MARSLIFE

SMITH SCIENCE REPORT

HASP 2012

Louisiana State University

12/14/2012

Table of Contents

1.0 MISSION DESCRIPTION

1.1 Mission Goal

The goal of the SMITH 2012 team was to design and construct a payload that samples microbial aerosols from the float altitude of 38 km. These samples shall be returned uncontaminated to the clean room facilities at LSU for analysis. Analysis of the samples will determine the total number of cells collected, and efforts will be made to culture, isolate, and characterize microorganisms from the samples.

1.2 Science Questions

I. Are there microbial aerosols present in the stratosphere?

Hypothesis: The number of cells per volume of air at 38 km will be less than those commonly found in the troposphere ($<10^4$ cells m^{-3}) due to decreases in water and nutrient availability, temperature, and pressure, and increased ultraviolet radiation (1). Microorganisms can be injected into the stratosphere by dust storms, volcanic activity, severe weather, and anthropogenic sources (4,9). Residency times for cells smaller than 10 µm can range from months to years due to the laminar flow of stratospheric winds.

II. Are the microorganisms collected at 38 km viable and can they be cultured in standard microbiological media?

Hypothesis: Given conditions of high UV fluence, low water availability, low temperature, and the potential presence of oxidative species (e.g, H_2O_2 and O_3) in the stratosphere, only spore-forming microbes or species with *Deinnococus*-like resistance would be capable of surviving.

1.3 Science Objectives

Objective	Success	Failure
Obtain an aerosol sample from a target altitude in the stratosphere.	X	
Evaluate the amount of microbial contamination associated with assembly, flight, recovery, and analysis.	X	
Determine the concentration of microbial cells collected at 38 km.	X	
Determine the viability of microorganisms collected at 38 km.	X	
Determine the environmental conditions from 1.5 to 38 km.	X	

The SMITH payload successfully sampled for microorganisms in the stratosphere and those samples were returned safely to the surface for laboratory analysis. Three identical systems were analyzed after flight. The sample filter actively pumped air through the system, while a second on board filter remained sealed. A third filter remained in the LSU clean room to serve as a control. Three filters allowed us to determine if cells were being introduced during transport and flight operations. Direct enumeration of cells visualized using nucleic acid stains allowed us to estimate the total number of cells collected in a given volume. The SMITH payload successfully monitored temperature, pressure, and relative humidity during flight.

1.4 Science Requirements

Requirement	Success	Failure
Sample at target altitude for the duration of float	X	
Minimize background contamination before flight	X	
Simultaneous assessment of laboratory and flight-associated contamination	X	
Direct enumeration of cells with microscopic analysis	X	
Isolate microorganisms from aerosol samples collected at 38 km		X

The SMITH payload successfully operated a pump at 1 mbar to collect microorganisms while minimizing contamination before and after flight. Decontamination procedures were successful at minimizing background contamination in the samples. Direct cell counts in the samples were performed. Attempts at isolating organisms recovered from the stratosphere were unsuccessful. Details of these analyses can be found below (Section 4.0).

1.5 Technical Requirements

Requirement	Success	Failure
Measure the temperature from -70 to +30° C	X	
Measure the pressure from 0-1000 mbar	X	
Measure the relative humidity from 0-100%	X	
Create a flow of air across a 0.2 μm filter	X	

2.0 PAYLOAD DESIGN AND OPERATION

2.1 Principle of Operation

Figure 1 shows the mechanical concept. The SMITH payload is bolted onto the High Altitude Student Platform (HASP) by four ¼" screws. HASP floats to ~38 km by means of an 11 x 10^6 ft^3 balloon. Once at float, SMITH will generate airflow (noted in Figure 1, solid black arrows) of ~25 mL min^{-1} (at STP) across a 0.2 μm polycarbonate filter (Sterlitech Corp., cat. no: PCT0247100). The dash-dot line notes the electrical power. The solid white arrow represents the mechanical connection by bolts to HASP. The filter sampling system is the system designed to collect microorganisms the size of prokaryotic cells from aerosols present in the stratosphere. A fully functional control system was also part of the payload and is used as a procedural blank.

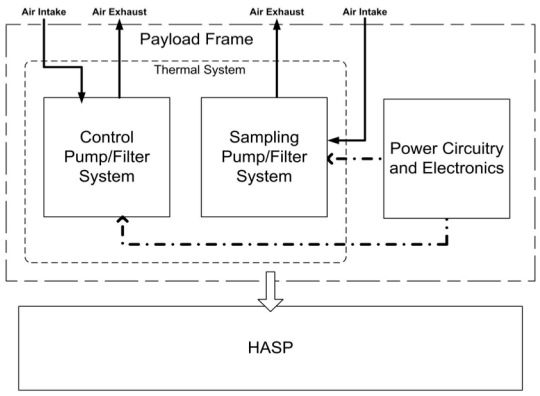


Figure 1: High level system diagram of the SMITH payload

2.2 Subsystem Design

The solid black arrows in Figure 2 represent the flow of air through the system. The dash-dot arrows represent the electrical power supplied by the SMITH power circuitry to the sampling pump system.

In Figure 3, electrical power (dash-dot line) was converted into crankshaft work represented (solid white arrow). The two-stroke engine was converted to the pull air through the filter. The exhaust to the atmosphere was located on the side opposite of the air intake tube. The engine was mounted onto a thermally conductive plate for heat dissipation by convection.

The filter housing contained the filter used to capture microorganisms, a manual valve, and two solenoid valves (Figure 4). The air was pulled from the atmosphere, through the filter system, and then to the pumping system. Electrical power, represented by the dash-dot line, was sent to open the solenoid valves and allow air to flow through the filter during the flight.

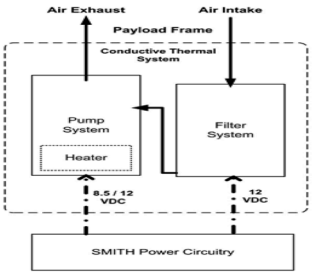


Figure 2: Pump/Filter System Block Diagram

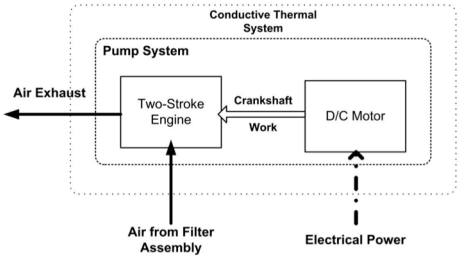


Figure 3: Pump System Block Diagram

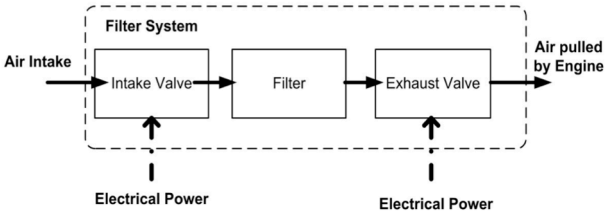


Figure 4: Filter System Block Diagram

3.0 SUMMARY OF SUBSYSTEMS

3.1 Mechanical

The mechanical requirements included a maximum payload mass of 20 kg, a maximum payload footprint of 38 cm x 30 cm, and a maximum payload height of 30 cm. The payload was able to withstand shock forces of 10 G vertical and 5 G horizontal and was capable of operating for a minimum of 12 hours. The payload was able to survive full flight time, and any changes in pressure and temperature associated with flight. These requirements drove the engineering specifications used to design the payload.

In the Figure 5, the sampling and control systems are shown. Each system consisted of an individual pump subsystem and filter subsystem mounted on plates in the main payload frame.

The pump system (Figure 6) consisted of a Leeson 12V D/C motor coupled to a Super Tigre G90 two-stroke model airplane engine. A coupler allowed the two-stroke engine to become a positive displacement pump powered by the D/C motor. A large cylinder head was installed on the engine to contain the oil needed to lubricate the engine throughout sampling. The filter system consisted of a stainless steel filter holder containing a Sterlitech 47 mm polycarbonate filter, two Omega Engineering 12VDC solenoid valves, and necessary stainless steel plumbing and aluminum alloy mounts.

The overall payload body (Figure 7) included the 16.5 cm x 30.5 cm x 30.5 cm main structural frame (A), 16.5 cm x 19 cm x 23.5 cm electronics box (B), and the 8.9 cm in diameter 0.5 farad starting capacitor (C). An 8.9 cm x 7.5 cm x 17.8 cm relay box (D) was mounted on the exhaust side of the main structural frame. The payload frame was built using mostly 6061 aluminum alloy and 8/32 chromium screws. Four ¼" chromium bolts were used to mount the payload body to the HASP interface plate.

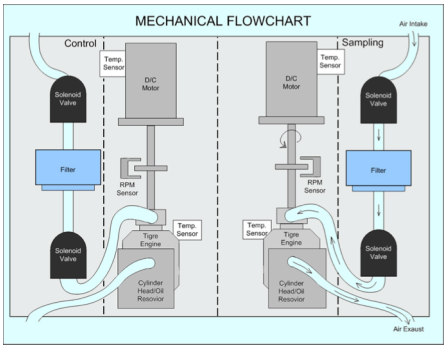


Figure 5: Overall Mechanical Diagram

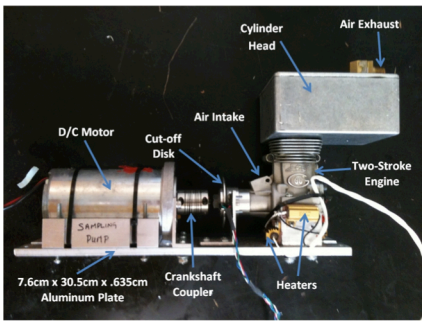


Figure 6: Labeled Pump Assembly Photograph

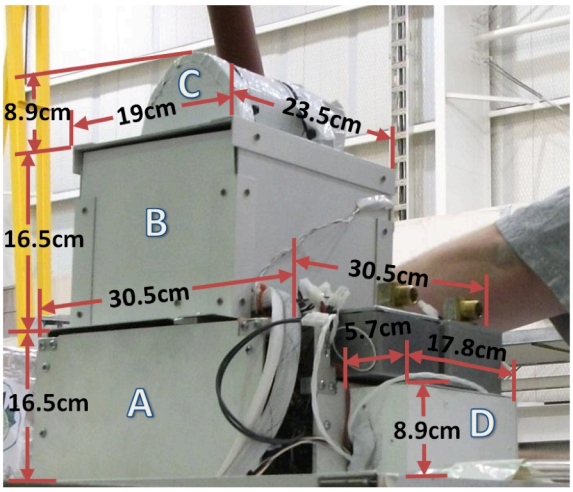


Figure 7: Dimensioned Photograph of Payload

3.2 Thermal

The thermal concerns included both the overheating of the engines and electronics at high temperatures (day time), and the seizing of the engine at cold temperatures (night time and inactivity). The outside panels were painted white to maximize heat emissivity during the day. In Figure 8, heat radiation is represented by the red arrows. The insides of the aluminum panels were buffed to allow for even distribution of heat on the panels at all times. Thermal padding was used between the engines and the engine mounts and between the D/C motors and their mounts to allow for maximum heat transfer to the payload frame (Figure 8, straight red arrows). A dry lubricant, Driconite, and a liquid lubricant, Mobil 1 Advanced Full Synthetic 5W-20 Motor Oil, were used to minimize loss of pumping power to friction. A preliminary vacuum test of the motor coupled to the engine without lubrication resulted in temperatures above 100° C and led to engine seizure. A cold test was performed on the DC motor and engine, this showed that the engine would seize if it was below -20° C. The DC motor's internal insulation is rated for 105° C. This sets the upper limit of the temperature range as the motor's coil windings will short circuit if the insulation melts. The DC motor was determined to function properly down to -50° C.

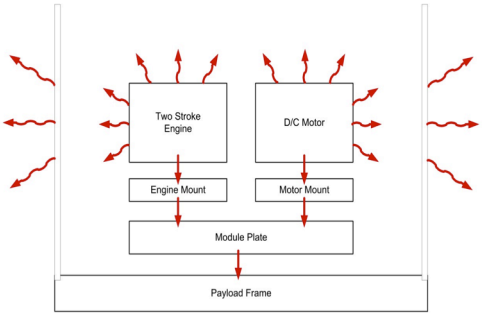


Figure 8: Heat Transfer Diagram

To produce heat as needed, a motor throttling system was put into place to create more friction. Large resistors were mounted onto the engine mounts to act as a heating system to be utilized if temperature dropped below the operational range of -20° C. Thermal pads were also used in between the resistors and engine mounts to maximize heat transfer.

3.3 Software

The overall payload's software goal was to collect environmental data and control and monitor the pumps. The payload's pump control subsystem provided control and monitoring of SMITH during ground testing and flight operations. The pump control subsystem describes both the hardware necessary to physically control SMITH and the software that provides the logic and control over the hardware. The high level general requirements for the payload's pump control subsystem were to: 1) monitor and record temperatures for the sample and control pumps, 2) collect pressure, temperature, and humidity readings of SMITH's environment from the environmental acquisition subsystem, 3) monitor the rotations of the payload's engines, 4) check the status of intake and exhaust valves, 5) receive, validate, and execute preset commands sent to the payload from ground control, and 6) downlink a time stamped data record to ground control through the HASP with the pump status, all environmental data, and responses and errors to the commands received.

The payload's environmental acquisition subsystem provided monitoring of SMITH's environment. The environmental acquisition subsystem contained both the hardware necessary to monitor SMITH's environment and the software that retrieves the payload's environmental data. This environmental data was sent to SMITH's pump control subsystem and then sent down in the data record. The high level general requirement for the payload's environmental acquisition subsystem was to collect pressure, temperature, and humidity readings. The general system that satisfies all the above requirements is shown in Figure 9.

The pump control subsystem and environmental acquisition subsystem was comprised of two independent microcontroller stacks: one to control and monitor the pump control subsystem and the other to collect environmental data via the environmental acquisition subsystem. Each stack ran its own software and they communicated with each other via two MAX3107s (connected by an RS-232 serial link). Both stacks used a BalloonSat board and a communications board. In addition, the pump control subsystem used a sensor board containing the circuitry necessary to monitor the pump system.

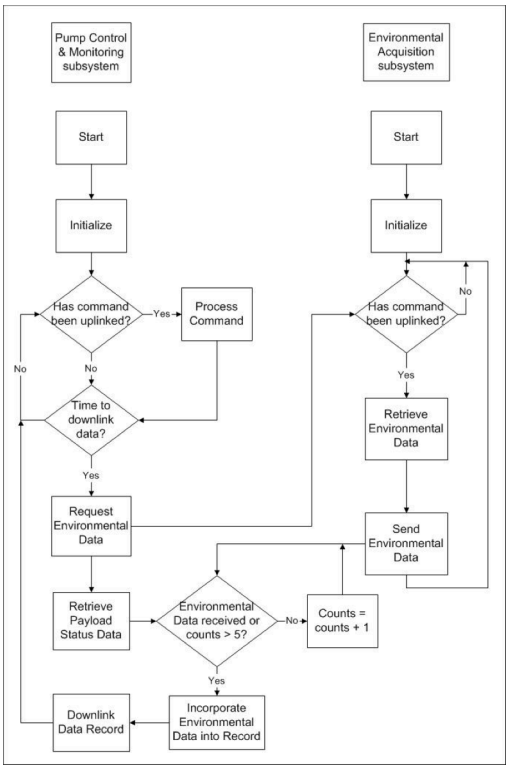


Figure 9: High level software design for both the pump and environmental microcontrollers

The environmental acquisition subsystem employed a board to collect environmental data. The pump control subsystem communicated with HASP via an RS-232 serial link to accept uplink commands and to downlink data. The microcontroller on board the BalloonSat boards was a Parallax BASIC Stamp. This BASIC Stamp was able to control different elements of SMITH: the rotations per second (RPS) sensors, recording data, and communicating with HASP through the communications board. The sensor board in the pump control subsystem allowed for temperatures of both pumps to be recorded and monitored. The sensor board in the environmental acquisition subsystem allowed for the environment's pressure, temperature, and humidity to be recorded.

Figure 9 illustrates the basic layout for the software of the pump control subsystem, the software of the environmental acquisition subsystem, and how the two subsystems interact. When powered on, the microcontrollers both initialized their MAX3107s and sensors, then the pump control software waited for incoming commands from HASP (while periodically sending data records to ground control) and the environmental acquisition software waited for incoming commands from the pump control microcontroller. When a command was received from HASP, the pump control software processed the command. After the command was processed, a data record was sent to the ground control. To do this, the pump control software sent a command to the environmental acquisition microcontroller to retrieve the environmental data. While the environmental acquisition software was retrieving the environmental data, the pump control software retrieved the payload status data and stored the data in variables. After the

environmental acquisition software retrieved the environmental data, it then sent the pressure, temperature, and humidity data to the pump control software. The pump control software stored the data in variables and sent the data record to ground control. If there was no environmental data to be received from the environmental acquisition subsystem after five loops, then the pump control subsystem sent a data record to ground control without the environmental data. The two stacks work together to achieve the overall software goal; however, each subsystem can achieve its own general requirements alone.

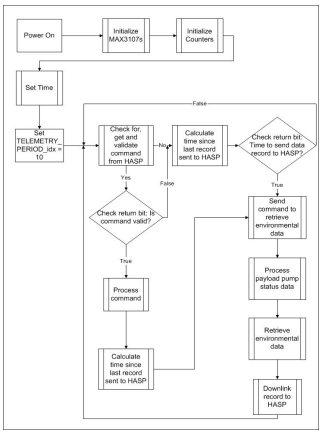


Figure 10: Pump Control Software

Figure 10 displays a high level diagram of the pump control software.**Error! Reference source not found.**. When powered, the controller first creates a buffer of 20 bytes to store the data records. (In the following flowcharts, each byte of the buffer is referred to by what the program stores in that byte of the buffer, not the byte number of the buffer.) After creating the buffer and other variables, the controller enters its initialization process as shown. After initialization of the MAX3107s and the counters, the microcontroller is sent to the Set Time subroutine. In this subroutine, the controller reads the time from the real time clock and stores it in variables. After completing and exiting this subroutine, the controller then initializes the telemetry period to ten seconds. Then the controller enters a loop where it checks for incoming commands from HASP, validates the incoming commands, and forces a data record if a command is received, valid or invalid. If no command is received, the loop will continue to calculate the time between the last data record sent to ground control and the current time. If this time difference exceeds the telemetry period, a data record is produced and downlinked to ground control. To do this, the pump control software sends a command to the environmental acquisition microcontroller requesting the environmental data. While the environmental acquisition software is retrieving the environmental data, the pump control software processes the payload status data. When the pump control software is done processing the payload status data, it retrieves the environmental data (temperature, pressure, and humidity) from the environmental acquisition software and then sends a data record to ground control. After this process, the loop repeats.

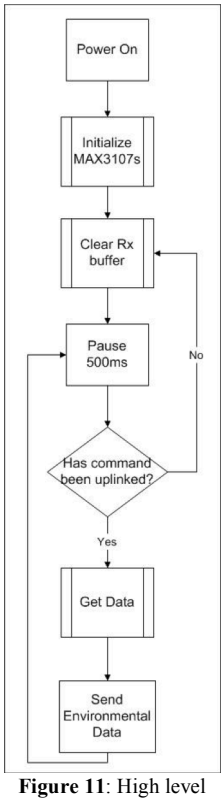


Figure 11: High level software design for the environmental acquisition microcontroller

When powered, the environmental acquisition software first initializes its MAX3107 and clears its RxFIFOLvl. After this, the program enters a loop as seen Figure 11. In this loop, the program first pauses for 500ms and then checks for a one byte command from the pump control subsystem. If there is a one byte command from pump control subsystem, the microcontroller reads in the command into a variable. If there is not a one byte command from pump control subsystem, the microcontroller clears the RxFIFOLvl and repeats the loop.

Once the command is read in, the BASIC Stamp validates the command. If the command to reinitialize the MAX3107 is sent, the microcontroller enters the initialize MAX3107 subroutine. If the command to retrieve environmental data is received, the microcontroller enters a subroutine called GetData. In this subroutine, the BASIC Stamp starts the conversation with the ADC chip to read the ADC channels. The microcontroller then reads the first channel of the ADC, which is the environmental temperature, stores it in a variable, and pauses 5ms. The BASIC Stamp then does the same process for the environmental pressure and humidity. Once all three channels are read, the controller sends these variables to the pump control subsystem in decimal format for the data record. It then exits the subroutine and returns to the main program. The final versions of the pump control software and the environmental acquisition software that flew on SMITH ran successfully. Overall the goals of the software subsystem were achieved.

3.4 Power and Electronics

The Electronics of SMITH was made up of three subsystems: pump control and monitoring subsystem, environmental acquisition subsystem, and the power distribution subsystem (Figure 12). The biological pump subsystem was controlled by the electronics and described in more detail in the mechanical section (3.1).

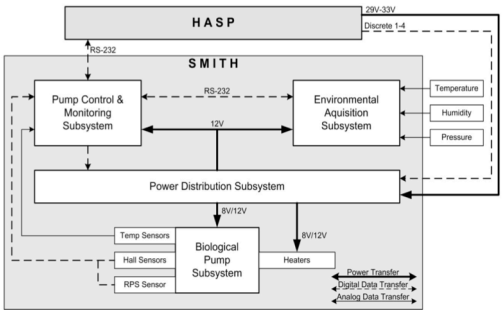


Figure 12: Electronics System Diagram

The pump control and monitoring subsystem received commands from HASP, sent data to HASP, received data from the environmental acquisition subsystem, controlled the pump subsystem, and monitored the pump subsystems state. To control the pump subsystem, a command was sent from HASP to the pump control and monitoring subsystem, and then to the power distribution subsystem, which then affects the motors, heaters, and valves. The pump subsystem was monitored with temperature sensors, rotation per second (RPS) sensors, and flow meters. The environmental acquisition subsystem collected data from the environment outside the SMITH payload and sent this data to the pump control and monitoring subsystem (to be sent to HASP). The power distribution subsystem converted the power from HASP to supply the rest of the payload. This included all boards, valves, and motors. The biological pump system consisted of the sampling pump system and the control pump system. Each system had its own temperature and RPS sensors.

3.4.1 Pump Control and Monitoring Subsystem

3.4.1.1 Controls

As shown in Figure 13, the pump subsystem was controlled through HASP by sending a signal to the Communication board through an RS-232 serial connection and then to the BASIC Stamp. The BASIC Stamp then sent a signal to the Power board to change the state of the valves or the motors/heaters. The relays also controlled the motors and heaters through HASP's discrete lines. Therefore to turn on a motor the command must be sent from HASP and the correct discrete signals must be sent.

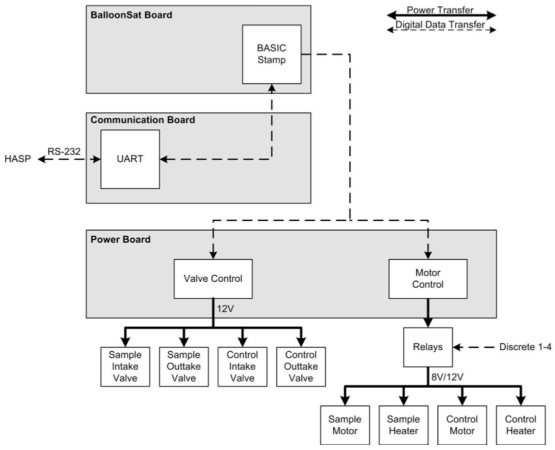


Figure 13: Controls System Diagram

3.4.1.2 Monitoring

The pump systems were monitored using photo-gate/RPS (rotations per second) sensors, and temperature sensors. There were two logic photo-gate sensors used to calculate the rotations per second of the motors (Figure 14). Four temperature sensors were used to make sure the pump system does not go over or under the operating range.

The photo-gate sensor output went to the photo-gate counter to record of the number of pulses from the photo-gate output. The temperature sensor outputted to an amplifying circuit on the temperature board and then to the ADC (analog to digital converter) on the BalloonSat board. The ADC converted the analog data from the temperature board to digital data and outputted to the BASIC Stamp. After the data from all these sensors reached the BASIC Stamp, it was sent to the communication board and then to HASP via RS-232.

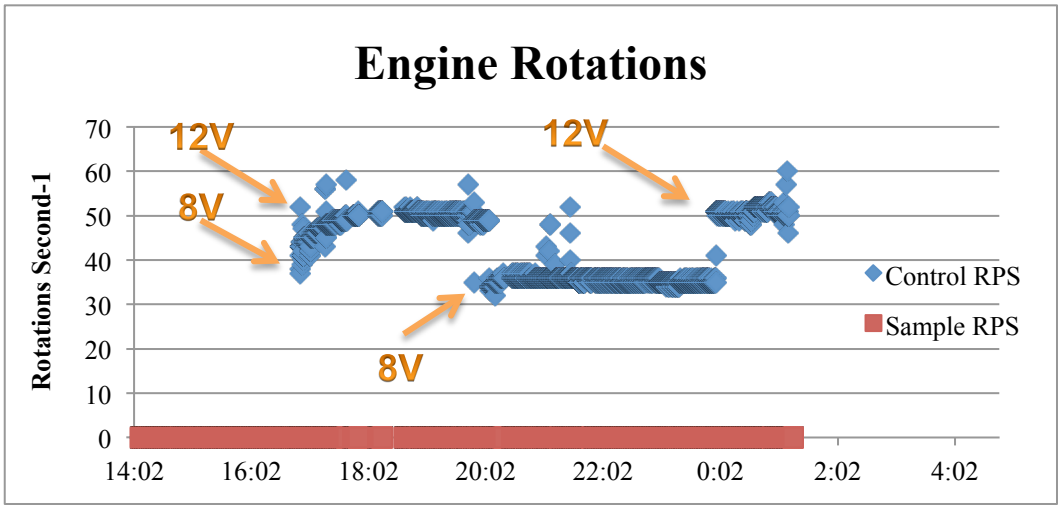


Figure 14: Rotations of the engines as monitored by SMITH 2012.

3.4.2 Communication

The pump control and monitoring communication board sent data to, and received commands from HASP, while also providing communication with the environmental acquisition subsystem. Once a command was received from HASP, the information was sent to the BASIC Stamp. The command was decoded and the requested process was initiated. The program also relayed to the BASIC Stamp to output a string of data at time intervals set by the telemetry to the communication board. This data string was then sent to HASP.

Figure 15 shows how data was sent through the pump control and monitoring communication board. When a command was sent from HASP, it was received by the transmitter/receiver (U3), and then sent to one of the UARTs (U1 or U2). After, it was sent to the buffer (U4), which then sent the command to the BASIC Stamp.

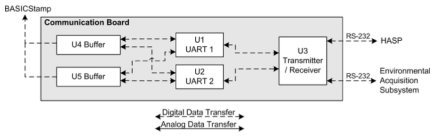


Figure 15: Communication System Diagram

3.4.3 Environmental Acquisition Subsystem

The environmental acquisition subsystem was used to measure the temperature, pressure and humidity during flight (Figure 16). All of the outputs were analog signals amplified on the environmental sensor board before being sent to the ADC on the environmental BalloonSat. The analog data were then converted to digital outputs and sent through the environmental communication board to the pump control and monitoring communication board. From the pump communication board, the data was sent to the Pump BASIC Stamp and then back to the pump communication board, to be sent to HASP.

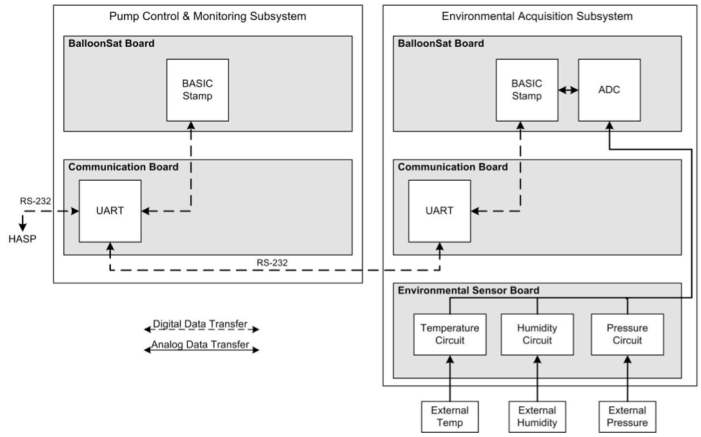


Figure 16: Environmental Acquisition System Diagram

3.4.4 Power Distribution Subsystem

As shown in Figure 17, the Power board distributed power to every component on SMITH as instructed by the BASIC Stamp. The power sent to the six other boards was not controlled and was activated when the power board was turned on. The control for the four valves and two motors were located on this board. Of the three DC to DC converters, two were located on this board. They were both 30V to

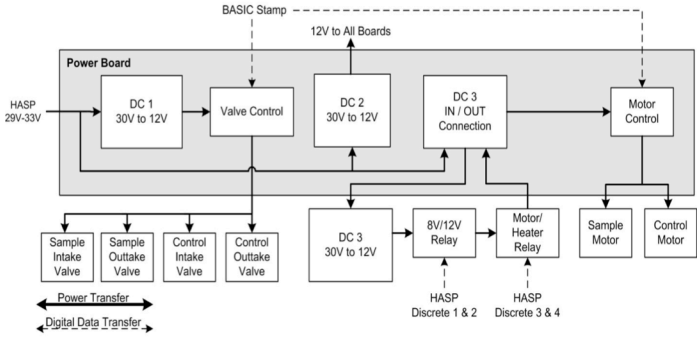


Figure 17: Power System Diagram

12V converters; one powered the boards and the other powered the valves. The DC-to-DC converter located off of the board was the adjustable converter that powered the motors. It was not located on the power board due to its size and was bolted to the bottom of the electronics housing along with the relays involved in the motor/heater relay circuit.

The purpose of the motor/heater circuit was to switch between 8V and 12V supplied to the motors and to switch between using the heaters or powering a motor. This switching occurred by using relays controlled by the discrete lines 1-4 to HASP through the EDAC cable.

3.4.5 Electrical Hardware

Figure 18 shows the hardware of the SMITH electronics. The boards related to each subsystem are outlined in red. In this image, the sensors and power were not connected, but the connection between the pump control and monitoring communication and the environmental acquisition communication can be seen at the top of the stack. Most problems encountered during testing were blown fuses. A fuse blows when either pump system draws too much power from the supply.

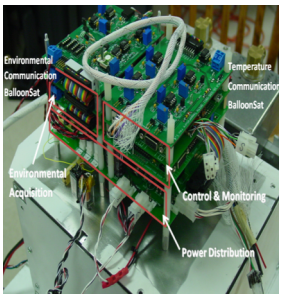


Figure 18: Electronics Hardware

3.5 Environmental Monitoring

The environmental data (Figure 19) collected by SMITH was read from ADC channels as counts. These counts were then converted to the appropriate units by the linear equation acquired when calibrating the environmental sensors. These numbers were then plotted against the data records timestamp to create the environmental data chart. Launch was at 14:19 GMT on 9/1/12 and float was reached at 16:28 GMT. The flight was terminated at 1:17 GMT. The average pressure at float was one mbar and the average relative humidity at float was two percent. The temperature at float was 34° C. Cut down was initiated at 1:17 GMT on 9/2/12 and the payload touched down at 2:07 GMT.

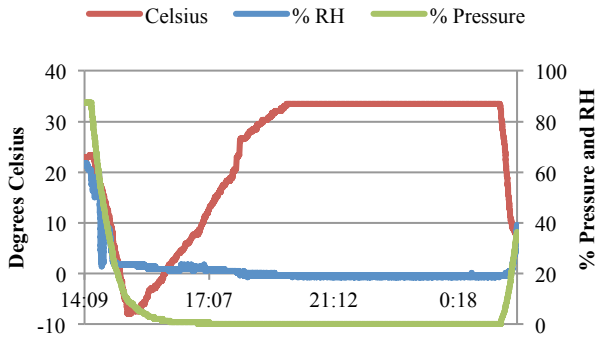


Figure 19: Environmental parameters measure by SMITH 2012

The altitude chart (Figure 20) was calculated by plotting HASP's altitude against HASP's timestamp from when the altitude was collected. This timestamp is recorded in GMT. The average altitude at float was ~38 km.

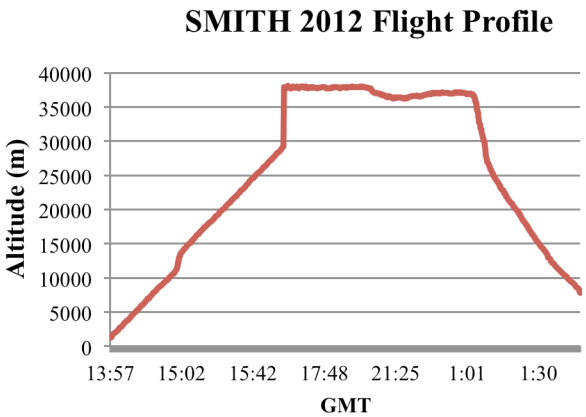


Figure 20: SMITH 2012 altitude during flight

The objective for the monitoring the physical environment during flight was met. Data was obtained for pressure, temperature, and humidity. This data allows us to better understand the environmental stresses faced by microorganisms to survive in the stratosphere.

4.0 Microbiological Analysis

4.1 Filter Assembly Decontamination

The payload sampled aerosols from 16:52:22 GMT on September 1, 2012 until 1:11:26 GMT on Septmenber 2, 2012. The payload was recovered and transported on ice to Fort Sumner, NM. The samples were transported on ice until return to LSU on September 8, 2012. At LSU, the side panels of the payload were removed. The filters were aseptically removed from the filter assemblies (Figure 21) under class 100 conditions and cut in half. One half of the filter was designated for determining the cellular ATP concentration. The remaining filter was cut in half again. One quarter was placed into liquid 1% R2A broth to attempt to recover any culturable microorganisms. The remaining quarter was stained and the cells present were directly counted via epifluorescence microscopy.

The decontaminating procedure of the filter assembly (Figure 21) used a series of techniques to kill and reduce microbial cell contamination. The payload was assembled in a class 100 clean hood housed within a class 10,000 clean room. All surfaces were exposed to germicidal UV-C (254 nm) for 20 minutes and then sprayed with 3% hydrogen peroxide (v/v) to oxidize biological macromolecules, including nucleic acids. The materials were then rinsed with a 70% ethanol (v/v). After drying, the 47 mm polycarbonate track etched filter (Sterlitech Corp., cat. no: PCT0247100) was loaded into the filter assembly. The filter unit was placed in a gas-porous sterilization pouch and exposed to ethylene oxide (EO) at a concentration of 450-650 Mg L^{-1} for 4 h. EO is effective for its bactericidal properties as well as its ability to inactivate spores and results in a 12-log reduction of the biological indicator, *Bacillus niger*, an endospore forming bacteria. Three identical chambers were constructed; two or which served as controls and allowed us to specifically measure the level of microbial contamination associated with assembly/laboratory analysis and flight/recovery. The entire assembly was sterilized with the solenoid valves closed, and EO penetrated the system via an open ball valve, which was opened before EO treatment and subsequently closed without removing the system from the sterilization pouch.

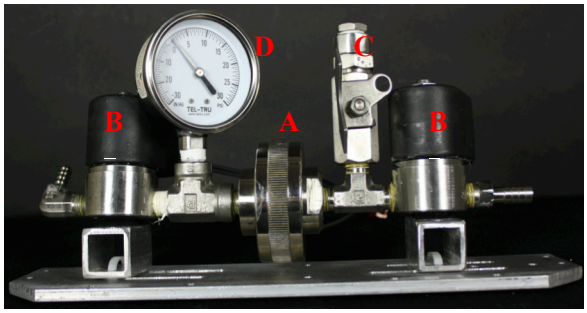


Figure 21: The filter housing (A) contains the 47 mm PCTE filter and is flanked by two solenoid valves (B). A manual ball valve (C) allows for opening the system for sterilization. The pressure gauge (D) was designed to verify the seal.

4.2 ATP Extraction and Measurement

Adenosine triphosphate (ATP) is an energy-carrying molecule present in all living cells. The average bacterial cell contains ~2.0 x 10^{-18} mol ATP and its concentration can be used as a proxy for microbial cell concentration and biomass. Endospores and difficult to lyse cells may be unaccounted for using this technique; therefore the numbers presented here represent a low

estimate for biomass. Studies have shown an increase in ATP signal only after germination has been induced in bacterial endospore samples (6, 7). Future experiments will determine if similar results can be produced from germinating aerosol samples.

The commercially available ATP Biomass Kit HS (BioThema, cat. no. 266-311) was used to quantify the amount of ATP released from cells collected on the filters. One half of the filter was placed into 1:1 solution of sterile 0.2 µm filtered, autoclaved water and Extractant B/S (provided by the manufacturer). Luciferase was added to the sample and the amount of light produced was recorded in relative light units (RLUs). The amount of light is directly proportional to the amount of ATP in a sample. A standard curve was created with a known concentration range of ATP (100 nmol/L) using the same sterile filtered, autoclaved water used for the samples (Figure 22).

Seven 100 µL subsamples were assayed for the total ATP concentration. Based on the standard curve, a concentration of 5.0×10^{-18} mol of ATP was the limit if detection for this assay. The lab control was not significantly greater than the limit of detection. Therefor, all measurements were below the limit of quantification. The total concentration of ATP on the backing filters was not significantly different from the lab control (data not shown) indicating there was no back contamination form the engine. The results imply there were less than 4.0×10^{2} cells on each filter.

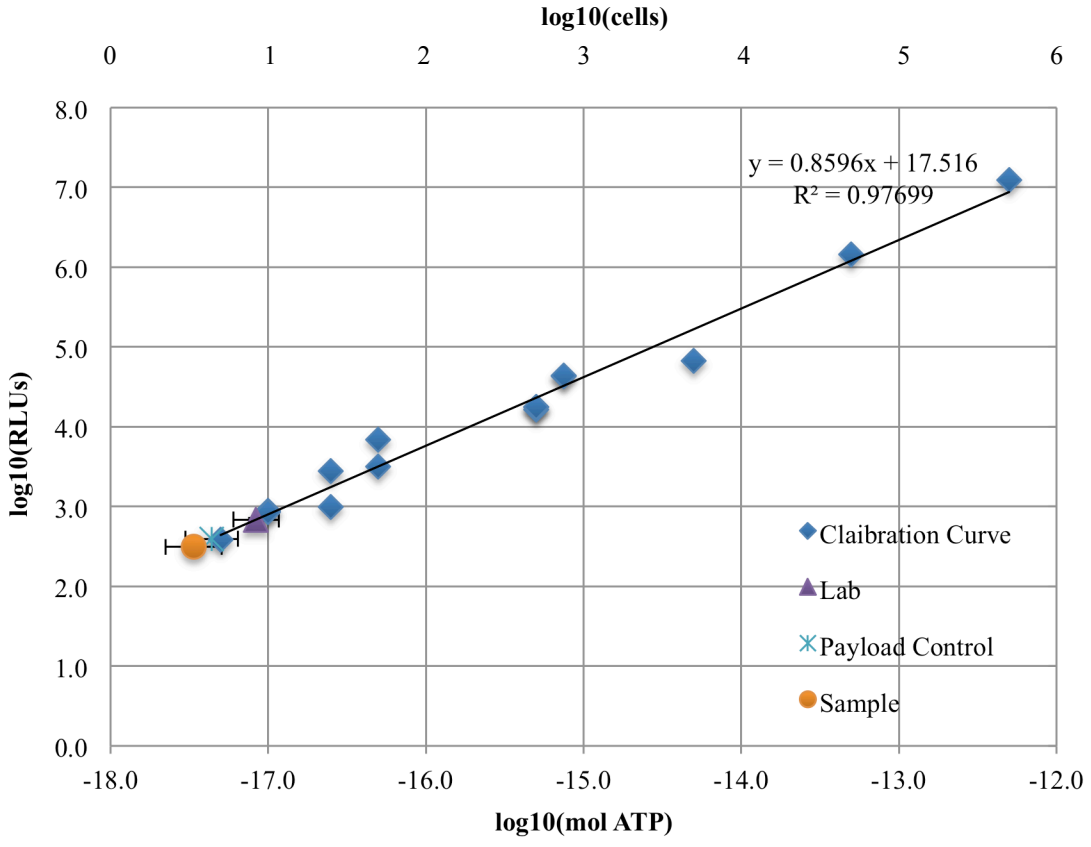


Figure 22: The total concentration of ATP was measured from one half of the filter. The sample and payload control filters were not above the lab control.

4.3 Microscopic Analysis

SYBR gold (Invitrogen, cat no. S-11494) is a fluorescent stain that can enter cells and binds to their DNA. Samples are viewed using epifluorescence microscopy and the cells present in a given area of the filter are enumerated. Previous analysis involved vortexing the entire filter in 10 mL of phosphate buffered saline to remove cells, and a 1 mL subsample was filtered and then counted. Based on results from the HASP 2011 flight, we expected to capture less than 5,000 cells on the filter. For the 2012 analysis, the stain was added directly to a quartered filter and 60 fields of view (1.7 x 10^6 μm^2) were examined. Statistical analysis (ANOVA) revealed there was no significant difference between the samples or controls (Figure 23). Visualizing DNA containing vegetative cells can confirm cell concentrations calculated from ATP measurements. Like the ATP extractions, this particular stain also fails to account for bacterial endospores. A minimum of 53 cells counted in 60 fields of view would have resulted in a signal 3σ above the control filter. There was no significant difference among the three filters visualized using epifluorescence microscopy. Although this technique is less sensitive than the ATP extraction (requiring an order of magnitude more cells on the filter), the failure to detect a signal agrees with the ATP data and the cell counts from 2011.

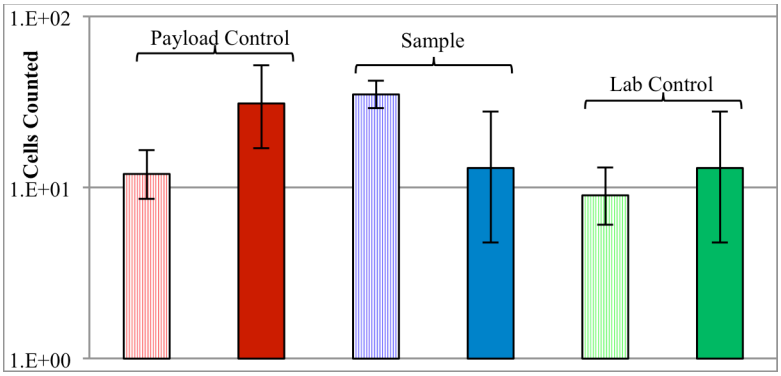


Figure 23: Total cells counted in 60 fields of view at 1000X magnification from two sampling missions. The hatched bars represent data from 2011; the solid bars represent 2012. Error bars represent 99% confidence intervals. The 2011 and 2012 cells counts were not statistically different ($p>0.05$, $df=2$, $F=3.05$).

4.4 Culturing

Previous stratospheric sampling missions have reported isolating microorganisms in the lab, but the existing data does little to account for background contamination incurred before or after flight (3, 5, 8, 9, 10). The majority of the isolates are endospore-forming *Bacillus,* but fungal isolates, as well as a single Gram-negative *Staphylococcus,* have also been reported (3, 5, 8, 9, 10). In an attempt to verify these results, we conducted culturing experiments on filter sections that represented the aerosols collected from ~1.3 x 10^{-2} m^3 of air. One quarter of the filter was placed into 1% R2A liquid media and incubated aerobically at 4° C. After 1 month of incubation, the samples were moved to 25° C. After an additional 45 days of incubations, there are no indications of growth (as measured by turbidity). We were unable to culture any microorganisms from the 2012 samples, as was the case in 2011. All controls for 2011 and 2012 were also negative for growth.

4.5 Conclusions

Our microbiological analysis utilized techniques with low levels of detection: ATP extraction (4.0×10^2 cells filter^{-1}), SYBR gold staining (5.0×10^3 cells filter^{-1}), and culturing (theoretically 1 cell filter^{-1}). Based on data from the 2011 campaign, we estimated that cell concentrations at 38 km were at least an order of magnitude lower than measurements collected from the troposphere (1). In 2012, we improved on this by not diluting the sample into a 10 mL volume of phosphate buffered saline. We reduced the number of measurements made in order to increase our chances of seeing a signal.

The flow rate for the pumps was verified in simulated 38 km conditions and approximated to function between 10 and 25 mL min^{-1} at STP. An estimate of 5.0×10^{-3} to 1.3×10^{-2} m^3 (7.2×10^{-3} to 1.8×10^{-2} m^3) of air was collected during the 2012 (2011) campaigns.

Had the filter collected as few as 1.0×10^3 cells m^{-3}, it would have registered a positive signal using the ATP analysis. For both 2011 and 2012 sampling missions, the samples and the controls were indistinguishable in each measurement. The lack of signal provides clear indication that the amount of contamination incurred during or after flight is not significant. The low pressure and relative humidity seen in Figure 19 indicate the few cells that may be present in the stratosphere are likely desiccated, highly damaged, and/or no longer viable. Future work with sounding balloon flights will allow us to work downwards and constrain the high altitude limit for microbial life.

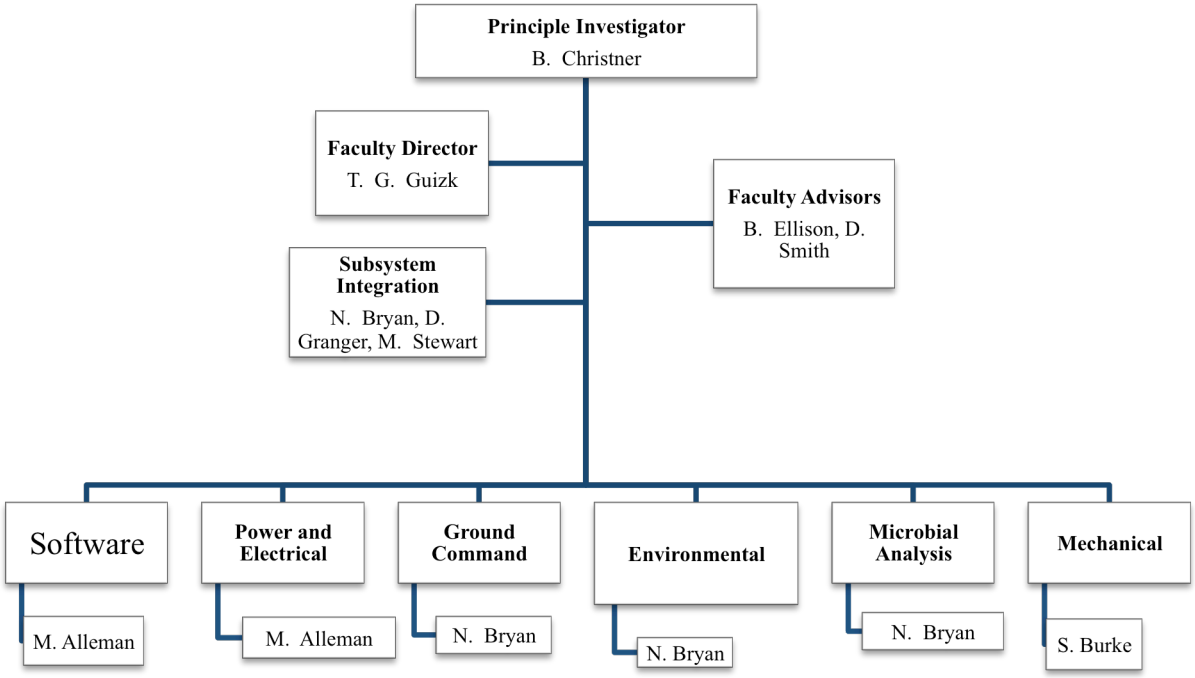
5.0 TEAM ORGANIZATION

5.1 Team Members

Name	Institution	Classification	Sex	Ethnicity
M. Alleman*	LSU	Undergraduate	F	Caucasian
A. Bordelon	LSU	Undergraduate	M	Caucasian
B. Broekhoven	LSU	Undergraduate	F	Caucasian
N. Bryan*	LSU	Graduate	F	Caucasian
S. Burke*	LSU	Undergraduate	M	Caucasian
R. Singh	LSU	Undergraduate	F	Indian
C. Toguem	LSU	Undergraduate	M	African
J. Zhou	LSU	Undergraduate	M	Asian

***Denotes 2011 and 2012 participation.**

5.2 Project Organization



6.0 Presentations and Outreach

1. Alleman, M. et al (2012) Ultraviolet Radiation in the Upper Atmosphere. Annual LaSPACE Council Meeting, poster presentation.
2. Branch, D. et al (2012) Life's Atmospheric Microbial Boundary. Annual LaSPACE Council Meeting, poster presentation.
3. Bryan, N. et al. (2012) Microbiological Sampling of the Atmosphere from 2-36 km Using Balloon Borne Payloads. 14th Annual LSU Biograds Symposium, poster presentation.
4. Bryan, N. et al. (2012) Microbiological Sampling of the Atmosphere from 2-36 km Using Balloon Borne Payloads. National Council of Space Grant Directors Meeting, oral presentation.
5. Bryan, N. et al. (2012) Microbiological Sampling of the Atmosphere from 2-36 km Using Balloon Borne Payloads. Annual LaSPACE Council Meeting, poster presentation.
6. Bryan, N. et al. (2012) Microbiological Sampling of the Atmosphere from 2-36 km Using Balloon Borne Payloads. LSU American Institute Aeronautics and Astronautics Chapter meeting, oral presentation.
7. Bryan, N. et al. (2012) Microbiological Sampling of the Atmosphere from 2-36 km Using Balloon Borne Payloads. Annual American Phytopathological Society Southern Division meeting, oral presentation.

8. Burke, S. (2012) Sampling for Microbiology in the Upper Atmosphere. Annual LaSPACE Council Meeting, poster presentation.
9. Christner, B.C. (2012) Cloudy with a chance of microbes (cover article). Microbe, 7:70-75.
10. Christner, B.C. (2012) Featured scientist in "Invisible", a documentary special for the History Channel (January 2013), Flight 33 Productions.
11. Life at the Edge of Space: Do high-flying microbes control Earth's weather? Cover article in April 2012 issue of Discover Magazine that features the MARSLIFE project.
12. The High Life. ScienceNews for Kids, November 2012
13. MARSLIFE students perform demonstrations at the annual Sally Ride Science Festival 22 sept 2012

7.0 References

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4. Griffin, D. (2007) Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. Clinical Microbiology Reviews, 20:459-477.
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9. Smith, D. J. et al. (2010) Stratospheric microbiology at 20 km over the Pacific Ocean. Aerobiologia, 26:35-46.
10. Wainwright, M. et al. (2003) Microorganisms cultured from stratospheric air samples obtained at 41 km. Federation of European Microbiological Societies Microbiology Letters, 218: 161-165.