ARES DemoSat Final Report

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1 Mission Overview

1.1 Introduction

Previous studies have shown that bacteria become more resistant to antibiotics in space. However, no conclusion as to why this happens has been made. Our team wants to examine a possible cause as to why bacteria are more antibiotic-resistant in space. We believe that bacteria become more antibiotic-resistant due to an increase in biofilm production. Biofilm is a dense microcolony of bacteria that helps the bacteria survive in unfavorable environmental conditions, such as in the presence of antibiotics. To observe how biofilm production may increase in space, we measured one of the products, ammonia, from a naturally occurring enzymatic reaction, shown in figure 1, in nonpathogenic Escherichia coli K12. While there are three products of this reaction, indole, ammonia, and pyruvate, only ammonia was measured due to the conditions that needed to be met for the Colorado Space Grant Consortium's (COSGC) DemoSat High Altitude Balloon Launch. The products of this reaction inversely correspond to the bacteria's biofilm formation rate: if the concentration of the products decreases in microgravity, the biofilm formation increases. If the biofilm formation increases in space, the bacteria may have a higher survival rate in the presence of antibiotics.

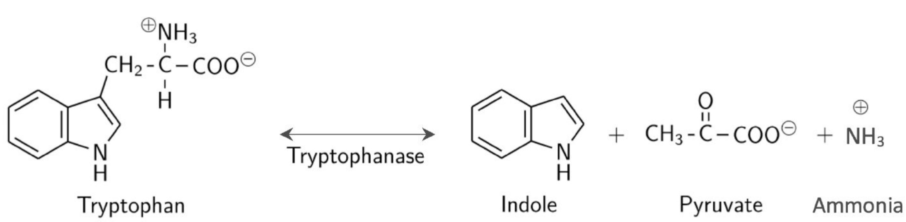

Figure 1: Enzymatic Reaction

1.2 Purpose and Goals

The purpose of this project is to give a possible explanation as to why bacteria are more antibiotic-resistant in space. We also want to examine the biological and chemical processes of bacteria in space, how they change, and how they could be related to antibiotic resistance. The discoveries of this project will hopefully serve as a gateway for further long term research on this topic, making future space travel safer.

1.3 Requirements

Below are the structural and interfacing requirements needed to launch. Structural Requirements:

- Mass does not exceed 1 kg
- Integration of washers on the outer top and bottom of the payload(where the flight tube will be fed through)
- Center of gravity of payload is as close to the flight string as possible

Hardware/Interfacing Requirements:

- All electrical components are self-contained
- Include an adequate thermal system for payload
- Integration of polyethylene flight tube with 3/8 " inner diameter

Structural, Environmental, and Functional testing were also required prior to launch. Structural testing consisted of the Whip Test, Drop Test, and Stair Pitch Test. Environmental testing consisted of the Cold Test and the Vacuum Test. Functional testing consisted of turning on all systems for the duration of the flight, to ensure they were functional for this time period.

2 Design

2.1 Structural Design

The structural design of our external payload was fairly simple. A foam core exterior shell containing paneled insulation was used to create a cubic design that would hold all experimental components. This design was based on earlier DemoSat flight payloads, and allowed for the easiest integration of all other elements. Holes were cut in the top and bottom panels to allow for a tube to run through, which eventually would act as a guide for the flight string. Two additional holes and switches were included in a side panel which controlled the independent heating unit, and the Arduino Mega to run all other systems. LEDs were put into the holes above the switches to determine whether the respective systems were being powered properly. Solidworks models of the design can be seen in figures 2, 3, and 4.

The design of our experimental containers was much more involved, since a greater number of variables such as pressure differences and internal electrical sensors were in play. Our final design used two polystyrene jars with a hermetic seal running through their respective lids. These seals allowed for us to run electrical sensors powered by an Arduino Mega through a theoretically airtight environment, letting us receive accurate Ammonia sensor readings. In order to make these containers as airtight as possible, we used a silicone seal and placed it under the lip of each jar in addition to hot gluing the sides and the internal and external attachments to the hermetic seals.

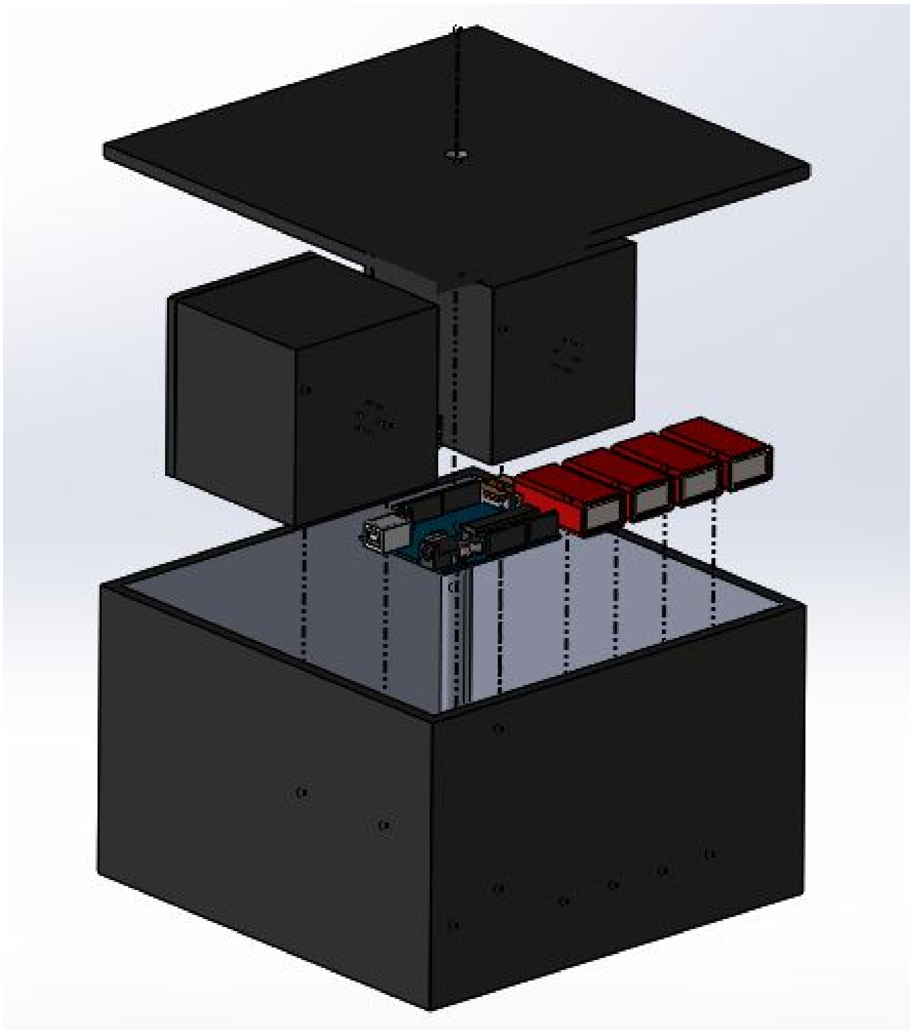

Figure 2: Early Solidworks Design of Payload

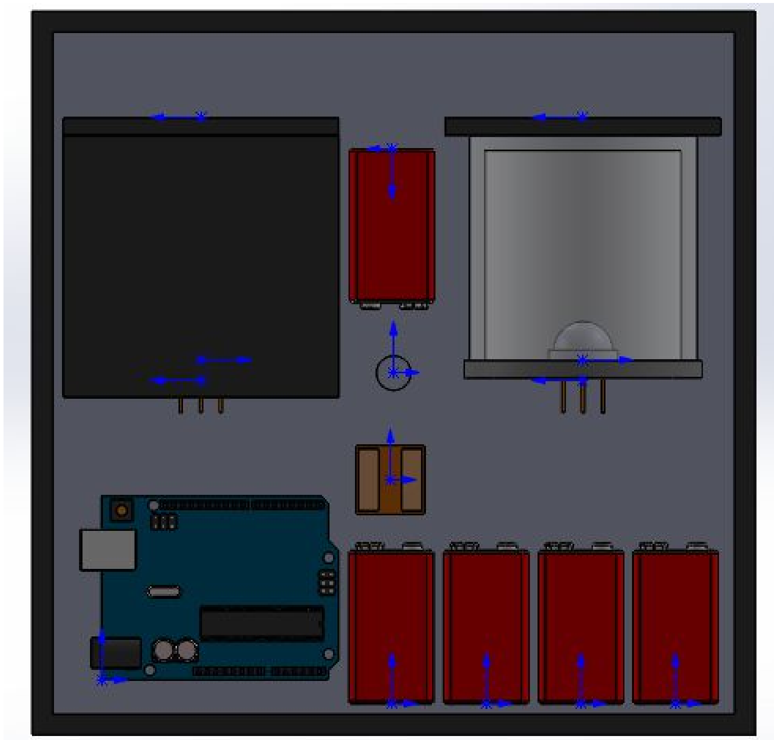

Figure 3: Top Down View of Early Solidworks Model

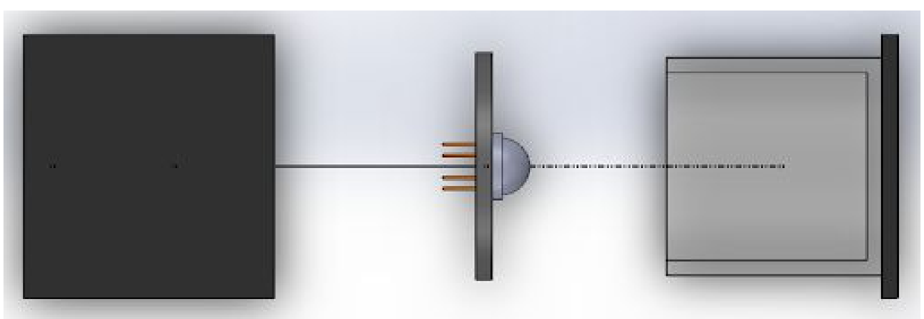

Figure 4: Early Solidworks Model of Experimental Containers

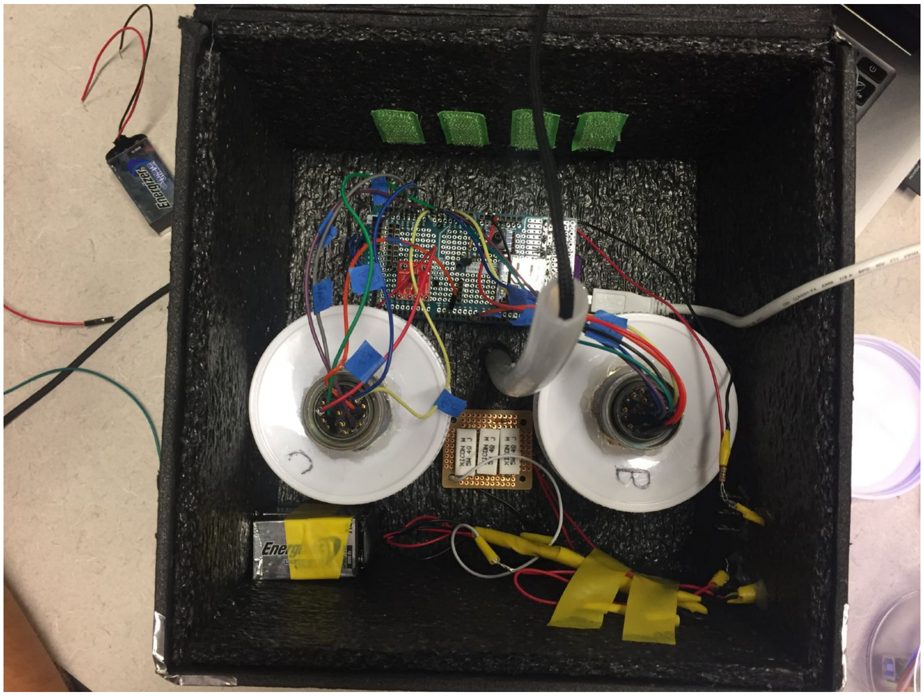

Figure 5: Systems integrated into Payload

2.2 Electrical Design

The electrical design of our experiment was broken up into three primary components: the electronics in the polystyrene jars, the external sensors included directly on the Arduino Mega shield, and the ceramic resistance heaters powered by three nine volt batteries. All systems were powered by an Arduino Mega. Beginning with the external sensors, an Arduino Mega Shield was created to house a pressure sensor, a temperature sensor, a three axis accelerometer, an SD card reader, and a Real Time Clock. This can be seen in figure 6. All of these were incorporated to give us general experimental readings and changes in payload conditions outside of the polystyrene jars. Each jar also contained a small electronics board with similar sensors powered by running wires through the respective hermetic seals and back to the Arduino.

In each container there was an Ammonia sensor, pressure sensor, temperature sensor, and a humidity sensor, shown in figure 7. These gave us readings on what the conditions were like in our pressurized containers, and whether or not they were suitable for the bacteria to produce ammonia. Finally, we included a separate power source for our ceramic heaters since they required more current than the Arduino outputted. This necessitated the addition of a second switch to the outside of the box, with the first describing the status of the Arduino and all sensors, and the second showing whether the heaters were powered and functional. Finally, the Arduino was powered by a rechargeable battery with an output of 10,000 mAh, more than enough to keep the system running for the three hour balloon flight.

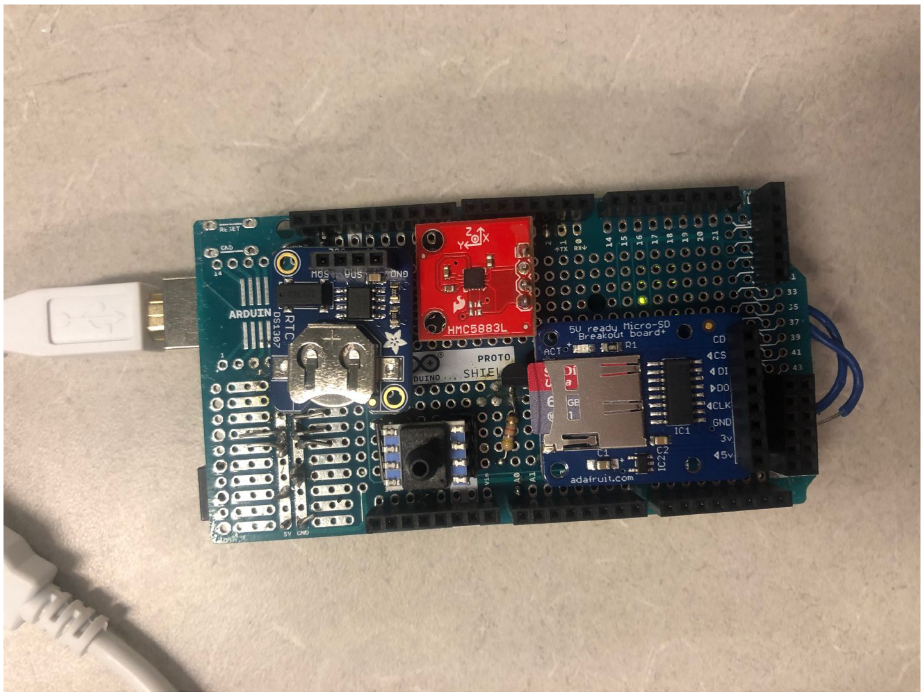

Figure 6: Sensors included on Arduino Mega Shield

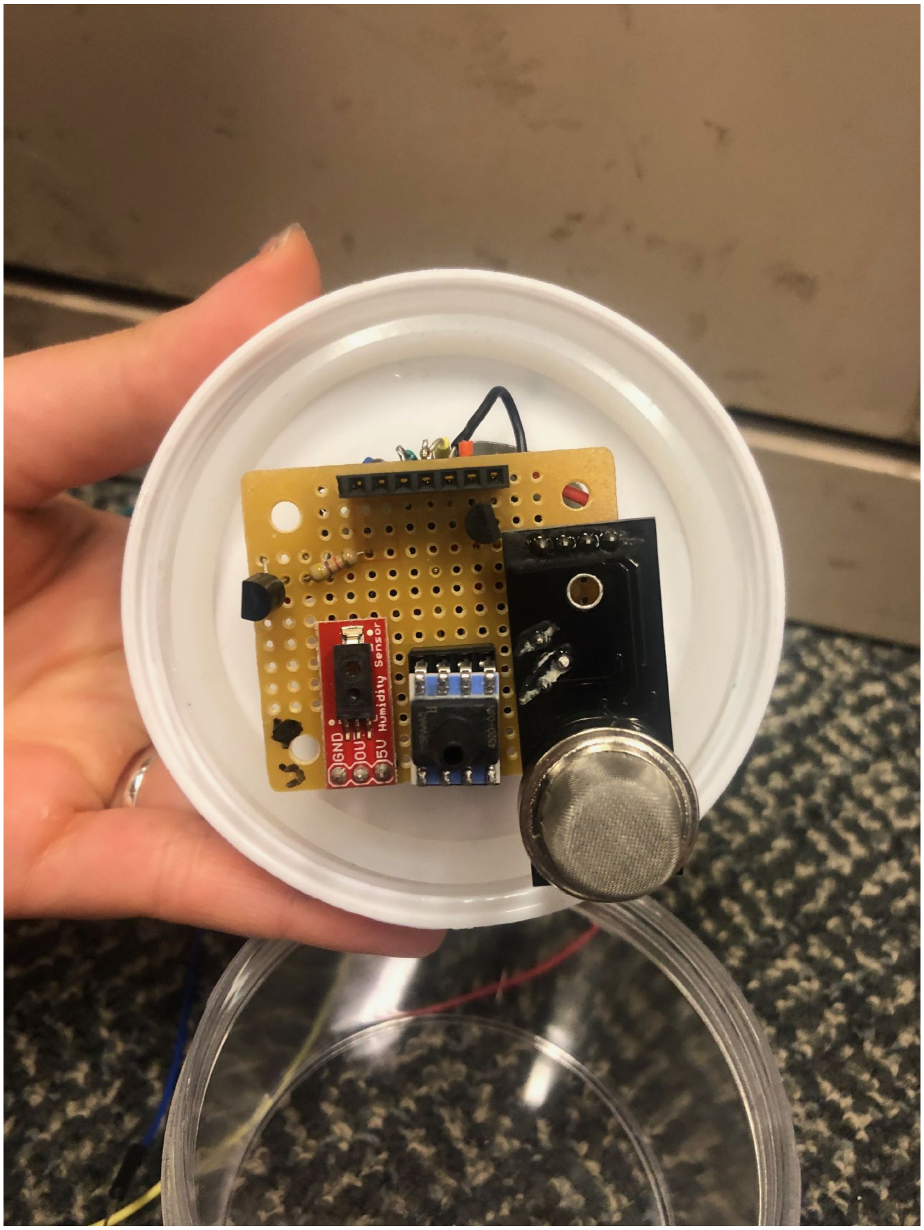

Figure 7: Sensors included within Polystyrene Jars

2.3 Software

Two types software were used in this experiment. The first was Arduino used to code the Arduino Mega and perform the experiment during flight. The second was MATLAB used to analyze the data received from the experiment. The code used for each sensor on the Mega shield and the smaller shields was basic. The most challenging sensors were the Ammonia sensors because they needed to be calibrated to fresh/clean air every time they were used. The code to calibrate the Ammonia sensors was simple, but the task of remembering to calibrate them was not. The ammonia sensors also had to preheat for at least 24 hours to get the most accurate results; The longer the duration the sensor preheated, the more accurate it was.

3 Testing

3.1 Bacteria Test Plan

65 μL of E. coli culture was added to 10 agar plates and incubated for 24 hours. These plates were used as the samples for the launch and testing prior to the launch. In the lab (68°F), ammonia production was measured from 6 of the plates for 3 hours/plate using two ammonia sensors. The bacteria was left out at room temperature before the ammonia testing began. After the 3 hours, the E.coli samples used were disposed of. After the ammonia production test, 2 of the samples were used for the cold test. For this test, the E. coli was removed from the incubator and sat at room temperature for 9.5 hours before being placed into the thermal chamber with dry ice for 4 hours. Following the cold test, a 10 hour functional test was completed using 1 of the E. coli samples that survived the cold test and an E. coli sample that was at room temperature for 9.5 hours prior to testing. The second sample of E. coli did not undergo the cold test.

3.2 Structural Test Plan

Three structural tests were performed on the payload to ensure it would survive during launch, burst, and landing. These tests included a drop test off of a balcony onto concrete to simulate a worst case scenario in landing, a stair test down multiple flights of stairs to represent a more gradual, rolling landing, and a whip test on a flight string to ensure the components inside the payload would not be damaged.

3.3 Environmental Test Plan

An important test that was performed was the pressure test. Multiple pressure tests were completed in the COSGC Bell Jar to determine if the biological containment units would hold the E. coli and ensure no ammonia would leak from the jars in a near vacuum.

A full systems test was performed to ensure all sensors and systems would function for the entirety of the launch. Two full systems tests were performed, the first one running for 4 hours, and the second one running for 10 hours, before both were manually shut off.

The cold test was also used to make sure all sensors and systems could function in extremely cold conditions. Four blocks of dry ice were put into a thermal chamber with the payload and all systems operating for four hours.

4 Test Results

4.1 Bacteria Test Results

Due to complications with the ammonia sensor in Jar B, data from the E. coli samples in Jar C (3 samples) were used for the results of the ammonia production test. The bacteria survived and produced ammonia throughout the entire test. The highest level of ammonia measured was 3.58 ppm (figure 10). During the cold test, the E. coli froze due to a malfunction with the heater failing to stay powered for the duration of the test. The inside of the thermal chamber reached a minimum temperature -36.5°C. The inside of the payload reached a minimum temperature of -7°C. When the E. coli was frozen, it did not produce ammonia. However, both samples survived the test. This conclusion was made when the plates thawed and the E. coli was able to produce ammonia again. Both samples of E. coli that were used for the functional test survived. Ammonia production was observed throughout the 10 hour test.

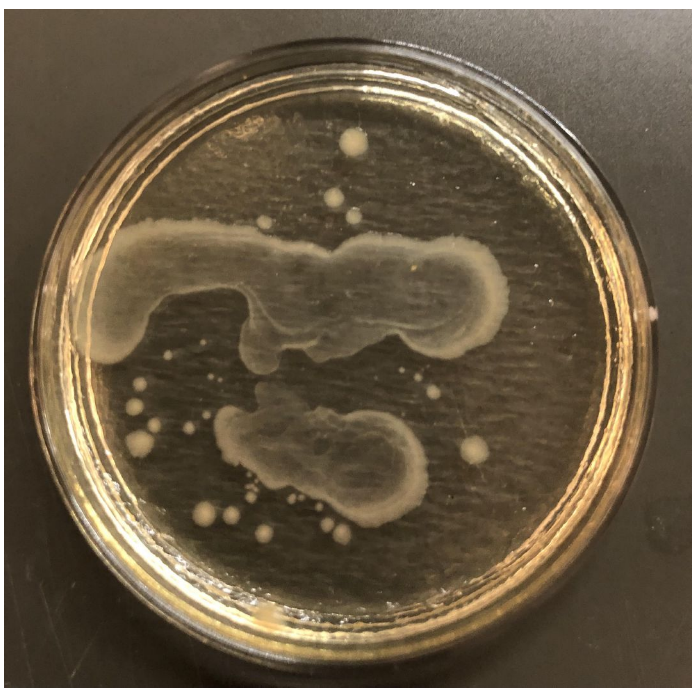

Figure 8: E. coli grown on Agar Plates

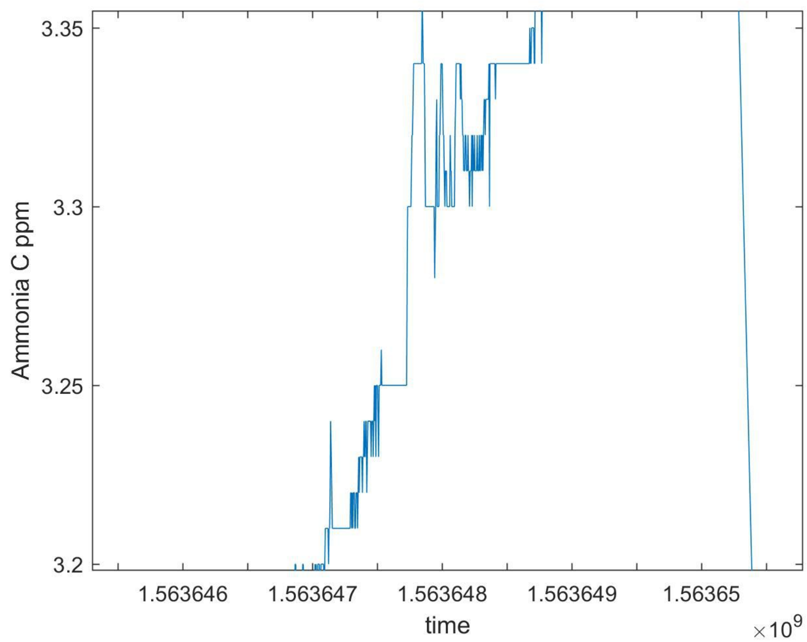

Figure 9: First graph of ammonia production from E. coli. Ammonia production was measured in ppm for 3 hours in a lab.

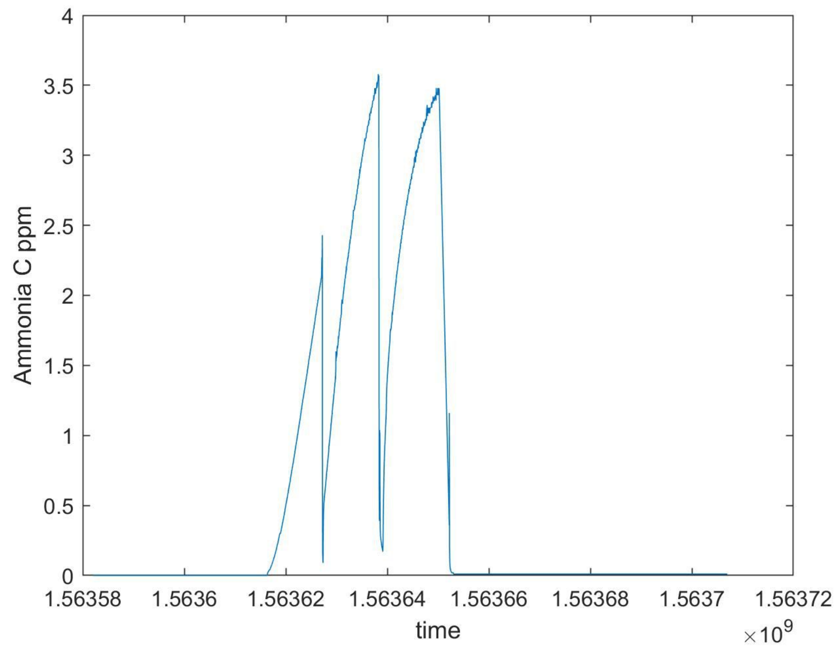

Figure 10: Second graph of ammonia production from E. coli. Ammonia production was measured in ppm for 3 hours in a lab.

Possible explanations of results of the ammonia production test include: the concentration of ammonia increased throughout the 3 hours because of ammonia buildup in the sealed jar or the E. coli produced ammonia as a waste product from another reaction. We would not be able to account for how much ammonia was produced from this other reaction. In the future, further testing needs to be done to draw a substantial conclusion about the ammonia test results.

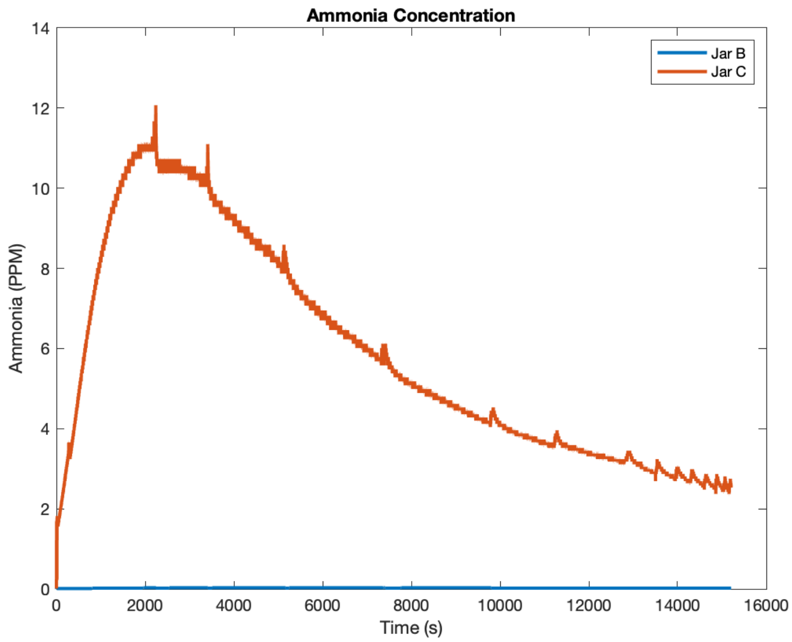

Figure 11: Ammonia production (measured in ppm) from the functional test. The E. coli that was used in the cold test was placed in Jar B and the E. coli that was not used in the cold test was placed in Jar C.

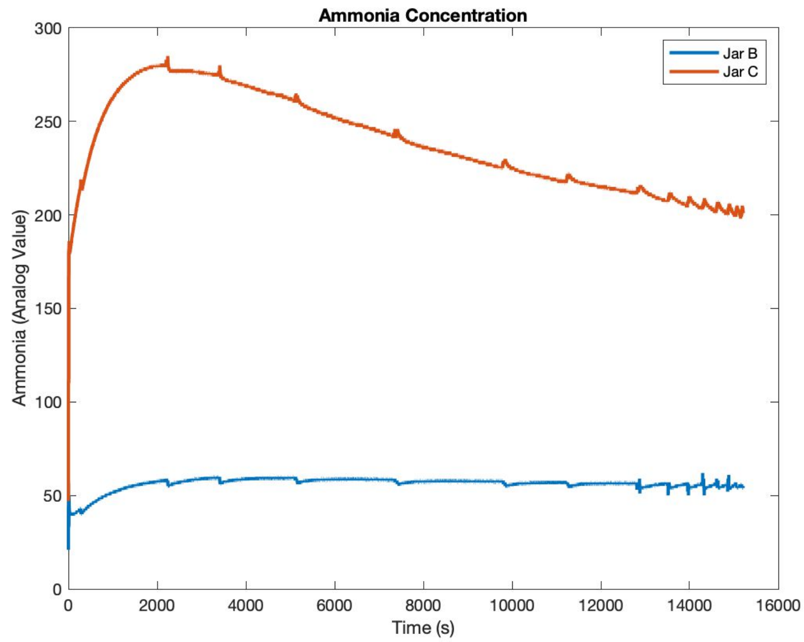

Figure 12: Raw data from the ammonia test.

Based on the data in Figure 11, it does not appear as though any ammonia was produced from the E. coli in jar B. However, based on the raw data shown in Figure 12, the E. coli did produce ammonia. These results suggest that the RO value used to calibrate the ammonia sensor in Jar B was not correct.

4.2 Structural Test Results

Our payload suffered minimal damage after the three structural tests were preformed. After the stair and drop test the payload bent in the corners. However, during the whip test, the systems remained intact. Images from all tests can be seen in figures 13, 14, and 15.

Figure 13: Damage from Stair Test

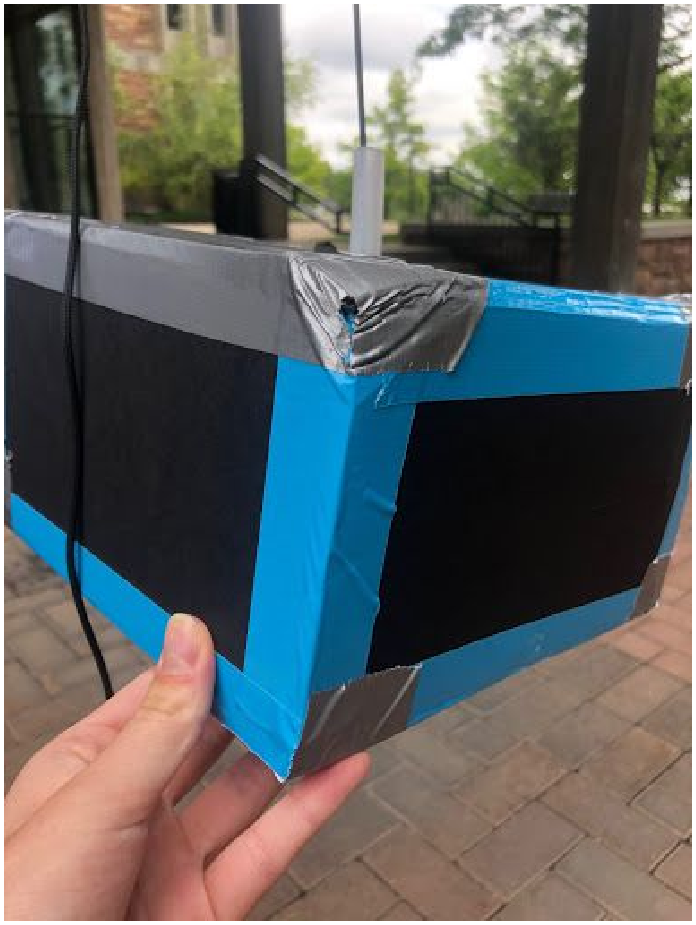

Figure 14: Damage from Drop Test

Figure 15: Whip Test

4.3 Environmental Test Results

During the vacuum test, all systems continued to work as the vacuum reached a low pressure of 0.2 psi. The polystyrene jars were not capable of being fully airtight, therefore internal pressure within the jars reached roughly 3.5 psi, and remained constant. This led to the conclusion that the polystyrene jars did leak some air, however, it did not allow any back into the sealed jar.

Multiple bell jar tests were completed with different methods to try to make the jars air tight, including silicone rings inside of the jar lids, o-rings placed between the hermetic seal and the lid of the jar, putting electrical tape and duct tape on the outside of the jars, seen in figure 16.

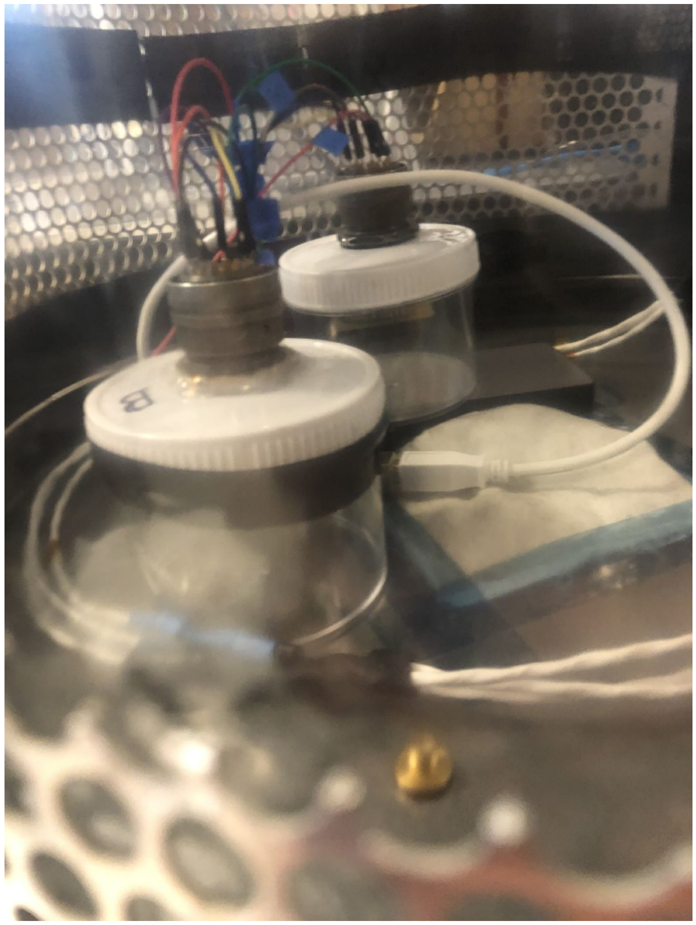

Figure 16: Jars with tape inside Bell Jar

One method that was tested was using petroleum jelly on the threads of the jar. This method worked very well but the lids were not strong enough to hold the pressure in the jars, so they cracked and this test failed as seen in figure 17.

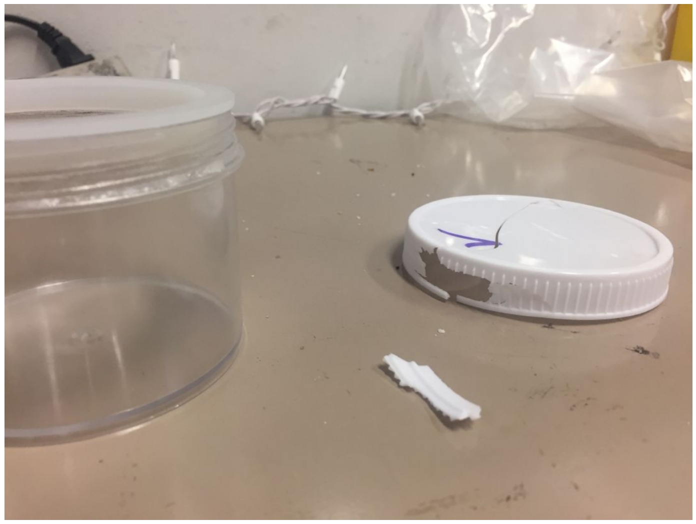

Figure 17: Jar lid failure

The final method of sealing was determined to be the silicone ring, o-rings, and hot glue around the hermetic seal and where the lid and jar meet, seen in figure 18. Though the petroleum jelly method worked the best, it was decided that air leakage was better than a chance of the lid failing, which would have caused mission failure.

Figure 18: Final method of sealing the jar

All systems worked for the duration of both functional tests. The first functional test used the ammonia packets to test the sensors. This test was four hours and used the portable battery to power everything except the heaters, which were not turned on. The second functional test was done overnight (10 hours) and used E. coli in both jars. This was done to see if we could get ammonia readings for extended periods of time. All sensors labeled "A" are on the Arduino, sensors labeled "B" are for the jar with the ammonia packet, and sensors labeled "C" are for the jar with the E. coli.

The graphs for the first test can be seen in figure 19.

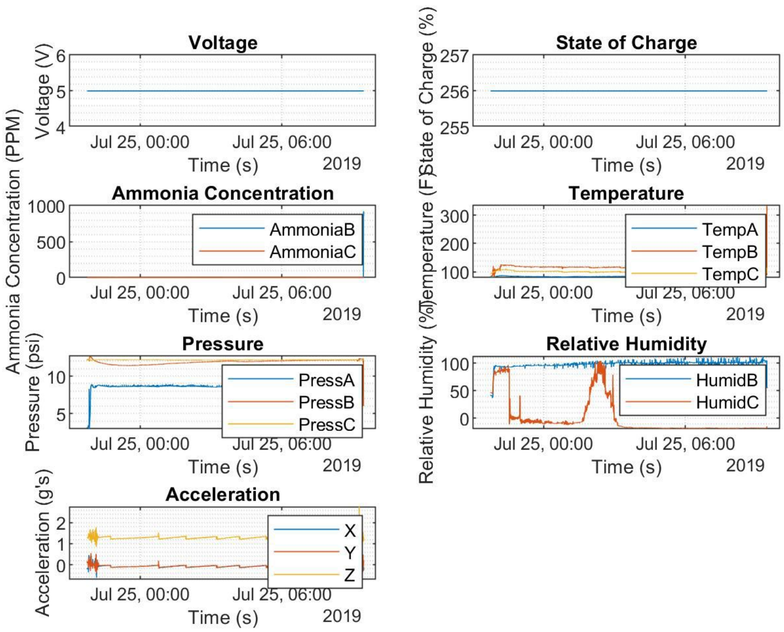

Figure 19: Graphs for Short Functional Test

The code converting the raw ammonia data to parts per million did not function properly but the raw data is shown in figure 20. Additionally, at this point, the team did not know the ammonia sensors needed to be calibrated each time they were used, and that the calibration value depends on both the sensor and how much it has been used. The same calibration value was being used for both sensors, providing the large difference in ammonia data shown.

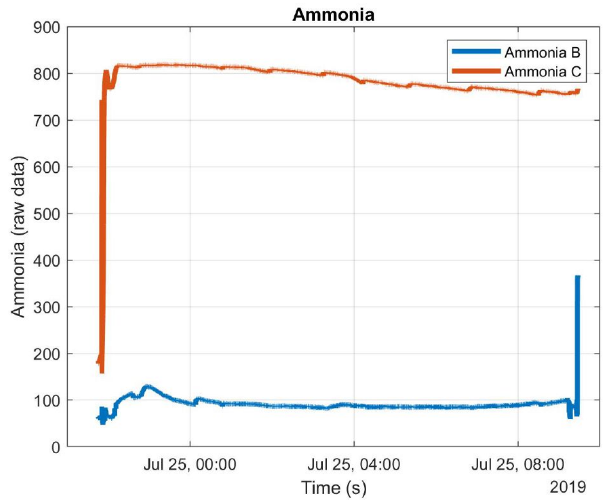

Figure 20: Raw Ammonia Data: Short Functional Test

The same graphs for the long functional test are shown in figure 21. The same ammonia code issue occurred so the raw ammonia data is shown in figure 12.

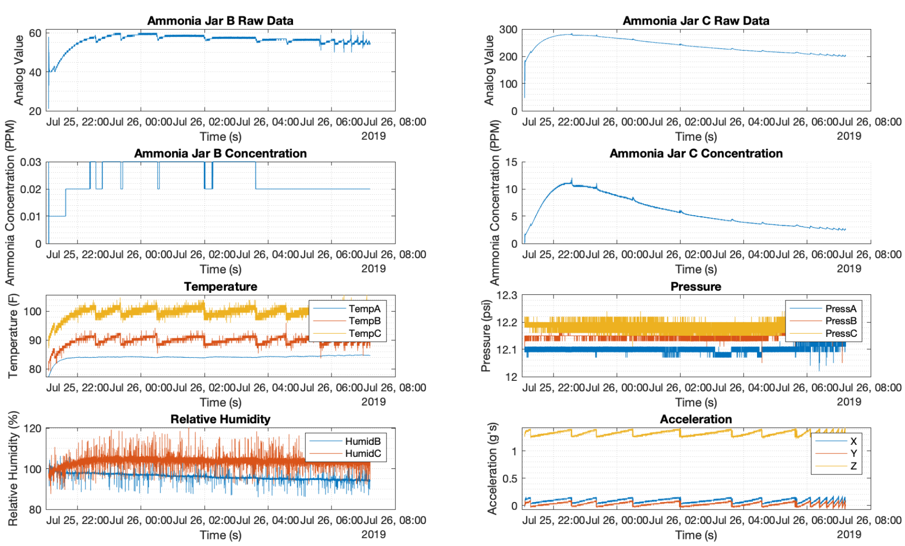

Figure 21: Graphs for the Long Functional Test

During the cold test, the thermal chamber reached a low of -36.5 °C. Within 15 minutes of testing, the batteries to the heater stopped working, causing our payload to not be heated throughout the majority of the test. Due to this, the bacteria samples and the real time clock froze, skewing data for this test. The readings for the ammonia sensors were reliable for the first 15 minutes of the test, however, the data was skewed after this time due to the bacteria samples freezing. The temperature reached a high of roughly 98 °F when the heater was powered. The pressure, humidity, and acceleration all remained constant throughout the duration of the cold test.

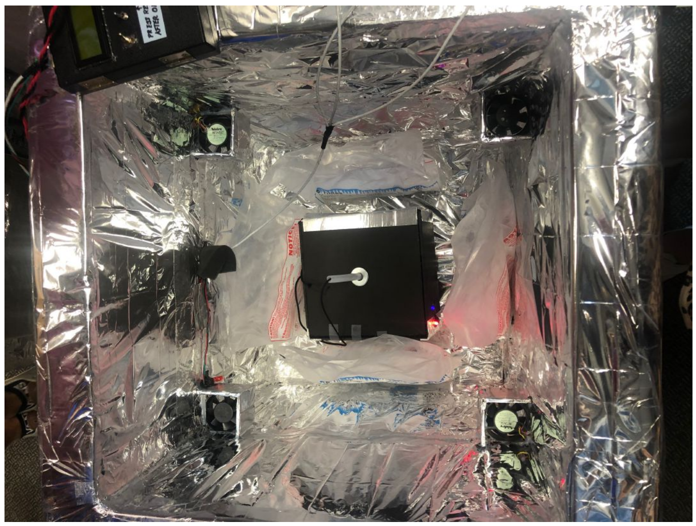

Figure 22: Payload in Thermal Chamber for Cold Test

5 Launch

The launch was on July 27, 2019 in Limon, Colorado. The balloon reached an altitude of 107,050 feet. Our payload was retrieved roughly three hours after launch in an Xcel Energy wind farm. Prior to the launch, the E. coli that would be used for the launch was removed from the incubator and placed in a sealed plastic bag at room temperature for 12 hours. This was done to mimic the conditions of the ammonia production test, cold test, and functional tests which also used E. coli that was at room temperature before testing began. This was also necessary due to the timing of the launch. The bacteria needed to be removed from the incubator the night before because we did not have access to the lab the morning of the launch.

Figure 23: Payload after Launch

Figure 24: Team with payload after launch

5.1 Launch Results

After the payload was retrieved, it was determined that all systems ran for the entirety of the flight. Immediate views of the internal and external structures revealed little to no damage, and all of the internal electrical components remained attached in the correct positions. Early analysis of the data revealed some interesting anomalies in the amounts of measured ammonia at different times in the flight. With the bacterial sample, large spikes occurred during burst, however, relatively constant values during both the release from the balloon and the impact with the ground were observed. When observing the raw collected ammonia data from both the bacterial jar and the jar with the ammonia packet, there were similar trends in ammonia production seen in both jars. More ground testing was needed to establish what might be causing these changes. With regards to the data received from other sensors, all the sensors worked well and produced data that was consistent with what we expected (based on previous weather balloon flight data). All of the plots from the flight are shown below.

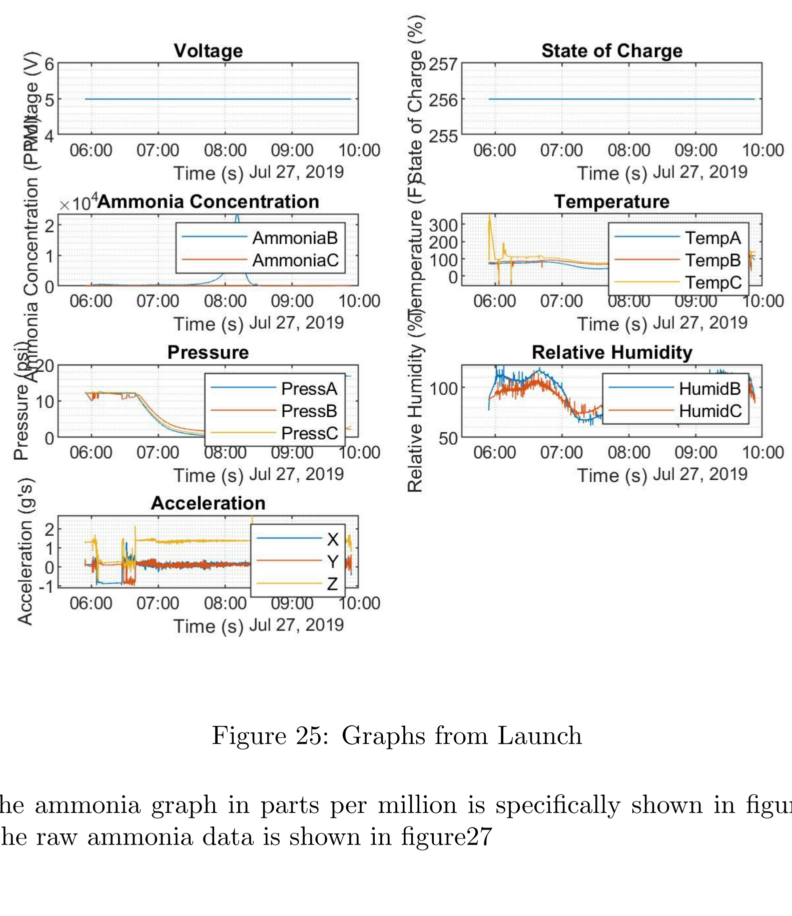

Figure 25: Graphs from Launch

The ammonia graph in parts per million is specifically shown in figure 26, and the raw ammonia data is shown in figure27

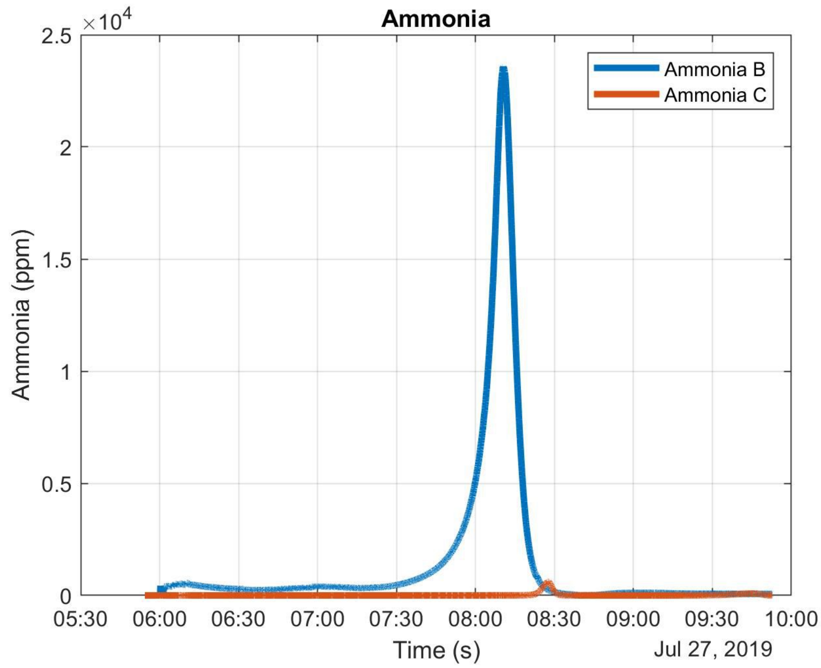

Figure 26: Ammonia Concentration (ppm) during Launch

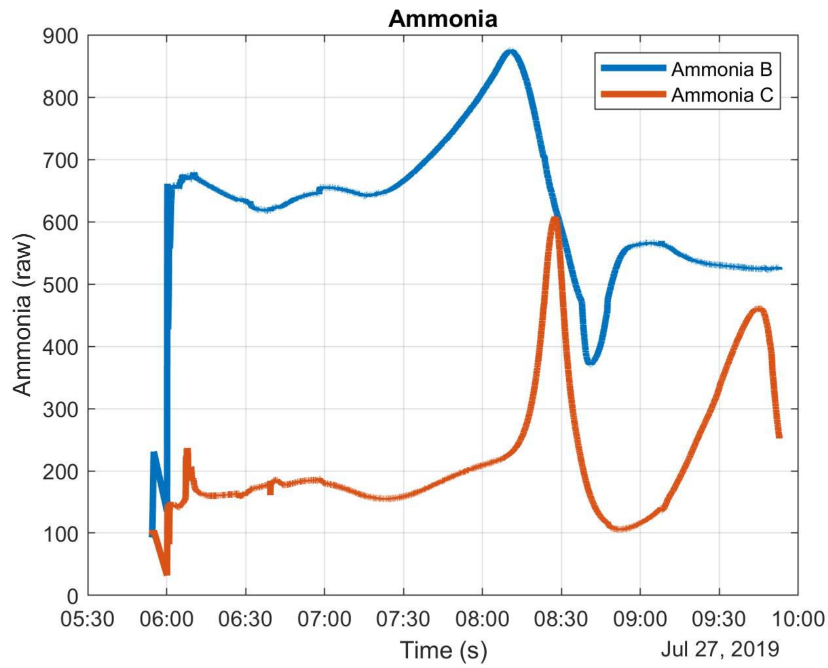

Figure 27: Ammonia Concentration (raw) during Launch

Comparing these graphs to the pre-launch testing shows that the E. coli, tested by ammonia sensor C, produced more ammonia during the launch.

The ammonia versus acceleration graph is shown in figure 28. The large spikes in the ammonia are around the same time as burst, between 8:00 and 8:30.

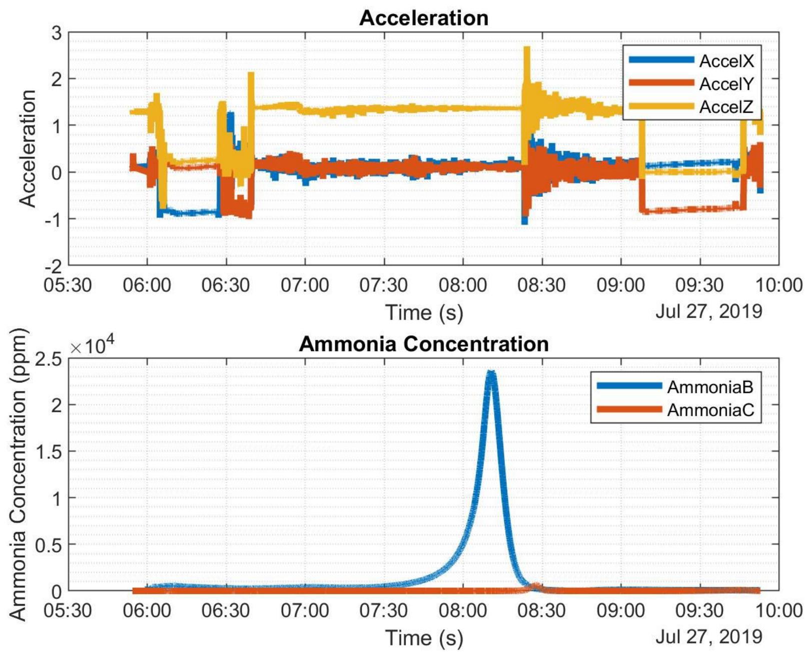

Figure 28: Ammonia compared to acceleration

6 Post Launch Testing

After launch, some testing was necessary to determine which, if any, of the environmental factors affected the ammonia sensors. Temperature, pressure, humidity, and shock tests using were performed to determine if any of these factors caused a significant change in the ammonia sensor readings. An ammonia packet was used for this test because it gave a constant ammonia reading. To get a constant reading, the packet was placed into the jar for 5 minutes prior to each test and then removed. In between tests, the jar was aired out for at least 10 minutes in order to prevent any ammonia build up.

The temperature testing was performed in the thermal chamber with dry ice alone followed by the heaters alone. This way the effects of extremely high and extremely low temperatures could be tested. The jars were left in the thermal chamber for 45 minutes per test. The pressure testing was performed in the COSGC Bell Jar. In this test, the pressure was brought down to 0.2 PSI then remained there for 30 minutes. The pressure and thermal test lengths were determined by the fact that the payload was not exposed to extremely high temperatures, low temperatures, or low pressure for extended periods of time due to the flight only lasting three hours total. Humidity testing was conducted in the thermal chamber in order to keep the humidity as constant as possible. A water diffuser was used to create the humidity. It was left on in the chamber with the jars for 20 minutes. The length of the humidity test was determined based on the fact that changes in humidity were seen 45 minutes into the DemoSat flight. The shock tests were very similar to the structural testing, the box was dropped down a flight of stairs and thrown off of a balcony. All post launch tests were performed three times.

Some difficulties arose when doing the post launch testing. The ammonia started corroding the wires after being tested so many times before, during and after launch and the results became inaccurate. More testing is planned to be done but the boards used in the jars need to be remade.

6.1 Post Launch Test Results

None of the tests yielded in significant changes in the ammonia sensor readings. The code for the conversion from the raw ammonia data to parts per million was not working, so the graphs below show raw ammonia concentration.

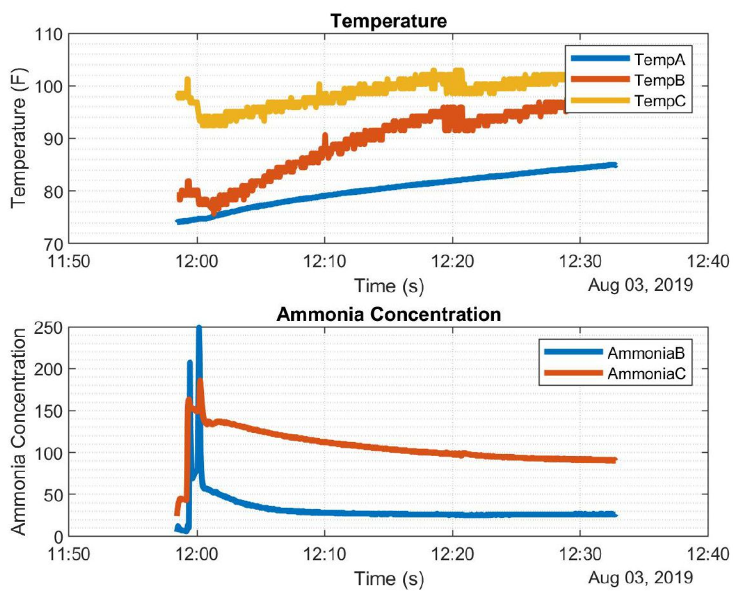

Figure 29: Temperature Test 1

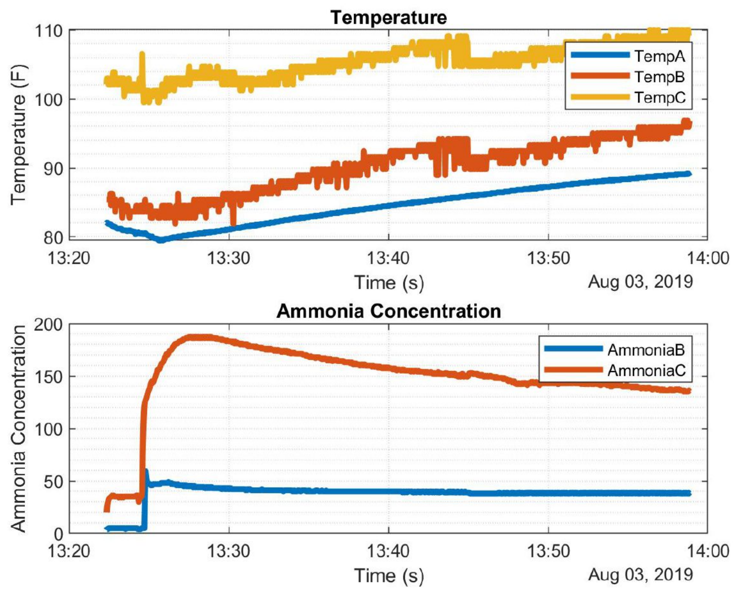

Figure 30: Temperature Test 2

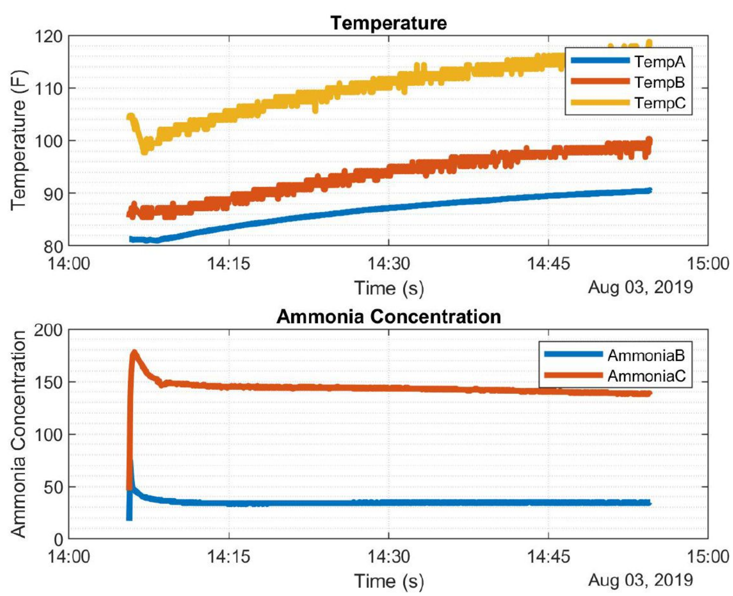

Figure 31: Temperature Test 3

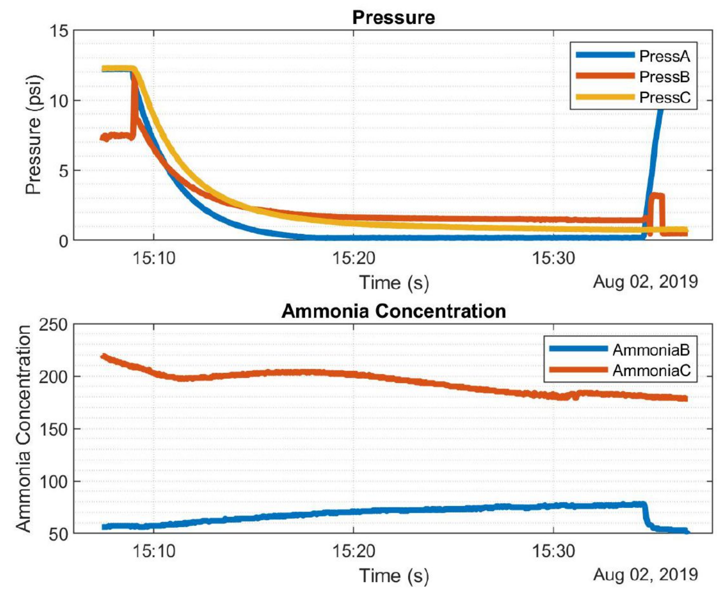

Figure 32: Pressure Test 1

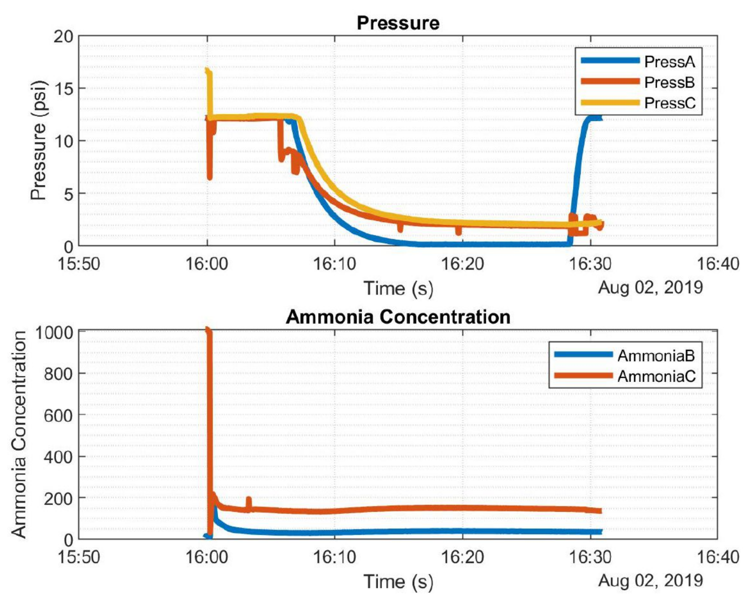

Figure 33: Pressure Test 2

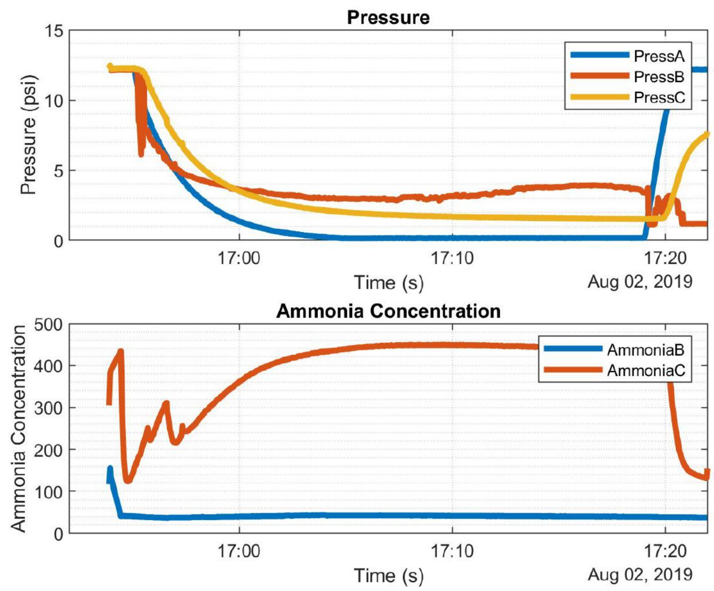

Figure 34: Pressure Test 3

7 Management

7.1 Team

The team had three different subsystems: Structural, Software and Electronics, and Science. The team breakdown is shown in figure 35.

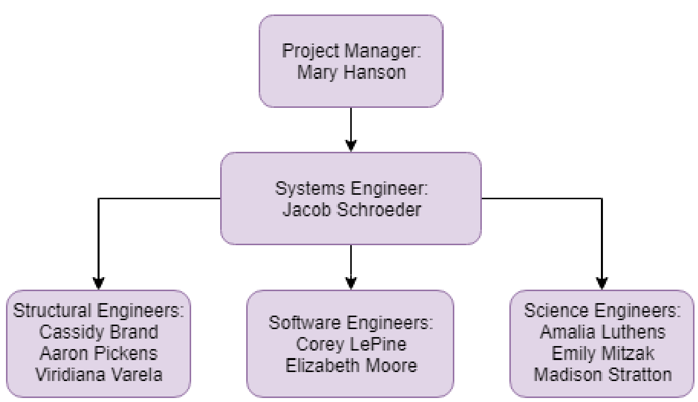

Figure 35: Team Member Breakdown

Though everyone had their own specific team, a lot of crossover happened to ensure the project was ready on time. All team members worked on many different aspects of the payload.

7.2 Budget

	Item	Price per Unit	Quantity	Total
BIOLOGY	E. coli	$29.99	1	$29.99
	Bacteria Loops	$11.00	1	$11.00
	Petri Dishes	$48.99	1	$48.99
HARDWARE	Ammonia Sensor	$38.99	2	$77.98
	SD Card	$11.99	1	$11.99
	Battery Boost Converter	$9.95	1	$9.95
	Battery	$29.95	1	$29.95
	Real Time Clock	$4.95	1	$4.95
	Breadboard	$5.90	1	$5.90
	SD Breakout	$7.50	1	$7.50
	Containment Jars	$15.99	1	$15.99
	Jar O Rings	$10.95	1	$10.95
	Clock Batteries	$13.03	1	$13.03
	Heater Switch	$0.50	1	$0.50
	Power Switch	$0.95	1	$0.95
	Arduino Shield	$4.93	1	$4.93
	Arduino Mega	$38.95	1	$38.95
	Humidity Sensor	$18.95	1	$18.95
	8-Pin Housing Socl	$0.50	1	$0.50
	Transistor	$0.50	1	$0.50
TOTAL				$366.40

Figure 36: Budget

7.3 Mass Budget

Component	Mass (grams)
Ammonia Sensor (x2)	13
Arduino	37
Real time clock	2
MicroSD and Shield	4
Portable Battery	178
Foam Core	255
Hermetic Seal (x2)	152
Jars (x2)	82
Heater	20
9V batteries x3	135
Atmospheric Sensors	2
Extra things: wires, tape, velcro	85
Total	**965**

Figure 37: Full Mass Budget

8 Conclusion

8.1 Future Directions

In order to draw a more substantial conclusion about the results of the balloon launch, the team came up with future directions and experiments that should be carried out. We were unable to complete the additional post launch testing (see section 6) with bacteria due to wires corroding in our payload, so repeating those tests with E. coli is our first priority. Additionally, we would like to observe biofilm under a microscope to confirm that the bacteria is producing one in stressful situations. Currently, we are researching ways to induce a biofilm in bacteria. We would like to observe how trends in ammonia production relate to its formation by doing ground testing. Also, we would like to isolate certain variables, for example, pressure and temperature, and observe how ammonia production and biofilm formation relate to each of those variables independently. Finally, we would like to complete an indole test to confirm that the E. coli is producing indole, one of the ohter products of the chemical reaction used as the basis of this experiment.

8.2 Message to Future Teams

Throughout the duration of this experiment, our team learned that we would need to control multiple variables, like temperature, throughout the DemoSat weather balloon flight. We discovered how difficult it was to conduct a biology experiment with insignificant extraneous variables under the conditions of this launch. While we were able to get data, there is still work that needs to be done in terms of analyzing what it means. We are hopeful that in the near future, another team is able to come up with a modified experiment that can serve as a continuation to this one. We advise future teams to use what we did discover, for example, the best way to seal the jar and how E. coli can survive a functional test overnight, to create their own experiment. Finally, we want teams to understand that the results they get may not always seem conclusive, but they can be used as a gateway to further experimentation.