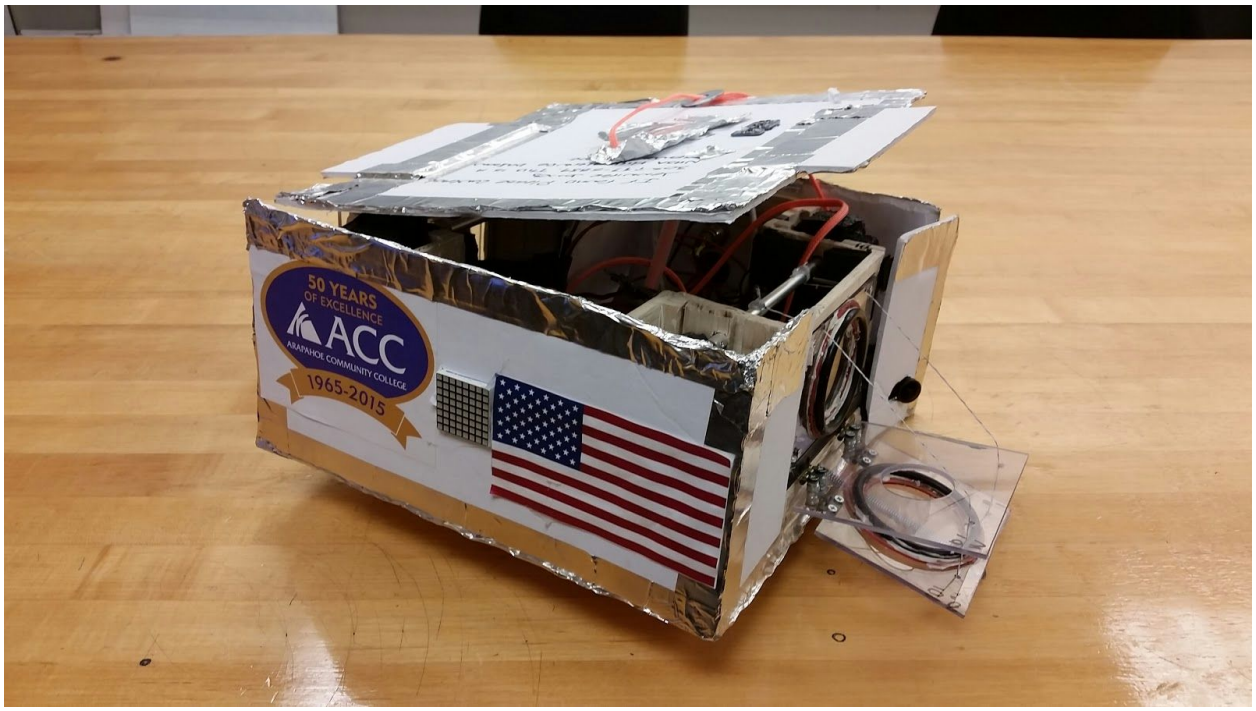
Test-Bed for High-Altitude Microbial Sample and Telemetry Data Collection



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Faculty: Hank Weigel, Jennifer Jones

Abstract

Our project contained two main parts: 1) The biology project served as a test platform for high-altitude microbial sample collection. This served as a proof-of-concept for future missions, and it provided experience towards a future mission in which we may discover microbial life in the upper troposphere/lower stratosphere. 2) The sensor project created a sensor package that captured in-flight navigation and environmental data that is precise enough to virtually simulate the exact launch. The flight computer controlled the mechanism to open the sample collection chambers. Though we could not fly sterile containment chambers due to a problem with the method of sterilizing the collection chambers. Isopropyl alcohol reacted with the plexiglass and cracked the collection chambers; however, the biology project was able to successfully provide proof-of-concept for a future sterile microbe collection mission. The sensor project successfully collected data, and though the data is still being analyzed as of December 2015, preliminary analysis showed that our sensor package not only matched the EOSS data, but it also provided enough data to successfully simulate the launch environment.

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1 Mission Overview

1.1 Primary Objective

Our primary goal was to collect microbial samples from various altitudes in the upper atmosphere and safely return the samples for analysis.

1.2 Secondary Objective

The payload was not limited to collecting life. A sensor package was designed to collect video, inertial navigation, and atmospheric data throughout the flight, which would be used digitally recreate the environment in which the payload flew, as well as the navigational path of the payload.

1.3 Learning Objectives

Through this project, we aimed to learn more about the distribution of life in the upper atmosphere, how to design and build a complex electronics package, and how to analyze data post-flight to develop a comprehensive narrative of the flight.

2 Design

The payload design was driven by the requirements. After determining the requirements, the payload and its components were designed to optimally fulfill these requirements.

2.1 Biology Payload

Evolution of Design

The microbial sampling aspect of this experiment underwent a great amount of change throughout the course of the experiment. And with this transformation came a great deal of new information that is critical to carrying out a more successful microbiological harvesting experiment in the future. Due to this, it is important to explain the evolution of this particular base of knowledge before explaining the data and conclusions made from it.
This experiment originally began with the idea to send bacteria up that were resistant to ultraviolet light in order to understand how varying concentrations of Ultraviolet light (as experienced during flight) might affect these samples. While conducting the research to determine the best way to carry out this experiment, an important problem arose. Namely, this experiment could not be carried out using the resources at our school, because any sufficiently UV-resistant microbe would not be attainable due to its virulence. And even if we could attain

such microbes, we would not be able to build the necessary pressure or temperature controlled environment on the payload with the resources available to us. This meant that if bacteria died or were affected, we would not know if it was a result of UV concentration, pressure, or temperature.
Further research lead us to articles discussing microbes recovered from the upper atmosphere for further study (Shivaji, 2009) (Smith, 2013). These articles explained how this information could be useful in determining the extent of earth's biosphere and what bacteria in the atmosphere could mean in terms of interplanetary dispersal of microbes (Smith, 2013).
Another article discussed how microbes in the atmosphere could affect the formation of weather due to its possible contribution as micronuclei in cloud formation.(Shen, 2013) We also came across articles that discussed the discovery of plankton on the surface of the international space station, and what that could mean about microbes in the atmosphere (Steadman, 2014).
With this information and the realization that sending up bacteria would not be practical, we decided to design a mechanism that would allow us to collect microbes from different elevations in the atmosphere and run tests to identify them.
The end goal of this is divided into two parts. The first was to design a door system that would allow us to collect and store microbes in a sterile environment. The second was to find a way to identify the collected microbes..

2.1.1 Collection Chamber

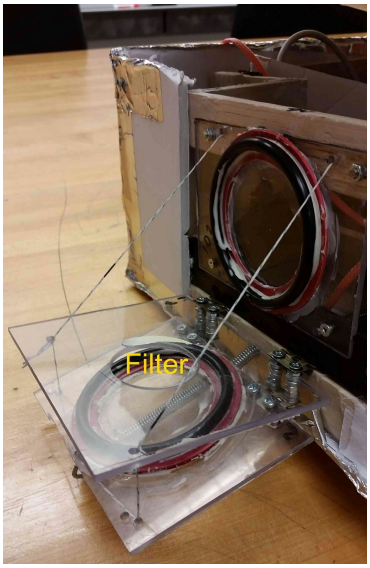
The collection chamber was designed to automatically open at a given altitude and and stay open for a specific span of time.

2.1.1.1 Requirement: Maintaining Sterility

Sterility was an important aspect of this experiment. We wanted to make sure that the filters we sent up were only exposed to microbes at the intended altitude. We would need to use a clean room to achieve this. The process would be to build the doors with a structure capable of keeping out microbes. The door would then be cleaned using a bleach solution, as that would not only kill any microbes present, it would also destroy any DNA left behind which would set up a clean basis for collection. We would then take doors to a clean room where filters would be installed before the launch. The clean room would provide a controlled environment which would contain less potential for contaminants and these contaminants could be accounted for more easily.

2.1.1.2 Requirement: Chamber Doors

When it came to designing the doors, many ideas were thrown around before a basic design of a door that would open outward, exposing an installed filter to a column of air, was decided upon. A structure of balsa wood for the main support and acrylic doors was decided upon as well. Balsa wood was meant to keep the payload as light as we could make it, and the acrylic doors were meant for the purpose of trying to maintain an area that could conceivably be kept more sterile.



2.1.1.3 Requirement: Chamber Seal

The remainder of the three weeks before launch was spent working on the design for the door. For the biological aspect of this, the focus was to see if we could find a way to seal the doors to keep microbes out. We initially started with rubber gaskets from the hardware store, one flat and a little more flexible the other raised but a bit sturdier. These were glued together and to the door using contact cement. It soon became clear that this particular design was not going to work. Tests using dry ice and a vacuum chamber showed that there clearly was a breach in the barrier that was letting material through. So again, the experiment was changed. We decided to spray the filters with alcohol before sending them up, acknowledging that this was in no way a viable experiment, but thinking that if we saw an excess of a particular microbe (based on agar growth) on either, it might be a result of prolonged exposure at a specific altitude.
We were ready to go with this hypothesis when a member of our group, Josh Fender, suggested that if we covered the barrier in a small amount of grease, it would not make an airtight, sterile environment, but it might help a little bit in keeping out unwanted microbes, more so than if we did not use it. After some deliberation we decided to use an antibacterial gel, similar to Neosporin, to make a seal, due to its antibiotic nature, which we thought might further prevent outside bacteria from entering the inner door environment. During a dry ice test, we placed dry ice inside the door and then closed it using the antibacterial gel as a seal and pressure built up for at least five seconds and then blew out a side of the seal. This showed us that the gel actually succeeded in make an airtight seal. Because of this, we decided to again revise the experiment to see if we could harvest live microbes in a sterile environment in order to grow them on agar.

2.1.1.4 Filter paper

The original concept for culture microbes that lived long enough to be cultured was inspired by the 2013 paper by Shivaji et al. In it, it is described that filters that were used to catch microbes

were divided in fourths and then placed on different agar substrates, in order to detect for different microbes (Shivaji, 2013). We used this method to culture microbes collected on our filter paper and our methods are explained in section 9.1.

2.2 Electronics/Software

2.2.1 Main Flight Computer

We performed various software tests with Netduino, Arduino Uno, and the Arduino Mega. Due to greater community support and the capabilities of the hardware, we decided to move forward with the Mega. With a 16 MHz single-core ATMega(9898), 32k flash storage, and 8Kb RAM, it gave us the power we needed to perform all of the mission requirements.

2.2.2 Software Overview

The requirements of our various objectives led to a necessity for concurrent program design. With a single core processor, our team ultimately designed an event-driven architecture that granted us concurrent execution coupled with a simplified and generally generic design that allowed any component(sensor, SD logger, etc) to both trigger an “event” and in turn respond to some “event” that was triggered elsewhere.

The main loop of the software kept track of time and triggered “events” at various time intervals (.1 s, .2s, 1s, 2s, etc). Other components used these events to collect and log sensor data, operate door motors, and log various flight data to a backup SD card.

All of the code is public domain and is available on GitHub at https://github.com/dcolclazier/demosat.

2.2.3 Inertial Navigation & Orientation Data Collection

We used an Adafruit 9-dof Orientation Sensor to collect inertial navigation and orientation data, mainly because of its post-processing capabilities. We updated our inertial navigation sensor data on a “.1s” event cycle update. Because of the single core nature of the architecture and delays associated with other actions the software performed, this led to an actual collection frequency of around 3Hz. This data included linear acceleration(removes gravitational acceleration), and gyroscope data, as well as euler and quaternion rotation vectors. Data collected could then be plugged into a program to create a visual representation of the payload as it flew.

2.2.4 Magnetic & Gravitational Field Data Collection

The same 9-dof Orientation sensor (BNO055) was used to collect magnetic and gravitational field data as it flew. We also collected on the same “.1s” event cycle for the entire flight. The

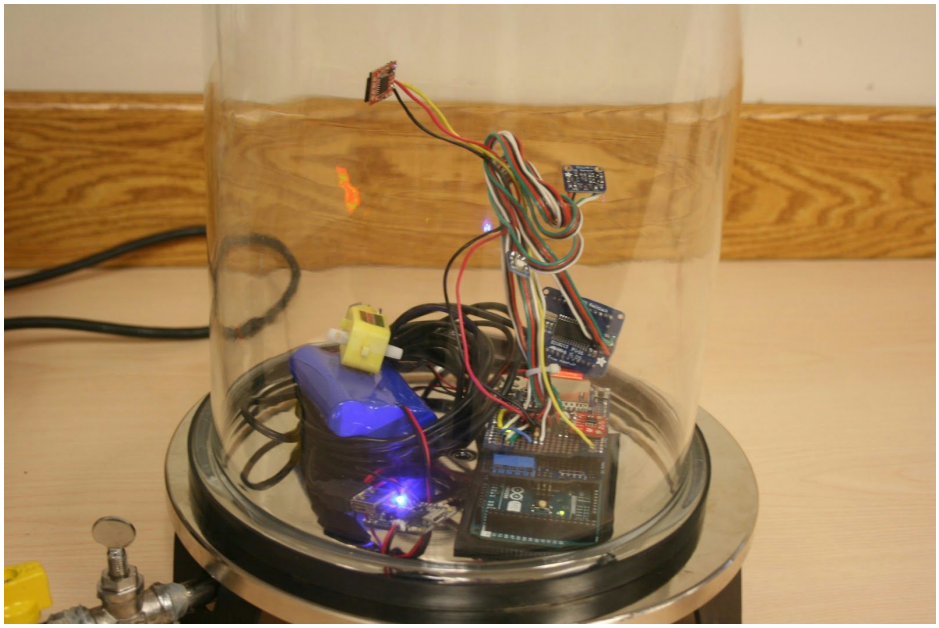
data sheet for the BNO055 can be found at this link.
http://www.adafruit.com/datasheets/BST_BNO055_DS000_12.pdf

2.2.5 Humidity Data Collection

The team decided on a Sparkfun HIH6130 to collect humidity data on a ".1s" event cycle update. Data will be collected for the entire flight. The datasheet for the HIH6130 can be found at this link. http://cdn.sparkfun.com/datasheets/Prototyping/1443945.pdf

2.2.6 Altitude/Pressure Data Collection

The team decided on an Adafruit BMP180 pressure sensor. Unfortunately the sensor is only rated down to a pressure of 300hPa (9000m), but based on previous results with similar experiments, we will still get relatively accurate data below that pressure. The datasheet for the BMP180 can be found at this link.
http://www.adafruit.com/datasheets/BST-BMP180-DS000-09.pdf



Payload undergoing pressure test

2.2.7 UV & Visible Light Data Collection

The team mounted two sensors to the exterior of the top of the payload, to maximize the amount of light the sensor is exposed to during flight. The Adafruit GUVA-S12SD UV sensor collects Ultraviolet light levels and calculates the "UV index". The datasheet for the GUVA-S12SD can be found at this link. http://www.adafruit.com/datasheets/1918guva.pdf
The Si1145 measured visible light and infrared light. We placed a (light filter) over the sensor, to increase the upper range at which the sensor can collect data. The datasheet for the si1145 can be found at this link.

http://www.adafruit.com/datasheets/Si1145-46-47.pdf

2.2.8 Temperature Data Collection

Both the Adafruit orientation and pressure sensors have internal temperature sensors, which we averaged for an internal temperature of the payload, collected on a ".1s" event cycle. The external temperature sensor we chose, (DS18B20), only updates on a .8s interval, so we only collected external temperature data on a "10s" event cycle update. The datasheet for the DS18B20 can be found here. http://cdn.sparkfun.com/datasheets/Sensors/Temp/DS18B20.pdf

Below is the assembled sensor package with flight computer in its prototyping stage.

3 Management

The team started with 12 students (Appendix 6). At launch the team had 9 students and 2 faculty advisors. The Biology team worked on everything to do with microbe collection from the filter paper. The Structure and Doors team designed and built the frame and the doors which held the microbe filter. The Electronics team coded and built the shields to control the doors. And the Organization team herded cats (kept the other students up to date with information from meetings).

3.1 Organization

Organization Chart Revision B:

- Biology team
    - Bekka Spidell, Josh Fender, Wesley Perkins
- Structure and Doors
    - Chris Littlefield, Joseph Hamvas, Magdalena Franchois, Valentine Stark
- Electronics and Software
    - David Colclazier, Wesley Perkins
- Organization
    - George Pandya, Wesley Perkins, Jamie Principato, David Colclazier

3.2 Zoho and Schedule

Zoho was used as a management site where all tasks were assigned, monitored, and completed. Documents could be uploaded to zoho and shared with the group in real time. One of the failures that was experienced with zoho was a follow through with communication. The full schedule and dates for teleconferences can be found in Appendix 8. The schedule was not always followed, so the day before deadline often became the day we worked, and less important meetings were not always seen by everyone on Zoho. Therefore, Zoho was helpful in

documenting the schedule and deadlines, but adhering to the schedule required calling and emailing people to set up meetings.

3.3 Budget/Parts List

The budget was managed through a spreadsheet that also managed the parts list. As parts requirements were determined, the name, link, cost, and other pertinent information was added for future reference. This allowed for accurate budget, weight, and power estimates for more efficient planning and reference. The total mass was expected to be 1,318 grams before the payload was sealed with aluminum tape. The real weight was 1,563 grams. The total Cost with shipping was $919.93 including parts shipping. Screenshots of the working parts list can be found in Appendix 10. The updated parts list can also be found on our Zoho page.

4 Testing

4.1 Potential Points of Failure

To better understand which tests to perform, the payload design was analyzed, and the following potential points of failure were determined

1. The collection chamber may not open at the desired altitude, and conversely the collection chamber may not close after collection has completed, thus contaminating the sample.
2. The collection chamber may be contaminated, thus contaminating the sample.
3. Heating system may fail, potentially rendering electronics inoperable.
4. Parachute may not deploy, potentially destroying payload on landing

4.2 Testing

The testing plans, were formulated in accordance with the potential points of failure to ensure that reliability was maximized and probability of failure was minimized.

4.2.1 Drop Test

In case the balloon’s parachute failed the payload must survive a crash landing. Hence, the payload was dropped so that it would fall at terminal velocity. The payload survived the test.

4.2.2 Cooling Test

Since the electronics need to operate at -80 C (the ambient temperature at maximum altitude), they were placed inside a cooler with dry ice to see if they could remain functional at that temperature. The electronics passed the test.

4.2.2 Field Dragging

During recovery the payload may be dragged and pitched. A test was conducted which verified that the payload was able to survive being dragged across a field and being pitched down a staircase

4.2.3 Vacuum Chamber

At maximum altitude, the ambient pressure is expected to drop to nearly vacuum levels. Since the electronics need to operate in such conditions, a vacuum chamber was used to perform the test, which the electronics passed.

4.2.4 Sterility Test

The collection chamber must be sterile to ensure bacteria collected is from upper atmosphere only. This test failed due to cracking in the door material as explained in section 7.1.

4.2.5 Balsa Wood Liquid Nails

Balsa wood with a density of 160 g/ft^3 proved to be an excellent lightweight material. All joints were cut with biscuit joints and glued using Liquid Nails. The Liquid Nails was tested by gluing two balsa wood pieces together, the sample was placed in dry ice overnight in a cooler. The glue bond was stronger than the balsa wood.

5 Plan for Microbe Analysis

For distinguishing the samples brought back on the payload, we decided to try and test the samples according to their DNA as we did not know if we would be able to keep any microbes alive on the way down to culture them. Initially, we had thought to send the samples to a third party coding source to see if they would be willing to code the DNA in exchange for the credit of doing so. To this end we would also prepare samples for PCR testing.

Sending samples to a third party gene sequencer turned out to not be a viable option for DNA testing due to expense and the uncertainty of results. However, we refined our experiment process using DNA. Knowing that with the resources we currently had available we decided that we would take each filter brought back, divide it into fourths and then do PCR testing so see if we could identify four basic types of microbes; bacteria, viruses, fungi, and protists. The thought was that we would send each filter through a PCR test and see if we received any results while testing for a specific gene for each microbe. We would not be able to establish specific genes for specific microbes, due to the fact that the nature of the testing made it so all the DNA on a specific filter would get mixed together. However, by establishing a DNA pattern we would at least be able to say where specific kinds of microbes could be found. For example, if we tested a filter collected at 20,000 feet and ran a Gel electrophoresis test for the 16s gene on a bacteria, if that test produced bars then we would be able to say that bacteria could be found in the atmosphere at 20,000 feet.

Our initial plan for testing this whole process would be to make a set of doors, sterilize them, add filters in the clean room, then leave them out in the open for a couple of days. We'd then take them back into the clean room to open them, swab them and see if we get any DNA results, then would compare those results with a control door left inside the clean room. If DNA on the inside of the built doors matched DNA found in the clean room, then we'd know that our door design was able to outside microbes from getting in. If there was other DNA not found in the clean room, then we would reconsider our design and try a different design.

We were hoping to get at least one test in, which would at least help us cut down on the number of potential errors. But at three weeks before launch, we re-assessed where we were and came across some problems. The first one was that we was still having trouble tracking down the exact procedure and materials needed to do PCR tests and electrophoresis. At the rate we were going we were not going to have time to test the design with these, much less have time to rebuild should there be a problem. In addition to this, we were still having trouble with a working model of the door design in general. After some deliberation we decided to change the experiment to being one where our main objective was just to see if we could construct a door that would open and close at altitude the way we needed it to in the event we wanted to pursue the microbe collection experiment in the future. As an extra little bit, we also decided that we would send up some filters on the payload, just on the off chance we could determine if the doors could create a sterile environment and just to see if it was possible to bring anything back and culture in on agar. We were not exactly expecting anything, but decided that it would not be too hard to see.

6 Expected Results

For the biological aspect, when we initially began the experiment, we were hoping to at least get results that correlated with previous studies. For example, one study successfully cultured bacteria that was harvested from 24-40 thousand feet (Shivaji, 2009). So, theoretically, and assuming that we managed to do a successful experiment as originally planned, we would hope to find bacterial DNA on filters collecting at any altitude within those parameters.

As the experiment progressed and it became more and more apparent that this experiment was going to be less precise than originally planned, we simply just expected to find out if our process and doors designs worked.

7 Flight Readiness

7.1 Bio Sterility De-scope

Before the launch we took the doors to a clean room so that we could put the filters into the structure. The night before we made sure to soak them in a solution of Clorox® in order to get

rid of the base layer of microbes present on them. Upon entering the clean room, we washed the doors down with an isopropyl alcohol solution as a last attempt to get anything off the doors. We then installed the filters, sealed the doors with gel, closed them back up securely and then placed them in a ziptop bag, just to be extra cautious about contamination.

The next day we were getting ready to get them onto the payload and found a new problem. We learned from this process that isopropyl alcohol dissociates into hydrogen gas and acetone, per the equation below:
$C_3H_8O_{(l)}$ --> $H_{2(g)}$ + $C_3H_6O_{(aq)}$

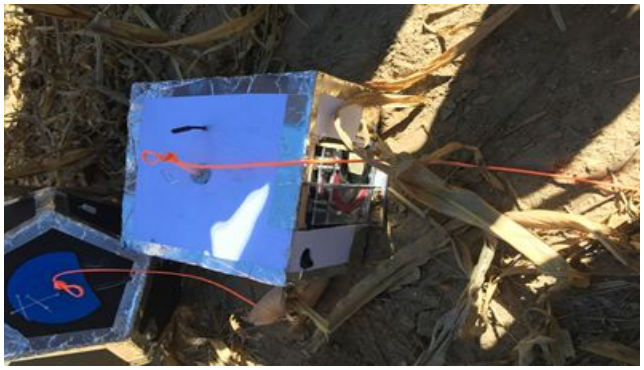
The formation of acetone dissolved the acrylic doors and the extra force put on them by the screws we used to keep them together caused them to crack. This happened two days before launch, so rebuilding the doors and replacing filters was not an option. This being taken into account, we simply rebuilt the doors and went back to the experiment of testing the door design and seeing if it was possible to being any microbes from altitude back alive.

8 Launch

ACC Demosat payload was launched from Deer Trail Colorado at 7:17 am on Saturday November 7, 2015

The balloon achieved an altitude of 95,263 ft. During the chase we stopped in Limon Co. and were able to track the balloon visually until burst.

We were able to observe landing approach. Our payload was recovered intact and undamaged.



9 Data Obtained

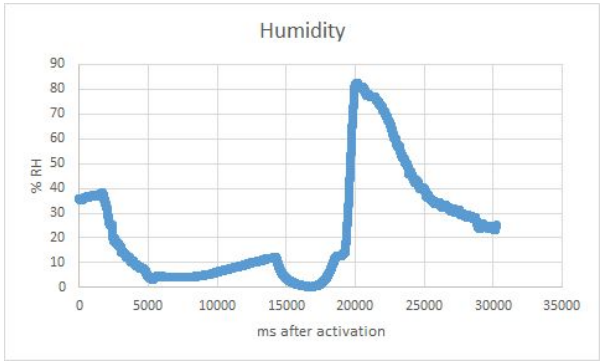
9.1 Microbe Harvesting

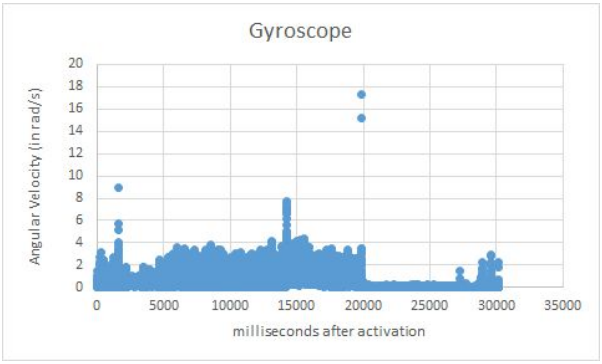
At the end of the experiment there were five filters our group decided to try and culture; one attached to the outside(outer filter) of the payload and exposed to all manner of microbes and environments, one attached inside(inner filter) one of the doors, which was exposed to the environment of the room it was opened in and more exposed to the altitude the door opened at, but still theoretically exposed in a sense to everything else since the door was not airtight, the filter in our control door(control filter) that was sealed and never opened (so it should have remained untouched), one filter(failure filter) from one of the doors that was sealed and then placed into a ziptop bag but then cracked and the ziptop bag was opened slightly, potentially exposing it to the environment for a small amount of time, and a filter(control filter) that in the clean room was opened from its sterile packaging and placed directly on an agar (meaning it theoretically should not have been exposed to anything except the environment of the clean room.) The filters were cut using an crafter's knife and soaked in isopropyl alcohol before and after every cutting. The agar we used were blood, nutrient, sugar, and salt. Below is a table recording whether or not each filter grew anything, and Rebekka Spidell recorded details on the appearance of each sample. (Voice recordings were made, but the results are in the Conclusion section)

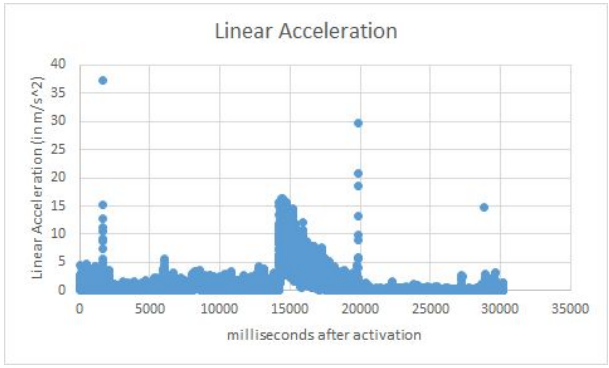
Agar-	Blood	Sugar	Nutrient	Salt
Control Filter	No	No	Yes	No
Failure Filter	Yes	No	No	Yes
Inner Filter	Yes	No	Yes (Pink)	Yes
Outer Filter	Yes (Nasty)	No	Yes	Yes
Sterile Filter	Yes	No	No	Yes (on agar)

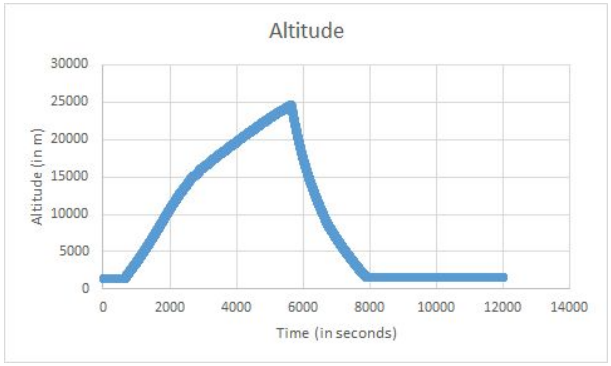
9.2 Navigation/Environmental Data

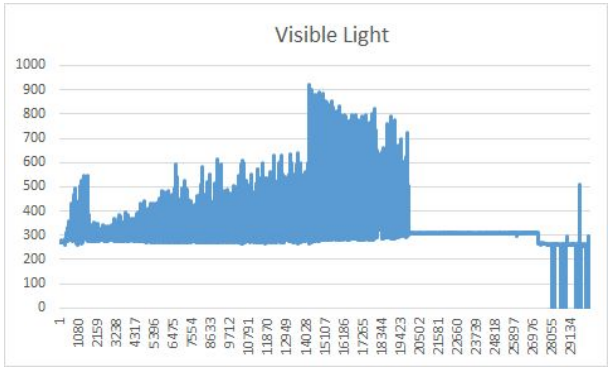
We were able to collect all of the desired data for the entirety of the flight, from the moment the payload was turned on till when our team powered it down after recovery. We are still processing some of the inertial navigation data, but most of the data we collected is viewable on the next page.

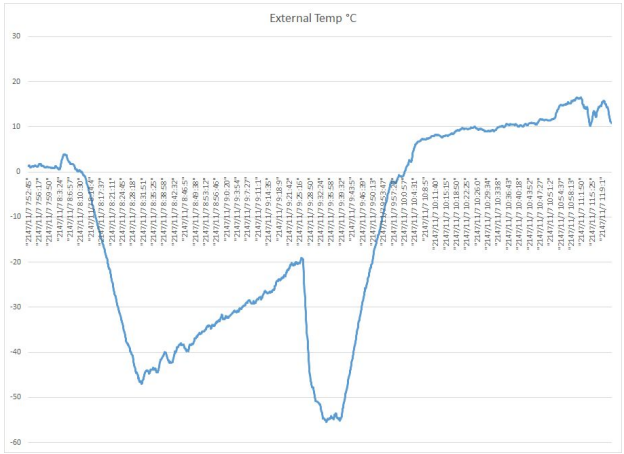


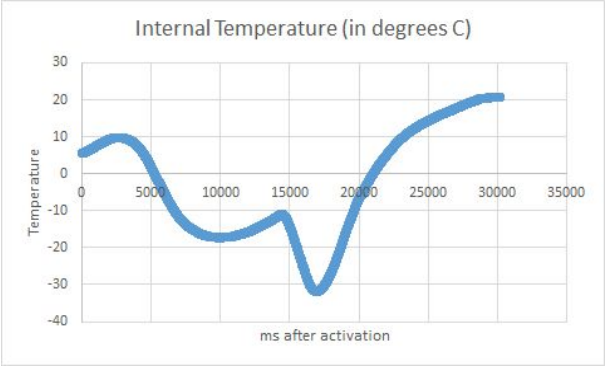


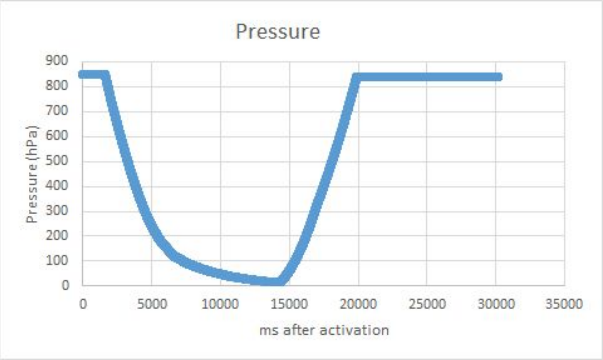












10 Conclusions

10.1 Biology

Right away we realized that there was either a problem with our method, the substrate, or the sterilized filter as there was growth of a milky white, oozing substance along the edges of the sterile filter in the blood agar. (See Appendix 5) At first we were afraid that it meant we could not trust the sterility of the filters. But after further discussion and observation, we concluded that it most likely had something to do with our cutting implement being contaminated since the growth happened around the edge of the filter. This result showed us that were we to do this experiment again, we would need to make absolutely sure our implements were clean, hence the decision that should we do this experiment again, we would use new blades and use one blade for only one filter.

On the blood agar of the filter that was on the outside of the payload, a virulent green, moss-like substance grew. (See Appendix 4) Due to the fact that this filter was exposed to anything, we do not know where it came from. We hypothesized that it was something picked up from the field the payload landed in, since that was a unique aspect of its handling. If the growth was from handling or environmental exposure, then the filter on the inner door would have the same growth on the blood substrate. (See Appendix 3) But for all we know it was because someone handled the outer filter differently than the inner payload filter.

Another growth that it seems important to mention are the growths on the outer payload nutrient and sugar agars and the growths on the cracked door salt and blood agars. (See Appendix 4) While studying the agars at about 5 days of incubation for a preliminary look, we noticed that some of the filters fell off of the agars. Apparently, when they were initially placed on the agar, they did not quite stick and then fell off when the agars were inverted for incubation purposes. So we pushed them back onto the agars. We knew that this would most likely lead to contamination, but we also had decided that there was a great deal of contamination happening already and wanted to see if they yielded anything. By 48 hours later there was a clear growth of some kind on the outer filter on the nutrient agar. This we hypothesized as being by

contamination by us touching it to put back on the agar as it spread all over the filter piece but did not extend much into the agar substrates. We saw a similar growth on the blood agar of the cracked doors. Due to the similarity of the growth on these substrates and the quickness with which they grew, we hypothesized that they were both contaminated by touch, but do not have any other information with which to support this. There was growth on the nutrient plate of the cracked door salt agar. It does not follow the same pattern of the growths on the cracked door blood agar or the outer door nutrient agar. It also appeared to more effect the agar more than the filter itself, so we hypothesized that it could have been due to something other than contamination. But again we have no other way of validating this. There was no growth on the outer filter MacConkey agar, though during the initial observation we noticed some small spots in the agar. We were not able to determine if they were growths of some kind or impurities in the agar though.

This experiment was the beginning in a process of learning. It took many turns and involved many errors. But through these errors much was learned that might not have been understood through simple information gathering alone. Therefore, while this experiment did not fully achieve the goal of harvesting microbes or successfully studying anything brought back it was beneficial in gaining more understanding in order to carry out a more successful experiment in the future.

11 Note to Future Teams

11.1 Biology

During analysis of the agar, a number of questions and concerns arose about the integrity of the culturing of the microbes. There were numerous questions which had not been thought before:

- How sterile are the agar substrates themselves?
- Does opening the agar affect its sterility?
- Does this mean that we need a clean room to study the agar substrates?

Other errors that came up were things that we could have foreseen coming, we just did not consider it when doing the experiment. Errors that fell into this category were how using alcohol on acrylic doors caused them to break, how we should have thought to make some control agars, including one that was never opened and one that was opened to the environment but had no filter placed on it, just to see what the agars did without external stimuli.
All of these questions we used to compile a new set of procedures to follow for the next experiment, and possibly add onto as other experiments are done. Below are lessons learned and best practices for the future.

- Finding time to test structure is key. If we do not have time for it then the experiment is simply our first test.

- When constructing, ensure that materials will stand up to both bleach and isopropyl alcohol and possibly an autoclave environment.
- Always have at least two people when experimenting.
    - This allows for better accountability.
- When experimenting, keep careful track of what is being done.
    - This allows for faster experimenting without compromising quality, better duplication in the future, and referencing in future analysis
        - This allow us to answer questions about who handled the inner door and outer payload filters and how, allowing us to more easily analyze the cultures.
- Research how to keep agar substrates sterile
    - Look into how to make agar plates (we might need to do it ourselves in order to ensure integrity of the experiment)
- Find out whether the culture study needs to be done in a clean room

11.2 Structure and Doors (Sterile Chambers)

The design of the structure was very sturdy and easy to build; however, to save weight, we may have been able to eliminate a lot of the balsa wood structure. The concern with not using the extra structure is that the doors/sterile chambers would fail to stay attached to the payload.

The design of the doors is viable, but they take a lot of time to build. Also, we were not able to confirm that the doors would keep a sterile environment after they had opened. A better option might be to find something premanufactured, like a flip top water bottle. (If the link is broken, this is a water bottle with a latch that releases the lid and the lid flips open. There is a rubber seal imbedded into the lid so when the lid is forced shut the latch catches. The rubber is then compressed, and it makes a waterproof seal.) A manufactured seal operated by servos should perform much better.

11.3 Electronics and Software

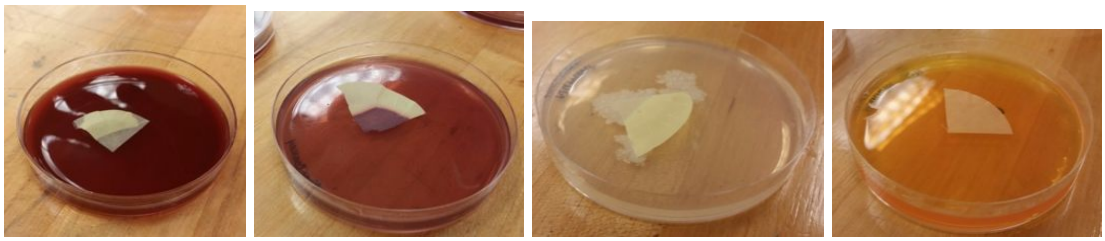
The Arduino MEGA proved too slow to handle events well. This could be resolved by using a Raspberry Pi; however, weight is a big concern. The other option is to use a non-object oriented programming system to simplify the code for the arduino.

Another problem was with the Adafruit motor shield and the way it was interfaced with the code and the sd card logger. There was a bug where everything would boot up if there were enough log files on the sd card, but if the card was empty the motors would fail to run. More research will be required to find the problem; however, adding extra log files and a delay in booting up the motor shield allowed us to work around the issue.

12 Appendix

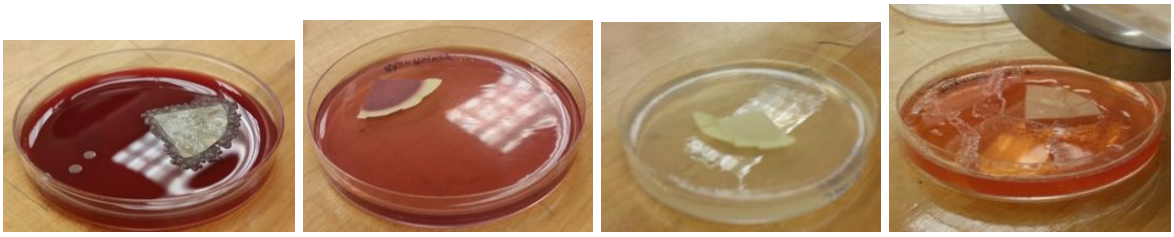
Appendix 1

Agar Plates of Control door filter from left to right: Blood, MacConkey Sugar, Nutrient, and Mannitol Salt after approximately seven days in the incubator.



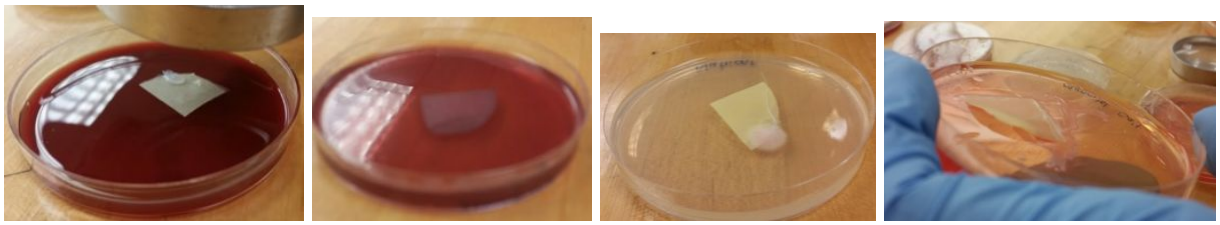
Appendix 2

Agar Plates of Cracked door filter top left to bottom right: Blood, MacConkey Sugar, Nutrient, and Mannitol Salt after approximately seven days in the incubator.



Appendix 3

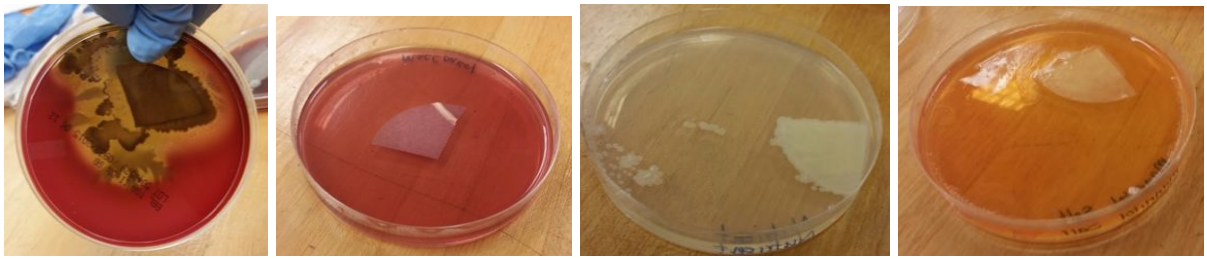
Figure 3: Agar Plates of Inner door filter left to right: Blood, MacConkey Sugar, Nutrient, and Mannitol Salt after approximately seven days in the incubator.



Appendix 4

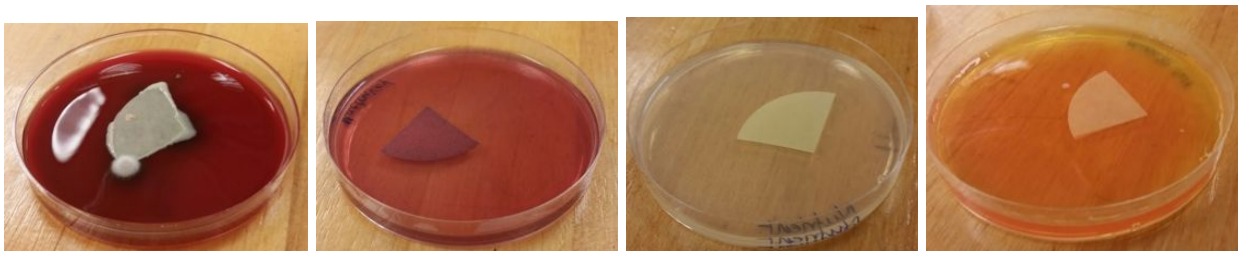
Agar Plates of Outside of Payload filter left to right: Blood, MacConkey Sugar, Nutrient, and

Mannitol Salt after approximately seven days in the incubator.



Appendix 5

Agar Plates of Sterile filter top left to bottom right: Blood, MacConkey Sugar, Nutrient, and Mannitol Salt after approximately seven days in the incubator.



Appendix 6: References

Shivaji, S., Chaturvedi, P., Begum, Z., Pindi, P. K., Manorama, R., Padmanaban, D. A., Shouche, Y. S., ... Narlikar, J. V. (January 01, 2009). Janibacter hoylei sp. nov., Bacillus isronensis sp. nov. and Bacillus aryabhattai sp. nov., isolated from cryotubes used for collecting air from the upper atmosphere. *International Journal of Systematic and Evolutionary Microbiology, 59,* 2977-86.

Smith, D. J. (January 01, 2013). Microbes in the upper atmosphere and unique opportunities for astrobiology research. *Astrobiology, 13,* 10, 981-90. DOI. 10.1089/ast.2013.1074

Shen, H. (January 28, 2013). High-flying bacteria spark interest in possible climate effects. *Nature.*

Steadman, I. (Aug 29-Sep 4, 2014) .The curious case of space plankton. *New Statesman,143,* 5225, 17.

Appendix 7: Images from Hack HD Camera

Figure 1: Launch

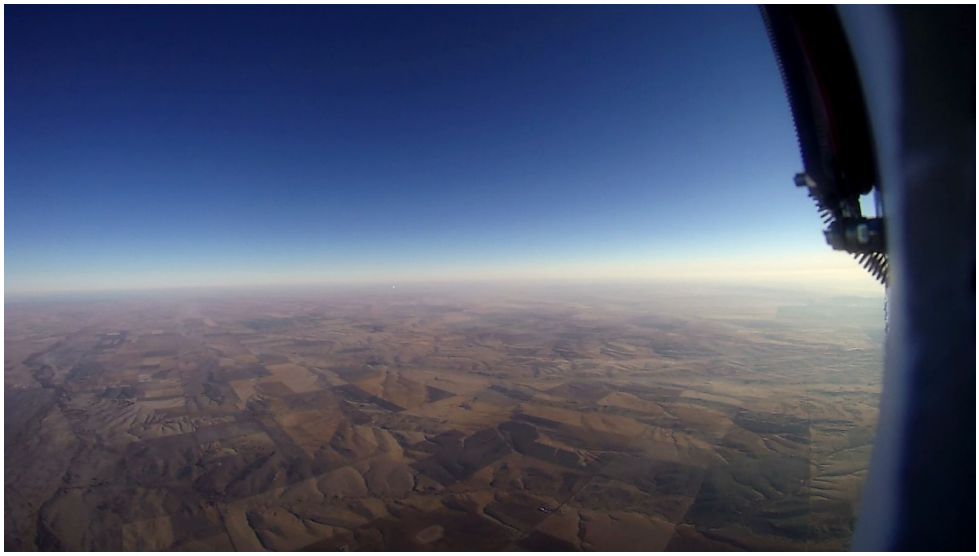


Figure 2: Ascent

Figure 3: Door open

Figure 4: Door Closed

Figure 5: Before Burst

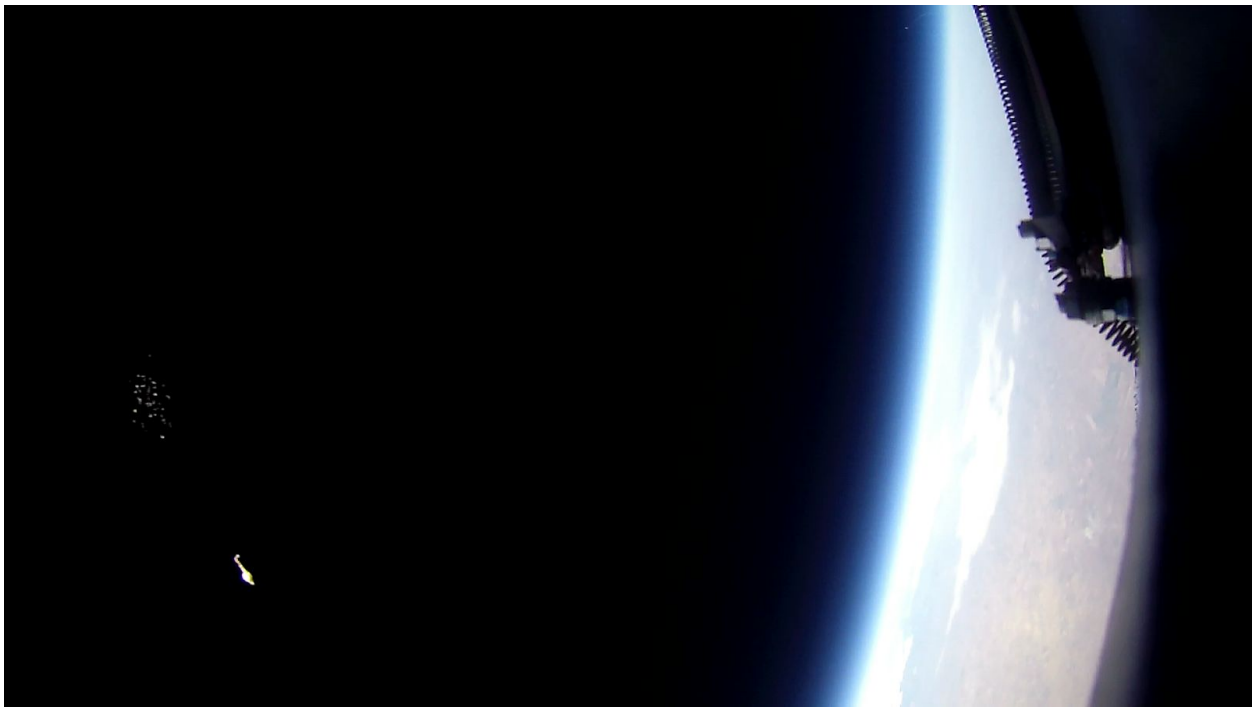


Figure 6: Balloon Pieces after Burst

Figure 7: Beginning Descent

Figure 8: Before landing

Figure 9: Before Landing 2

Figure 10: Touchdown

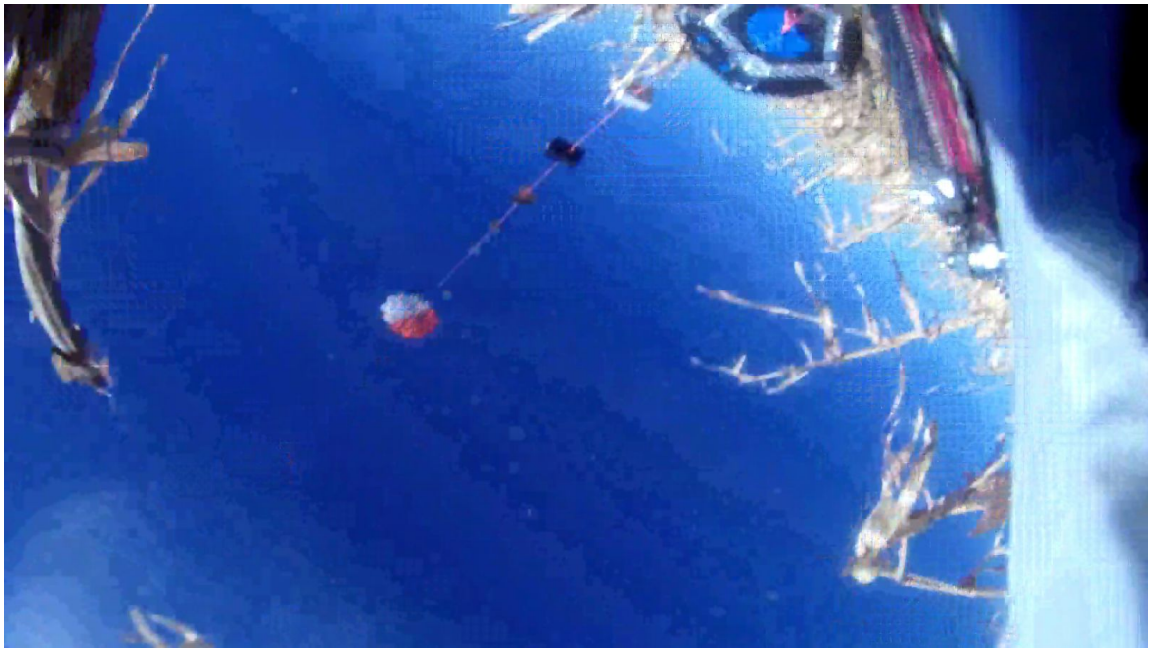
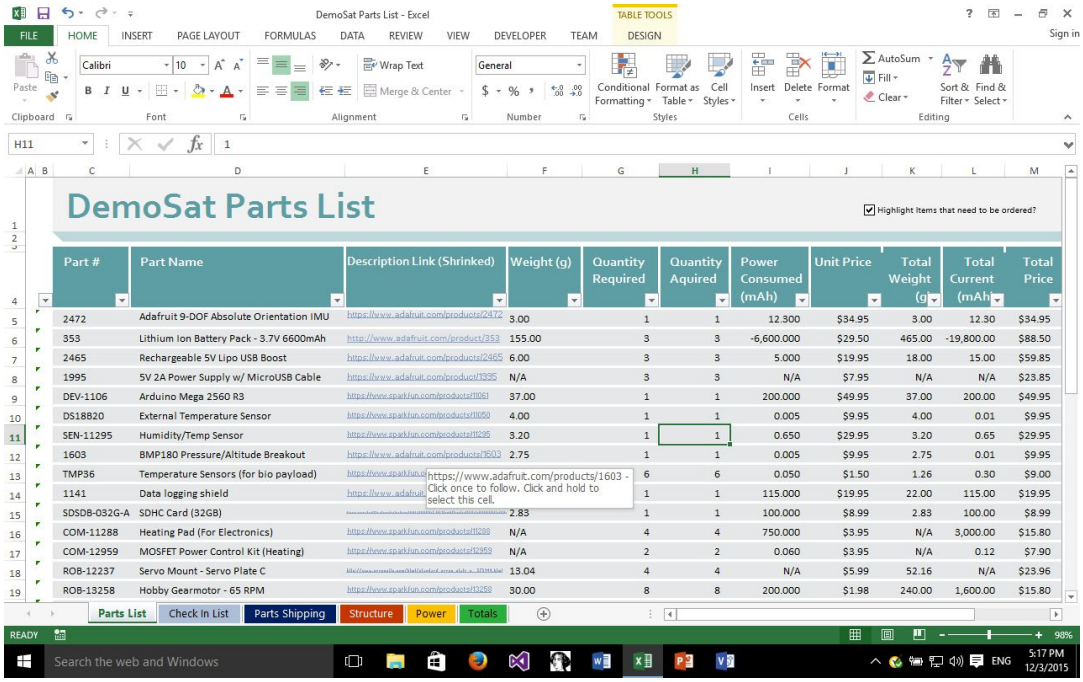


Figure 12: Parachute

Appendix 8: Parts list and Weights

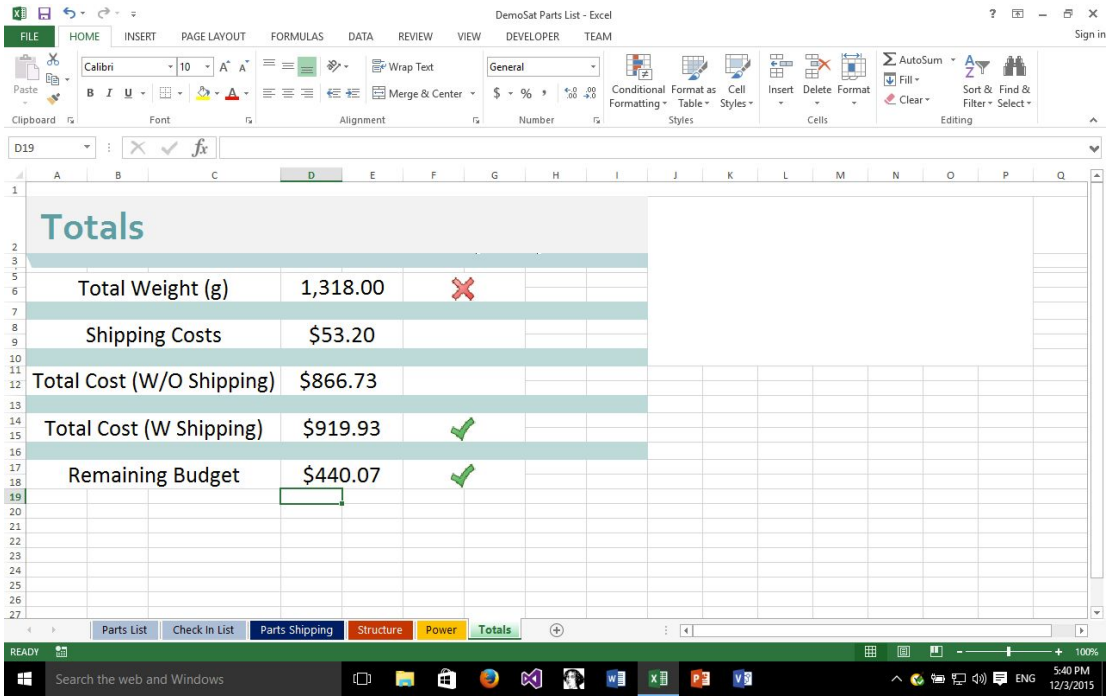
This is piece of our parts list. It does not entirely account for costs and weights, but it provides a sample of our accounting.



DemoSat Parts List

Highlight Items that need to be ordered?

Part #	Part Name	Description Link (Shrinked)	Weight (g)	Quantity Required	Quantity Aquired	Power Consumed (mAh)	Unit Price	Total Weight (g	Total Current (mAh	Total Price
2472	Adafruit 9-DOF Absolute Orientation IMU	https://www.adafruit.com/products/2472	3.00	1	1	12.300	$34.95	3.00	12.30	$34.95
353	Lithium Ion Battery Pack - 3.7V 6600mAh	http://www.adafruit.com/product/353	155.00	3	3	-6,600.000	$29.50	465.00	-19,800.00	$88.50
2465	Rechargeable 5V Lipo USB Boost	https://www.adafruit.com/products/2465	6.00	3	3	5.000	$19.95	18.00	15.00	$59.85
1995	5V 2A Power Supply w/ MicroUSB Cable	https://www.adafruit.com/product/1995	N/A	3	3	N/A	$7.95	N/A	N/A	$23.85
DEV-1106	Arduino Mega 2560 R3	https://www.sparkfun.com/products/11061	37.00	1	1	200.000	$49.95	37.00	200.00	$49.95
DS18B20	External Temperature Sensor	https://www.sparkfun.com/products/11050	4.00	1	1	0.005	$9.95	4.00	0.01	$9.95
SEN-11295	Humidity/Temp Sensor	https://www.sparkfun.com/products/11295	3.20	1	1	0.650	$29.95	3.20	0.65	$29.95
1603	BMP180 Pressure/Altitude Breakout	https://www.adafruit.com/products/1603	2.75	1	1	0.005	$9.95	2.75	0.01	$9.95
TMP36	Temperature Sensors (for bio payload)	https://www.sparkfun.c	6	6	0.050	$1.50	1.26	0.30	$9.00
1141	Data logging shield	https://www.adafruit	1	1	115.000	$19.95	22.00	115.00	$19.95
SDSDB-032G-A	SDHC Card (32GB)		2.83	1	1	100.000	$8.99	2.83	100.00	$8.99
COM-11288	Heating Pad (For Electronics)	https://www.sparkfun.com/products/11288	N/A	4	4	750.000	$3.95	N/A	3,000.00	$15.80
COM-12959	MOSFET Power Control Kit (Heating)	https://www.sparkfun.com/products/12959	N/A	2	2	0.060	$3.95	N/A	0.12	$7.90
ROB-12237	Servo Mount - Servo Plate C		13.04	4	4	N/A	$5.99	52.16	N/A	$23.96
ROB-13258	Hobby Gearmotor - 65 RPM	https://www.sparkfun.com/products/13258	30.00	8	8	200.000	$1.98	240.00	1,600.00	$15.80


DemoSat Parts List - Excel
Totals
Total Weight (g) 1,318.00
Shipping Costs $53.20
Total Cost (W/O Shipping) $866.73
Total Cost (W Shipping) $919.93
Remaining Budget $440.07
Parts List
Check In List
Parts Shipping
Structure
Power
Totals
READY
Search the web and Windows
5:40 PM
12/3/2015

ACC DemoSat Fall 2016