Real-Time Temperature Controlled Microalgae Incubator

A Major Qualifying Project Report

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Abstract

This project aimed to develop a low-cost miniature incubator (Mincubator) that provides a stable environment to grow phototrophic cells. The Mincubator is designed to augment phototrophic cell research with controlled lighting and temperature based on user input. The hardware is designed to be used in an incubator or separately on lab benches and sends real-time data to an online spreadsheet at specified intervals. The Mincubator simplifies the research process, enabling more sample variations to be tested in the same amount of time. Cultivation tests show that the Mincubator can maintain a set temperature and light intensity, surpassing the unregulated sample in transmittance percentage. Further work is needed to solidify the build quality and user experience of the Mincubator.

Acknowledgements

The team would like to thank Professor Stephen Bitar for his invaluable advice, guidance, and support as we worked to overcome the several obstacles during the formulation of this project.

The team would also like to thank William Appleyard and Lisa Wall for their help and support in completing this project.

Executive Summary

The world's coral reefs are under increasing threat from anthropological impacts such as climate change, pollution, and overfishing, leading to coral bleaching and increased mortality rates. The Coral Triangle, comprising almost 76% of the world's coral reef species located in the western Pacific, exemplifies the interconnectivity between coral degradation and its impact on the livelihoods of those who depend on it. Over 370 million people reside in the Coral Triangle region, with at least 120 million people directly benefiting from the region's natural goods and services with coastal communities highly dependent on marine and coastal resources for income, food security, and storm protection. Swift and concrete action is required to restore and preserve coral reefs, which are critical to human life and prosperity.

The goal of this project was to produce a miniature incubator that would augment the research experience for scientists studying phototrophic microorganisms by introducing reliable and automated features to accelerate the cultivation process. Phototrophic microorganisms were selected due to the team’s motivation towards addressing the deteriorating health of the global coral reef population exacerbated due to climate change. As a result, the team strived to create a functional, user-friendly incubator prototype, allowing the user to control the light intensity and temperature of a standard T25 culture flask through a seamless human-machine interface.

There were several constraints identified for the design of the prototype. To increase the manufacturability and affordability of the device, the materials, and by extension, the costs of the materials needed to be taken into consideration. This was necessary to ensure that the product had the capability for larger scale production. Accessibility of the culture flask was also a big factor, as it must be placed in a location where the user can remove the flask and take measurements of the contents inside on a regular basis. Another important constraint was ensuring that the temperature the flask was exposed to could be reliably maintained and controlled per user input. Lastly, due to the difficulty of sourcing zooxanthellae for functional testing, the phototrophic microorganisms found inside coral, an accessible

alternative, chlorella vulgaris, was used to evaluate the success of the system. Therefore, the device was configured to replicate the optimal conditions for the cultivation of chlorella vulgaris cells.

To design the device, the team divided the project into three main sections: electronics, software, and hardware design. The electronics consisted of the electrical components for the system function and transmit the information collected by the sensors. The software referred to the software needed for the microcontroller to interface with the sensors as well as the human-machine interface that the user interacts with to control the overall system. Lastly, the hardware design is the physical housing necessary to store and regulate the culture flask in a secure and accessible environment.

For the electronics, a 15V power supply was selected as the LEDs were powered at that voltage. A polyimide heater powered at 15V was chosen to regulate the temperature of the system, as it was capable of maintaining the 25-30 °C target temperature range. To monitor the heat emitted by the heater, a DS18B20 temperature sensor was utilized, with an accuracy of ± 0.5 °C. Lastly, an ESP32 was selected as the microcontroller of choice due to its low cost and built-in Wi-Fi capabilities, which allows for telemetry to be transmitted to an external server in the format of a JSON package.

When it comes to the software of the Mincubator system, the code used for the operations of the microcontroller was completed in Arduino code. This included the sensor monitoring functionalities, data packaging, and JSON transmission features. The choice of Arduino code was due to the extensive documentation and libraries from other programmers online, thus reducing the software development process of the project. For the human-machine interface, the web server was written in HTTP and CSS. This was hosted locally on the microcontroller, thus allowing the user to

Figure 1. HMI Web Server

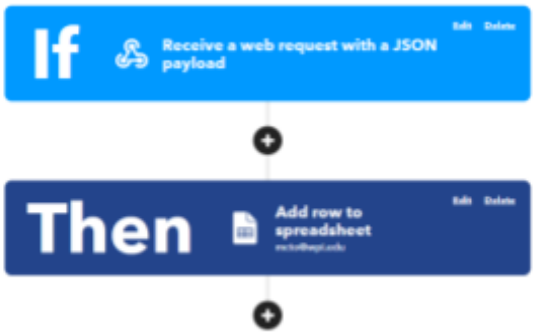

Figure 2. IFTTT Website Interface

directly change the measurement parameters of the system (See Figure 1). Lastly, the telemetry was received using a free platform called IFTTT, where it converts the incoming JSON packages into inputs on a Google Sheet spreadsheet (See Figure 2).

The hardware needed to contain the T25 cell culture flask and provide supporting infrastructure to regulate temperature and lighting, therefore it was broken into two subsections. Firstly, to provide thermal regulation, an aluminum heat plate was designed to be placed below the flask, with a cut out for a polyimide heater that could distribute the heat evenly. This was manufactured using CAD/CAM processes, and designed to fit within the enclosure. The enclosure itself was designed with angled front retaining walls to constrain the flask into the proper position. It also includes a wire channel cutout for the temperature sensor and the polyimide heater wires, as well as a mounting feature that advantageously holds the temperature sensor in-place without contacting the sensitive IC silicon packaging. The enclosure was manufactured with a 3D printer using a thermoplastic monomer called PLA.

Prior to assembling all the subsystems, a series of tests were performed on each of the core functionalities to reduce the risk of unforeseen technical errors. These tests were used to ensure that the key features of each system were operating within nominal parameters. Upon satisfying the integration tests, the hardware and electronics were assembled to complete the Mincubator system.

After performing basic integration testing, it was decided to test the operational capabilities of the Mincubator by cultivating a phototrophic microorganism. To facilitate this experiment, a common phototrophic microalgae, chlorella vulgaris, was utilized and the microalgae was observed over the course of a 10-day period to evaluate the performance of the system. The microalgae cultivated within the Mincubator was compared to a sample that was left out in unregulated conditions. To evaluate the effectiveness of the system, a spectrophotometer was used to obtain daily measurements of the opacity of the samples at 680 nm

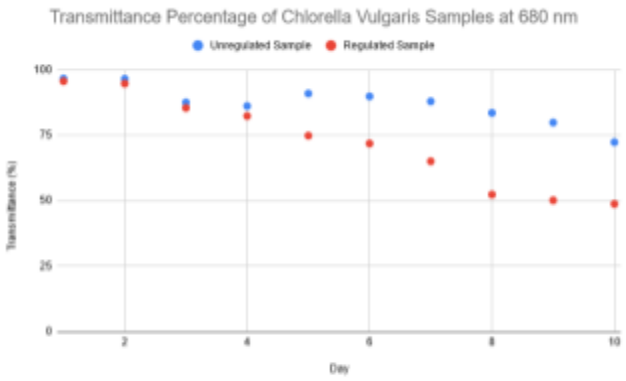

Figure 3. Experimental Transmittance Results at 680 nm.

and 750 nm respectively based on the absorption of chlorophyll cells. This was quantified through recording the transmittance values outputted by the spectrophotometer (See Figure 3). From comparing the unregulated and regulated samples, the regulated one that was grown within the Mincubator system exhibited an average 15.02% transmittance rate difference over the one grown in uncontrolled conditions.

The team was able to accomplish the design objectives established at the start of the project. The team successfully produced a prototype that automated the cultivation process of phototrophic microorganisms. Additionally, user centered design principles were considered throughout the production process of the physical hardware design and the software interface, such as an accessible enclosure and real-time parameter adjustment features.

The team identified a few avenues towards improving the design of the device with future iterations. To enable better thermal insulation, a steel-based hardware encasing was proposed. In addition, to mitigate electrical short-circuits, a custom PCB was suggested to facilitate the integration of all the components onto a single board. Lastly, a greater array of troubleshooting mechanisms were proposed, such as a notification system when an anomaly is detected or live telemetry values on the human-machine interface web server.

The team successfully produced a functional miniature incubator. The Mincubator allowed for temperature regulation and the adjustment of light intensity based on user input. The design was made to facilitate modular production of multiple Mincubator systems, with the software capable of interfacing with multiple setups. Overall, the completion of the project automated the research process of the phototrophic microorganism that the team conducted an experiment with.

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Introduction

Phototrophic cells are typically grown in incubators that provide a controlled environment to promote optimal growth conditions. The incubator's environment is typically regulated in terms of temperature, humidity, and gas exchange. Phototrophic cells are grown in a culture medium that provides the necessary nutrients for their growth and development. Incubators used for growing phototrophic cells are equipped with specialized lighting that provides the cells with the required light intensity and spectrum, which varies depending on the type of cells being grown. The lighting is typically set to a specific time period to simulate day and night cycles, which is essential for the cells' growth and development. The goal of this project is to provide culture specific temperature and lighting regulation. The team aims to produce a low-cost miniature incubator that functions as an accessory to be used within a standard laboratory incubator to create individual micro-environments and enable researchers to test more variables in the same amount of time.

Discussed herein is the relationship of phototrophic cell cultivation and incubators, the fundamentals of incubators, and existing accessories currently used. The report also lists the design objectives and constraints of the Mincubator platform, as well as the hardware and software design involved in the manufacturing of the platform. The implementation and testing results of the proof-of-concept are documented.

Background

This section provides the fundamental context and understanding for the environmental and economic need behind the project. An overview is outlined in regard to existing cultivation methods of phototrophic cells. This section also details the mechanisms of current incubators on the market and some of the competing accessory products that align with our design objectives. Lastly, this section will identify the design opportunities identified based on market research and begin to lay the groundwork for them to be refined in subsequent chapters.

2.1 Coral Ecosystems and Anthropological Impacts

Coral reefs across the globe are facing increasing challenges from anthropological impacts such as sea temperature fluctuations due to climate change, pollution, and overfishing. Coral bleaching and increased mortality rates caused by gradually increasing sea temperatures impact the coral reefs' surrounding ecosystems. Due to its abundant biodiversity, and nearly 76% of the world's coral reef species located in the western pacific, the Coral triangle is an excellent example of the interconnective relationship between coral degradation and the impacts on the livelihoods of those that depend on it.[1]

[1] Green, A., & Cros, A. (2008). Coral Triangle facts, figures, and calculations. Part II: Patterns of biodiversity and endemism. *The Nature Conservancy*.

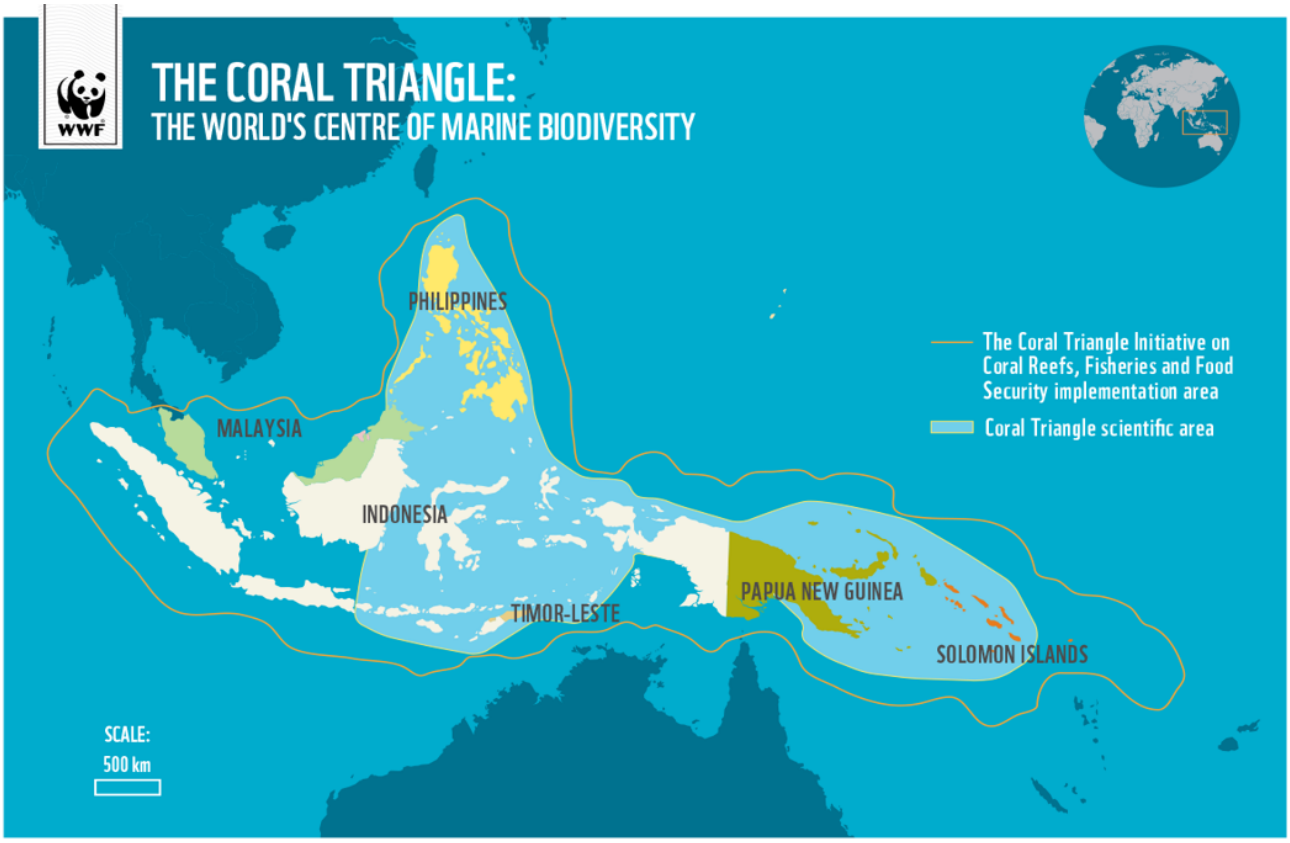

Figure 4: Map of Coral Triangle.[2]

There are over 370 million people residing in the Coral Triangle region, and at least 120 million people who directly benefit from the region's natural goods and services.[3] It is estimated that the annual monetary value of the ecosystems, natural habitats, and mangrove protection the Coral Triangle provides totals to $13.9 billion, in addition to an immeasurable amount of cultural and social benefits.[4] Individuals residing in coastal communities in this region have a greater dependency on marine and coastal resources including income, food security, and storm protection.

By understanding the interrelationship between coral ecosystems and anthropological activity, a correlation between communities dependent on the natural resources of coral ecosystems and their

[2] WWF. (n.d.). *The Coral Triangle: The world's center of marine biodiversity*.
[3] The David and Lucile Packard Foundation. (2018). *Trends in Marine Resources and Fisheries Management in Indonesia*.
[4] *UN Environment, ISU, ICRI, & Trucost. (2018). The Coral Reef Economy: The business case for investment in the protection, preservation, and enhancement of coral reef health.*

vulnerability to disruptions by global climates can be established. The Coral Triangle is a key example in understanding the impacts of anthropological climate change on coral reefs around the world; it solidifies that swift, concrete action must be taken to restore and preserve coral reefs thus sustaining marine ecosystems critical to human life and prosperity.

2.2 Phototrophic Cells Cultivation

Zooxanthellae are phototrophic dinoflagellates which have a symbiotic relationship with corals. Categorized as a genus under the phylum of dinoflagellates, *Symbiodinium*, commonly referred to as zooxanthellae, are phototrophic organisms that produce up to 95% of the organic photosynthetic compounds (glucose, glycerol, amino acids, O2, and lipids) for the host. In return, the coral polyps provide the microalgae with a protected environment and inorganic nutrients (carbon dioxide, ammonia, and phosphate) necessary for photosynthesis.[5] Anthropogenic threats causing ocean temperature increases have overwhelmed zooxanthellae's photosynthetic systems leading to an overproduction of reactive oxygen species that leak out of the endosymbionts and cause host cell damage.[6]

Although zooxanthellae are known to be sensitive to high solar irradiance and irregular water temperature fluctuations, this delicate symbiotic relationship has persevered for the past 400 million years.[7,8] Therefore, understanding the thermal tolerances of zooxanthellae will be crucial in modifying or adapting them to withstand higher temperatures. The rapidly changing environmental landscape in the 21st century demonstrates that it is critical to develop mitigation and restoration strategies to strengthen the resiliency of corals against the dynamic ecosystem. A method to approach this issue is to understand

[5] Fransolet, D., Roberty, S., & Plumier, J.-C. (2012). Establishment of endosymbiosis: The case of cnidarians and Symbiodinium. *Journal of Experimental Marine Biology and Ecology*, *420–421*, 1–7. https://doi.org/10.1016/j.jembe.2012.03.015
[6] Nielsen, D.A., Petrou, K. & Gates, R.D. Coral bleaching from a single cell perspective. *ISME J* 12, 1558–1567 (2018). https://doi.org/10.1038/s41396-018-0080-6
[7] Takahashi, S., Whitney, S., Itoh, S., Maruyama, T., & Badger, M. (2008). Heat stress causes inhibition of the *de novo* synthesis of antenna proteins and photobleaching in cultured *Symbiodinium*. *Proceedings of the National Academy of Sciences*, *105*(11), 4203–4208. https://doi.org/10.1073/pnas.0708554105
[8] Turgeon, D. D., Asch, R. G., Causey, B., Dodge, R. E., & Jaap, W. (n.d.). *The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States: 2002*. 279.

the existing cultivation methods of zooxanthellae cells in the research community to identify opportunities in improving the research process.

Phototrophic cells can be cultivated in incubators using different methods, including batch culture, continuous culture, and semi-continuous culture. The specific conditions required for cultivating phototrophic cells depend on the species being grown and the desired outcome, however, most lab-scale cultivation methods are similar to the following: [9,10]

1. Inoculation: A small amount of the culture is added to the growth medium, and the culture is allowed to grow to a certain density before being transferred to the incubator.
2. Growth medium: A suitable growth medium is selected for the specific species being grown. The growth medium typically contains a carbon source, nitrogen source, and essential minerals, along with vitamins and trace elements required for growth.
3. Temperature: The incubator is set to a temperature optimal for the specific species being grown.
4. Light: Phototrophic cells require light for growth. Therefore, an appropriate light source, such as LED lights, is installed in the incubator to provide the necessary light for the growth of the cells. The intensity and duration of light exposure are adjusted to optimize the growth of the cells.
5. Agitation: Agitation is used to ensure that the cells are evenly distributed in the growth medium and receive adequate light exposure. Different methods such as shaking, stirring, or bubbling air into the medium can be used to agitate the cells.

2.3 Fundamentals of Incubators

A laboratory incubator is an insulated chamber used to grow and/or maintain microbial cell cultures. Commonly manufactured in rectangular forms, laboratory incubators control the temperature,

[9] Hoshina, S., Sekizuka, T., Kataoka, M., Hasegawa, H., Hamada, H., Kuroda, M., & Katano, H. (2016). Profile of Exosomal and Intracellular microRNA in Gamma-Herpesvirus-Infected Lymphoma Cell Lines. PLOS ONE, 11(9), e0162574. https://doi.org/10.1371/journal.pone.0162574

[10] Davis, R., Markham, J., Kinchin, C., Grundl, N., Tan, E. C. D., & Humbird, D. (2016). *Process Design and Economics for the Production of Algal Biomass: Algal Biomass Production in Open Pond Systems and Processing Through Dewatering for Downstream Conversion* (NREL/TP--5100-64772, 1239893; p. NREL/TP--5100-64772, 1239893). https://doi.org/10.2172/1239893

humidity, and at times the gas contents of the contents within.[11] Additionally, incubators have basic programmable timers to allow the user to specify the environmental conditions within the chamber. Larger incubators include internal power outlets for scientific equipment to be powered which is a desired feature by most researchers.

Figure 5. Standard Temperature and Humidity Control Incubator.

A temperature controlling laboratory incubator relies on thermostats, sensors, and microprocessors to monitor and alter the conditions of the chamber. One method of raising the temperature is by turning on a heating element, such as a heating bulb, then using internal fans to circulate that air throughout the system. Laboratory incubators that control humidity often also have a separate water tray to modify the humidity levels inside the container.[12]

Since the deliverable of this project could be used in an incubator, it was decided to specify the intended application of the prototype to be specifically for incubators that have internal power outlets. By narrowing the scope, this ensures a continuous supply of electricity and avoids the need for battery-powered solutions for the power management system.

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[11] *Laboratory Incubators | What is a lab incubator?* (n.d.). Froilabo. Retrieved December 27, 2022, from https://www.froilabo.com/blog/what-is-a-laboratory-incubator/

[12] Shrestha, A. (2022, August 19). *Laboratory Incubator: Principle, Parts, Types, and Uses*. Microbe Online. https://microbeonline.com/laboratory-incubator-principle-parts-types-and-uses/

The purpose of a laboratory incubator is to provide a controlled and sanitary environment for cell cultures to grow. There are various types of incubators, such as bioreactors and shaking incubators. After analyzing the methodologies of literature published in the marine microalgal and zooxanthellae community, it was determined that researchers primarily use laboratory incubators as temperature control to conduct their research, with little to no mentions of the incubator controlling other parameters.[13,14] This matches existing understanding as marine microalgal cells such as zooxanthellae are highly sensitive to the surrounding temperatures, and the ability for zooxanthellae cells' acclimation to higher temperatures is an active subject area of research among marine biologists.[15,16]

2.4 Existing Incubator Accessories

To determine the design opportunities for this project, an understanding of existing incubator accessories and similar products on the market must be established. Three distinct incubator accessories were selected to determine their respective marketing strategies and intended applications.

Benchmark Scientific - BioMixer 3D Rocker

The BioMixer™ 3D rocker manufactured by Benchmark Scientific is a shaker designed to provide multi-dimensional shaking motion for scientific equipment and is safe for incubator use.

[13] Buerger, P., Vanstone, R. T., Maire, J., & van Oppen, M. J. H. (2022). Long-Term Heat Selection of the Coral Endosymbiont Cladocopium C1acro (Symbiodiniaceae) Stabilizes Associated Bacterial Communities. *International Journal of Molecular Sciences*, *23*(9), 4913. https://doi.org/10.3390/ijms23094913

[14] Gibbin, E. M., Putnam, H. M., Davy, S. K., & Gates, R. D. (2014). Intracellular pH and its response to CO2-driven seawater acidification in symbiotic *versus* non-symbiotic coral cells. *Journal of Experimental Biology*, jeb.099549. https://doi.org/10.1242/jeb.099549

[15] Buerger, P., Alvarez-Roa, C., Coppin, C. W., Pearce, S. L., Chakravarti, L. J., Oakeshott, J. G., Edwards, O. R., & van Oppen, M. J. H. (2020). Heat-evolved microalgal symbionts increase coral bleaching tolerance. *Science Advances*, *6*(20), eaba2498. https://doi.org/10.1126/sciadv.aba2498

[16] Berkelmans, R., & van Oppen, M. J. H. (2006). The role of zooxanthellae in the thermal tolerance of corals: A 'nugget of hope' for coral reefs in an era of climate change. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1599), 2305–2312. https://doi.org/10.1098/rspb.2006.3567

Figure 6. Promotional Images of BioMixer 3D Rocker.

The rocker contains a 12x12 inch dimpled mat to set equipment such as centrifuge tubes onto the surface to be mixed evenly, with additional flat mats available. According to Benchmark Scientific's website, the base unit costs $587.[17] In regard to notable technical specifications, the shaker weighs 2 kilograms and can support the same amount of weight on the mat. Additionally, it is powered by an electrical outlet at 115V or 230V. The power management of this product is significant as it requires an internal electrical outlet for it to be used inside the incubator, which aligns and justifies the product requirements for this project.

Phi Lab - Holomonitor Live Cell Imaging System

The Holomonitor is a live cell imaging system that incorporates a compact microscope into a module intended to be fit into an incubator. Using a micro-USB connection, the Holomonitor transmits live imaging data onto a computer, which interfaces with Phi Lab's App Suite software for further processing. It should be noted that the instruction manual of this product suggests the power and data wires to be routed through the access port of the incubator, which based on previous research, isn't a feature that most incubators have.

[17] *BioMixer™ 3D Rockers Product Description*. (n.d.). Benchmark Scientific. Retrieved December 29, 2022, from https://www.benchmarkscientific.com/product/b3d1020-group/

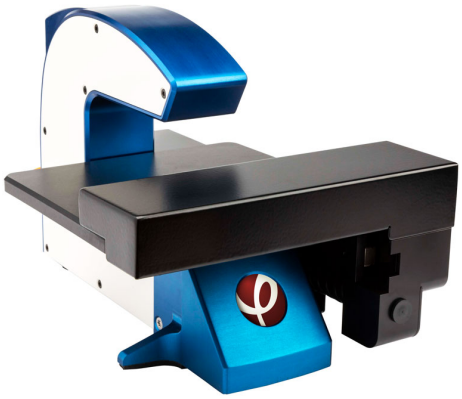

Figure 7. Promotional Image of Holomonitor.

With a starting price of $27,000, the Holomonitor was designed for researchers to consistently monitor the culture vessel placed onto the platform. This is similar to the design objective of this project, as instead of transmitting telemetry data from the sensors to the user, the Holomonitor sends live imaging data. The Holomontor's imaging sensor transmits the live feed with a resolution of 1024x1024 pixels per second, and supports a host of standard laboratory equipment, such as petri dishes, 24-well, and 96-well microplates. Lastly, it should be noted that two power supplies are necessary to power the device, as one is for the main unit, while the other is for the laser microscope.[18]

Olympus Provi - CM20 Incubation Monitor System

The CM20 is a cell culture incubator monitoring system designed to periodically scan the culture vessels placed on top of the platform for key metrics and transmit them remotely. The device records quantitative measurements such as cell count and confluency percentage using an optical sensor, which enables users to identify abnormalities without physically opening the incubator and disturbing environmental conditions of the incubator.

[18] *Holomonitor M4 Setup and Operation Manual*. (2018). Phase Holographic Imaging PHI AB.

Figure 8. Promotional Image of CM20.

The CM20 starts at $12,600, which includes the base unit, as well as the accompanying software that provides data processing capabilities. In regard to wireless transmission of scientific data, it was shown on Olympus Provi's website that a USB 3.0 connection from the CM20 hardware to a WiFi-enabled computer must be established. Afterwards, a local host is generated for other devices in the WiFi network to access the CM20 and its data.[19] The CM20 supports a host of culturing vessels, such as the petri dish, multi-layered flasks, and 6-well or 12-well microplates, and transmits 1280x960 sized images to the software interface. Lastly, the CM20 system is powered by a single USB 3.0 port, which is plugged into a designated computer, thus assuming that the incubator has an access panel for it to be connected externally.

Through market research, it was determined that there are several competing solutions available on the market when it comes to cell monitoring systems for incubator applications. Although existing solutions offer advanced features such as live imaging and cell count capabilities, they also come at a high price tag, which limits their market appeal to institutions or organizations that don't have plentiful financial resources. Additionally, it was a challenge to find a comprehensive lighting and sensor solution capable of facilitating phototrophic cell development. Therefore, the proposed product will have a competitive advantage if a lower cost all encompassing sensor suite and phototrophic cell compatible lighting solution can be produced.

[19] *OLYMPUS Provi CM20 | Incubation Monitoring System | Olympus LS*. (n.d.). Retrieved December 29, 2022, from https://www.olympus-lifescience.com/en/cell_culture_solution/cm20/

2.4 Background Summary

In summary, the symbiotic relationship between coral and zooxanthellae was discussed, in addition to the detrimental effects anthropogenic environmental changes have on the symbiotic relationship, through a process called coral bleaching. The current understanding of coral bleaching was discussed. This was intended to highlight the limited knowledge that exists about this microalgae and why further research on them is crucial. Then, the growth conditions for phototrophic cells were discussed. Phototrophic cells tend to enjoy warm temperatures within the 22-27°C range, with a typical 12 hour light cycle that mimics the daylight hours in temperate climates. This provided the foundational understanding as to how microalgae cells are cultivated in a laboratory setting. Afterwards, the mechanisms of how incubators work was discussed. This was necessary in identifying the design opportunities in this market, in which our team determined the need for intelligent temperature regulated systems within a larger incubator setting.

Methodology

The following section discusses the steps taken to design and create the incubator device. The design objective criteria were identified and justified based on the research conducted. Additionally, the research performed in determining each of the components of the system was listed.

3.1 Design Objectives

Product Requirement	**Description**	**Justification**
Functionality	Measure and transmit key environmental parameters	The novelty of this product is the ability to actively monitor the environmental conditions of the biological substance on a digital platform. Therefore, it is critical for the product to have sensors measuring key parameters that are valuable to phototrophic cell researchers in the system.
	Artificial lighting and lighting cycles	To facilitate phototrophic cell growth, it is essential for the phototrophic cells to receive sufficient artificial lighting. In addition, the duration of which the light source is turned on should be controlled, as some phototrophic cells require a day and night cycle
Cost	Affordable	Since this product is designed to be a supplemental solution for phototrophic cell researchers, the materials selected should be inexpensive whilst not compromising on the functionality of the product. In addition, the device should remain in the MQP's allocated budget for the project
Product Lifespan	Durable and sturdy	Although this product is meant to function as a proof-of-concept, the build quality should be durable and can perform its function within the environmental conditions of the incubator
Safety	Water hazard	Since the device will be subject to varying temperature and humidity levels, the electronics of the system should be protected from the exposure to these conditions
	Electrical hazard	The connections between components in the system should be insulated to protect any potential short circuits or electric shocks when in use or when the user interacts with the system
Size	Compact form factor	The system should be a lab-scale prototype capable of being used inside an incubator setting
	Accommodating	This product aims to be a universal platform for phototrophic cell

	to various cultivation equipment	researchers using incubators for cell cultivation. Therefore, it is essential for the researchers to place their cultivation equipment of choice onto the platform, eg. culture flasks or petri dishes.

3.2 System Architecture

The system has three key aspects: the sensor suite, LEDs, and the digital platform as shown in Figure 9.

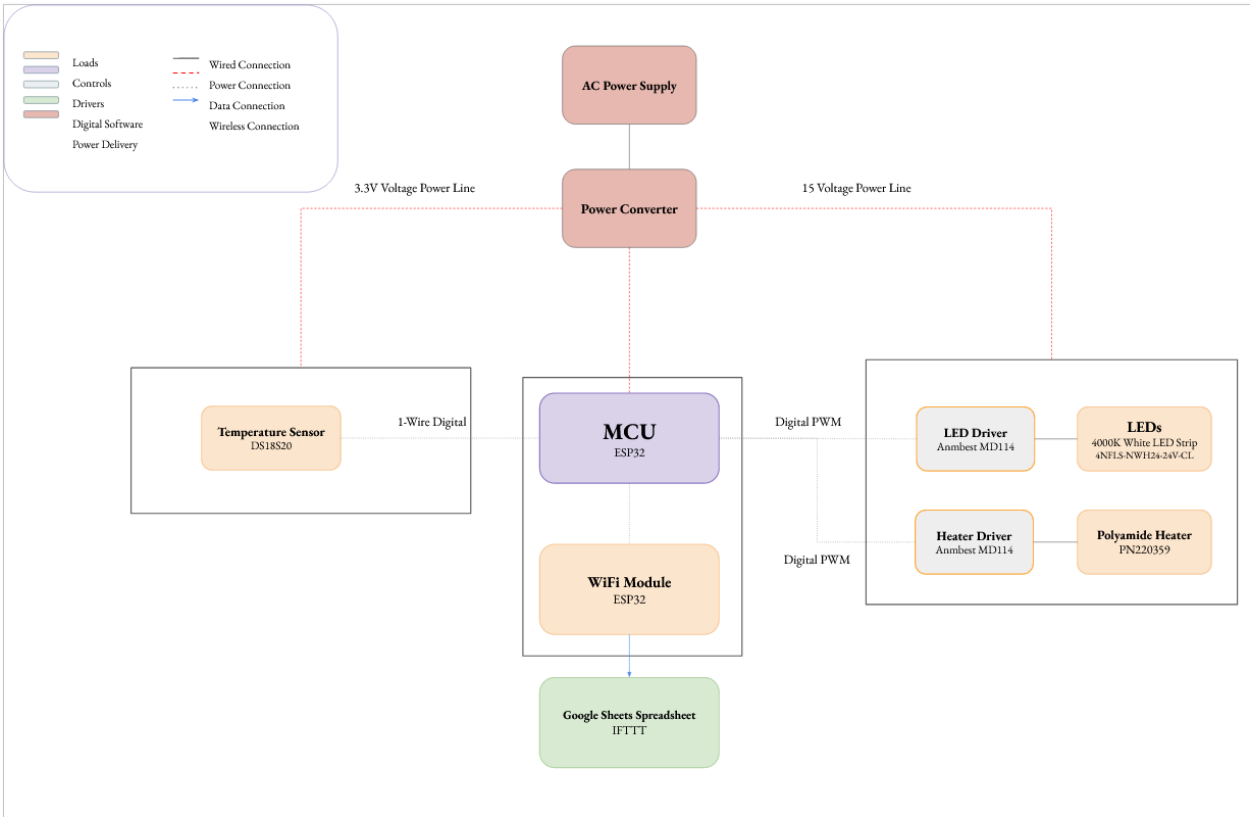

Figure 9. System Block Diagram of Platform

The sensor suite includes one heating device communicated over digital PWM to control the heat and cooling of the system, followed by two temperature sensors transmitting temperature data over 1-wire digital protocol.

The LEDs and heater module are controlled by a MOSFET driver that communicates with the microcontroller via a digital PWM. This driver is capable of controlling the duty cycle of the two modules

in addition to the on-off state of the lights and heater. The LEDs, heater and MOSFET drivers are powered by a 15V DC connection from the power converter.

To transmit the relevant scientific information onto a digital platform, a WiFi module was necessary for the microcontroller. This component was built onto the microcontroller and enables information to be sent wirelessly via sending HTTP Post messages that include a JSON package, where it is received by the digital platform for further processing.

3.3 Component Research

3.3.1 Heating Module

Although the system will be placed into an incubator, integrating an active heating component will be necessary to prove that a temperature control can be maintained.

Various methods and heating/cooling systems are used for temperature control when working with biology. For instance, in a thesis publication from California Polytechnic State University, researchers outlined how they were able to vary the temperature of their system from 9°C to 39°C using an off-the-shelf aquarium chiller and aquarium heater.[20] In the aforementioned application a more archaic cooling system was used which relied upon an operator spraying water on the hardware to maintain thermal equilibrium. In another paper there was a mention of "jacket heating" which allows the system to be encapsulated in adhesive silicone heating pads that can provide thermal energy to the system.

Initially the use of a polyimide film flexible heater, also known as a Kapton heater, was discussed to follow a similar principle to the "jacket heating" method outlined earlier. These heaters provide precise heat distribution and a thin profile to be integrated into small spaces. This lightweight flexible heater has a low operating cost and can supply heat to maintain extreme temperatures from as low as -200°C to as high as 200°C.

[20]Mehlitz, T. H. (2009). *Temperature Influence and Heat Management Requirements of Microalgae Cultivation in Photobioreactors* [California Polytechnic State University]. https://doi.org/10.15368/theses.2009.15

However, the use of a Kapton heater neglects the fact that the system could require cooling capabilities beyond maintaining a specific temperature.

To determine which heater is best suited for this application, its wattage needs to be compared against the project requirements. The following formula is used to determine wattage:[21]

$$P = \frac{(m \times C_p \times \Delta T)}{3412} \times h$$

Equation 1. Power Requirements for Heater.

Where P = the power requirement in kilowatts; m= the weight of the material to be heated, in pounds. Cp = the specific heat of the material to be heated, in BTU/lb°F; Δ T = Temperature Rise, in °F; 3412 = Conversion Factor, BTU/kWh; and h = how long it should take to reach the temperature set point, in hours.

3.3.2 Temperature Sensor

Temperature sensors are electronic devices used to measure fluctuations in temperature through changes in resistance. Temperature sensors are integrated into various applications including the monitoring of water heaters, thermometers, microwaves, geotechnical structures, etc. With so many different applications, there is also an established need for different types of temperature sensors of various monitoring capabilities. Usually temperature sensors are categorized into contact and non-contact. The former, as the name implies, requires direct contact to the object in order to measure temperature, and the latter measures the thermal radiation released by the heat source.

One type of contact temperature sensor is a thermistor, which due to drastic changes in resistance, can accurately display small changes in temperature. There are two types of thermistors, Negative Temperature Coefficients (NTC), which account for most of the thermistors in production, and Positive Temperature Coefficients (PTC). Thermistors with an NTC decrease their resistance when there is an increase in temperature, while ones with a PTC do the inverse. Non-contact temperature sensors include

[21] *How to calculate heater wattage to get to the right temperature?* (n.d.). Https://Www.Omega.Com/En-Us/. Retrieved December 20, 2022, from https://www.omega.com/en-us/resources/how-calculate-heater-wattage

semiconductor-based ones; these IC sensors contain internal circuitry capable of measuring the voltage difference between two internal diode terminals which is then used to calculate temperature.

Digital vs Analog

Temperature sensors with analog outputs use the transfer function to determine the temperature, whereas ones with digital outputs do not require a programmed function to determine the temperature. Analog sensors will usually require an analog to digital converter (ADC) to digitize the output. Digital sensors typically have a better system integration complexity and also better performance. On the other hand, analog sensors are known to have higher accuracies, but as a trade off also have higher noise.

Requirements

The following requirements are outlined to ensure the performance of the thermal management system is properly monitored: size, accuracy, water resistivity, and operating range will be necessary factors when selecting a temperature sensor for the system.

Size

The first product requirement is that the size of the temperature sensor needs to be relatively small. This product is meant to fit inside an incubator as one of many sensors, therefore the temperature sensors should therefore be integrated in a way to occupy minimal space.

Accuracy

A sensor that is capable of logging temperature data to a +/- 0.5℃ range will be necessary. Since the thermal management system is responsible for maintaining a constant temperature for the biology, the temperature sensor should be capable of reporting temperature changes within 1℃ to regulate those fluctuations.

Water Resistant

The temperature sensor will be placed to monitor the environmental conditions of the compartment. Since the humidity of the incubator is often controlled during experiments, a highly humid environment should be expected. Therefore, it is imperative that the sensor be impervious to water so that it is not damaged from exposure to droplets or high humidity. Researching temperature sensor probes that offer an extension to the sensor so that the IC itself is not located inside of the module could be practical for this requirement.

Temperature Range

The temperature range that the microalgae will be exposed to will fluctuate around 12℃ to 40℃, therefore finding a sensor that is capable of performing, and logging data, in those conditions will be necessary.

Price

The temperature sensor selected needs to be affordable, ideally below $30. This is an important product requirement since some temperature sensors for industrial applications can get expensive, and while they might be a perfect fit for monitoring temperature due to their accuracy, the goal of this project is to achieve a system proof of concept.

Sensor Selection

Digital Temperature Sensor

Temperature sensor ICs have a more linear response than NTC thermistors, often NTC thermistors also require temperature-voltage curves that need to be characterized and can have higher power consumption since the current draw varies over temperature[22].

The digital sensor selected was the Dallas (now acquired by Maxim and Analog Devices) DS18B20. Because it is a digital temperature sensor, there is no signal degradation over time, or over

[22] Texas Instruments. (2015, June 2). Why Use Temperature Sensor ICs vs. Thermistors in Power-Sensitive Applications. *EEWeb*. https://www.eeweb.com/why-use-temperature-sensor-ics-vs-thermistors-in-power-sensitive-applications/

distance. This temperature sensor provides a 9-bit to 12-bit Celsius temperature measurement and requires only one data line. The latest revision of this sensor is also able to derive parasitic power from the data line if needed. Each DS18B20 has a unique 64-bit serial code, which allows multiple DS18B20s to be communicated with on the same 1-Wire bus, making it ideal for distributing a few sensors over a large area. It is also capable of measuring temperatures from -55°C to 125°C. The digital sensor itself is contained within a sealed waterproof casing and can be submerged for long durations, making it an ideal candidate for this application.

Infrared Temperature Sensor

The use of an infrared (IR) temperature sensor was disqualified as a possibility after not scoring highly in each of the product requirements. IR temperature sensors rely on the radiation of heat waves emitted by a hot object and absorbed by a colder object which occurs in the infrared region of the electromagnetic spectrum[23]. Depending on the object's thickness, materials, and the distance from which the temperature is being measured, the accuracy of the temperature reading is affected. Moreover, the ratio of the distance to surface area measured and needs to accurately be maintained, and if this ratio is disrupted it can result in inaccurate temperature readings. Another consideration is that depending on the thermal management system implemented, i.e. if heaters are used and attached to the hardware, it is likely that the field of view would be obstructed and thus the IR temperature sensor would not be able to read the internal temperature of the modules.

Combinational Temperature and Humidity

Combinational temperature and humidity sensors were also considered for this application. There are three types of humidity sensors: capacitive, resistive and thermal. Capacitive ones measure relative humidity through a thin metal oxide strip between two electrodes. However, these sensors require a complex circuit and regular calibration. Resistive ones use ions in salts to measure the electrical impedance in an environment and translate that into humidity changes. Finally a thermal one requires two

[23] John Gyorki. (2009, September 14). *Infrared Temperature Sensor: What is it and how does it work?* https://www.sensortips.com/temperature/infrared-temperature-sensor/

sensors that conduct electricity based on water vapor in the air between the two of them, translating that into humidity. In general, some types of humidity sensors are known to often fall out of sync which could provide unreliable measurements. Thus this combinational sensor does not make a good fit for the application because of the maintenance and calibration that could be required. In addition, it is expected that there will be water vapor in the chambers, and having a sensor that is measuring humidity, when that parameter is not needed, would be a waste of electricity and space.

Resistance Temperature Detection Sensor

Establishing size, accuracy, water resistivity, and operating range as product requirements for the temperature sensor allowed for the selection of a component that best meets the thermal management system requirements.

3.3.3 LED Lighting Solution

Light emitting diodes (LEDs) are diodes that specialize in emitting light when sufficient forward current is passing through it. A light source is necessary for the system to cultivate microalgal cells, as they require light to perform photosynthesis. Since this is a prototype intended to be used in a laboratory setting, artificial light generation is needed. LEDs were selected as the artificial light source of choice as they are compact for the application of being placed inside an incubator, allowing greater flexibility when it comes to designing the proof-of-concept. Additionally, LEDs are cost effective and more energy efficient compared to incandescent or other lighting technologies.

Requirements

There are several requirements for the LEDs, with the most prominent ones being the color spectrum, intensity, and size.

Color Spectrum

The color spectrum of the LEDs must cover the visible light spectrum. It is shown that although chlorophyll, the photosynthetic pigments responsible for light absorption, reacts to blue (425-475 nm) and

red (640 nm) lights the most, green light (510 nm) enables the light rays to travel further into the cells.[24] Therefore, to simulate natural sunlight conditions, white light will be utilized for this prototype.

Intensity

The intensity of the LEDs must be suitable for cultivating microalgae cells. The light intensity of the commercial grow-lights used in academic research studies are around 60 μmol photons m^{-2} s^{-1}.[25] Using that as a benchmark, the intensity of the LEDs should match or exceed that brightness.

Size

Due to the compact form factor of the proof-of-concept, a flexible LED strip is the preferable type of form factor to enable greater design versatility.

The LEDs were selected based on the requirements for an LED solution that mimicked the wavelength of natural sunlight and sufficient light intensity to facilitate cell growth. Using the previously stated light intensity as reference, an LED strip emitting a color of 4000K, or natural white light, with 70 μmol/s was selected. The 24V LED strip can be cut every two inches, which makes it suitable for the compact form factor of the system. Additionally, the LED strip has 3M double coated tape on the rear side, which is rated to be resistant to high humidity, temperature cycling, and water resistance conditions, thus making it suitable for the application.[26]

To effectively control the LED strips from the MCU, a MOSFET with PWM adjustment capabilities was necessary. Based on the power requirements of the LED strip, a MOSFET capable of supporting high voltage and current applications, in this case at least 24V, was required. As a result, the Anmbest MD114 driver was selected.

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[24]Baidya, A., Akter, T., Islam, Md. R., Shah, A. K. M. A., Hossain, Md. A., Salam, M. A., & Paul, S. I. (2021). Effect of different wavelengths of LED light on the growth, chlorophyll, β-carotene content and proximate composition of Chlorella ellipsoidea. *Heliyon*, *7*(12), e08525. https://doi.org/10.1016/j.heliyon.2021.e08525

[25] Benstein, R. M., Çebi, Z., Podola, B., & Melkonian, M. (2014). Immobilized Growth of the Peridinin-Producing Marine Dinoflagellate Symbiodinium in a Simple Biofilm Photobioreactor. *Marine Biotechnology*, *16*(6), 621–628.

[26] 3M. (2008). *3M High Strength Double Coated Tape with Adhesive 300LSE Datasheet*.

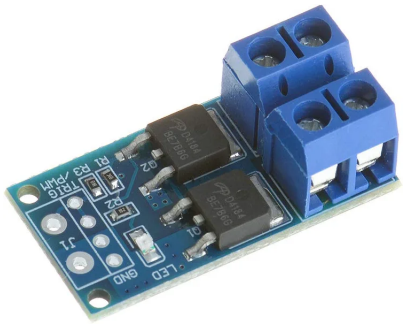

Figure 10. MOSFET PWM Driver.

The driver has a dual MOSFET layout and is rated with a maximum voltage of 36V and 30A, thus making it applicable for the desired use case. Additionally, it supports up to 20kHz for the PWM, which is beyond the maximum frequency of 980 Hz emitted by the PWM pins of the Arduino Uno, which was the chosen MCU.

3.3.4 Microcontroller Unit

The ESP32 was chosen as the microprocessor unit for the project. This decision was made based on its versatility when it comes to accommodating digital and analog input signals with an onboard ADC, in addition to a built-in WiFi chip for wireless data transmission. There are a total of 34 GPIO pins on the ESP32, with all of them available to be used for both digital and analog domains. This is essential for the integration of the pH sensor which exclusively provides an analog output.

Figure 11. ESP32 Microprocessor Unit.

The ESP32 also offers 3.3V and 5V power supplies, which will be sufficient in powering the drivers for the LEDs in this system. It also operates on low power, with an onboard voltage regulator that controls the

input voltage to be from 2.2V to 3.6V. Its small size also contributes to the overall selection of this product as it reduces the physical footprint of the system.

The Wi-Fi chip is a hardware component built into the ESP32 that transmits the relevant telemetry data collected from the sensors to a Google Sheets document. The chip itself is part of the MCU, which is a key reason why the ESP32 was selected.

3.3.5 Digital Platform

A platform called IFTTT was selected to relay information between the Arduino Uno and the digital database. IFTTT, formally known as "if this then that", is a free-to-use application that connects two or more applications together upon satisfying a specific condition, similar to an "if" statement in code.[27] This platform was selected because of its compatibility with the HTTP callback application Webhook, which allows users to send small JSON packets of information to a designated link in the form of an HTTP POST request.[28] The format of the JSON package is in the form of three name and value pairs, which matches the intended application of sending the temperature, pH value, and the dissolved oxygen value if necessary.[29] In addition, IFTTT automatically registers the time when each message is received, which is convenient for data processing. Once the telemetry data is sent to the link, a Google Sheets document records the date and contents of the message, which then in turn isolates the values and produces representative plots of each parameter.

[27] *How does IFTTT work?* (n.d.). IFTTT Help Center. Retrieved December 27, 2022, from https://help.ifttt.com/hc/en-us/articles/115010158167-How-does-IFTTT-work-
[28] *What's a Webhook? | Twilio SendGrid*. (2014, June 24). SendGrid. https://sendgrid.com/blog/whats-webhook/
[29] IFTTT. (n.d.). *Webhooks—Receive a web request with a JSON payload*. IFTTT. Retrieved December 27, 2022, from https://ifttt.com/maker_webhooks/triggers/json_event

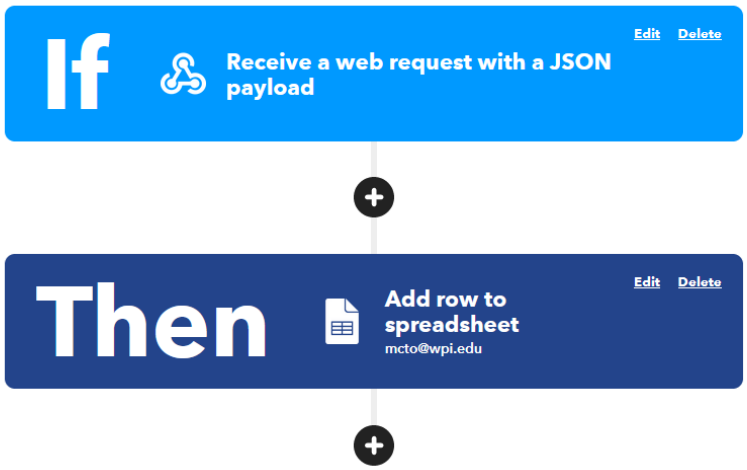

Figure 12. IFTTT Website Interface.

The main factors that contributed to the usage of IFTTT and Google services for the project were accessibility and cost. In regards to accessibility, IFTTT offers free access to its basic services such as triggering two applications upon successful activation of the initial conditions, in this case a HTTP POST message sent to the designated website. In addition, IFTTT is compatible with Google Sheets hence it was a straightforward choice to use IFTTT to transmit the JSON package from the hardware device to an online database. Similarly, Google's Sheets is also free and easy to use from both the end-user and the developer's point of view This design approach is suitable for the application required, as the data source is periodically updated, which is crucial towards understanding the status of the cultures inside the incubator.

Implementation and Results

In order to perform a functional test of the device, the individual components of the device were tested in stages. By conducting separate tests, this allowed for easier debugging and the ability to perform redesigns if necessary.

4.1 Component Testing

4.1.1 Temperature Sensor

To measure the variance of the temperature sensors, the following test experiment was devised. Three testing conditions were established, room temperature water, cold water, and hot water. The room temperature variant acted as the control, with the two other variants demonstrating the effectiveness of the temperature sensor when reacting to different temperatures. Each of the trials were conducted for two minutes, with the first ten seconds recording the room temperature before transitioning to the hot or cold water solutions.

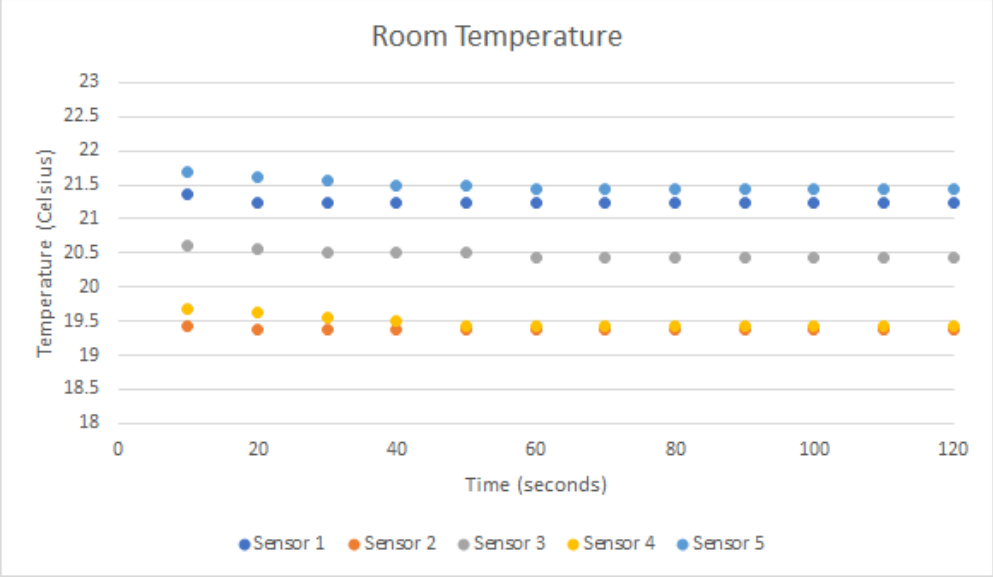

Figure 13. Temperature Testing Data for Room Temperature Trial.

To increase the temperature of the water, a microwave was used to heat the water for 4 minutes to increase the temperature of the room temperature water to a near-boiling state. Then, the temperature probes were placed into the cup for the duration of the trial. As for the cold water variant, the same cup was refilled with room temperature water and placed in the freezer to rapidly cool the water for 15 minutes.

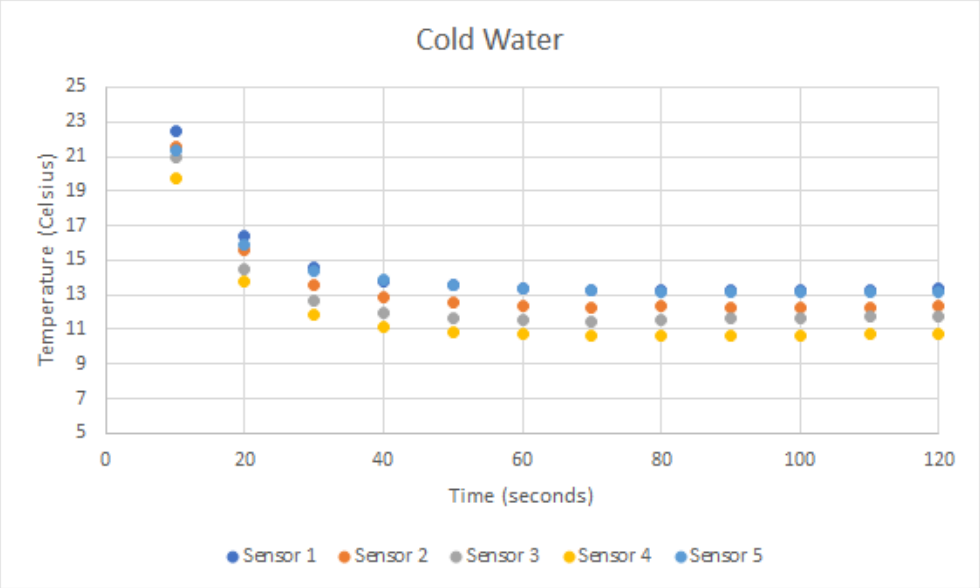

Figure 14. Temperature Testing Data for Cold Temperature Trial.

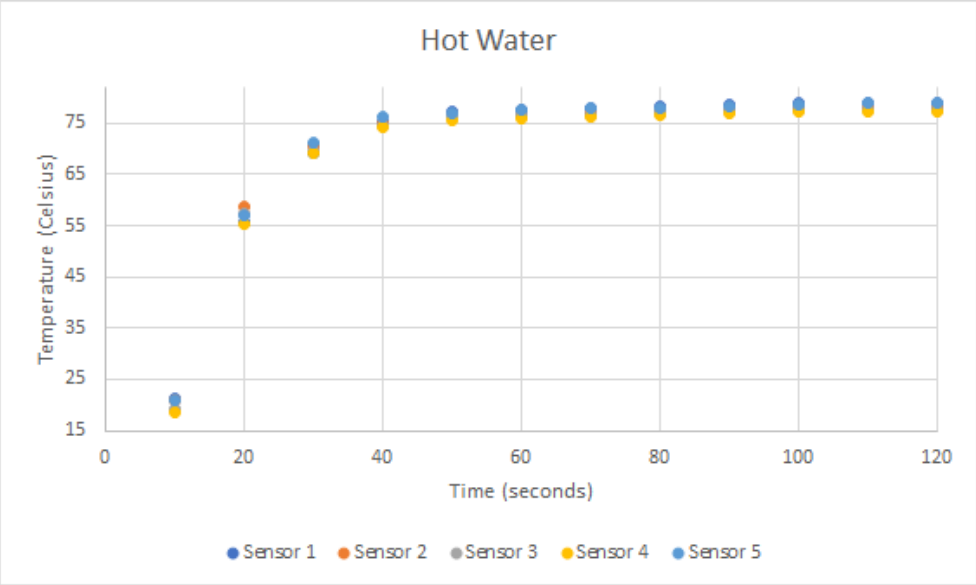

Figure 15. Temperature Testing Data for Hot Temperature Trial.

As shown from the temperature graphs above, the temperature sensor requires approximately 30 seconds for the sensors to acclimate to the hot and cold temperatures. It should also be noted that a variance between the five sensors was recorded, as shown from the room temperature graph in Figure 15. The discrepancy between the rated values of ± 0.5 ºC is most prominent for the room temperature data, with a 2.5 ºC difference shown between sensor 2 and sensor 5. A reason for this difference in temperature may be attributed to faulty hardware and insufficient quality control. To alleviate this systematic error affecting the results of the system, the two probes with the least amount of variance amongst the five temperature probes were selected to minimize temperature inaccuracies, with one of the two being the backup for the prototype.

4.1.2 LEDs

To test the LEDs, a 15V power supply was attached to the LED strip. The preliminary test involved driving the brightness of the LEDs by supplying a higher voltage/current and monitoring the

intensity of the LEDs. The lux sensor was used to establish the change in intensity of the LEDs also mapped to a change in lux as the voltage increased.

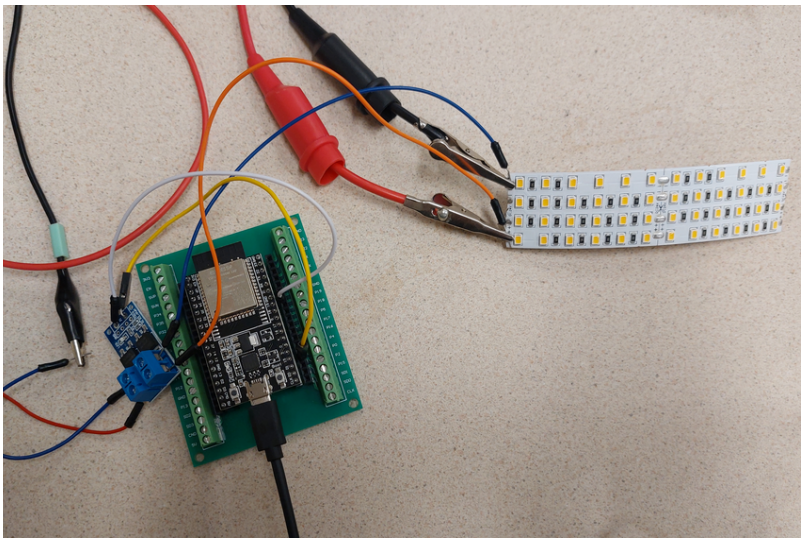

Figure 16. Hardware Setup for LED Testing.

Next, the LED drivers were integrated with the LEDs and the MCU to demonstrate that the MCU can control the voltage and on-off state of the LEDs. The LED strip was wired to the PWM driver, with the signal sent to the ESP32 MCU in the form of PWM. Using the built-in peripheral, LED control (LEDC), the on and off time of the LEDs were established, with the intervals controlled via software. The light intensity of the LEDs were controlled by creating an ordinal scale of integer values from one to ten. Upon modifying the integer value, this changes the duty cycle sent to the LED driver, thus decreasing the intensity of the light emitted. Additionally, to increase the accessibility of the system, the serial monitor on the ESP32 actively checks if the user chooses to change the light intensity by inputting an accepted integer. This enables the user to modify the light intensity in real time instead of having it be a static value hard coded onto the microcontroller unit.

Next is to produce a light intensity profile of the LEDs in relation to the voltage being fed to the LED strips. The light intensity profile accomplishes the objective of translating the strength of the light source into a controllable variable by basing the duty cycle of the LEDs on it, thus allowing the user to select a rating based on the data from the light intensity profile. First, the distance between the LEDs and

the bottom of the T25 culture flask was measured using calipers, resulting in a separation value of 32 mm. With that measurement, a photon meter was positioned at that length to determine the light intensity of each increment. To minimize the effects of external lighting, the measurements were conducted in an unilluminated room.

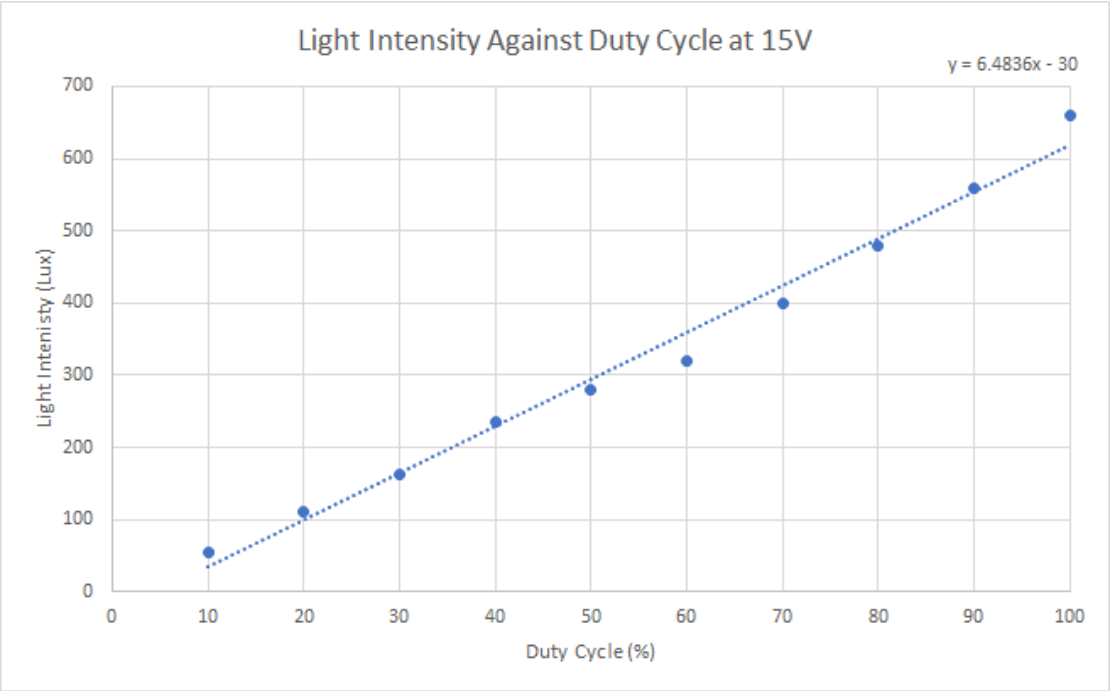

Figure 17. Light Intensity Profile Measurements.

As shown in Figure 17, the light intensity of the LED strips display a highly linear relationship. When the light intensity was set to 10% duty cycle, 55 lux (lx) was measured. Meanwhile, a lux value of 660 was recorded at 100%. It should be noted that the test was conducted with 15V of supply voltage, as it was experimentally shown that the illumination threshold for the LEDs were at 15V.

4.1.3 Kapton Heater

To test the kapton heater placed beneath the culture flask, the heating and cooling properties of the heater were evaluated. This preliminary test was done by placing the temperature sensor in contact with the kapton heater and measuring the ambient temperature in the room prior to activation and then heating the kapton heater to a set temperature. The kapton heater was supplied by a 15V power supply,

which is controlled by a PWM driver running at 100% duty cycle that is connected to the ESP32. The base temperature was 21°C , with the objective being to record the time it took to hit 32°C as well as the time necessary for it to cool down. This experiment was repeated by placing the metal sheet in between the temperature sensor and the kapton heater to simulate the prototype's conditions. This metal sheet was selected on the basis that it will be further processed and manufactured upon the completion of the CAD model for the design. Since at the time of testing the final CAD design was not available, the entire metallic piece was used to determine the difference in heating and cooling times.

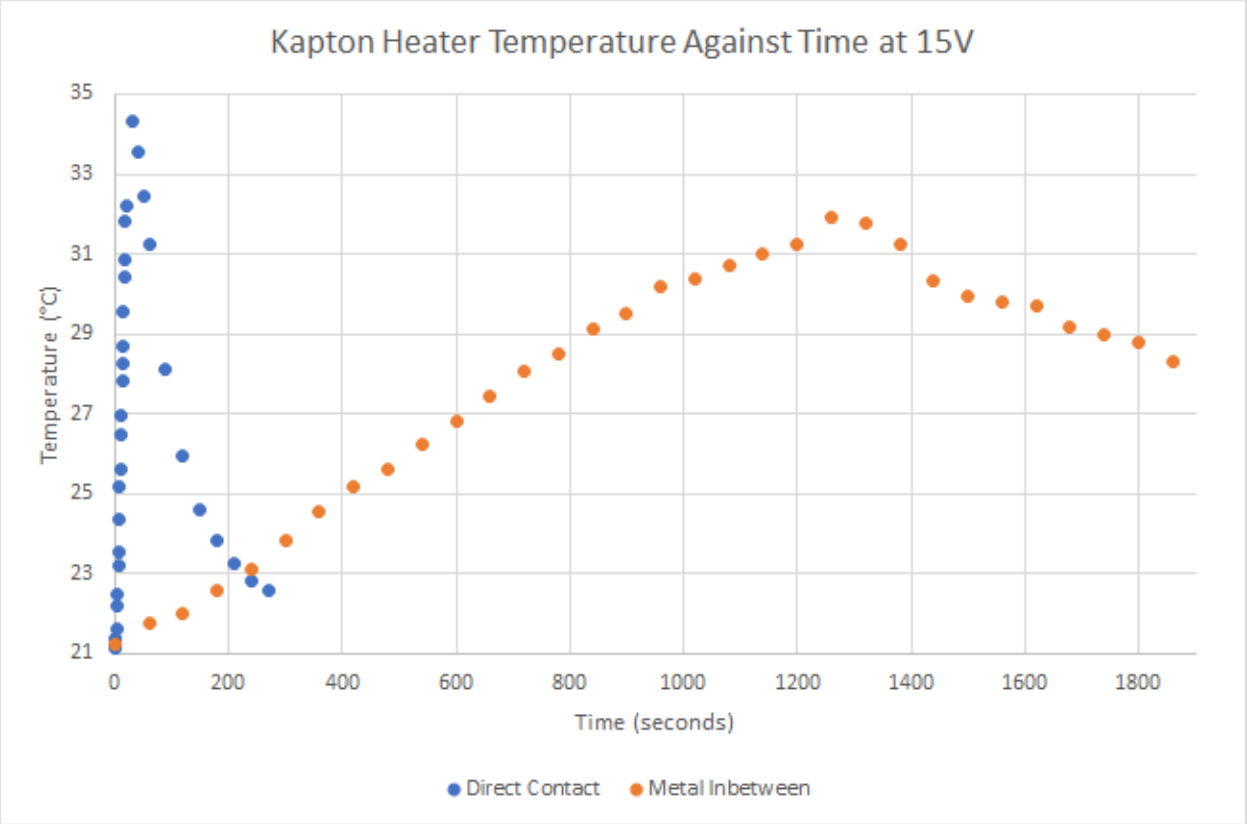

Figure 18. Heating Properties of Kapton Heater.

As seen in Figure 18, the curve with a direct contact between the temperature sensor and the kapton heater shows a rapid increase in temperature, requiring merely 20 seconds to reach the target temperature. This is followed by a slow cooldown time of at least 240 seconds for it to approach the initial temperature. One notable factor is that after reaching the target temperature, the kapton heater proceeds to exceed the

expected temperature value, thus suggesting that a damping mechanism should be implemented, such as reducing the target temperature to compensate for the residual heat in the system.

Comparing the direct contact plot against the plot with a metal, placing the metal in between the two components requires a considerably more amount of time to heat up. This makes sense as the specific heat capacity of metallic elements is typically much higher than air. Plus since the metal piece used for this experiment has not been cut to the actual size of the metallic component, the total volume that the heater needed to heat up was much more than directly contacting the temperature sensor with the heater. Likewise, the ability for the metal sheet to retain heat was much stronger than the one exposed to air.

4.1.4 WiFi and Data Transmission

The data transmission process involved sending the telemetry values to a specific IFTTT address with an HTTP POST request. Before performing any HTTP POST requests, it was essential to verify the ability for the ESP32 to establish a WiFi connection with the WiFi chip on board. To simplify the testing process, a mobile hotspot network was used. Then, using the included WiFi library, the credentials of the network were inputted onto the ESP32, resulting in the ESP32 being able to connect to the network.

Next, the HTTPClient library was used to verify the transmission of HTTP POST requests. This was accomplished by first setting up the connection from the IFTTT backend to transmit any HTTP POST requests to a Google Sheets document. In addition, on a separate page, the values were then processed and labeled to indicate the variable names of each. Afterwards, mock variable values were used on the ESP32 to simulate live telemetry data with a new request being sent upon pressing the enable (EN) button.

Live Telemetry Data		
Date	Temperature Sensor 1	Temperature Sensor 2
1/9/2023 13:42:00	23.7	24.8
1/9/2023 13:42:00	23.4	24.6
1/9/2023 13:44:00	23.6	24.4

Figure 19. Processed Mock Telemetry Data on Google Sheets Document.

4.2 Hardware Design

The hardware design consisted of two different design concepts, the first would accommodate a PSBR-like system and the final would accommodate the Mincubator system which was ultimately selected as the system of choice.

4.2.1 Version 1

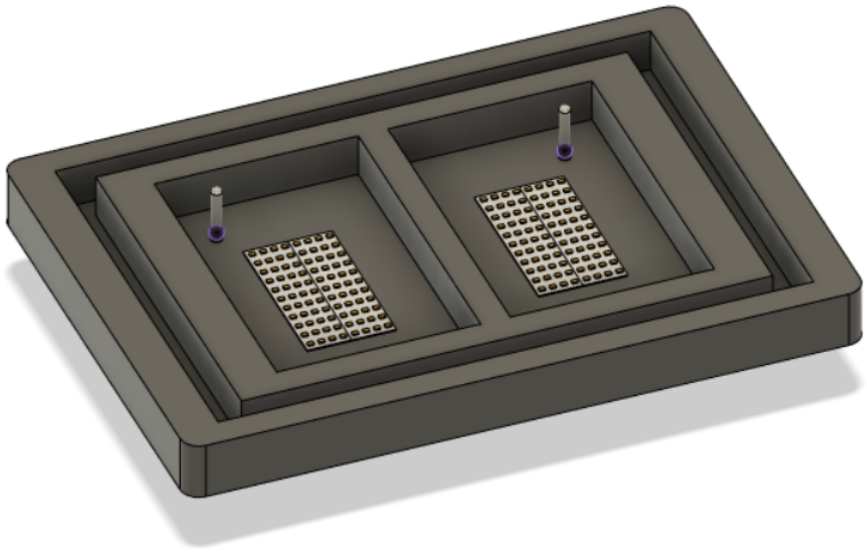

Figure 20. V1 Top Cover

The version 1 top cover contains two separate slots for two LED strips to fit into each. This would allow for two different units, and therefore two different experiments, contained within the same package. Each side would also have a DS18B20 temperature sensor running through from the outside into each respective chamber as pictured in Figure 20.

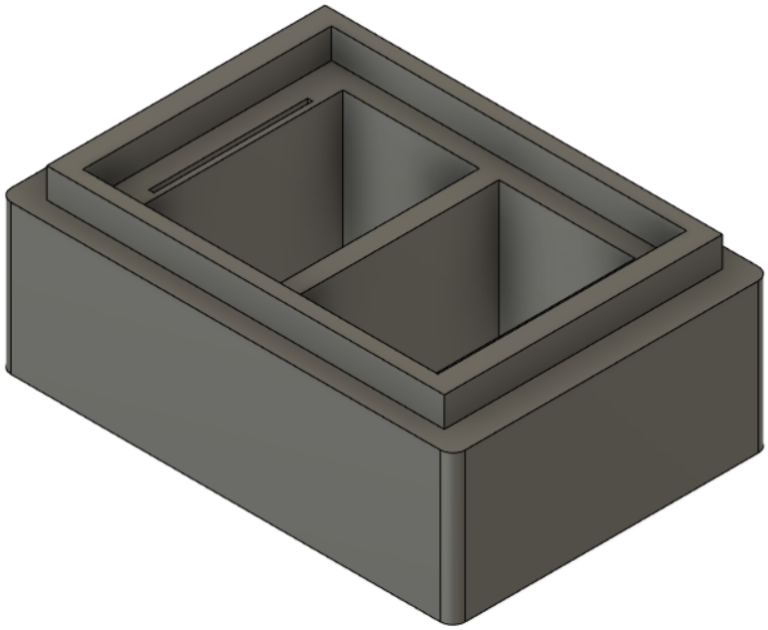

Figure 21. V1 Bottom Housing

The bottom housing would contain the porous substrate, microalgae and culture media, with two chambers to accommodate two different experiments. On either side of the chamber there were inserts to contain a flexible polyimide heater that would heat the chambers to different temperatures. The bottom and top parts would fit together through a continuous extrusion around the entire perimeter of the design. This would enable a tight seal so that no air or light could escape, and the two chambers could be discrete from one another.

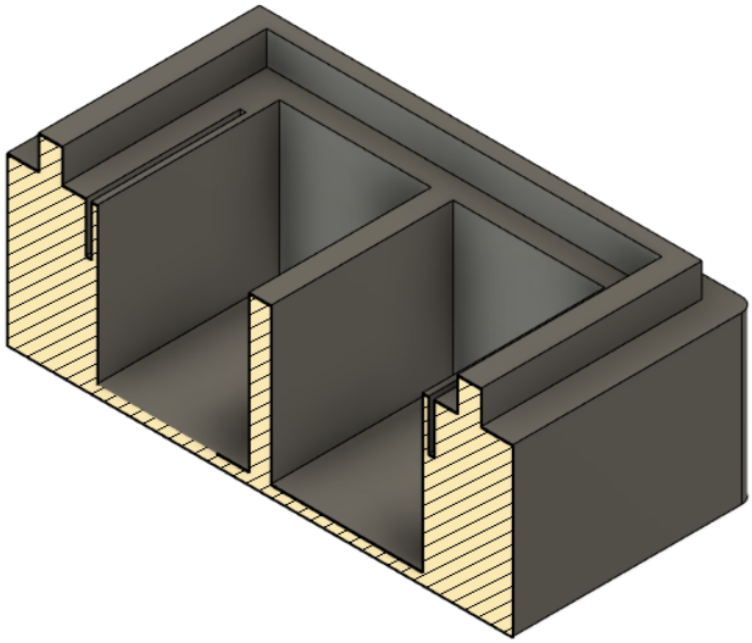

Figure 22. V1 Bottom Part Section Analysis

Figure 22 displays the distinct slots where the heaters would be inserted relative to the porous substrate chambers. The material properties of the system were also considered, where the entire bottom housing would have been machined out of steel, over aluminum, to provide better thermal insulation. Steel has a higher heat resistance while aluminum conducts and dissipates heat up to 15 times faster.[30]

[30]Bedarkar, D. S. S., & Ratio, S.-W. (2021). *Aluminium casting vs. Steel casting: How to choose the right alloy for your cast product*.

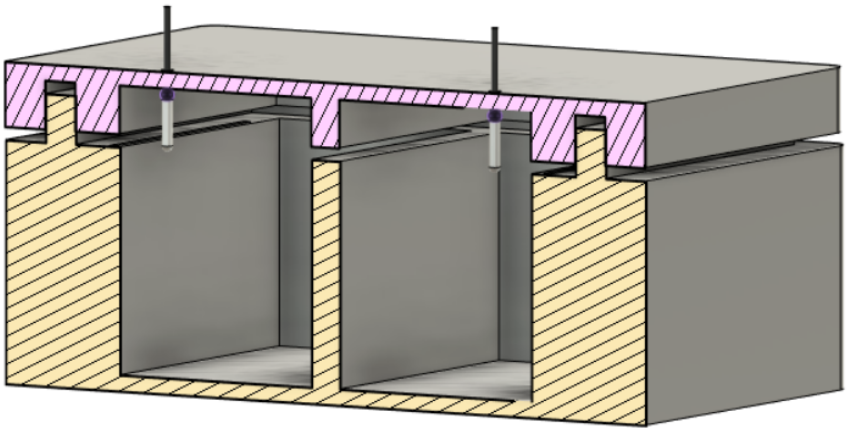

Figure 23. Overall Assembly Section analysis

Although not displayed herein, the system would rely on the use of peristaltic pumps to provide continuous flow and filtration of culture media. This would lead to complications including requiring massive reservoirs for both waste and fresh culture media for a truly autonomous system. Ultimately, the v1 design was not feasible nor practical for working and growing microalgae and did not provide many benefits than already existing systems did.

4.2.2 Final Version

The final version that was created included a much more elaborate and useful system. Instead of focusing on providing continuous flow for culturing microalgae, the focus was to provide stable environments where that microalgae could grow. The design therefore consisted of a single unit that could accommodate one cell culture flask at a time, ensuring stable light and temperature control for the units. The system needed to be versatile enough so that multiple culture flasks could be swapped in and out of the platform.

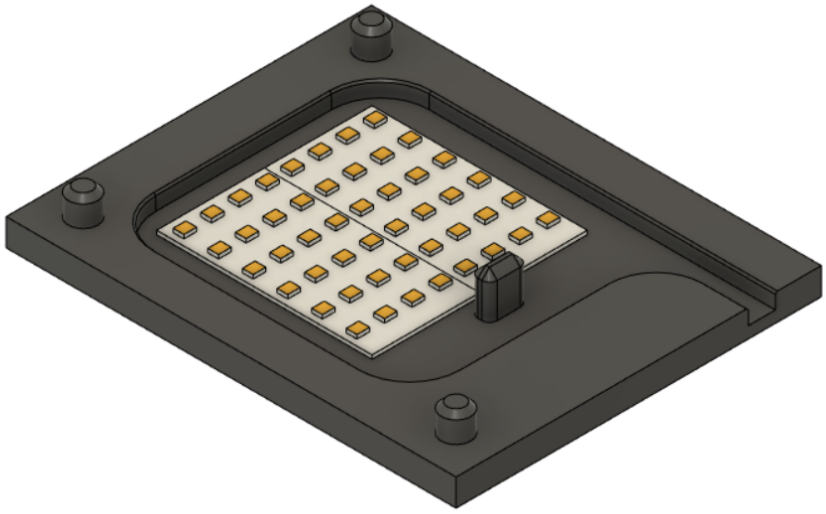

Figure 24. Final Version Top Cover.

The top cover has an inner cutout to house two LED array panels. The panels are wired in series, with power cables running through the thin cutout that runs to the side of the top cover. Three locating bosses are positioned on the bottom surface of the top-cutout to create a mating interface with the bottom housing. Another locating boss is positioned in the inner cutout of the top cover and used to properly position the cell culturing flask within the bottom housing when the top cover is closed onto the bottom housing. This locating boss mates with the back edge of the cell culturing flask and pushes it into alignment with the front retaining walls of the bottom housing. The chamfered edges on all locating bosses allow for smooth insertion into the rest of the system and remove possibilities for edge to edge interferences.

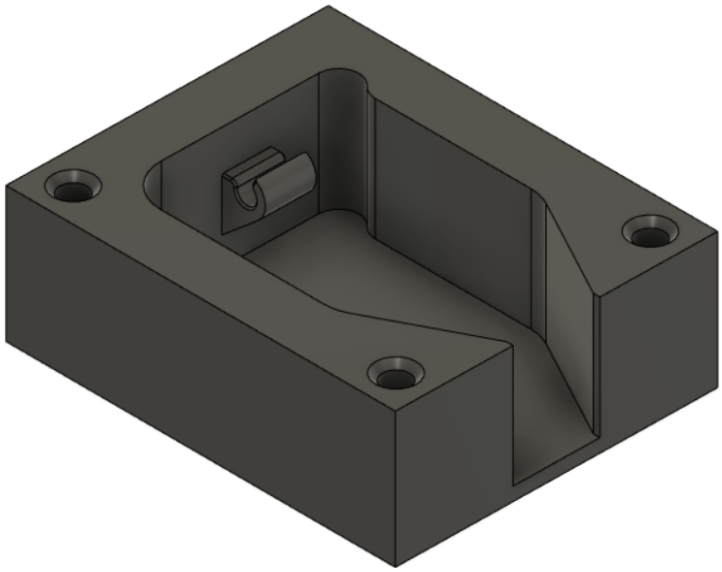

Figure 25. Final Version Bottom Housing.

The bottom housing consists of a central cutout to house the heating plate and kapton heater assembly, the flask and the temperature sensor. The angled front retaining walls of the bottom housing act to constrain the angled walls of the flask into the proper position. When the top cover is secured and aligned onto the bottom housing via the three locating bosses/ holes, the position of the flask aligning boss in the top cover forces the front of the flask into full contact with the front retaining walls of the bottom housing. Another feature includes the wire channel cutout for the temperature sensor and the polyimide heater wires. The exit of this channel aligns with the exit channel of the top cover wire cutout to reduce the number of wire outlets. The temperature sensor wires will run through the cutout and subsequently through the temperature sensor mounting feature to properly suspend the temperature sensor within the inner cutout volume. The section of wires that runs through the mounting feature will be surrounded by a rubberized c-profile plug, which will snap into place inside the circular section of the mounting feature. This advantageously holds the temperature sensor in-place without contacting the sensitive IC silicon packaging of the temperature sensor.

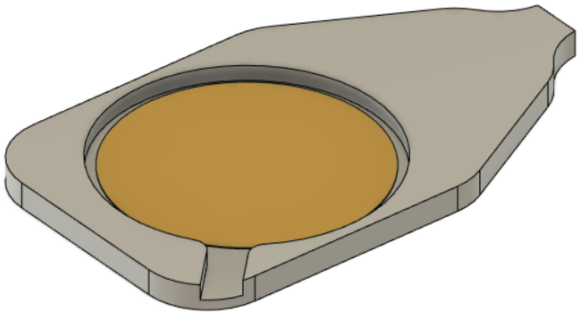

Figure 26. Heating plate and Polyimide Heater Assembly.

An aluminum heating plate consists of a recess to house a polyimide heater with an associated wire routing cutout that outputs the wires to the wire channel cutout in the bottom housing. The external profile of the heating plate allows it to be positioned into the central cutout of the bottom housing. The position of the polyimide heater recess within the external profile situates it directly beneath the cell culture flask. This, along with the high thermal conductivity of the aluminum plate, effectively distributes the heat from the polyimide heater to the flask in a uniform manner.

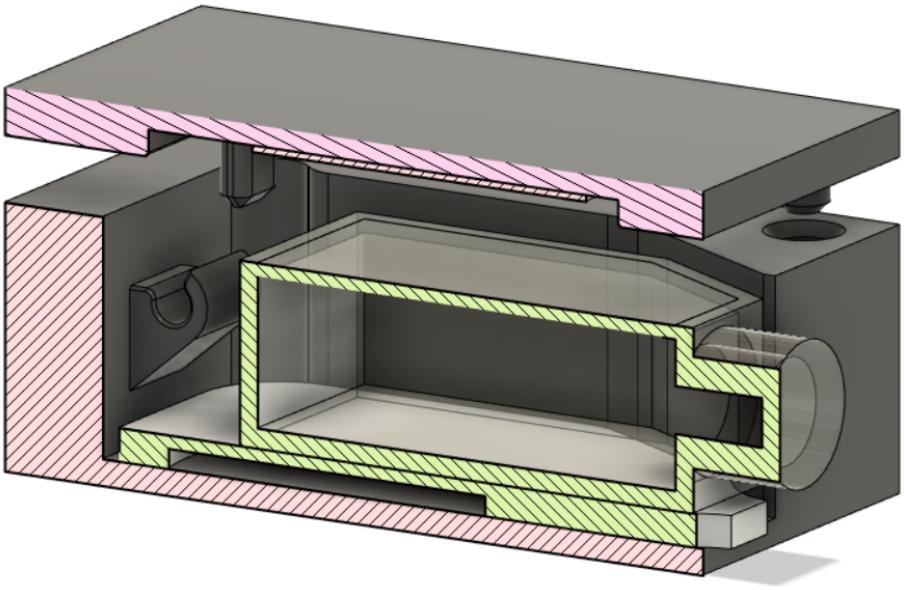

Figure 27. Section Analysis of Overall Assembly.

Figure 27 displays a section analysis of the entire assembly. Through this system, stable temperature and light control will be provided for each individual unit.

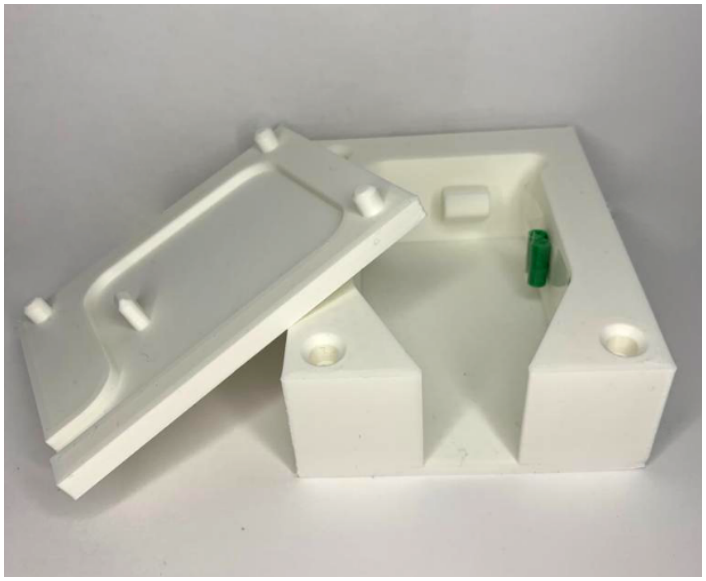

Figure 28. 3D Printed Encasing.

4.3 Hardware Manufacturing

4.3.1 Aluminium Heat Plate

The aluminum heat plate outlined in the Design section plays a crucial role in the temperature regulation of a Mincubator. The plate is wedged between the housing part of the overall system and the culture flask, so that the culture flask is resting on it. To manufacture the part, the CAD model of the plate was converted into a CAM model in Fusion 360. CAM essentially provides information to machines through the use of software to automate machining of a part. The Aluminum heat plate was then manufactured in the Washburn Manufacturing Lab using stock already existing at the Manufacturing Lab.

Figure 29. Aluminum Plate in the Haas Super Mini Mill 2.

Utilizing the Haas Super Mini Mill 2, the aluminum stock was cut to the shape of the aluminum plate on the CAD model. Additional post-processing was necessary to complete the manufacturing process. This included using a bandsaw to remove the supports that hold the final piece together to prevent the plate from moving during manufacturing, as well as sanding off the minor imperfections of the milling process.

4.4 Software Design

In order to facilitate the communication stream between sending and reading back information, the team developed a HTML website which could be launched from the ESP32. This would enable a user to communicate with the system remotely, given it is connected to the same network as the ESP32. The microcontroller would then be able to launch a website with different features that a user could interact with.

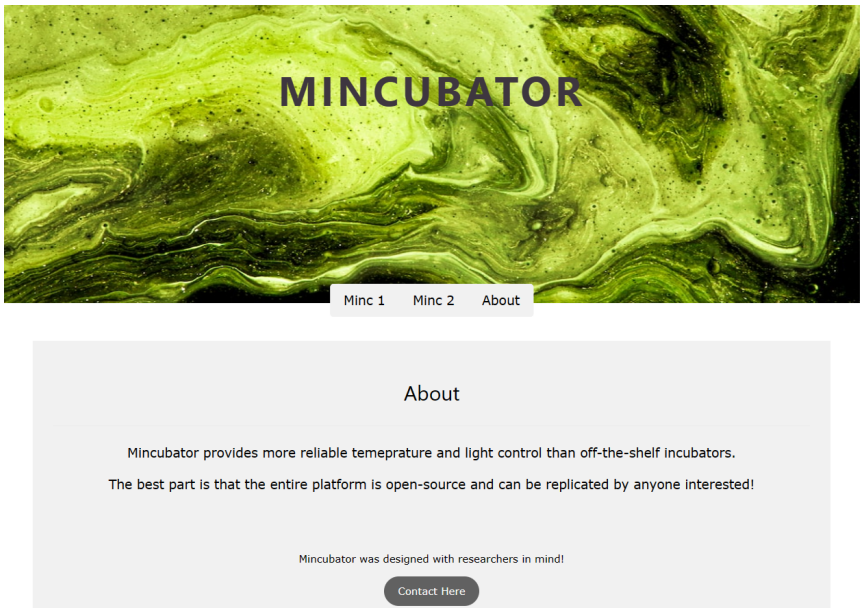

Figure 30. Landing Page.

The top of the landing page contains the banner of the website with the name of the product, followed by a navigation bar that lists Mincubators currently connected to the platform. All the UI and styling was created from scratch through HTML and with the use of an already available CSS.

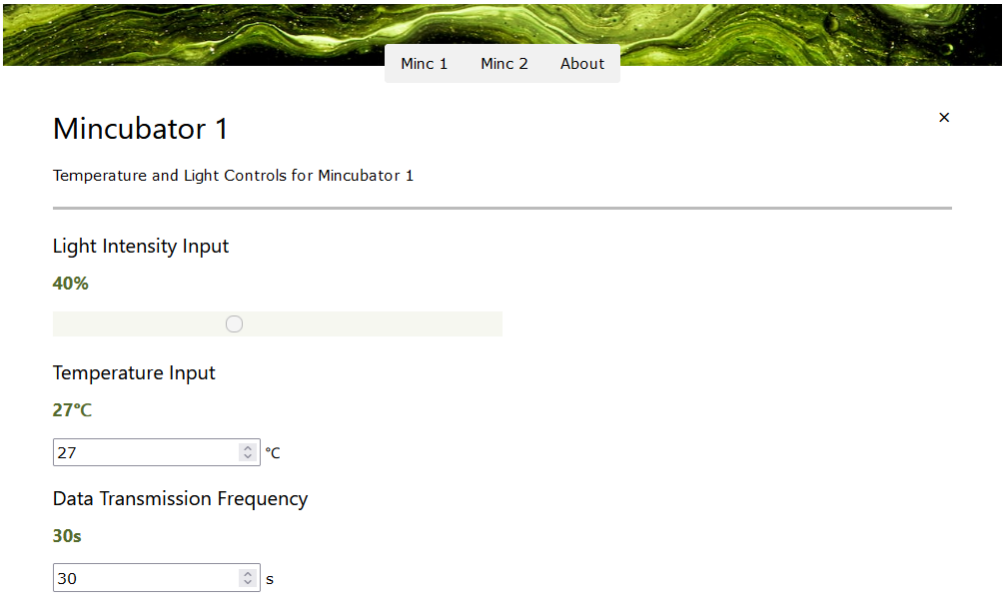

Figure 31. Setting Mincubator temperature and light parameters

The navigation bar on the landing page would allow for multiple Mincubators to be added, which the user would then be able to select. When selecting any of the navigation bar elements, a new page appears which displays all of the relevant information and inputs for that Mincubator. In this case, the Mincubator 1 info page contains inputs for light intensity and as well temperature. Eventually another section would be implemented which would contain live temperature feedback/ plots for the Mincubator unit. The user can then exit out of that page by clicking the exit button on the top right of the screen. The user may also switch through different Mincubator pages by simply selecting them on the navigation bar.

Another improvement to the webpage would be the addition of more features and elements for each Mincubator. The website, in its current state, simply includes crucial features required for core functionality control. The overall goal was to create a webpage which had an aesthetic appeal to it, however without overcomplicating what the user interacted with. The website was designed to be user friendly without any required onboarding steps, a sharp contrast to a lot of already existing software which interfaces to hardware.

4.5 Functional Testing

4.5.1 Laboratory Procedure

To validate the functionality of the system, it was decided to cultivate an abundant algae species inside of one of the Mincubators and compare it against a control variable where the system is not implemented. This choice was made as the team struggled to identify a method to access zooxanthellae cells within a reasonable logistical and economic timeframe. As a result, chlorella vulgaris was selected as the algae species to emulate the effects of the cultivation system.

Chlorella vulgaris was selected due to numerous reasons. Firstly, chlorella vulgaris is widely available and can be obtained from many scientific supply companies. Additionally, it is a representative species commonly found in natural sources such as freshwater ponds and streams. Secondly, chlorella vulgaris is a fast-growing species of algae, with a doubling time of around 10-12 hours under optimal conditions. This makes it ideal for conducting experiments that require observing changes in growth over time. Thirdly, chlorella vulgaris is a unicellular organism, which makes it easy to work with in the lab. It can be easily cultured in a small volume of liquid, and individual cells can be easily observed under a microscope, which makes it highly desirable for it to be cultured inside a T25 culturing flask. Fourthly, chlorella vulgaris is known to have relatively simple nutritional requirements, which makes it easy to culture in a laboratory setting. Overall, chlorella vulgaris is a versatile and easy-to-work-with algae species that can be used in a wide range of experiments in a laboratory setting. [31]

With Chlorella Vulgaris as the selected algae species, a laboratory procedure was created to ensure that proper laboratory standards were followed during the testing phase of the system.

Materials:

[31] Allaguvatova, R., Myasina, Y., Zakharenko, V., & Gaysina, L. (2019). A simple method for the cultivation of algae Chlorella vulgaris Bejerinck. IOP Conference Series: Earth and Environmental Science, 390(1), 012020. https://doi.org/10.1088/1755-1315/390/1/012020

- 1x 50g Culturing salts
- 30x 1.5mL Cuvettes
- 1 mL Dense Chlorella vulgaris culture
- 500 mL deionized water
- 1 mL F/2 growth medium
- 2x T25 culturing flasks
- 1x Mincubator
- 1x Spectrophotometer
- 7x Sterile pipettes

Procedure:

1. Sterilize all equipment and materials that will come into contact with the Chlorella vulgaris culture to prevent contamination and compromise the microalgae.
2. Place 500 mL of distilled water into the beaker, then insert the package of culturing salts and the F/2 growth medium.
3. Using a sterile pipette, transfer 0.5mL of the Chlorella vulgaris culture into the T25 culture flask containing the growth medium. Close the cap upon completion. Repeat the same process to establish a control variable.
4. Incubate the culture in the Mincubator at a constant temperature of 27°C at light intensity 60% with a 12 hour lighting cycle.
5. After 24 hours, prepare the calibration process for the spectrophotometer by first setting the spectrophotometer to measure 680 nm and filling a cuvette with 1.5mL of deionized water.
6. Place the cuvette into the spectrophotometer and press the calibrate button until the displayed values are zeroed.
7. Thoroughly shake the two samples and pour 1.5 mL of each solution into their respective cuvettes.

8. Insert one of the samples into the spectrophotometer and wait until the output value stabilizes
9. Record the absorption value and the transmittance percentage on a spreadsheet and repeat the same process for the other solution.
10. Repeat steps 5-9 upon calibrating the spectrophotometer for the 750 nm wavelength.
11. Repeat steps 4-10 every 24 hours to monitor the growth rate of the culture over ten days.

Safety Precautions:

1. Follow proper aseptic technique to prevent contamination of the culture.
2. Wear gloves and a lab coat for protection when handling the chlorella vulgaris culture.
3. Dispose of all used cuvettes, culture vessels, and materials in a biohazard bag for proper disposal.

Spectrophotometer Overview

Spectrophotometers are instruments used to quantify the amount of light that is transmitted from a material, particularly by measuring the absorption of the sample at a specific wavelength. For this application, the spectrophotometer is being used to evaluate the growth of biological cells in the experiment as it allows for a quick and accurate measurement of cell concentrations in a sample. As microalgae cells grow and divide, the number of cells in the culture increases thus causing an increase in the overall absorbance of light. Through measuring the absorbance of the culture at regular intervals, it is possible to track the growth of the cells in order to determine the rate of growth of the sample.

Spectrophotometers work by measuring the amount of light that is absorbed at a specific wavelength by a sample. A light source at the top of the device emits a broad spectrum of light, usually from ultraviolet to visible wavelength part of the spectrum. That light then passes through a monochromator, or dispersion device, that produces a single light beam that goes through a wavelength selector, usually between 200 and 800 nanometers. The amount of light absorbed by a sample is determined through comparing the intensity of light that passes through the sample to the intensity of the light initially emitted by the light source which is readout on a digital display.

Spectrophotometer functionality depends on the Beer-Lambert law which states that when "a beam of light incidents on [a] homogenous solution, [it] reflects some fraction of incident light, absorbs some light and transmits the remaining light through the solution." [32] This law relates the amount of light absorbed by a sample to the concentration of the absorbing species in a sample, and quantifies that concentration. The equation for the Beer-Lambert law is displayed in Equation 2. Where A is the absorbance of the sample, ε is the molar absorptivity, c is the concentration of the absorbing species in the sample and l is the path length the light takes through the sample.

$$A = \varepsilon c l$$

Equation 2. Beer-Lambert Law.

The Beer-lambert law provides the theoretical basis for quantitative analysis when using a spectrophotometer. Transmittance and absorbance are related through a logarithmic relationship with absorbance being proportional to the logarithm of the ratio of incident light intensity to transmitted light intensity. By taking the logarithm of this ratio it's possible to convert a non-linear relationship between absorbance and concentration into a linear relationship which allows for the quantification of a concentration within a sample.

4.5.2 Experimental Results

In the results section, the absorption and transmittance of the two samples, both the unregulated and the regulated, are shown.

[32] N, S. (2019, December 11). What is a Spectrophotometer? Definition, Principle, Types & Components. *Biology Reader*. https://biologyreader.com/spectrophotometer.html

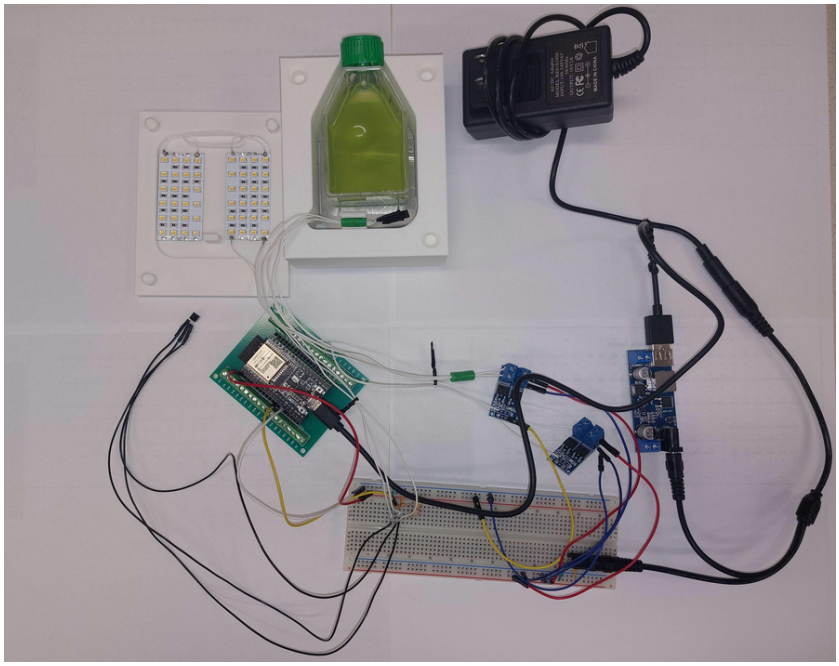

Figure 32. Mincubator Top-Down Physical Setup.

The data was taken at a wavelength of 680 nm and 750 nm. Wavelength is an important parameter as it determines the specific color or frequency of light that is used to measure the absorbance/transmittance of the sample. As different molecules and compounds absorb light at different wavelengths, when selecting the appropriate one it is possible to selectively measure the concentration of a specific compound in the sample. The wavelength values were selected based on existing scientific literature for the most representative wavelengths of chlorophyll cells, which are the green pigments responsible for photosynthesis. [33] The following were the results collected from the 10-day experiment based on the procedure established in the previous subsection.

[33]Belianin, V. N., Spirov, V. V., & Furiaev, E. A. (1975). Spektrofotometriia otdel'nykh kletok khlorelly [Spectrophotometry of individual Chlorella cells]. *Biofizika*, *20*(5), 848–852.

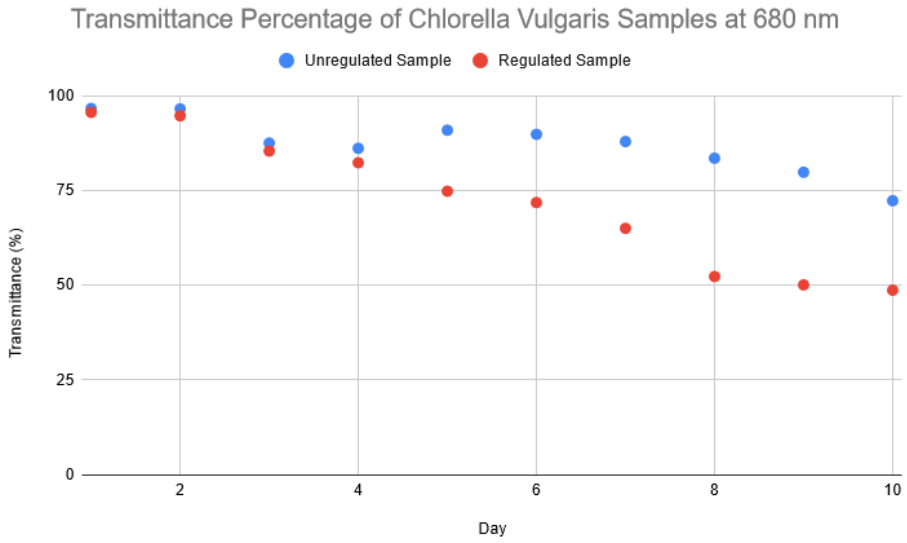

Figure 33. Experimental Transmittance Results at 680 nm.

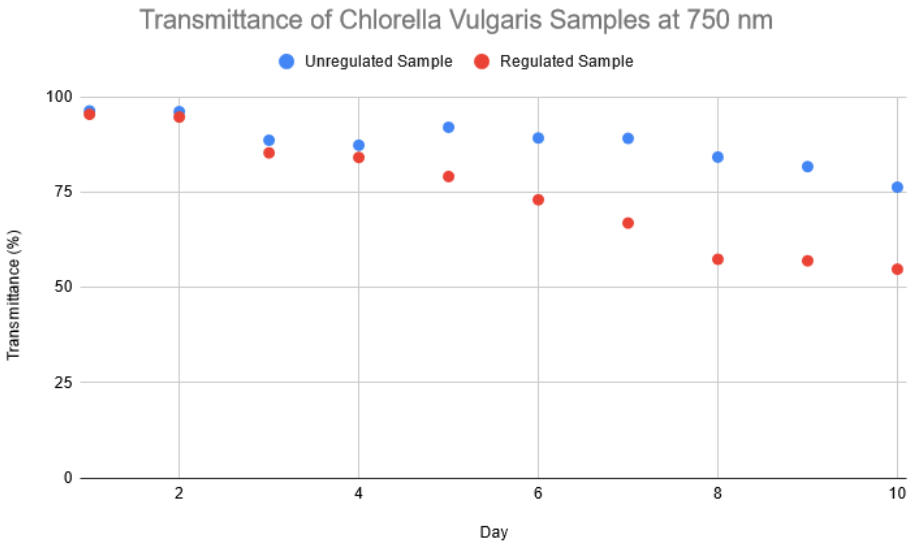

Figure 34. Experimental Transmittance Results at 750 nm.

In this 10-day experiment, chlorella vulgaris was cultivated and its transmittance was measured at 680 nm and 750 nm under unregulated and regulated conditions, with the regulated conditions referring to the sample placed inside the Mincubator system. The data shows a noticeable decrease in transmittance at 680 nm and 750 nm under regulated conditions, with the regulated sample showing lower transmittance values compared to the unregulated sample. This observation indicates that chlorella vulgaris is sensitive

to changes in light conditions, and that the application of regulated light may have led to decreased photosynthetic activity in the cells. This decrease in photosynthetic activity may be due to a number of factors, such as changes in light intensity, wavelength, or duration of exposure. Furthermore, the data shows a consistent trend of decreasing transmittance values over the course of the 10-day experiment, indicating that the cells were able to successfully grow and in turn block out the light measured by the spectrophotometer.

Figure 35. Comparison between Unregulated (C) and Regulated (T) Flasks after Experiment.

Temperature is another important factor that may have affected the results of this experiment. The regulated sample was maintained at a stable temperature of 27 degrees throughout the experiment, while the unregulated sample was susceptible to changes in ambient temperature. Temperature can have a significant impact on the growth and photosynthetic activity of chlorella vulgaris, as it is a thermophilic species that thrives in warm environments. Therefore, it is possible that the regulated sample may have exhibited lower transmittance values due to the constant temperature, as the cells may have been under more comfortable and warmer growing conditions. Conversely, the unregulated sample was exposed to

much lower temperatures at certain times during the experiment, which could have led to the cells being stressed due to their environment and higher transmittance values.

Additionally, the temperature values of the temperature inside the Mincubator and the ambient temperature were collected at a frequency of 30 minutes per datapoint and is shown in Figure 36.

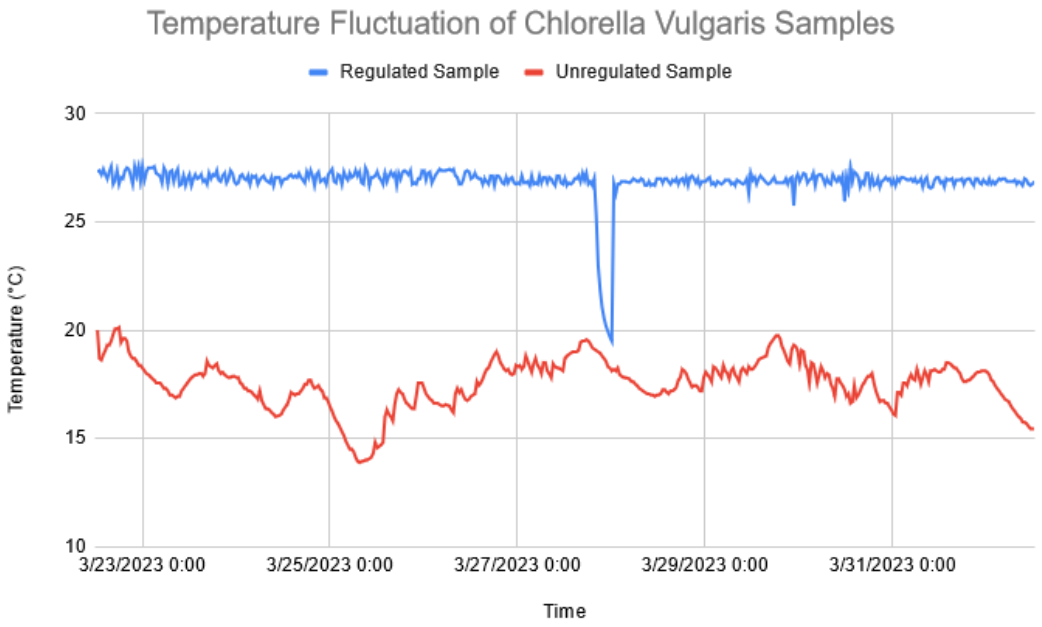

Figure 36. Experimental Temperature Data.

With the target temperature set at 27 °C, the Mincubator was able to self-correct and maintain the temperature of the system for the majority of the experiment. The only notable exception was when an electrical malfunction occurred on the fifth day of the experiment. Although the system was untouched during the data collection process except when taking daily measurements, this was suspected to be a wiring issue as it did not occur shortly after taking measurements from the culture samples.

When comparing the regulated temperature values to the ambient temperature, a noticeable difference was identified. Firstly, the average ambient temperature was at 17.5 °C, which is far below the regulated sample's 27 °C. Additionally, the temperature fluctuates depending on the time of day as well as

the weather. The effects of this are evident when comparing the transmittances between the two samples with the regulated sample having a 31.2% difference as compared to the unregulated sample for the 680 nm measurement on the eighth day of the experiment. Overall, these findings suggest that careful regulation of light conditions and temperature lead to a decrease in transmittance, thus higher cell concentration in the cultivation of chlorella vulgaris.

Discussion

5.1 Manufacturability and Cost

The intent of this project was to make this device open-source and simple to manufacture. The modular design and simplistic electrical components make this device a viable product for batch scaled production. During the development and production phase, the aluminum heat plate was the component that required the most integration. Both of these concerns were primarily due to the unfamiliarity of the components or the machinery necessary to manufacture them. As the manufacturing process is standardized for batch production, it is anticipated that both of these timesinks can be alleviated and the manufacturability of the Mincuabator would increase.

The cost of production for the Mincubator prototype is shown in Table 1.

	Component	**Cost of 1 Unit**	**Total Units**	**Total**
Electronics	LEDs	$45	1	$45
	Microcontroller	$9	1	$9
	Temperature Sensor	$1.67	2	$3.34
	Kapton Heater	$28.05	1	$28.05
	MOSFET PWM Drivers	$2	2	$4
	Power Supply	$13	1	$13
	5.5 mm Power Supply Splitter	$7	1	$7
	5.5 mm DC Barrel Jack	$0.95	1	$0.95
	DC-DC Buck Converter	$9	1	$9
Hardware	Aluminum Heat Plate		1	~$20
	3D Printed Encapsulation		1	~$40

Total				$179.34

Table 1. Final Bill of Materials.

The total cost of the Mincubator prototype was $179.34. When the materials are purchased in larger quantities for batch scale production, it is anticipated that this value can be further decreased.

5.2 Recommendations

5.2.1 Interface Upgrades

During the functional testing process, several features were identified to be beneficial if they were implemented in a future version of the web server interface.

Firstly, one of the features that would have increased the user accessibility of the system is a notification system when the temperature parameters fall below the expected range. A notification system is a crucial improvement for the Mincubator system, as it can alert the user in real-time if the temperature parameters fall below the expected range. This feature helps to ensure that the user is aware of any potential issues with the temperature inside the container, which is crucial for the cultivation process where the temperature needs to be maintained within a specific range to avoid damage or excessive stress on the cells. Without a notification system, the tester was not aware of the temperature drop that occurred on the fifth day of the experiment until several hours later, resulting in a potential increase in cell stress. Therefore, the implementation of a notification system is an important improvement to ensure the proper functioning and protection of temperature-sensitive microorganisms.

Another design upgrade would be the addition of live telemetry on the web server itself. Having live telemetry displayed on a web server interface would be beneficial to monitoring and troubleshooting the system in real-time. It allows the user to view the status of various parameters such as temperature, light intensity, and data transmission frequency, and track any changes or fluctuations that may occur. Currently, the temperature information is only sent to the spreadsheet via a JSON package based on the data transmission frequency inputted by the user. By having access to live telemetry, the user can quickly

identify any issues or abnormalities and take immediate action to rectify them instead of waiting for when the next package is due to be sent, thus preventing potential failures or downtime. Moreover, it doubles as a feature suitable for remote monitoring, which is particularly useful in cases where the user is unavailable or is not physically close to the hardware. Hence, having live telemetry displayed on a web server interface is an essential tool for efficient and effective system monitoring.

Lastly, to facilitate stronger troubleshooting capabilities, the ability to instantly send a JSON package of the most up-to-date telemetry to the spreadsheet on the web server would be a great addition. When monitoring systems or processes, any delay in data acquisition or processing could result in a missed data point when conducting an academic study. Having the ability to instantly ping telemetry data ensures that the data transmission feature is functioning properly, enabling the user to rest assured that the data sending process is operational. By including an instant ping system on the web server interface, the user can respond quickly to changes or anomalies, preventing system failures or downtime and ensuring the smooth functioning of the Mincubator.

5.2.2 Electronics

One of the anomalies during the functional testing process was the suspected electrical malfunction that occurred midway of the cultivation experiment. In the prototype model, a breadboard was used as the primary method to establish connections between the interfaces as a proof-of-concept, for future models. A method to alleviate this risk is to implement a custom printed circuit board (PCB) to reduce the number of wires in the system.

A custom PCB is a significant improvement over a breadboard solution in many ways. Firstly, a custom PCB provides a more permanent and robust solution as it is designed to be mounted directly onto the system or equipment it serves. In contrast, a breadboard is a temporary solution that is intended for prototyping or testing. Secondly, a custom PCB can be designed to meet specific requirements, such as size, shape, and functionality, which can be optimized to reduce the overall size of the electronics compartment. This results in a more compact and efficient solution with a reduced footprint. Thirdly, a

custom PCB can be designed with built-in features, such as connectors, power supplies, and signal conditioning, which can simplify the installation process and reduce the need for external components. Lastly, a custom PCB can offer a more reliable and error-free solution as it eliminates the risk of loose connections or accidental short circuits, which are more common in breadboard solutions. Overall, a custom PCB is a significant improvement over a breadboard solution as it offers a more permanent, optimized, and reliable solution that is tailor-made to meet specific requirements.

In addition another review of the electronics should be conducted to simplify the system further. For example the PWM Drivers selected occupy quite a bit of space and could be simplified, instead of having a unique breakout board for the drivers the components on the board could be integrated into the custom PCB. Smaller transistors that meet the design criteria could also be selected to minimize the size of the system and the terminal block connectors are not necessary to maintain the electrical interface as they could be replaced with a single wire connector unit with multiple inputs.

Moreover, it would be worthwhile to explore active cooling elements for the system. While the team can reliably control heating to a certain temperature, there is no control on decreasing the temperature of the system. Earlier in the report the use of a peltier module to aid in both active heating and cooling was discussed, however that was deemed to be too unstable and inefficient. It would however be interesting to explore what changes to the design could be made to contribute to system cooling, whether that be something analogous to the peltier or not.

Another possible improvement would be condensing all the wires interfacing to the Mincubator to a single connector. This connector could then be plugged into the bottom part of the Mincubator assembly and internally there could be a power distribution unit that routed all the required signals to the correct components without the need for wires that extrude out of the design. This would improve the overall design because it would decrease the number of unfettered wires.

5.2.3 Hardware Materials

With future design iterations the material that the assembly was manufactured with would change from PLA to steel. This would allow for better thermal insulation of the internal Mincubator chamber thus requiring less power to keep the system thermally stable. An alternative solution could be to use a different plastic over PLA to 3D print the system. This would need to be a material with thermal properties that would not allow for heat to dissipate out of the T25 culture flask chamber. Another possible design improvement would be to allow for slots where a material such as aerogel could be placed to act as a barrier between the internal chamber and the external environment. Aerogel's excellent thermal insulation properties come from its unique structure that doesn't allow for any air pockets on a nanoparticle level. It also has low thermal conductivity which makes it an excellent insulator while also not allowing for any conduction of electrical energy. These improvements would be done to enhance the insulation of the system to allow for less heat loss and therefore not require the heater to be less of an electrical load on the system.

In terms of hardware design an area the team would consider further improving upon would be designing some form of socket so that multiple units can be connected to, or stacked upon, one another. This would allow for a more complete system that could be condensed to one space and be secure enough that things don't bump into one another and come apart. Therefore if a user would like multiple incubator systems they could all interface with one another in a stationary and secure manner.

Finally adding hinges to the system would be beneficial so that a user doesn't need to remove the top lid of the hardware each time they would like to replace a flask or remove one and put another one in for data collection. This way the wires could stay put as well each time the chamber is opened and would improve the overall user experience.

Conclusion

The goal of this project was to create a low-cost incubator accessory capable of providing temperature regulation and lighting to facilitate the growth of phototrophic cells adaptable to a larger carbon dioxide incubator setup or on a lab bench. This goal was met as the final design of the project was able to allow the user to control the inputs of the Mincubator from a digital interface. The design specifications for the system were formed based on researching commercial incubator solutions and insights from scholars pioneering in the field of marine microalgae. When the user selects a set temperature and light intensity for their experiment, the Mincubator was able to maintain a set temperature by generating adequate heating and outputting a certain brightness by controlling the PWM of the two subcomponents. This user interface also allowed the user to control the rate of which data was being transmitted to a specific spreadsheet document, thus providing an accurate record of the state of the system at any period in time.

Without the Mincubator, researchers utilizing a standard incubator would not have the ability to micro-manage specific cultures to test more variables in the same batch. As there wasn't a flask-specific solution to control the temperature and light intensity received by the phototrophic cells. The Mincubator accomplishes this by offering an intelligent incubator accessory that allows for precise temperature control and monitoring, thus facilitating and potentially increasing the productivity of researchers in investigating phototrophic cells.

Although the Mincubator met the overall design objectives of the project, there were several areas that can be improved upon for better user experience and functionality enhancements. If the limitations and recommendations outlined are to be resolved, future versions of the Mincubator may be applicable to a wider range of design applications as a regulated incubator accessory and contribute towards the goal of accelerating academic cultivation and research processes.

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Appendix

Appendix A: Experimental Data

The following table shows the experimental results of the measurements taken by recording the absorption and transmittance values at 680 nm and 750 nm.

		Absorption				Transmittance (%)			
		Unregulated Sample		Regulated Sample		Unregulated Sample		Regulated Sample	
Day	Date	680nm	750nm	680nm	750nm	680nm	750nm	680nm	750nm
1	3/23/2023	0.015	0.016	0.021	0.022	96.6	96.3	95.6	95.4
2	3/24/2023	0.016	0.017	0.023	0.023	96.5	96.1	94.7	94.7
3	3/25/2023	0.058	0.053	0.068	0.069	87.5	88.6	85.4	85.3
4	3/26/2023	0.065	0.058	0.085	0.076	86.1	87.3	82.3	84.1
5	3/27/2023	0.041	0.036	0.126	0.102	90.9	92	74.8	79.1
6	3/28/2023	0.048	0.05	0.144	0.137	89.8	89.2	71.8	73
7	3/29/2023	0.056	0.051	0.187	0.174	87.9	89.1	65	66.9
8	3/30/2023	0.078	0.075	0.282	0.24	83.5	84.2	52.3	57.4
9	3/31/2023	0.099	0.088	0.301	0.244	79.8	81.7	50.1	57
10	4/1/2023	0.14	0.117	0.312	0.259	72.3	76.3	48.7	54.8

Appendix B: Software Code

```
// WiFi and IoT libraries
#include <WiFi.h>
#include <AsyncTCP.h>
#include <ESPAsyncWebServer.h>
#include "SPIFFS.h"
#include <HTTPClient.h>

// Temperature sensor libraries
#include <OneWire.h>
#include <DallasTemperature.h>



// WiFi and IoT variables
const char* ssid = "NETGEAR87";
const char* password =  "unusualbutter356";
const char* telemetryServer =
"https://maker.ifttt.com/trigger/telemetry/json/with/key/cHTEwjPfFYDMqEMYk
KZean";
unsigned long lastTime = 0;
unsigned long intervalTime = 1800000; //30 mins

// Temperature sensor variables
const int TempPin = 14;  // Arduino pin connected to DS18B20 sensor's DQ
pin
OneWire oneWire(TempPin);             // setup a oneWire instance
DallasTemperature tempSensor(&oneWire);  // pass oneWire to
DallasTemperature library
float tempCelsius1;// temperature 1 var
float tempCelsius2; //ambient temp var

const int LEDPin = 16;

unsigned int relayState = LOW;
long onTime = 43200000; //in milliseconds
long offTime = 43200000;
unsigned long previousMillis=0;

// HTML interface variables
```

```
String sliderValue = "0";
String tempValue = "27";
String timeValue = "1800000";

// setting PWM properties
const int PWMFreq = 5000;
const int PWMChannel = 0;
const int PWMResolution = 8;
const int MAX_DUTY_CYCLE = (int)(pow(2, PWMResolution) - 1);
unsigned int intensityVal = 5;
unsigned int intensity = 0;

const int PWMFreqHeat = 5000; /* 5 KHz */
const int PWMChannelHeat = 1;
const int PWMResolutionHeat = 8; //max val of 255
const int MAX_DUTY_CYCLE_HEAT = (int)(pow(2, PWMResolutionHeat) - 1);
// Heater variables
int desiredTemp = 27;
const int HeatPin =  18;

const char* PARAM_INPUT = "value";

// Create AsyncWebServer object on port 80
AsyncWebServer server(80);

// Replaces with button section in your web page
String processor(const String& var){
  //Serial.println(var);
  if (var == "SLIDERVALUE"){
    return sliderValue;
  }
  return String();
}

void setup(){
  // Serial port for debugging purposes
  Serial.begin(115200);

  tempSensor.begin();
```

```
  // configure LED PWM functionalities
  ledcSetup(PWMChannel, PWMFreq, PWMResolution);
  ledcAttachPin(LEDPin, PWMChannel);
  ledcWrite(PWMChannel, sliderValue.toInt());

  ledcSetup(PWMChannelHeat, PWMFreqHeat, PWMResolutionHeat);
  ledcAttachPin(HeatPin, PWMChannelHeat);

  if(!SPIFFS.begin()){
        Serial.println("An Error has occurred while mounting SPIFFS");
        return;
  }

  // Connect to Wi-Fi
  WiFi.begin(ssid, password);
  while (WiFi.status() != WL_CONNECTED) {
    delay(1000);
    Serial.println("Connecting to WiFi..");
  }

  // Print ESP Local IP Address
  Serial.println(WiFi.localIP());

  // Route for root / web page
  server.on("/mincubator.html", HTTP_GET, [](AsyncWebServerRequest
*request){
    request->send(SPIFFS, "/mincubator.html", "text/html");
    // request->send(SPIFFS, "/algae.jpg", "image/jpeg");
  });

  // Send a GET request to <ESP_IP>/slider?value=<inputMessage>
  server.on("/slider", HTTP_GET, [] (AsyncWebServerRequest *request) {
    String inputMessage;
    // GET input1 value on <ESP_IP>/slider?value=<inputMessage>
    if (request->hasParam(PARAM_INPUT)) {
      inputMessage = request->getParam(PARAM_INPUT)->value();
      sliderValue = inputMessage;
      intensity = sliderValue.toInt();
      ledcWrite(PWMChannel,intensity);
    }
```

```
    else {
      inputMessage = "No message sent";
    }
    Serial.println(inputMessage);
    request->send(200, "text/plain", "OK");
  });

    // Send a GET request to <ESP_IP>/slider?value=<inputMessage>
  server.on("/tempin", HTTP_GET, [] (AsyncWebServerRequest *request) {
    String inputMessage1;
    // GET input1 value on <ESP_IP>/slider?value=<inputMessage>
    if (request->hasParam(PARAM_INPUT)) {
      inputMessage1 = request->getParam(PARAM_INPUT)->value();
      tempValue = inputMessage1;
      desiredTemp =  tempValue.toInt();
      Serial.print(desiredTemp);
    }
    else {
      inputMessage1 = "No message sent";
    }
    request->send(200, "text/plain", "OK");
  });

  server.on("/timein", HTTP_GET, [] (AsyncWebServerRequest *request) {
    String inputMessage2;
    // GET input2 value on <ESP_IP>/slider?value=<inputMessage>
    if (request->hasParam(PARAM_INPUT)) {
      inputMessage2 = request->getParam(PARAM_INPUT)->value();
      timeValue = inputMessage2;
      intervalTime =  timeValue.toInt() *1000;
      Serial.print(intervalTime);
    }
    else {
      inputMessage2 = "No message sent";
    }
    request->send(200, "text/plain", "OK");
  });

  // Start server
  server.begin();
```

```
}

void loop() {
  // put your main code here, to run repeatedly:
  LEDControl();
  HeatControl();
}

void WiFiTransmission() {
  if (WiFi.status() == WL_CONNECTED) { //Check WiFi connection status
    if ((millis() - lastTime) > intervalTime) {
      HTTPClient http;

      http.begin(telemetryServer);  //Specify destination for HTTP request
      http.addHeader("Content-Type", "application/json");
//Specify content-type header

      String httpRequestData = "{\"temperature sensor 1\":\"" +
String(tempCelsius1) + "\",\"temperature sensor 2\":\"" +
String(tempCelsius2) + "\"}";
      // Send HTTP POST request
      int httpResponseCode = http.POST(httpRequestData);

      if (httpResponseCode > 0) {
        String response = http.getString();                       //Get
the response to the request
        Serial.println(httpResponseCode);   //Print return code
        Serial.println(response);           //Print request answer
      } else {
        Serial.print("Error on sending POST message: ");
        Serial.println(httpResponseCode);
      }
      http.end();  //Free resources
      lastTime = millis();
    }
  } else {
    Serial.println("Error in WiFi connection");
  }
}
```

```
void LEDControl(){
  if(Serial.available() > 2) {
    intensityVal = Serial.parseInt();
    Serial.println("Intensity:" +String(intensityVal));
  }
  long currentMillis = millis();
  if ((relayState==HIGH)&&(currentMillis-previousMillis>=onTime))
  {
    relayState = LOW;// turn it off
    previousMillis = currentMillis;   // Remember the time
    ledcWrite(PWMChannel, 0);     // Update the actual relay
  }
 else if((relayState==LOW)&&(currentMillis-previousMillis>=offTime))
  {
    relayState=HIGH;  // Turn it on
    previousMillis = currentMillis;  // Remember the time
    // intensity = (intensityVal*0.1)*MAX_DUTY_CYCLE;
    Serial.println(intensity);
    ledcWrite(PWMChannel, intensity);  // Update the actual relay
  }
}

void HeatControl(){
  tempSensor.requestTemperatures();
  tempCelsius1 = tempSensor.getTempCByIndex(0);
  tempCelsius2 = tempSensor.getTempCByIndex(1);
  WiFiTransmission();
  Serial.println(tempCelsius1);

  if (tempCelsius1 > desiredTemp -0.2)
  {
    ledcWrite(PWMChannelHeat, 0);     // Update the actual relay
  }
 else if (tempCelsius1 < desiredTemp -0.25)
  {
    Serial.println("Heating!");
    ledcWrite(PWMChannelHeat, 0.75*MAX_DUTY_CYCLE_HEAT);  // Update the
actual relay
  }
}
```