

SPS Chapter Research Award Interim Report

Name of SchoolUniversity of Tennessee – KnoxvilleSPS Chapter Number7158Total Amount Awarded\$2000Project LeaderPeter E. Tarlé	Project Title	High Altitude Rocket Assisted MicroOrganism Capture (HARAMOC)
SPS Chapter Number7158Total Amount Awarded\$2000Project LeaderPeter E. Tarlé	Name of School	University of Tennessee – Knoxville
Total Amount Awarded \$2000 Project Leader Peter E. Tarlé	SPS Chapter Number	7158
Project Leader Peter E. Tarlé	Total Amount Awarded	\$2000
	Project Leader	Peter E. Tarlé

<u>Abstract</u>

The Society of Physics Students at the University of Tennessee Knoxville is seeking to use a high powered transonic rocket to collect high altitude bacteria and viruses known as bioaerosols. These poorly understood microorganisms may play an important role in cloud seeding and atmospheric chemical processes which have climate implications.

Statement of Activity

Interim Assessment

Bioaerosols are microscopic biological material suspended in the air. Among these bioaerosols are a largely unstudied class of microbial life, some of which are believed to have evolved independent of life on the ground to survive long periods of aerial life. In addition to the potentially unique biology of these microbes, they are also believed to have a significant impact on the environment, playing an important role in cloud seeding. Studying these microbes could provide insight into potential life on other planets, owing to the harsh environment these microbes live in with low oxygen levels, low air pressure, limited nutrients and elevated levels of ultraviolet radiation.

HARAMOC (High-Altitude Rocket-Assisted Micro-Organism Capture) is a joint venture between the Tickle College of Engineering, the Physics Department and the Center for Environmental Biotechnology with the goal of capturing samples of these microorganisms for study in a lab, including genetic sequencing. The payload itself is comprised of a sealed and sterilized compartment with access panels opened and closed by a mechanism driven by a stepper motor. HARAMOC is designed to be flown on a transonic or supersonic sounding rocket, allowing high speed airflows to sterilize the outside of the payload. At apogee, the maximum altitude achieved during the flight, HARAMOC deploys the access panels to expose the collector surface to air at the desired sampling altitude.

Several prototypes have been developed for various payload subsystems, including panels for exposing and sealing capture media. The HARAMOC payload has been developed and is preparing for flight testing, with the goal of capturing our first samples. Initial prototypes used a centrifugal pump and a 0.2 micron electrospun filter, though testing showed the pressure gradient required for effective use of this filter media was simply too great to achieve at an altitude of nearly 15,000 feet above sea level. Subsequent iterations of the design have utilized a passive sample collection method, in which panels are opened on the airframe opposite the direction of travel, creating a low pressure region behind the panels in which bioaerosols can be concentrated and then collected on open-cell melamine foam blocks. The new collection method shows lower capture efficiency but substantially increased airflow, allowing for a larger sample to be returned to ground for examination. The first in-flight test is now scheduled for June 20th at Spaceport America in Truth or Consequences, New Mexico.

There are roughly a dozen University of Tennessee students collaborating in this endeavor. Anthony Condra, Robert Nickel, and Peter Tarlé are SPS national members and members of our local chapter. Peter Alley, Nicholas Crowder, Matt Demorat, Sam Sexton, and Maggie Spangler are local chapter members. These members have worked extensively in developing the payload mechanism and integrating it into the rocket's airframe. Dr. Steven Ripp and Veronica Brown, who work with the Center for Environmental Biotechnology, will be more involved in the sequencing of samples secured by the rocket's payload.

For this project to be successful, it is absolutely essential for design constraints to be understood by all. For instance, any mechanism for opening and closing the payload has to allow the rocket to fly nominally, but it must also be sealed and re-sealed tightly enough to ensure sterility. When in operation, there has to be sufficient airflow through the collection media to capture our sample, but pumps would have to fit in the airframe, which limits their size and, therefore, efficiency. Capture media selection proved difficult in that the media must allow enough airflow through to capture enough material for gene sequencing, but is selective for the particle size desired. While the initial electrospun filter elements were capable of capturing even the smallest of microbial life expected, it required an 80 psi pressure differential and was thus impractical for our purposes. Work on this research project has forced students to make critical design choices and compromise at every level and requires a deep knowledge of all aspects of the integrated system. This whole-picture mentality is important to budding physicists who might not consider the limitations inherent in real world manufacturing. The interdisciplinary collaboration required for the success of this capture mechanism has proven invaluable.

Updated Background for Proposed Project

Bioaerosols are believed to play an important role in the Earth's climate, one example being the possibility of these aerosols seeding cloud formation; however, not much is known about microbial life suspended in the upper

troposphere [1]. Current sampling has been limited to either mountain-based passive collection stations or atmospheric balloons [2,6]. Aircraft have been used to collect these bacteria, but there were issues with ground contamination stirred up by powerful storms [6]. In all of these studies, contamination of samples has proven to be their greatest challenge [1,3]. Our proposed sampling technique employs the use of an experimental sounding rocket which, through transonic flight, will sterilize the outer surface of the payload.

The planned processing of samples collected by this sounding rocket involves gene sequencing to compare bioaerosols collected during the flight with samples collected on the ground surrounding the launch site [1,3,6]. We will collect our own samples in an autoclave-sterilized melamine foam block. By flying in the transonic regime and using aerodynamic heating and high velocity airflow to sterilize the surface of the rocket, we will be able to overcome challenges faced by some of the other studies performed on bioaerosols, an example being a study performed by Smith [5] where the majority of the culturable samples were from the fuselage and not their collection media.

DNA sequencing is an extremely useful tool to identify bacteria. For this experiment, we will sequence the 16S rRNA portion of the bacterial DNA. The 16s rRNA gene is a sequence of DNA that encodes for a subunit that is the RNA portion of a ribosome, the protein-producing molecular machine. A portion of this gene is highly conserved across all species of bacteria with a variable segment specific for each bacterial species. The variable portion allows for ease of identification of the specific bacteria collected. The data taken from this sequencing will be compared against other known 16S rRNA sequences stored in public databases, giving us a definite identity of the bacteria. It is likely some microbes sampled will be new to science and will greatly differ from their terrestrial counterparts due to their extended suspension in air. Next year, we wish to examine the possibility of building a low pressure circulated air bioreactor to attempt to grow collected bacteria under similar conditions to their native habitat. Due to time constraints and currently available funding, we have chosen to forgo this option for the time being.

Description of Research - Methods, Design, and Procedures

Previous studies have used aircraft, balloons, and mountain top collects to recover samples of these microbes, but have difficulty reducing contamination. Mountain top capture methods introduce ground contamination. Balloon gondola methods introduce contamination during the filling stage, where the balloon is laid out on the ground yet remains above the collection media for the remainder of the flight. The study using hurricane chase planes noted significant amounts of storm blown human and animal waste. The rocket for this year's competition is capable of reaching between 10,000 and 12,000 feet above ground level, which is the lower bound of where these organisms have been reported. By next year our team should have the infrastructure in place to enter into the 30,000 foot competition allowing for an order of magnitude increase in the time of collection of bioaerosols as per rules regarding drogue decent rate for the rocket. There is a minimum descent rate of 75 feet per second before main deployment to ensure that the rocket does not drift outside of the prescribed waiver cylinder (roughly 15,000 ft radius with column height of 30,000 feet).

The first sampling mission will be performed June 20th at the Spaceport America Cup in Truth or Consequences, New Mexico. A sample of dirt from the base of the launch pad and the landing site will be taken for experimental control. The rocket will launch to an altitude of approximately 10,000 feet AGL, at which point the rocket will deploy a drogue parachute to stabilize its descent. A few seconds later after the rocket has stabilized an altimeter will signal an arduino to unlock the payload access panels and drive a stepper motor to actuate the panels. At an altitude of 5,000 feet AGL, the payload panels will close and lock in place to avoid contamination upon landing. When the rocket is located after its flight, the payload will be separated from the rocket and the outside of the payload will be washed with antimicrobial solution and sealed in a vacuum bag. The bag will then be put into a cooler filled with ice to keep the temperature low during the 22-hour drive back to UT's campus. After the first launch we will open the payload in the clean room and cut the melamine foam capture media into several small pieces, using half for immediate genetic sequencing and trying various methods of growth media for biological amplification for subsequent sequencing. Additional sampling missions will be conducted in Hearne, Texas following the competition launch.

Initial Results

The payload opening mechanism was flown in South Charleston, Ohio to stress-test components. This flight pushed our rocket and payload harder and faster than the competition flight, flying in excess of Mach 1. The payload

easily withstood the enormous stresses endured during the flight, proving it is capable of enduring future flights. Tracking and recovery systems were certified and the payload was recovered sealed 3.5 miles away from the launch site.

The entire payload subsystem was ground tested and was triggered to open the panels from the internal flight computer, showing that all components function as planned; however, additional electronics necessary for proper operation of the payload assembly required more space than anticipated and once assembled, the electronics assembly was too long for the carbon fiber airframe that was on hand. Luckily, a new fiberglass tube of appropriate size was graciously donated by one of our sponsors, Imperia Aerospace, and the payload is now being prepped for flight testing. These preparations should be finished by June 4th. Full sterilization of the payload is planned for the week of June 10th-14th in preparation for its first sampling mission. There are a limited number of locations and dates that have the proper FAA waivers for these launches to 10,000 ft or higher.

Project Timeline

- SPS Grant Submission November 15th, 2018
- Spaceport America Cup Competition Entry Form Submission November 16th, 2018
- Motor and Payload Preliminary Design Complete November 17th, 2018
- First Prototype Pump Test November 18th, 2018
- Competition Team Acceptance Announcement December 3rd, 2018
- AIP first check is received by UTK January 14th, 2019
- Filter test on pump shows inadequate airflow January 16th, 2019
- First Draft of Payload Bay door opening mechanism fabricated January 17th, 2019
- First Competition Progress Update January 25th, 2019
- Second competition progress update March 8th, 2019
- First AIP check processed by Office of Research and Engagement at UTK March 23th, 2019
- First parts for the payload arrive March 27th, 2019
- Construction of payload begins April 19th, 2019
- Payload bay door mechanism completion April 26th, 2019.
- Non-sampling hardware shakedown flight test April 27th, 2019
- Flight Ready Rocket Motor Completion April 30th, 2019
- Rocket Motor Static Test Fire May 15th, 2019
- Payload Electronics completed and ground tested May 17th, 2019
- Final Competition Deliverables Due May 17th, 2019
- SPS Interim Report May 31st, 2019
- Reassembly in longer airframe June 4th, 2019
- Sterilization of Payload and Sealing for June 19th, 2019
- Competition Week June 17th through 22nd, 2019
- Further flights Hearne, Texas June 24th, 2019 through August 24th, 2019
- Specimen Analysis June 24th, 2019 through August 24th, 2019
- SPS Final Report December 31st, 2019

Statement of Next Steps

Plan for Carrying Out Remainder of Project (including Timeline)

- The key milestones and the dates by which important steps need to be completed in order to finish the project on time (by December 31).
- Personnel Who will be involved in the remaining research activities and in what way? How many participants are likely to be SPS members? Are there SPS members or others with special expertise that will help to ensure success?
- Personnel As of this moment, there are three national SPS members on our team; there are also 5 non-SPS national members working on the project. There are an additional dozen students on the rocketry team as well. There are still roughly a dozen additional SPS members that have shown a minor interest in working on this.
- Expertise Many of our team members have experience that will prove invaluable to our experiment: Robert
 Nickel and Peter Tarlé both hold Level 2 High Power Rocketry certifications through the Tripoli Rocketry
 Association and years of experience with high power rocketry, and Maggie Spangler is a senior in microbiology
 who will be assisting the team. In addition, three of our members hold at least General Class amateur radio
 licenses and experience with APRS beacons, helping to ensure the rocket and payload will be located after
 landing.
- Research Space All research space has been graciously provided by The Center for Environmental Biotechnology where Dr. Ripp and Veronica Brown will conduct all genetic testing and oversee and direct DNA extraction from our samples with our team.
- Contributions of Faculty Advisors We have three faculty members working closely with us on this project; Maxim Lavrentovich, who serves as our SPS chapter faculty advisor, Dr. Evans Lyne, who is overseeing the design, construction and testing of our launch vehicle, and Dr. Steven Ripp, who is assisting us with gene sequencing and consolidation of data once we collect our samples from the payload.

Future Timeline

- Reassembly in longer airframe June 4th, 2019
- Sterilization of Payload and Sealing for June 19th, 2019
- Competition Week June 17th through 22nd, 2019
- Further flights Hearne, Texas June 24th, 2019 through August 24th, 2019
- Specimen Analysis June 24th, 2019 through August 24th, 2019
- SPS Final Report December 31st, 2019

Bibliography

- [1] Deleon-Rodriguez, N., Lathem, T. L., Rodriguez-R, L. M., Barazesh, J. M., Anderson, B. E., Beyersdorf, A. J., ... Konstantinidis, K. T. (2013). Microbiome of the upper troposphere: Species composition and prevalence, effects of tropical storms, and atmospheric implications. *Proceedings of the National Academy of Sciences*, 110(7), 2575-2580. doi:10.1073/pnas.1212089110
- [2] Maki, T., Hara, K., Kobayashi, F., Kurosaki, Y., Kakikawa, M., Matsuki, A., . . . Iwasaka, Y. (2015). Vertical distribution of airborne bacterial communities in an Asian-dust downwind area, Noto Peninsula. *Atmospheric Environment*, 119, 282-293. doi:10.1016/j.atmosenv.2015.08.052

- [3] Reche, I., D'Orta, G., Mladenov, N., Winget, D. M., & Suttle, C. A. (2018). Deposition rates of viruses and bacteria above the atmospheric boundary layer. *The ISME Journal*, 1154-1162. doi:10.1038/s41396-017-0042-4
- [4] Smets, W., Moretti, S., Denys, S., & Lebeer, S. (2016). Airborne bacteria in the atmosphere: Presence, purpose, and potential. Atmospheric Environment, 139, 214-221. doi:10.1016/j.atmosenv.2016.05.038
- [5] Smith, D. J., & Griffin, D. W. (2013). Inadequate methods and questionable conclusions in atmospheric life study. *Proceedings of the National Academy of Sciences*, *110*(23). doi:10.1073/pnas.1302612110
 [6] Spring, A. M., Docherty, K. M., Domingue, K. D., Kerber, T. V., Mooney, M. M., & Lemmer, K. M. (2018). A Method for Collecting Atmospheric Microbial Samples From Set Altitudes for Use With Next-Generation Sequencing Techniques to Characterize Communities. *Air, Soil and Water Research*, *11*, 117862211878887. doi:10.1177/1178622118788871

Appendix

- Figure 1 Front view of payload electronics
- Figure 2 Side view of payload electronics
- Figure 3 Completed rocket with prototype payload