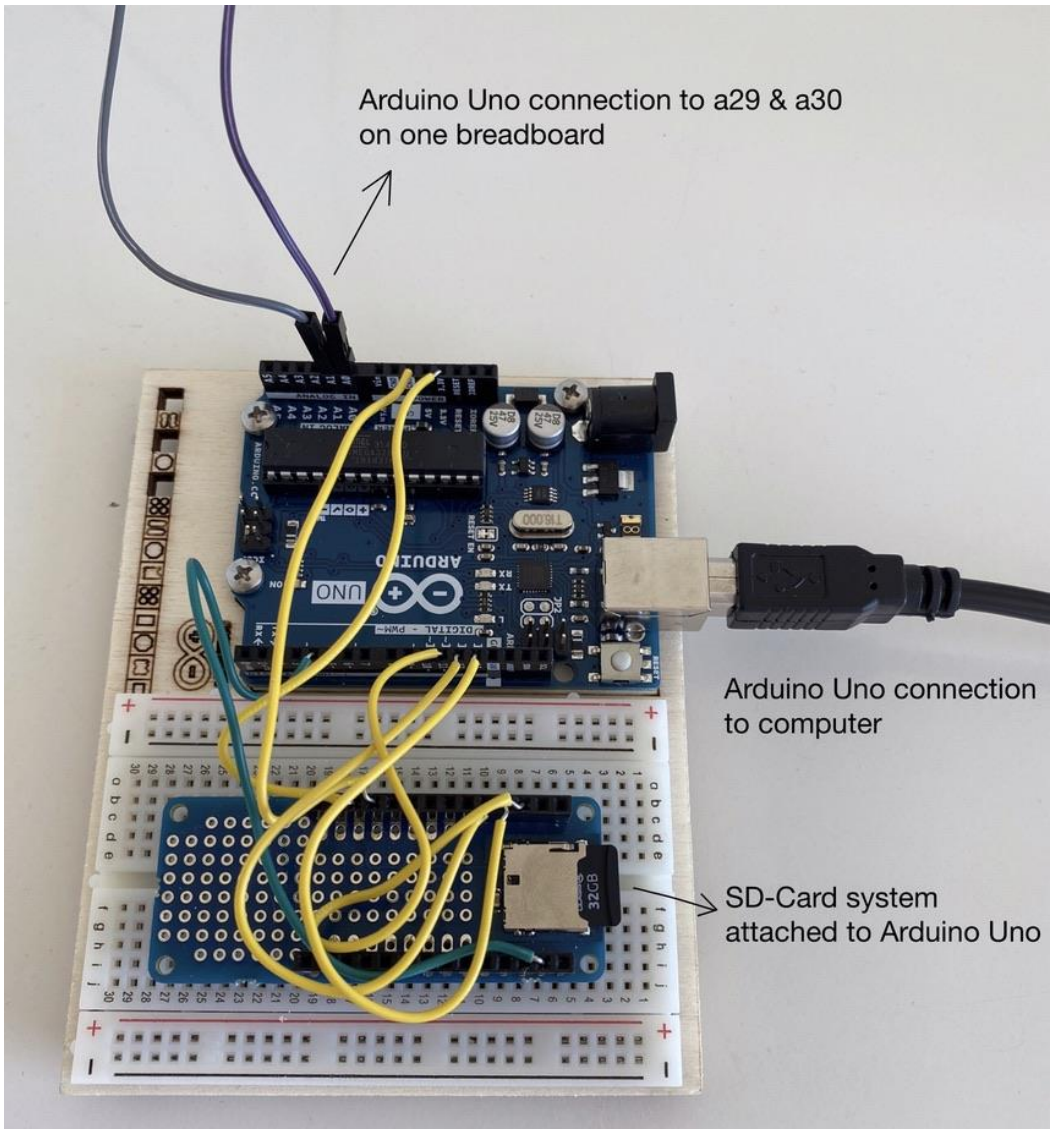


Image 5: Arduino Uno connection to computer and measuring device

[14] Refer to https://www.arduino.cc last accessed 12.12.20
[15] Refer to "Arduino Uno Gauging code" & "Measuring code" in Appendix

In Image 5, the connection to the computer enables the activation of the microcontroller. As previously stated about Image 2, the voltage can be obtained by measuring the difference between a29 and a30. In Image 5, each of the two jump wires connects to either a29 or a30 and therefore closes the parallel circuit visualised in Image 4. The whole measuring system including the measuring device, the Arduino Uno microcontroller and the computer is illustrated in Image 6.

Image 6: The whole measuring system

Gauging Process

Previous to the measurement series, the instrument had to be gauged in order to enhance the understanding of the later obtained data. For the gauging process to be performed under standardised conditions, the device was placed in a lightproof plastic box located in a windowless, dark room, so that no external interference signals could distort the data collection.

The Arduino Uno is able to reliably detect a voltage ranging from zero to five volt. However, its hardware displays this voltage range as numbers from zero to 1023. Luckily, the two ranges are linear proportional and can therefore be easily converted into the other. In the following the number range of the Arduino Uno hardware is used.

The aim of the gauging was to use a light source with known power and corresponding voltage, placing it within the measuring device and relate the obtained values ranging from 0-1023 to the known values of the light source in order to determine the conversion factor of the instrument. This later enables to convert the obtained voltage values into corresponding power values, which helps to determine the light intensity of the algae. The chosen light source consisted of three blue LED's as they emitted similar light waves as the algae. Their efficiency factor was according to a local electrician 50%, meaning that half of the invested energy is turned into light.

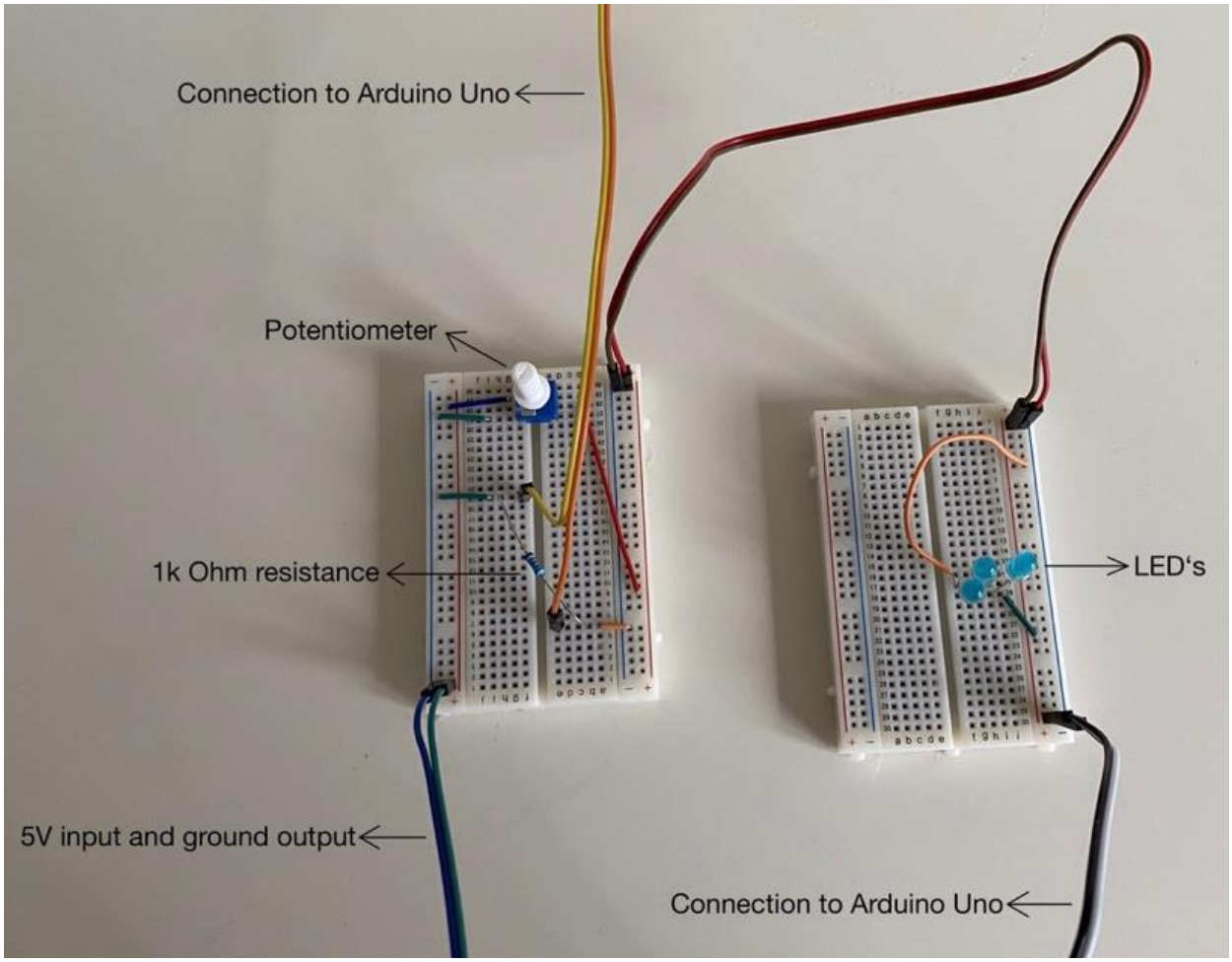


Image 7: LED gauging setup

Image 7 shows the gauging setup used for this study. It is a serial circuit consisting of three blue LED's, a 1 kilo Ohm resistance and a potentiometer. The latter is a tool to manually dim the LED's. The two jump wires in the lower left part of the picture are attached to the Arduino Uno and act as the power supply of 5V. As can be seen in Image 5, the Arduino Uno can connect six jump wires, hence it can measure the voltage difference at three different spots. One spot was used for the diodes and the others were used for the remaining two connections in Image 7. While the yellow and orange jump wire submit the voltage difference at the resistance, the black and white jump wire concern the voltage difference at the LED's. Having connected the wires correctly to the Arduino Uno and the instrument, the left breadboard in Image 7 was situated within the instrument, while the right one was outside of it. This enabled the manual diming of the LED's without distorting the gauging measurements. The goal of the gauging was to obtain a so-called gauging graph that gives for each voltage value from the diodes a corresponding power value for the investigated light source. The setup can be seen as a simple serial circuit consisting of a light source (LED's) and a resistance. The sum of the voltage at the light source and at the resistance corresponds to the total voltage which can be regulated via the potentiometer ranging from zero to five volt. Further, it must be noted that the amperage is constant throughout the circuit. Hence, the following relationships can be established:

$$U_{[V]} = R_{[\Omega]} * I_{[A]}$$
$$P_{[W]} = U_{[V]} * I_{[A]}$$

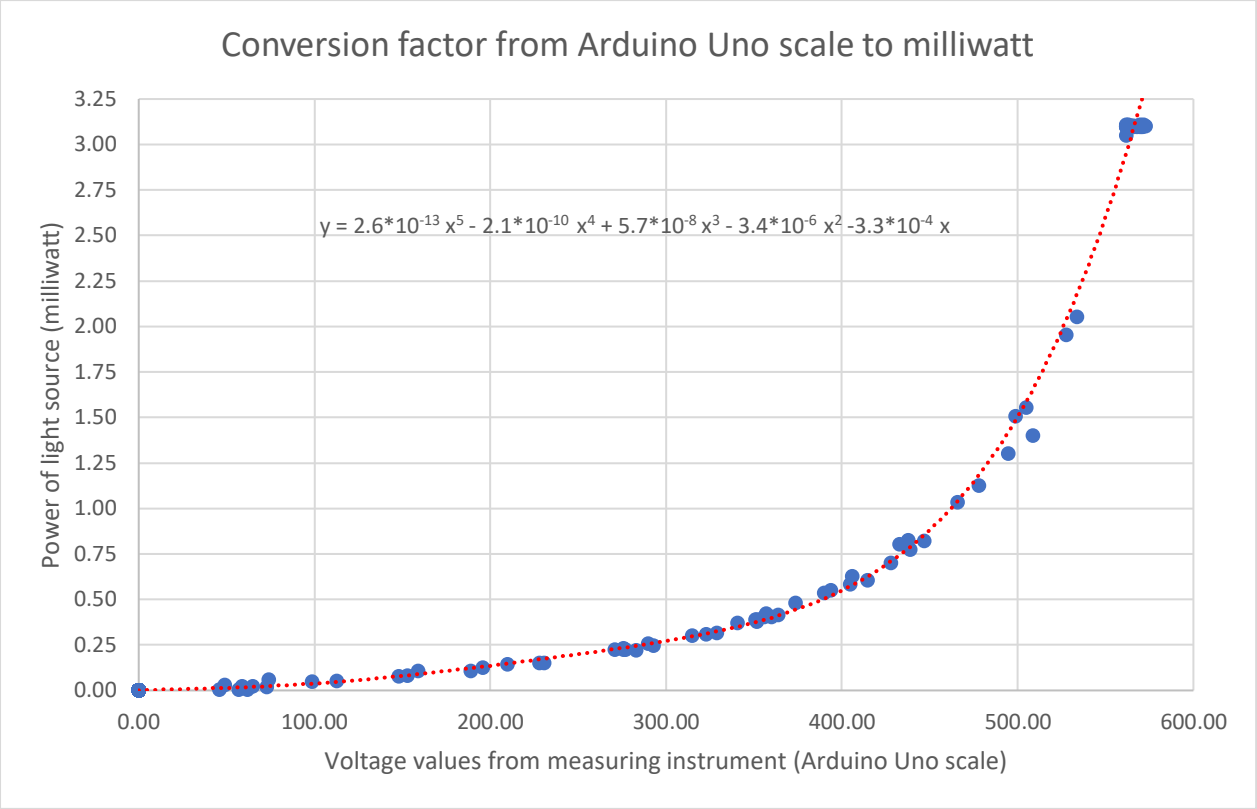
With the help of the upper formulas, the power (P_{LED}) of the LED's can be calculated. The voltage (U) in the following formula is in the scale of the Arduino Uno and therefore needs to be multiplied with the factor of $\frac{5}{1023}$ to get voltage as a result.

$$P_{LED} = U_{LED} * I = U_{LED} * \frac{U_{resistance}}{R_{resistance}} * \left(\frac{5}{1023}\right)^2$$

According to those relationships, a programme was written that recorded the needed values[16]. Having done this, the gauging process was started. While the current was steadily increased

[16] Refer to "Arduino Uno Gauging code" in Appendix

from 0 to 5V with the help of the potentiometer, the Arduino Uno recorded the dynamic voltage differences from the photodiodes, the 1 kilo Ohm resistance and the LED's[17]. Each voltage value measured at the photodiodes was assigned the corresponding power value of the LED's which resulted in the general gauging graph labelled as Graph 1.



Graph 1: General gauging graph

As visible in Graph 1, the relationship between the measured values at the photodiodes and the corresponding power of the light source is correlating in an exponential fashion. This interesting finding can be explained with a potential decrease in the photodiode's sensitivity when light intensity increases. However, for small values below 300 one may argue that the graph resembles a straight line and the gradient only increases after this threshold. The general gauging graph is later used in the analysis section as a reference.

[17] Refer to URL-link in Appendix; within "RAW DATA" excel workbook focus on sheets labelled with "Gauging"

Measurement Series

This study focuses on the research question of whether *Pyrcoystis fusiformis* could present an energy-efficient and sustainable light source in terms of light intensity, performance duration and repetition. Knowing the limitations of the built measuring device enabled the reliable designing of different experiments to investigate the stated research question. The following list shows the used material.

- *Pyrcoystis fusiformis* population
- Measuring system as visible in Image 6
- Magnetic stirrer[18]
- Dark, closable plastic box (55cm*40cm*30cm)
- Cardboard box (50cm*35cm*25cm)

Prior to the experiment, six algae populations were separately placed within different closable and transparent beakers[19]. As they were used in separate experiments, the lids were numbered from one to six and populations one to three got red lids while the others got blue ones. The beakers were located so that sunlight would allow photosynthesis, but would prevent overheating, as this could harm the sensitive algae. The lids were opened in the morning and stayed so during the day to facilitate the airflow. Using a clean plastic spoon to mix the algae once a day, enhanced this flow. All six algae populations were fed every week with 20ml of the prescribed nutrient solution[20] and their exact volume was noted in an Excel sheet[21]. Other so-called controlled variables such as temperature and exposure to sunlight were regularly checked and if needed adapted to ensure standardised conditions. Having carefully treated the algae, a standardised experiment setup was designed to enable a consistent data collection.

[18] https://www.expondo.co.uk/steinberg-systems-mini-magnetic-stirrer-2-000-rpm-10030444 (integrated LED lights were removed previous to the experiment) last accessed 12.12.20
[19] Refer to Image 10 & 11 in Appendix
[20] Refer to Image 12 in Appendix
[21] Refer to Table 7 in Appendix

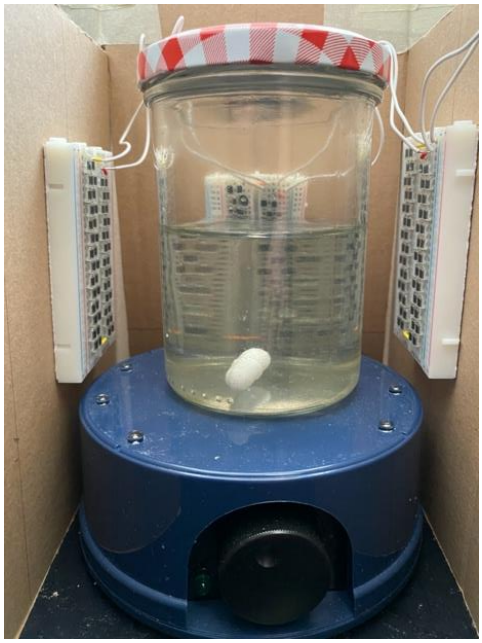


Image 8: Beaker containing algae placed on magnetic stirrer including white stir bar

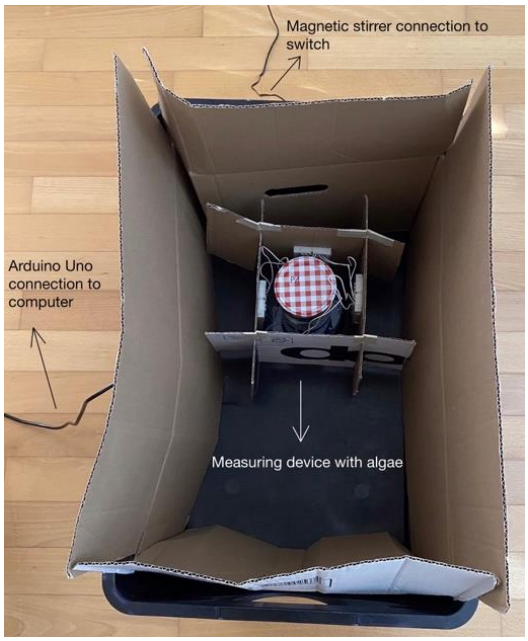


Image 9: Standardized experiment setup

Image 8 shows the magnetic stirrer and the stir bar that produce an external force to stimulate the algae. Image 9 illustrates the whole setup and how it is placed within the dark box. The cardboard can be closed and acts as an additional tool to minimise the influence of external light sources. As the Arduino Uno has integrated lights that blink when the microcontroller is used, it is situated behind the cardboard wall on the left. The magnetic stirrer is connected to a switch which enables its external operation without interfering with the measuring device. With this experiment setup the light intensity, performance duration and repetition were analysed every night over the course of two weeks. A fitting code for the Arduino Uno was written[22] and the measuring procedure was as follows.

1. Beaker containing algae was treated as in Image 8. The starting time of the first experiment (later denoted as Series 1) was noted in an excel sheet[23].
2. The cardboard was closed, and the box was covered with a lid.
3. The switch for the magnetic stirrer was turned on so that the maximal revolution of 2'000 rpm was reached and the values from the photodiodes were permanently checked on the computer screen. The constant stimulation enabled the investigation

[22] Refer to "Measuring code" in Appendix
[23] Refer to Table 7 in Appendix

of the decrease in light intensity with time. Hence it is a measure of the algae's efficiency.

4. After the fixed measuring time of one minute and forty-five seconds was over, the stirrer was turned off and the beaker was removed.
5. Step 1-4 were consecutively performed for beakers 1, 2 & 3.
6. Having treated the first three beakers, the others were tested on how fast they recover from stimulation and thus their ability of repeating their bioluminescent activity.
7. The beaker was placed as in Image 8.
8. The stirrer was now activated for 30 seconds and then switched off for a minute (experiment later denoted as Series 3). This was repeated five times and the exact time was regulated using a stopwatch. In order to record this data, the running time of the measuring code was increased to eight minutes.
9. After having performed steps 7 & 8 for the beakers 4, 5 & 6, a third experiment was conducted to see whether the algae recover over a longer period of time of exactly 40 minutes.
10. After 40 minutes had passed from the starting time, the first three beakers were again treated as in steps 1-4 to analyse their recovery time more closely (experiment later denoted as Series 2).
11. Potential errors that may have occurred during the data collection were added to the previously stated excel sheet.

This procedure was performed on 14 consecutive days and the measuring device was checked and recalibrated after each measurement. It was found that an unavoidable background noise with a value of 20 in the Arduino Uno scale was present if the algae stayed unstimulated. As this value is intrinsic to the system, the goal was to maintain a controllable accuracy by keeping it constant throughout the two weeks data collection. This was done by checking the intricate electrical tools every measuring day. Each photodiode was tested in a time-consuming process and the jump wires and regular wires were replaced every two days as their oxidation from the usage increased the background noise.

Results

Raw Data

The data for each measurement was saved in a separate file on the SD-card[24]. The faulty data sets[25] were removed while the others were transferred to individual excel sheets so that each excel sheet contained the data of one measurement. A sheet consisted of two columns. The first one displayed the time values in milliseconds and the second one the corresponding values measured by the photodiodes. The following Table 1 shows an extract of such an excel sheet.

Table 1: Extract of the raw data sheet for population 2 in the first measurement (Series 1) on the eight measuring day

Time (ms)	Value (Ard. Scale)
37	33
562	1
1062	2
1563	85
2064	199
2566	158
3066	163
3567	146
4069	147
4570	169
5071	174
5572	164
6073	148
6575	143
7075	133
7577	115
8078	47
8579	111
9080	112
9581	117
10083	114
10584	115
11085	108
11586	100
12088	100
12589	99
13090	95
13591	90

[24] For an example refer to Table 8 in Appendix
[25] Refer to Table 7 in Appendix

Table 1 shows that the values from the photodiodes at 37 and 8078 milliseconds do not fit the overall pattern of the whole table. Such incidents of minor distortions may influence the general interpretation of the data and therefore, must be taken into consideration. To reduce the impact of bias, the raw data was processed using mathematical filters.

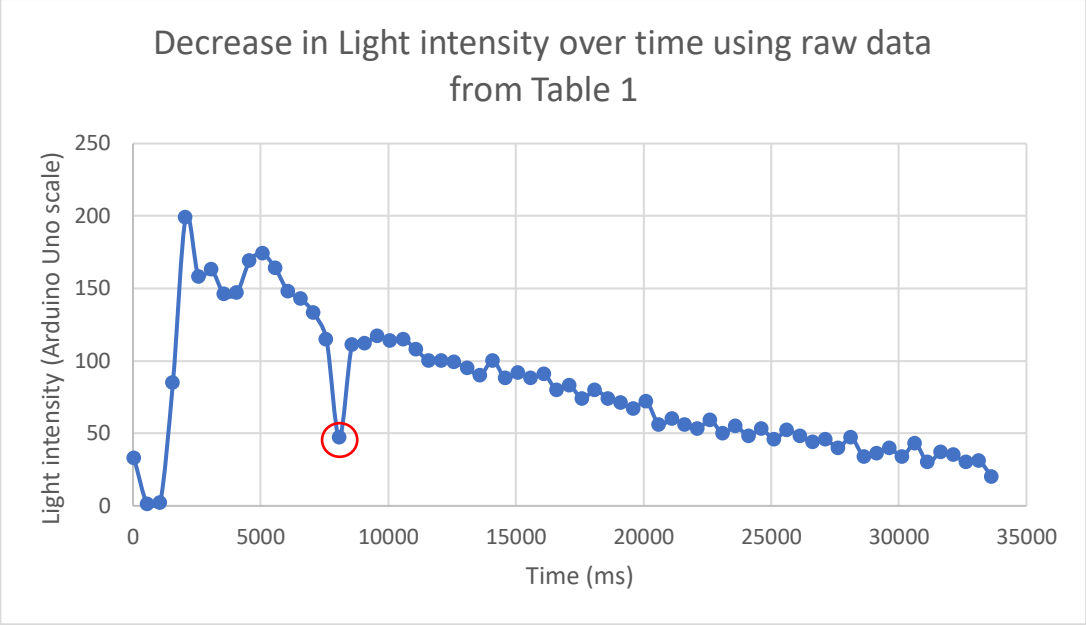
Processed Data

As this study focuses on two main experiments to analyse the light intensity of the algae and their ability to repeat their bioluminescent activity after different recovery times, the raw data was distinguished based on the performed experiments. The raw data obtained using the first three populations was differently treated from the other three. In the following, the data procession of the first three populations will be explained.

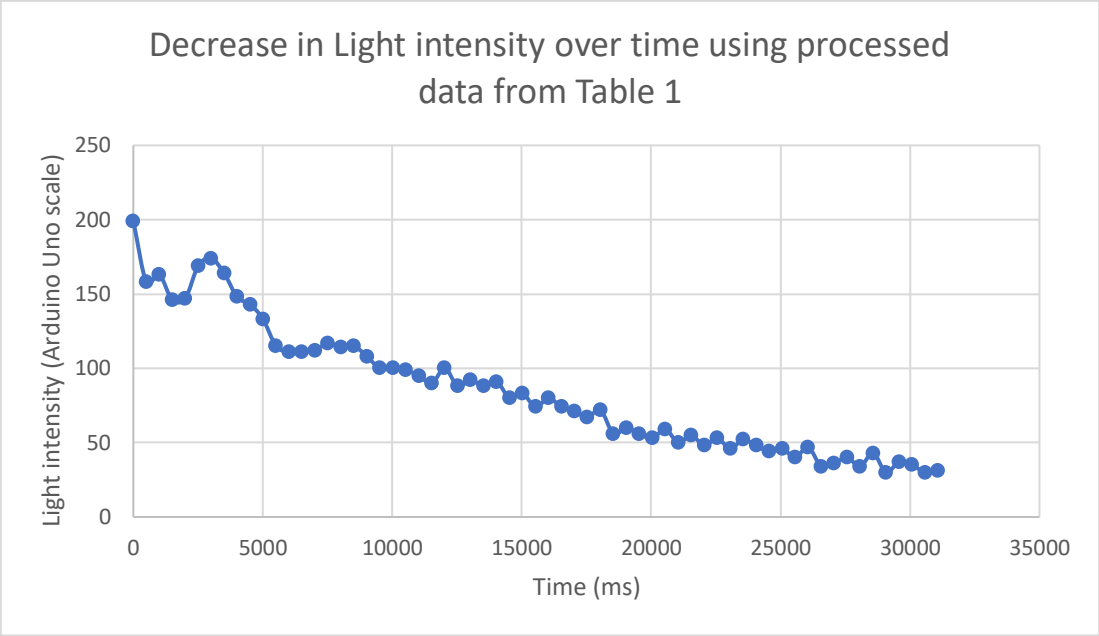
Populations 1 to 3

The data from the first three populations was treated using a median filter from three consecutive photodiode values. However, the filter was only applied when the value was potentially distorting the data as the two in Table 1. If the value was not equal to zero and neither the maximum value it had to satisfy the following condition: The value of the cell times two either had to be smaller than the average of the two vertically adjacent cells or it had to be bigger than the sum of the two vertically adjacent cells. This resulted in a new column with processed data.

The first three populations were used to identify a maximum light intensity and the corresponding decline in bioluminescent activity. To identify this timespan the time point at the maximum value was set as an origin and the endpoint was marked by the first time the measuring device recorded the background noise. The subsequent Graphs 2 & 3 visualise the data before and after the application of the median filter and identification of the previously stated timespan.



Graph 2: Visualisation of the raw data from Table 1



Graph 3: Visualisation of the processed data from Table 1

The difference indicated by the red circle in Graph 2 shows the impact of processing the raw data to enable an efficient data analysis. The red circle in Graph 2 shows how the median filter effectively spotted outliers and corrected them according to the overall trend of the data set. However, the data procession includes further steps. As Graph 3 only shows the absolute time span from the maximum value to the detection of the background noise, one may better understand the data by looking at a relative decline in bioluminescent activity. For this purpose, the period from the maximum up to a point where the measuring device detected a value equal or lower as a fourth of the maximum value was identified and noted in a separate column

within the excel sheet. From this excel sheet the maximum value of light intensity in Arduino Uno scale and the equivalent milliwatt, the time span from the maximum value to the detection of the background noise and the timespan from the maximum value to one-fourth of the latter were transferred to a separate excel sheet. One-fourth was chosen to put the decline in bioluminescent activity into a reasonable relation to the maximum value.

Further, an approximation for the energy emitted during a period of bioluminescent activity was calculated using the known relationship between watt and joule, being that joule equals watt-seconds. The sum of the joule values at each time step indicated an approximation for the total of released energy. Having done this data procession for all measurements involving the first three populations enabled to take the average of the values from the collected data for each population in order to arrive at a more accurate meaning of the vast amount of the data. The following tables summarise the processed data, starting with Tables 2 & 3 concerning the absolute time.

Table 2: Processed data concerning the absolute time span in experiment Series 1 showing the average values from the two weeks measuring period

Series 1						
Population	Time (ms)	st.dev.	Max. Light Int. (mW)	st.dev.	Energy (mJ)	st.dev.
1	44262	10304	0.149	0.052	1.428	0.489
2	39934	7335	0.155	0.051	1.463	0.734
3	40226	20994	0.139	0.048	1.197	0.891
Average	**41310**	**14055**	**0.148**	**0.049**	**1.359**	**0.724**

Table 3: Processed data concerning the absolute time span in experiment Series 2 showing the average values from the two weeks measuring period

Series 2						
Population	Time (ms)	st.dev.	Max. Light Int. (mW)	st.dev.	Energy (mJ)	st.dev.
1	13602	4973	0.049	0.023	0.216	0.100
2	10094	5811	0.078	0.060	0.173	0.090
3	9235	5720	0.044	0.026	0.150	0.114
Average	**10977**	**5578**	**0.058**	**0.043**	**0.180**	**0.101**

Tables 4 & 5 show the processed data for the relative time span from the maximum value to one-fourth of the latter.

Table 4: Processed data concerning the relative time span in experiment Series 1 showing the average values from the two weeks measuring period

Series 1						
Population	Time (ms)	st.dev.	Max. Light Int. (mW)	st.dev.	Energy (mJ)	st.dev.
1	24912	2799	0.149	0.052	1.205	0.359
2	22139	6330	0.155	0.051	1.243	0.684
3	21508	7891	0.139	0.048	1.033	0.742
Average	**22769**	**6129**	**0.148**	**0.049**	**1.161**	**0.615**

Table 5: Processed data concerning the relative time span in experiment Series 2 showing the average values from the two weeks measuring period

Series 2						
Population	Time (ms)	st.dev.	Max. Light Int. (mW)	st.dev.	Energy (mJ)	st.dev.
1	9808	3168	0.049	0.023	0.186	0.074
2	6801	3271	0.078	0.060	0.144	0.065
3	6657	2416	0.044	0.026	0.125	0.075
Average	**7755**	**3192**	**0.058**	**0.043**	**0.152**	**0.073**

Populations 4 to 6

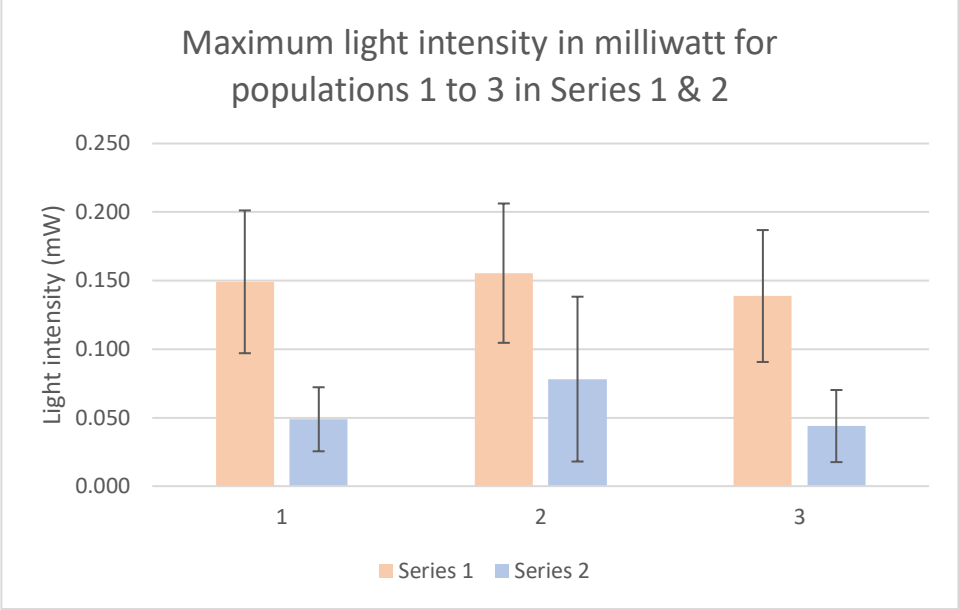
The data from the last three populations was treated in a similar way, as previously discussed. The effect of potential data distortion within measurements that functioned properly was reduced by applying the same median filter. However, the data procession involved further analysis of the data. The experiment conducted with populations 4 to 6 focused on investigating the ability of the algae to recover their light emission capacity over a short time period of 1 minute. An efficient way to analyse this capability is by comparing the peak values in every thirty seconds lasting stimulation round. Those values were obtained by applying a filter that sorted out these values for each stimulation round. As only the peak values of the first three rounds were observed to be above the background noise, the other two were neglected. The three obtained values were transferred into a separate excel sheet and for each population the average of those values over the whole measurement period was calculated. Table 6 contains these data points.

Table 6: Processed data concerning the recovery capability in experiment Series 3 showing the mean peak values (1,2,3) from the two weeks measuring period

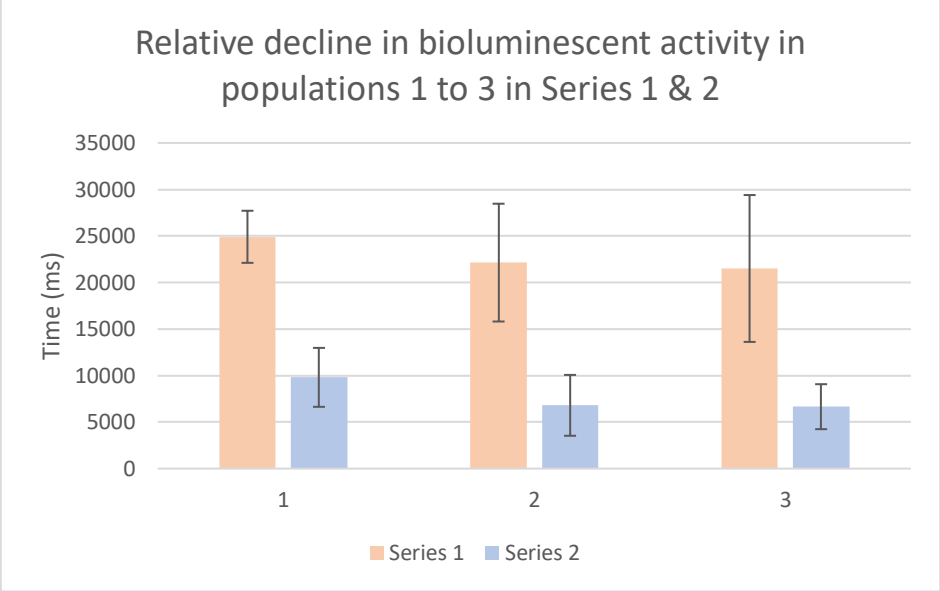
Series 3						
Population	Max. Light Int. 1 (mW)	st.dev.	Max. Light Int. 2	st.dev.	Max. Light Int. 3	st.dev.
4	0.099	0.016	0.010	0.001	0.009	0.001
5	0.136	0.053	0.032	0.069	0.008	0.005
6	0.143	0.059	0.014	0.011	0.010	0.006
Average	**0.131**	**0.052**	**0.021**	**0.046**	**0.009**	**0.005**

Visualisation of Processed Data

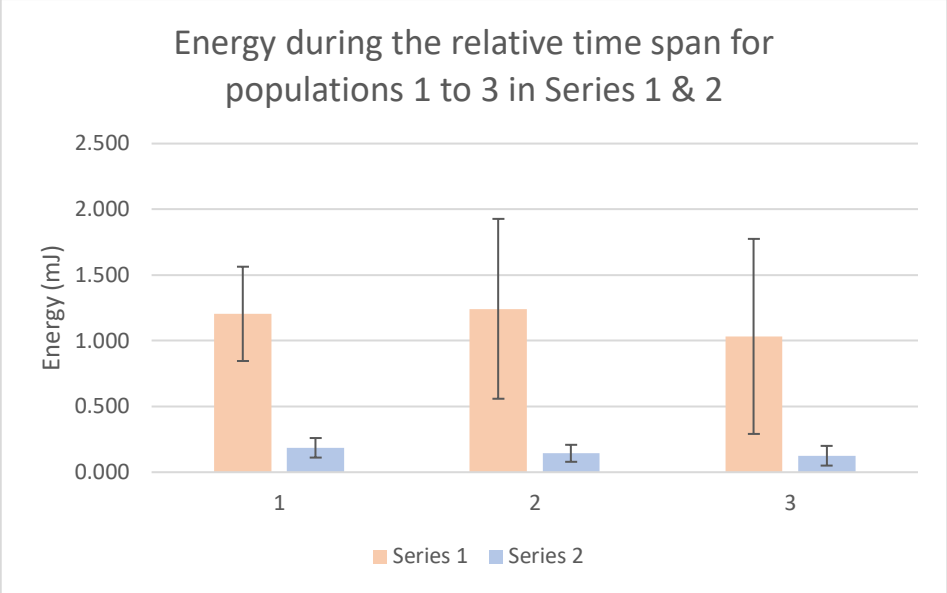
Combining the data points from the preceding tables allows one to visualise the important findings of this study. For the data concerning the first three populations, the following graphs were established.



Graph 4: Light intensity values concerning the first three populations in Series 1 & 2 including ± the concerning standard deviations

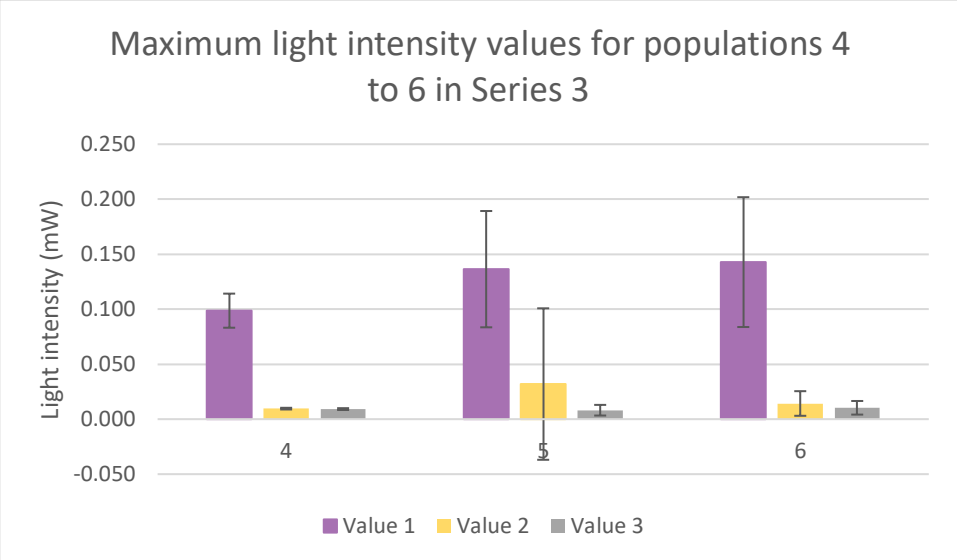


Graph 5: Relative time span of the bioluminescent behaviour in the first three populations in Series 1 & 2 including ± the concerning standard deviations

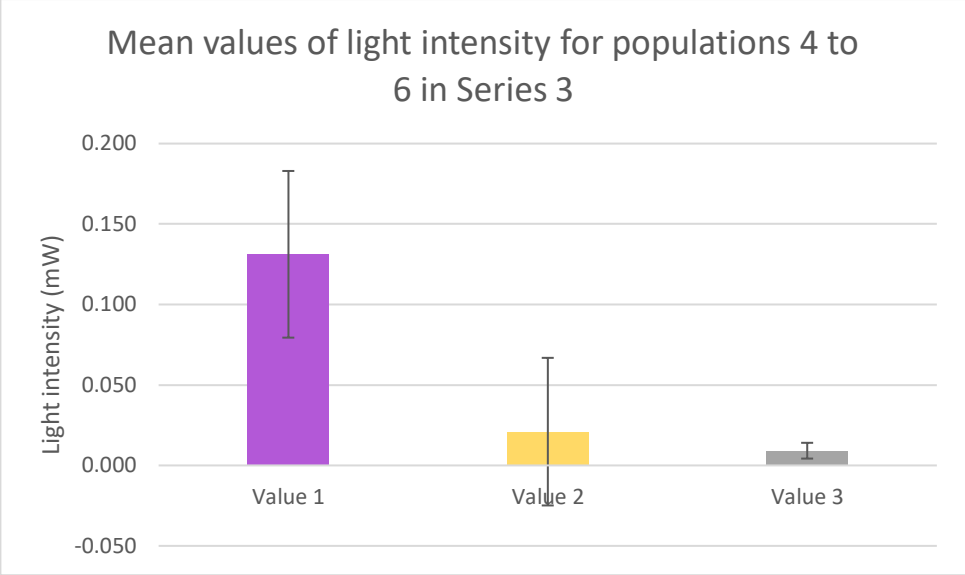


Graph 6: Estimated energy emitted during the relative time span for the first three populations in Series 1 & 2 including ± the concerning standard deviations

The following two graphs concern the data for the populations 4 to 6 from Table 6.



Graph 7: The maximum light intensity value during the first three stimulation periods of the populations 4 to 6 in Series 3 including ± the concerning standard deviations



Graph 8: The mean values for each stimulation from Graph 7 including ± the concerning standard deviations

Discussion

The aim of this study was to investigate the potential of the bioluminescent *Pyrocystis fusiformis* algae to act as an energy efficient light source by analysing their light intensity, performance duration and recovery ability. With the help of the self-designed sensitive measuring device and the three different experiment series, those characteristics were closely studied.

This setup enabled the estimation of the light intensity stemming from the bioluminescent behaviour of the investigated algae populations. The light orange bars in Graph 4 show the average values of maximum light intensity for each of the first three populations in the experimental Series 1. During the two weeks of data collection, it was observed that the daily light intensity maximum for a population stayed constant around a certain value with some minor fluctuations[26]. This indicates that the periodical stimulation of the algae did not result in an increase in the production of *Luciferin* and hence the increase of bioluminescent activity. As visible in Graph 4, the light orange bars behave similarly concerning the value and spread of the data points shown by the standard deviation. These characteristics in combination with the fact that those three populations were treated equally, allows one to estimate an average maximum light intensity for a population of that size, treated under the stated conditions. This study managed to determine this maximum light intensity via the produced current and its value is according to Table 2 around 0.148 milliwatts. At such an intensity, structural changes

[26] Refer to "RAW DATA" excel workbook, sheets "Data Series 1 & 2" and "Data Series 3"

in the surface of near objects became visible[27]. Having found an estimation for the light intensity, the next step was to determine the performance duration via the relative decline in bioluminescent activity during constant stimulation. Graph 5 shows this data for the first three populations in the Series 1 & 2. Analogous to the maximum light intensity, the relative decline in bioluminescent activity for each population behaved in a similar fashion. Using the idea of an average value succeeded in estimating how long a *Pyrocystis fusiformis* population treated under the standardised conditions would emit light until the intensity dropped to one-fourth of its maximum. Graph 5 allows a hint at the range of this value which is according to the calculations performed for Table 4, 22'769 milliseconds. Hence, a standardised population from this study on average emits a detectable amount of light for around 22 seconds when constantly stimulated. To get a better understanding of the scale of the investigated values, Graph 6 depicts the calculated amount of energy emitted through the bioluminescent activity. The average energy emitted during Series 1 is according to Table 4, 1.161 millijoules.

The third investigated characteristic was the ability of the algae to recover from stimulation. If *Pyrocystis fusiformis* should act as alternative light sources, they have to be capable of fast recovering from stimulation to allow a repeated stimulation in a short period of time. The experimental Series 3 was used to analyse this potential ability. Graph 7 shows the maximum light intensity values for populations 4 to 6 each with a minute recovery time gap. In all three populations the values drastically decreased from the first to the third value. The standard deviations for those values were not as similar to one another as in the previous graphs. Although the data spread for the population 5, Value 2 is bigger than the value itself, the overall trend indicated by the graph still holds true as it is clearly visible. To enhance the understanding of this data, Graph 8 was established using the averages of the same-colour bars. This last graph shows that the general trend is a decrease in maximum light intensity per stimulation round. This indicates that a short recovery period of one minute does not suffice to equalise the decrease in maximum light intensity. While the experimental Series 3 showed that a short amount of time does not have an impact, closely looking at Series 2 in this context unfolds an interesting finding. Series 2 was performed 40 minutes after Series 1 and at the end of the first series the populations bioluminescent activity had ceased due to exhaustion. However, looking at Graph 4, one can see that the bioluminescent activity returned as the measuring device was

[27] Refer to Image 2 (visible structure of sensors in proximity to the algae jar)

able to detect a significant light intensity for all three populations. Comparing the values for the first two series, one is able to see that for populations 1 & 3, the maximum light intensity is again around 30% of the one from the first experimental series and in population 2 it even reached one half of the prior maximum light intensity. Graph 5 shows the corresponding relative decline in bioluminescent activity, which the sensitive measuring device was able to record. For the second and third population, the reached time span was around 30% of the one from Series 1, while population 1 managed to even reach a close to 40% of the initial relative time span. Although the populations managed to respond to a second stimulation round after a recovery time, the calculated energy values for those experimental Series 2 shown in Graph 6 relativise this capacity. For the first population this energy is around 15% of the energy emitted in Series 1 and for the other two populations the value is even smaller with a percentage of 10%. As the Series 2 experiments showed, the *Pyrocystis fusiformis* algae seem to have the capacity of recovering parts of their bioluminescent activity after complete exhaustion stemming from the constant stimulation in Series 1. A 40 minutes recovery time sufficed to return a significant level of bioluminescent activity in the populations 1,2 & 3. Edith A. Widder and his colleague James F. Case observed a similar result in their collaboration from 1981. With the help of the underlying chemical processes this rather counterintuitive phenomena in the bioluminescent activity of the algae may be explained.

As Roger G. Christianson pointed out in his publication, *Dinoflagellates* are autotrophic algae that live according to a circadian rhythm. This rhythm involves "photosynthesis" and the bioluminescent activity that he calls "glowing". The prior takes places while sunlight is present to convert radiation into chemical energy. The latter is used in the production of the compound called *Luciferin* which is known for its potential to oxidise to *Oxyluciferin* in an exergonic reaction by radiating visible light. As spontaneous reactions may harm the cell, it is performed in the *Scintillon*[28] and the enzyme called *Luciferase* is involved in catalysing the oxidation. Although this enzyme is present in an inactive state, a drop in pH to around 6 may activate it and therefore promote the chemical processes that result in the bioluminescent activity. As John Lee explained in his book "*Bioluminescence, the Nature of Light"*, external stress is a possibility to increase the acidic content within a cell. In Series 1, the algae were constantly

[28] Refer to Figure 2

stimulated meaning that the pH within the *Scintillons* was at a persistent low. The *Luciferase* was therefore assumed to continuously catalyse exergonic oxidations until all *Luciferin* was converted to *Oxyluciferin*. To reduce *Oxyluciferin* to *Luciferin* again, the algae need the energy from photosynthesis. However, as the experiments were performed in the night and the *Pyrocystis fusiformis* were kept in complete darkness during the recovery time, they were unable to photosynthesise. Respecting those facts, the ability of *Pyrocystis fusiformis* to recover parts of their bioluminescent activity within 40 minutes may be explained with the idea of excessive energy within the cell. Based on the findings of Edith A. Widder and James F. Case one could state that the algae may have stored surplus chemical energy gained from prior photosynthesis and used it during the recovery time to reconvert the *Oxyluciferin* compounds into *Luciferin*.

Evaluation

The research question of the present investigation was analysed with the help of a self-designed measuring device. The application of photodiodes enabled an accurate quantification of minute amounts of light coming from the bioluminescent algae. By performing a gauging process, the data values could be related to a general norm. The latter made it possible to analyse the values in a differentiated way. Within this framework, the obtained data has shown key findings concerning the investigated properties. To drive the scientific exploration in this aspiring field of research ahead, the performed experiment and its methodology may be used as a template for further development.

Taking the experiment setup into close consideration, one could improve its accuracy with the help of a few adaptations. To increase the significance of the arguments made about the obtained data, one could expand the number of involved samples. Further, the standardisation of the algae samples could be improved by investigating the number of algae present within a sample. The provider of the *Pyrocystis fusiformis* does not state this number which presents a particular bias to the study. As further research may focus on the bioluminescent ability of a single alga, a way to estimate the number of algae within a sample is required. A method to accurately count the number of algae per volume such as a so-called Hemocytometer could eliminate the corresponding bias. Having analysed ways of standardising the samples more effectively, the next step is to adapt the methodology.

Prior to the experiment, a gauging process was performed that involved placing an LED within the measuring setup. This light source had a smaller surface area than the jar containing the *Pyrocystis fusiformis,* hence influencing its light intensity. Assuming that the jar with the algae and the LED would emit the same amount of light, the LED would have a higher light intensity because its surface area is smaller than the one of the jar. Further, it may be stated that the distance from the light source to the sensors of the measuring device depends on the size and diameter of the prior. If the jar is placed in the apparatus, this distance is smaller than if the LED is placed within it. The amount of light and therefore, the detected light intensity decreases with increasing distance between the sensors and the light source. According to governing physical laws this light intensity is inversely proportional to the distance squared[29], which indicates an observable bias. The impact of this bias could be reduced by either using smaller jars for the algae or by constructing a gauging light source that has an equivalent surface area and diameter as the investigated probe. With this adaptation made to the gauging process the actual experiment can be analysed.

The latter was performed on fourteen consecutive days. Similar to the sample size, an increase in the number of measuring days would make the study more accurate by decreasing the bias of distorting data. Focusing on the implications of the data shows that certain behaviours of the algae, such as their ability of recovering from stimulation need more in-depth research. To get a better understanding of this particular finding, it may be useful to increase the number of investigated recovery times, as only three different ones were studied in this experiment (30 seconds, 40 minutes and 24 hours). Further, one could look at the underlying variables of this investigation as they in general strongly influence the outcome of an experiment. By doing so it becomes apparent that in order to obtain clear results, the present investigation focused on the duration of exposure to stimulation and therefore the factor of time. The impact of other variables was controlled via standardised procedures and thus, their potential importance stayed unobserved. To increase the scientific insights, future research in this field may focus on the impact of other variables, one being temperature. The latter plays a crucial role in the development of maritime organisms, as they currently face global climate change. It may therefore be beneficial to analyse the effect temperature has on the performance of

[29] https://openoregon.pressbooks.pub/radsafety130/chapter/inverse-square-law/ last accessed 12.12.20

bioluminescent activity in *Pyrocystis fusiformis*. As the acidification of our oceans is linked to global climate change, the role of pH in this setting may be of importance as well. Jaquelyn Craig et al. published a paper in 2003 that combined those two factors and focused on how pH and temperature affect the bioluminescent activity of the closely related *Pyrocystis lunula* species. The results of this study may function as a base for future research in this field.

A third variable of potential importance is the energy obtained from photosynthesis. As stated in the Introduction, the investigated algae species is autotrophic and receives its energy from photosynthesis. If *Pyrocystis fusiformis* should act as a sustainable light source, they have to show an effective conversion of energy obtained from photosynthesis into bioluminescent light. Hence, further research may focus on the impact of photosynthesis on their bioluminescent activity by exposing the samples to controllable grow lights and using a CO_2 measuring device to measure their performance.

Conclusion

This study presented a new approach to investigating the bioluminescent activity in *Pyrocystis fusiformis.* Using the self-designed measuring device enabled the exploration of the research question stated in the Introduction. On the basis of the scientific background, it was hypothesised that the standardised *Pyrocystis fusiformis* population presents an energy-efficient and sustainable light source in terms of light intensity, performance duration and recovery ability. The important results for analysing the hypothesis show an average maximum light intensity of 0.148 milliwatts that stayed nearly constant during the two weeks measuring series and a mean relative performance duration of 22’769 milliseconds. Concerning the emitted energy during the relative decline in bioluminescent activity, a value of around 1.161 millijoules was identified, and the experiment further showed that the algae have a rather fast recovery ability as they regained 30-40 % of their initial light intensity during a 40 minutes recovery period directly after their bioluminescent activity had ceased entirely. Now the question arises whether these values suffice to accept the hypothesis?

Closely looking at the obtained data reveals, that the innovative idea of using the *Pyrocystis fusiformis* algae as an energy-efficient sustainable light source is not ready yet and the hypothesis must therefore be rejected.

The light of a standardised algae sample slightly illuminated the close proximity so that structural changes in the surface of near objects became visible to a certain degree, but the intensity showed neither a remarkable increase nor decrease over the two weeks. Hence, it seems that without increasing the concentration of algae in a sample, the light cannot be intensified. This indicates that a single alga is unable to boost the intensity of its bioluminescent light which in turn poses borders to the exploitation of the latter. Further, the observed performance duration is too short to actually work as a reliable light source as it is roughly around 22 seconds. In addition to those two points, there exists a large imbalance between the energy used to stimulate the algae and the minute amount that is regained as light. This means that the algae are not energy-efficient because the obtained light does not equalise the energy required for stimulation. However, despite those facts, the study revealed that the algae seem to possess the remarkable ability of fast recovery from stimulation hinting at some hidden potential of the *Pyrocystis fusiformis.*

When rejecting the hypothesis, one has to keep in mind that this field of research is still in its beginnings and the present study delivered basic findings that build a foundation for future research. The latter may go into the direction of attempting to increase the light intensity artificially and elongating the performance duration, not forgetting about the goal to create an energy-efficient stimulation in relation to the output. Therefore, future improvements in the implementation of bioluminescent algae as a light source, may one day still prove the now rejected hypothesis right.

References

Chen, Antony K., et al. (2007) *"Evidence for the role of G-proteins in flow stimulation of dinoflagellate bioluminescence"*, American Journal of Physiology-Regulatory, Integrative and Comparative Physiology
https://journals.physiology.org/doi/full/10.1152/ajpregu.00649.2006
Last accessed 12.12.20

Christianson, Roger Gordon (1976) *"Studies on the circadian rhythm of bioluminescence in the marine dinoflagellate Gonyaulax polyedra"*, University of California Press

Craig, Jaquelyn et al. (2003) "*Effects of salinity, pH and temperature on the re-establishment of bioluminescence and copper or SDS toxicity in the marine dinoflagellate* Pyrocystis lunula *using bioluminescence as an endpoint*", University of Louisiana at Lafayette
https://pubmed.ncbi.nlm.nih.gov/12810320/
Last accessed 12.12.20

Eckstein, Jens W. Living Light (1991) *"Untersuchungen zum enzymatischen Reaktionsmechanismus der bakteriellen Biolumineszenz"*, University of Constance

Elbrächter, M. and Drebes, G. (1978) *"Life cycles, phylogeny and taxonomy of Dissodinium and Pyrocystis (Dinophyta)"*, Helgoland Marine Research
https://hmr.biomedcentral.com/articles/10.1007/BF02189487
Last accessed 12.12.20

Fajardo, Marcos et al. (2020) *"New Perspectives Related to the Bioluminescent System in Dinoflagellates: Pyrocystis Lunula, a Case Study"*, International Journal of Molecular Sciences
https://www.mdpi.com/1422-0067/21/5/1784/htm
Last accessed 12.12.20

Fogel, Margaret and Hastings, J.W (1972) *"Bioluminescence: Mechanism and Mode of Control of Scintillon Activity"*, Proceedings of the National Academy of Sciences of the United States of America
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC426536/
Last accessed 12.12.20

Lee, John. (2015). *"Bioluminescence, the Nature of the Light"*, University of Georgia

Maldonado, Elisa M, and Latz, Michael I. (2007) *"Shear-stress dependence of dinoflagellate bioluminescence"*, The Biological bulletin
https://www.researchgate.net/publication/6272174_Shear-Stress_Dependence_of_Dinoflagellate_Bioluminescence
Last accessed 12.12.20

Rodriguez, Juan D. et al. (2017) *"Identification of a vacuolar proton channel that triggers the bioluminescent flash in dinoflagellates"*, PLOS ONE
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0171594
Last accessed 12.12.20

Seo, K.S. and Fritz, L. (2001) *"Cell ultrastructural changes correlate with circadian rhythms in Pyrocystis lunula (Pyrrophyta)"*, Journal of Phycology
https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1529-8817.2000.99196.x
Last accessed 12.12.20

Smith, Susan ME, et al. (2011) *"Voltage-gated proton channel in a dinoflagellate"*, Proceedings of the National Academy of Sciences of the United States of America
https://pubmed.ncbi.nlm.nih.gov/22006335/
Last accessed 12.12.20

Valiadi, Martha and Iglesias-Rodriguez, Debora (2013) *"Understanding Bioluminescence in Dinoflagellates-how Far Have We Come?"*, Department of Evolutionary Ecology, Max Planck Institute for Evolutionary Biology
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5029497/#B19-microorganisms-01-00003
Last accessed 12.12.20

von Dassow P, Latz MI. (2002) *"The role of Ca(2+) in stimulated bioluminescence of the dinoflagellate Lingulodinium polyedrum"*, Journal of Experimental Biology
https://jeb.biologists.org/content/205/19/2971
Last accessed 12.12.20

Widder, Edith A. and James F. Case (1981) *"Two flash forms in the bioluminescent dinoflagellate, Pyrocystis fusiformis"* Journal of comparative physiology
https://link.springer.com/article/10.1007%2FBF00606067
Last accessed 12.12.20

Widder, Edith. A. (2002) "*Bioluminescence and the Pelagic Visual Environment*" Marine and Freshwater Behaviour and Physiology
https://www.researchgate.net/publication/232550670_Bioluminescence_and_the_Pelagic_Visual_Environment
Last accessed 12.12.20

Appendix

Table 7: Qualitative Data

			Series 1			Series 2			Series 3									
		Population	1	2	3	1	2	3	4	5	6		Background noise					
Date	MD											Volume in Jar (ml)	Series 1	Series 2	Series 3	Experiment Start	Experiment Finish	Usable Data
09.09.20	1											200	±11		±16	22:12	22:54	Faulty Data
10.09.20	2											200	±15		±14	21:15	22:20	Feeding Day
11.09.20	3					Not measured there						200	±20		±12	23:50	00:30	
12.09.20	4											200	±6		±20	22:00	23:00	
13.09.20	5											200	±20		±21	21:40	22:20	
14.09.20	6											220	±20	±23	±20	21:20	22:20	
15.09.20	7											220	±19	±20	±20	21:42	21:30	
16.09.20	8											220	±20	±20	±22	23:40	00:30	
17.09.20	9											220	±23	±23	±17	21:30	22:30	
18.09.20	10											220	±20	±22	±20	22:00	22:50	
19.09.20	11											220	±20	±10	±10	01:00	01:40	
20.09.20	12											240	±20	±15	±13	21:00	22:00	
21.09.20	13											240	±11	±10	±10	22:00	23:00	
22.09.20	14											240	±24	±25	±20	20:30	21:00	
23.09.20	15											240						
24.09.20	16					Not measured there, but on MD 19 again as interested whether any effect occured during the 4 days break						240						
25.09.20	17											240						
26.09.20	18											260						
27.09.20	19											260	±25	±20	±20	22:00	23:00	

Table 8: Extract of excel sheet with raw data for the population 2 (Series 1) on the eight measuring day

Measuring Day	8		Corrections	Median filter		x0	x1	x2	x3	x4	x5				Quartile			
Population	2		6	2.0		0	0.00033869	-3.43842E-06	5.72024E-08	-2.09682E-10	2.6077E-13							
Series	1							Noise		Timing	Value	mW	Energy	Quartile		Timing	Value	mW	Energy
File number	49				5	2064		20		31078	199	0.135	1.118	0.25		21551	199	0.135	1.005
						timing	value		1	Timing	FINAL	Mwatt	mW*sec	1		Timing	FINAL	Mwatt	mW*sec
37	33		1	17	5	0	199		1	0	199	0.135		1		0	199	0.135	
562	1		-	2	6	502	158		1	502	158	0.088	68	1		502	158	0.088	68
1062	2		-	2	7	1002	163		1	1002	163	0.094	44	1		1002	163	0.094	44
1563	85		-	85	8	1503	146		1	1503	146	0.076	47	1		1503	146	0.076	47
2064	199		-	199	9	2005	147		1	2005	147	0.077	38	1		2005	147	0.077	38
2566	158		-	158	10	2506	169		1	2506	169	0.100	39	1		2506	169	0.100	39
3066	163		-	163	11	3007	174		1	3007	174	0.106	50	1		3007	174	0.106	50
3567	146		-	146	12	3508	164		1	3508	164	0.095	53	1		3508	164	0.095	53
4069	147		-	147	13	4009	148		1	4009	148	0.078	47	1		4009	148	0.078	47
4570	169		-	169	14	4511	143		1	4511	143	0.073	39	1		4511	143	0.073	39
5071	174		-	174	15	5011	133		1	5011	133	0.064	37	1		5011	133	0.064	37
5572	164		-	164	16	5513	115		1	5513	115	0.049	32	1		5513	115	0.049	32
6073	148		-	148	17	6014	111		1	6014	111	0.046	25	1		6014	111	0.046	25
6575	143		-	143	18	6515	111		1	6515	111	0.046	23	1		6515	111	0.046	23
7075	133		-	133	19	7016	112		1	7016	112	0.047	23	1		7016	112	0.047	23
7577	115		-	115	20	7517	117		1	7517	117	0.051	23	1		7517	117	0.051	23
8078	47		-	111	21	8019	114		1	8019	114	0.048	25	1		8019	114	0.048	25
8579	111		-	111	22	8520	115		1	8520	115	0.049	24	1		8520	115	0.049	24
9080	112		-	112	23	9021	108		1	9021	108	0.044	25	1		9021	108	0.044	25
9581	117		-	117	24	9522	100		1	9522	100	0.038	22	1		9522	100	0.038	22
10083	114		-	114	25	10024	100		1	10024	100	0.038	19	1		10024	100	0.038	19
10584	115		-	115	26	10525	99		1	10525	99	0.038	19	1		10525	99	0.038	19
11085	108		-	108	27	11026	95		1	11026	95	0.035	19	1		11026	95	0.035	19
11586	100		-	100	28	11527	90		1	11527	90	0.032	18	1		11527	90	0.032	18
12088	100		-	100	29	12029	100		1	12029	100	0.038	16	1		12029	100	0.038	16
12589	99		-	99	30	12530	88		1	12530	88	0.031	19	1		12530	88	0.031	19
13090	95		-	95	31	13031	92		1	13031	92	0.033	16	1		13031	92	0.033	16
13591	90		-	90	32	13532	88		1	13532	88	0.031	17	1		13532	88	0.031	17
14093	100		-	100	33	14033	91		1	14033	91	0.033	16	1		14033	91	0.033	16
14594	88		-	88	34	14535	80		1	14535	80	0.027	16	1		14535	80	0.027	16
15095	92		-	92	35	15035	83		1	15035	83	0.028	13	1		15035	83	0.028	13
15596	88		-	88	36	15537	74		1	15537	74	0.024	14	1		15537	74	0.024	14
16097	91		-	91	37	16038	80		1	16038	80	0.027	12	1		16038	80	0.027	12
16599	80		-	80	38	16540	74		1	16540	74	0.024	13	1		16540	74	0.024	13
17099	83		-	83	39	17040	71		1	17040	71	0.022	12	1		17040	71	0.022	12
17601	74		-	74	40	17542	67		1	17542	67	0.021	11	1		17542	67	0.021	11
18102	80		-	80	41	18043	72		1	18043	72	0.023	10	1		18043	72	0.023	10
18604	74		-	74	42	18544	56		1	18544	56	0.016	11	1		18544	56	0.016	11
19104	71		-	71	43	19045	60		1	19045	60	0.018	8	1		19045	60	0.018	8
19606	67		-	67	44	19546	56		1	19546	56	0.016	9	1		19546	56	0.016	9
20107	72		-	72	45	20048	53		1	20048	53	0.015	8	1		20048	53	0.015	8
20608	56		-	56	46	20548	59		1	20548	59	0.017	8	1		20548	59	0.017	8
21109	60		-	60	47	21050	50		1	21050	50	0.014	9	1		21050	50	0.014	9
21610	56		-	56	48	21551	55		1	21551	55	0.016	7	1		21551	55	0.016	7
22112	53		-	53	49	22053	48		1	22053	48	0.014	8	0		0	0	-	-
22612	59		-	59	50	22553	53		1	22553	53	0.015	7	0		0	0	-	-
23114	50		-	50	51	23054	46		1	23054	46	0.013	8	0		0	0	-	-
23615	55		-	55	52	23556	52		1	23556	52	0.015	7	0		0	0	-	-
24117	48		-	48	53	24062	48		1	24062	48	0.014	8	0		0	0	-	-
24617	53		-	53	54	24564	44		1	24564	44	0.012	7	0		0	0	-	-
25118	46		-	46	55	25064	46		1	25064	46	0.013	6	0		0	0	-	-
25620	52		-	52	56	25565	40		1	25565	40	0.011	7	0		0	0	-	-
26126	48		-	48	57	26067	47		1	26067	47	0.013	6	0		0	0	-	-
26628	44		-	44	58	26568	34		1	26568	34	0.010	7	0		0	0	-	-
27128	46		-	46	59	27069	36		1	27069	36	0.010	5	0		0	0	-	-
27629	40		-	40	60	27570	40		1	27570	40	0.011	5	0		0	0	-	-
28131	47		-	47	61	28072	34		1	28072	34	0.010	6	0		0	0	-	-
28632	34		-	34	62	28573	43		1	28573	43	0.012	5	0		0	0	-	-
29133	36		-	36	63	29074	30		1	29074	30	0.008	6	0		0	0	-	-
29634	40		-	40	64	29575	37		1	29575	37	0.010	4	0		0	0	-	-
30136	34		-	34	65	30077	35		1	30077	35	0.010	5	0		0	0	-	-
30637	43		-	43	66	30578	30		1	30578	30	0.008	5	0		0	0	-	-
31138	30		-	30	67	31078	31		1	31078	31	0.009	4	0		0	0	-	-
31639	37		-	37	68	31580	20		0	0	0	-	-	0		0	0	-	-
32141	35		-	35	69	32081	30		0	0	0	-	-	0		0	0	-	-
32642	30		-	30	70	32583	31		0	0	0	-	-	0		0	0	-	-
33142	31		-	31	71	33083	40		0	0	0	-	-	0		0	0	-	-
33644	20		-	20	72	33585	33		0	0	0	-	-	0		0	0	-	-
34145	30		-	30	73	34086	30		0	0	0	-	-	0		0	0	-	-

URL-link to excel workbook with raw data:
https://balloon.tam.ch/share/e87b350fb0b5bce7d6340762be49b72f

Image 10: All six standardised algae samples

Image 11: Close up of a Pyrocystis fusiformis *sample (algae visible as brownish dots)*

Image 12: Nutrient solution for Pyrocystis fusiformis

Arduino Uno Gauging code:

```
#include <SPI.h>
#include <SD.h>
#include <stdlib.h>
#include <stdio.h>
#include <Wire.h>

int light;
int schundspannung;
int ledspannung;
int var;
unsigned long zeit;
File myFile;

void setup() {
var = 1;
Serial.begin(9600);
  while (!Serial) {
   ;
 }
```

```
 Serial.print("Initializing SD card...");
   pinMode(10, OUTPUT);
  if (!SD.begin(4)) {
   Serial.println("initialization failed!");
   return;
  }
  Serial.println("initialization done.");
  myFile = SD.open("mdXpY.txt", FILE_WRITE);
}

void loop() {
while (var<1001) {
  light = analogRead (A1)-analogRead(A0);
  schundspannung = analogRead(A3)-analogRead(A2);
  ledspannung = analogRead(A5)-analogRead(A4);
  zeit = millis();
  Serial.print(var);
  Serial.print(";");
  Serial.print(zeit);
  Serial.print(";");
  Serial.print(light);
  Serial.print(";");
  Serial.print(schundspannung);
  Serial.print(";");
  Serial.println(ledspannung);

  myFile.print(zeit);
  myFile.print(";");
  myFile.print(light);
  myFile.print(";");
  myFile.print(schundspannung);
  myFile.print(";");
  myFile.println(ledspannung);
  delay(500);
  var = var+5;
  }
  myFile.close();
}
```

Measuring code:

```
#include <SPI.h>
#include <SD.h>
#include <stdlib.h>
#include <stdio.h>
#include <Wire.h>

int light;
int var;
unsigned long zeit;
File myFile;

void setup() {
var = 1;
Serial.begin(9600);
   while (!Serial) {
    ;
  }
   Serial.print("Initializing SD card...");
   pinMode(10, OUTPUT);
  if (!SD.begin(4)) {
    Serial.println("initialization failed!");
    return;
  }
  Serial.println("initialization done.");
  myFile = SD.open("mdXpY.txt", FILE_WRITE);
}

void loop() {
while (var<1001) {
  light = analogRead (A1)-analogRead(A0);
  zeit = millis();
  Serial.print(var);
  Serial.print(";");
  Serial.print(zeit);
  Serial.print(";");
  Serial.println(light);

  myFile.print(zeit);
  myFile.print(";");
  myFile.println(light);
  delay(500);
  var = var+5;
  }
  myFile.close();
}
```