

Designing Biological Experimentation Systems for High Altitude Ballooning

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The prevalence of bacteria in the atmosphere has been well-established in relevant literature, suggesting that airborne bacteria can influence atmospheric characteristics including the development of clouds. Studies have also demonstrated that the atmospheric biological profile is influenced by the underlying terrestrial biomes. An understanding of the complex interplay of factors that can influence the atmospheric biological profile, not to mention developing a biological census of the atmosphere, requires a cost-effective experimental system capable of generating reproducible results with reliable data. However, as a recent balloon payload launched by JAXA demonstrated, these payloads are both complex and cost prohibitive. This paper discusses the evolution of experimental payloads for high-altitude ballooning for biological experiments that are within the means of most student-run experimental programs. Two proof-of-concept payloads, PHANTOM (Probe for High Altitude Numeration and Tracking of Microorganisms, a payload for the capture of aerial microorganisms at multiple altitudes in order to characterize the biological composition of the upper atmosphere) and ATOMIC (Atmospheric Thindown Originating Mutagenesis Investigational Capsule, which seeks to evaluate bacterial mutagenesis due to radiation), have undergone flight trials. The goal of this project is to develop a self-contained payload capable of real-time telemetry/telecommand and the measurement of atmospheric parameters related to bacterial fluxes.

Nomenclature

HAB = High Altitude Balloon
Aetherophile = Microbiota that are capable of surviving at high altitudes. *

I. Introduction

UNTIL recently, the atmosphere – particularly the parts of it that lie above the troposphere - has been viewed as only a means of transporting biological life, rather than a thriving biome in its own right. However, airborne bacteria have been discovered at altitudes of 8 to 15 km and above, with evidence suggesting that the micro-organisms may exist as permanently established bacterial populations at high altitudes. A 1935 study by Meier [1] reported evidence of microbial life at even higher altitudes in the range of 70-80km. These stable populations of high-altitude bacteria (henceforth referred to as ‘aetherophiles’) have been uncharacterized for the most part due to the limited scope of previous sampling of the microbiome. Most research has focused on low-altitude bacterial populations, whose numbers and compositions are constantly in flux and easily influenced by changes in weather and temperature.

Evidence suggests that the aerial microbiome and aetherophile populations are closely related to the characteristics of the underlying terrestrial biosphere, with microbes being introduced into the atmosphere when aerosolized particulates such as such as respired droplets, dust, and other organic matter are ejected into the atmosphere and are transported distances of over 8 km [2]. The size of the average bacterium, typically in the order of a few micrometers in length, allows airborne microbiota to remain in the atmosphere significantly longer than most other particles. Burrows et. al. [3] identified the mean atmospheric retention time for micro-organisms to be approximately a week, which is sufficient time for a single bacterium to multiply numerous times. The locations of individual cells remain subject to air currents and other atmospheric dynamics during this time period and thereby allow the dispersal of cells to geographic locations several kilometres from their points of origin. Understanding the nature of these nomadic microbes has significant

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*The term was coined by the author; it derives from the Latin root aether (‘upper air’) and -philus (‘liking’).

implications for public health and unmanned aerial flight, given the role aerosol dispersal plays in both the transmission of disease and potentially in developing micro aerial vehicles that are capable of harnessing microbiotic migration pathways to travel long distances.

In contrast to other airborne bacteria, aetherophiles are capable of being active agents of meteorological change in addition to being influenced by atmospheric phenomena. The high-altitude environment in which they exist uniquely positions them to interact with a part of the atmosphere that is highly sensitive to changes. Aetherophiles are capable of acting as nucleation sites for cloud formation, determinants of cloud type, and as manipulators of the chemical composition of the atmosphere through their metabolic activity and interactions with organic molecules. Understanding the manner in which bacteria are capable of inducing environmental changes and the ways in which the health of the terrestrial biome of a particular area can alter weather conditions is also of critical importance to the aerospace community when designing and operating aerial devices, for which weather can be the determining factor in the success of a mission.

Given the unique combination of conditions that characterize the near-space environment – including reduced pressure, lower temperatures (as low as -80°C at 100,000 ft), decreased availability of respirable gases, and increased radiation levels – developing a biological profile of the atmosphere is of great interest from a biological perspective as well. Evolutionary pressures undoubtedly have altered the physiology of aetherophiles to increase their survivability in the harsh conditions of the upper atmosphere, providing for an intriguing juxtaposition against their terrestrial counterparts. In particular, analyzing the mutations and genetic variations that appear in bacteria in relation to altitude may provide better insight into the mutagenesis of terrestrial pathogens.

A long-term biological sampling mechanism also offers a unique window into the vicissitudes of the atmosphere, and has the potential to serve as a tool for environmental health monitoring and possibly predictions of atmospheric behavior, along with a number of alternative applications in both the fields of biology and aerospace. There are, however, a number of challenges that have impeded progress in this promising area of research. For instance, reaching the high altitudes needed for sampling is a non-trivial matter, and the existing options for transporting a scientific instrument to heights of over 30 km are rather limited. High-altitude ballooning is typically the best platform in these cases, given the higher-weight loads it can bear and the altitudes that can be attained on a standard flight. Its optimality as a vehicle for the transport of instrumentation nonetheless does not fail to preclude the omnipresence of microbial life from posing a constant contamination risk to any sort of atmospheric biological sampling mechanism. Without careful precaution, terrestrial bacteria carried on the surface of the mechanism can intermix with aerial samples. Although pre-existing designs for contamination-free devices do exist, implementing them is often a costly and time-intensive endeavour that is prohibitive to smaller research programs and student-run groups. Additionally, even once samples are collected, there is often difficulty in maintaining live cultures of aetherophiles due to their poor adaptability to terrestrial conditions and the rapid growth of more adaptable terrestrial species such as *Escherichia coli*.

This paper seeks to describe methods by which systems designers may overcome these and other obstacles when developing, constructing, and testing systems for biological sampling and experimentation. In particular, this discussion focuses on two different payloads that were designed to be lightweight, cost-effective, and compatible with the high-altitude ballooning platform.

II. Biological Payloads in the Context of High Altitude Ballooning

A. Standard Features of High Altitude Balloon Flights

The platform of a high altitude balloon (HAB) provides a unique and accessible means of studying the atmosphere and near-space environment, particularly through a biological lens. In terms of accessibility, the cost of a single high altitude balloon launch can be as low as \$1000 – well within the means of small research groups and some student-run teams - depending on the size of balloon used and the amount of lift needed for the payload string. HAB flights also do not rely on any active propulsion that might cause excessive disturbances in the pre-existing atmospheric biome (e.g. turbulence), a key characteristic for observational payloads with a biological focus.

HABs have the additional benefit of being highly modularized and easily customizable. Unlike with other aerial vehicles, it is fairly trivial to alter a number of flight-critical variables (e.g. target burst altitude, lift capacity, etc.) within certain tolerances on the launchpad of a HAB flight if necessary, or to interchange various flight-critical parts with spares in the case of an unexpected failure (e.g. inadvertent and/or premature termination of the balloon). This is well-suited for biological experimental systems, where one may need to alter the speed at which the balloon ascends or the altitude at which the balloon bursts in order to ensure the payload's exposure to conditions of experimental interest.

A standard high altitude balloon flight using a 1600 g latex balloon, filled with 13 m³ of helium and with a payload string approximately 5 kg in weight can last two to three hours on average, and can reach 25 km to over 30 km in altitude. The bulk of the flight is the ascent, during which the balloon climbs at a rate of around 5 m/s (if one assumes around 2 kg of free lift). This provides adequate time for a biological payload to receive exposure to near-space conditions and sufficient airflow to collect samples during ascent. As a passive vehicle, the balloon is also highly subject to the types of powerful air currents that move large quantities of airborne bacteria, and thus has the capability of following the direction of microbial migration during its journey to the stratosphere. HABs like the one described herein are capable of traveling an average of 72 km downrange from the initial launch site, and their flight patterns describe sprawling arcs that can cover a wide geographical area and a number of different terrestrial biomes.

B. Challenges of HAB Flights

Despite the numerous benefits of using high-altitude ballooning as a platform for launching an aerobiological payload, there are a number of constraints and challenges that have to be considered when designing a biological experimentation system for flight.

Most notable among these constraints are weight and dimensions, which in turn limit the construction materials and parts that can be used. Although foam core board is a structural material of choice for payload exteriors, using easily degradable material is restrictive for biological payloads that require repeated sterilization with liquid disinfectants. Thin sheets of aluminium alloy or other such lightweight metals are a viable option, but are more difficult to work with and take time to machine. Liquids of any sort are also not permissible in payloads due to the sub-freezing temperatures experienced during flight and the potential for spillage or container rupture.

The potential for mechanical damage must also be taken into account during payload design. During flights, payloads experience dramatic shifts in environmental conditions while in the air, and need the capability to endure a gamut of environmental conditions that might otherwise stress the external structure of the payload and threaten sensitive items inside the payload. Pressure changes, for instance, can place stresses on the box that can cause it to either explode or implode if it is not properly vented, something which poses a particular issue to a biological payload that needs to minimize or entirely eliminate outside contamination. In addition, payloads need to be able to withstand high-velocity wind speeds of close to 70 m/s during descent prior to parachute deployment, and can impact the ground or other terrestrially-based obstacle at a speed of 5-10 m/s [4].

Arguably the most imperative requirement of any payload, however, is to adhere to financial constraints. The financial burden associated with either buying or fabricating parts for a payload is not insignificant, especially for payloads with more complex designs or housing sophisticated equipment. This particularly affects biological payloads, where standard experimental equipment such as the biological air samplers recommended by ISO 14698 [5] can cost upwards of \$1500 – more than the operational cost of some HAB launches. In order to keep a biological payload within the means of a student-operated ballooning group, such costly equipment has to instead be substituted with innovative and unconventional solutions that harness the unique environmental conditions associated with HAB flights in an experimentally viable manner.

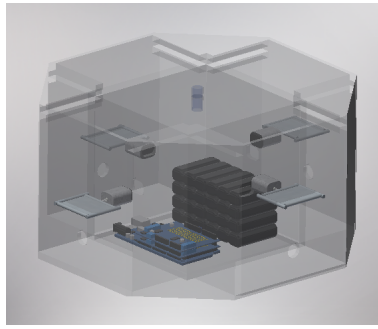
Even with the challenges that face payloads designed for HAB flights, it is nonetheless possible to design, construct, and test biological experimental systems capable of obtaining scientifically viable data and samples. The two payloads whose evolution and designs are described herein are a testament to the feasibility of weight, space, and cost-effective biological payloads.

III. Case Studies in Biological Payload Design

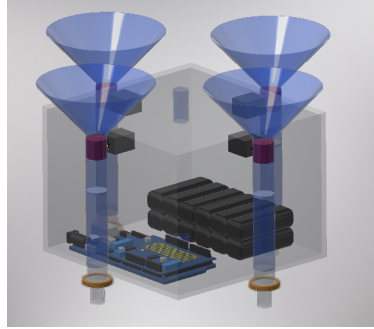
A. Probe for High Altitude Numeration and Tracking of Micro-organisms (PHANTOM)

As aforementioned, the microbiome of the upper atmosphere – in particular, the upper troposphere and stratosphere – has only recently started to be documented. The longstanding assumption that the microbiome of this region is transient, with its denizens primarily dictated by air currents, has meant that very little if any longitudinal information on the distribution and health of aetherophile populations exists in the literature.

PHANTOM was designed with the intention of facilitating the reconnaissance and identification of micro-organismal species across various altitudes in a cost-effective, lightweight, and spatially conservative system. With long-term use, such a system is capable of collecting a sufficiently large data sample with enough samples from different points within any one geographical region and over a sufficient span of time to allow for the density of different aetherophilic species



(a) PHANTOM v. 0.2 featured retractable doors at the top of the payload that would expose rotating pieces of filter paper to the atmosphere.



(b) PHANTOM 1.0 featured four funnels that shunted air into tubes that were opened and closed via solenoid valves (colored black and red in the image); the air would then be forced through syringe filters (orange).



(c) An image of PHANTOM 2.0 at a launch (bottom payload on string).

Fig. 1 The evolution of PHANTOM over time.

to be mapped across various altitudes, independent of launch location.

To achieve this goal, PHANTOM relies on active air sampling mechanisms that activate mid-flight at predetermined altitudes through pre-programmed, self-contained electronic control systems. Studies involving air sampling methods for sterility testing of hospital operating theatres and other areas in which sterility is of paramount importance have found that active sampling methods (which involve drawing particulates present in the air over a collection surface) [6] are superior to passive collection methods (which use gravity to collect airborne bacteria that settle on the collection surface). In particular, the preferred method for air monitoring is active sampling via impaction, which involves accelerating particulates present in the air – including micro-organisms – onto an agar plate that fosters bacterial growth [7]. This method has been found to be the most effective at collecting bacteria and at avoiding false positives, with the Andersen 6-stage and its derivatives being by far the most effective designs.

The first design for PHANTOM was in essence an adaptation of the airflow-based techniques used in standard impactors for high altitude flight. While commercial-grade impactors are expensive and prohibitively heavy (often up to 3.9 kg in weight) [8], the adapted design replaced the continuous duty vacuum pump from the Andersen-style impactor [9] with small fans that would draw air into a chamber through small vents covered in two layers of lab-grade filter paper (the top one with a larger pore size to filter contaminating debris, and the second one rated for biological filtration with a $<1 \mu\text{m}$ pore size to capture micro-organisms). The design featured four such chambers, segregated from each other with the foam-core material used to construct and insulate the box, and with the fans activated based on altitude measurements read in from a BMP280 sensor connected to an Arduino Mega.

Unlike impactors, however, PHANTOM is not intended to continuously process air for sample collection. As an altitude-based payload with the goal of developing a biological profile of the atmosphere, it is essential that samples from different altitudes be appropriately segregated to avoid cross-contamination. In the initial design, the top layer of filter paper was constantly exposed to the atmosphere; it was therefore possible for bacteria to collect on the paper even when the fan systems were deactivated, and given the rapid ascent rate of HABs, it was more than likely that the force of air rushing past would be sufficient to push contaminated air into the interior of the box.

PHANTOM was subsequently redesigned to include collection mechanisms that were located in regions separated from the rest of the environment. While a design with linearly actuated retractable doors and rotating collector arrays of filter paper was considered (Fig. 1a), the cost and weight of linear actuators of the desired dimensions was prohibitive. Instead, the design for the first PHANTOM prototype (dubbed PHANTOM 1.0) integrated four solenoid valves controlled by the same Arduino Mega/BMP280-based system as the previous design (Fig. 1b). The solenoids were embedded in the walls of the payload box such that the valve inlets faced upwards, with plastic funnels forcing air through the valves and into syringe filters secured over the outlets. The payload was programmed so that each valve opened at a specific altitude (one at 5.00 km, one at 10.00 km, one at 15.00 km and one at 25.00 km) for ten seconds, during which time bacterial samples would theoretically accumulate in the syringe filter. The on-board Arduino was also programmed to log the real-time clock and BMP280 sensor data (altitude, humidity, temperature, and pressure) once per second during

the entire flight. The entire system ran off of a 1200 mAh 12V LiPo battery connected to a low-voltage cutoff circuit.

The launch of PHANTOM 1.0 provided an opportunity to test the functionality of the payload's electronic circuitry under near-space conditions, especially since the valves were exposed during flight. Based on the logs retrieved post-flight, the valves successfully activated at the appropriate altitude despite the interior of the payload experiencing temperatures below -6°C. A vulnerability was discovered where the syringe filters – which were affixed to the valves using a Luer-locking joint supplemented with epoxy and hot glue – were susceptible to being forcibly detached from the rest of the payloads on impact during landing. Due to PHANTOM's location at the bottom of the payload string, it also took the brunt of the impact, a fact that was taken into account during the design and launch of PHANTOM 2.0.

PHANTOM 2.0 (Fig.1c) saw a radical change in design, with the four-valve design being replaced in favour of one that did not involve any mission-critical parts projecting beyond the walls of the payload box. Instead, the new prototype featured a two-compartment design, with the bottom compartment being reserved for the same Arduino-based electronics system as before and with the top compartment containing the actual collector array. Rather than using a valve to restrict airflow to any particular piece of filter paper, the new system utilized a stepper motor affixed to two CDs with lab-grade filter paper sandwiched between them. The top CD had 4 evenly spaced holes, each approximately 3 cm in diameter; when a sample was to be collected, the stepper would rotate the CD so that one of the holes in the CD, and the filter paper sandwiched beneath it, would be exposed to the atmosphere via a hole in the box's lid that aligned with it. To close the payload, the stepper merely rotated until it reached a part of the CD without a hole; the air therefore did not come in contact with the filter paper except during the intended time periods.

PHANTOM 2.0 has flown on two separate HAB flights, and was successful each time. The filter paper from the first flight, when immersed in nutritional media and left to incubate at 37°C, appeared to be successful in collecting samples, as the media became cloudy with what appeared to be a mix of bacterial species after incubating overnight. The control filter paper – kept in one of the parts of the collector disk that did not receive exposure – was negative for growth, indicating a successful test.

Based on observations of from PHANTOM 2.0's launches, a new design has evolved for the next iteration of PHANTOM. One of the issues faced when culturing the viable samples from PHANTOM 2.0 was the ease with which more common bacterial species – for instance, *E. coli* – were able to outcompete aetherophiles and thereby overgrow the media. The new design integrated agar gel – which permits any collected specimens to grow in separate locations from one another, instead of being mixed in a single liquid culture – in order to facilitate the capture and culturing of microbiological samples. Additionally, it maintains the two-compartment design from PHANTOM 2.0 – with the collection agar set up on the inside of servo-powered drawbridge-style doors in the bottom section, and the Arduino electronics system located in the top compartment – in order to minimize or entirely eliminate the need to open the payload on the launchpad. The need for benzalkonium chloride and ethanol-based disinfectant to repeatedly sterilize the interior of the box, as was necessary for PHANTOM 2.0, has also been eliminated.

PHANTOM 3.0 is still being prototyped, but will in theory take advantage of the downward air flow during the balloon's ascent to force particulates onto the agar hydrogel. The servos will be used to open and close the doors bearing the agar gel, at times determined by the Arduino script and altitude sensor data. In its current iteration, PHANTOM 3.0 still lacks telemetry/telecommand capabilities, but it is expected that future versions will include some ground to payload control capabilities in order to allow user input when taking samples. This has the potential to allow for samples to only be taken over specific biomes, or within specific geographical regions, in order to verify the claim that the biome has a significant effect on the aetherophile populations in the atmosphere above.

B. Atmospheric Thindown Originating Mutagenesis Investigational Capsule (ATOMIC)

High-energy ionizing radiation has been confirmed as a mutagen that is capable of damaging or breaking DNA bonds. Although radiation can be lethal to some bacteria – hence the efficacy of UV light in sterilizing surfaces – in lower doses, radiation-induced mutations allow bacteria to evolve traits more rapidly than normal. In the atmosphere, most ionizing radiation is found in the ionosphere, normally located at an altitude that is beyond the reach of HABs. However, drops in temperature – such as those seen during night-time and total solar eclipses – have the potential to lower the altitude of the ionosphere and increase the amount of ionizing radiation that is able to reach the upper troposphere and stratosphere. ATOMIC (Atmospheric Thindown Originating Mutagenesis Investigational Capsule) seeks to investigate how much of an effect naturally occurring doses of ionizing radiation might have on the mutation rates of common bacterial species.

ATOMIC, at its core, is a fairly straightforward experiment – it simply involves placing bacteria in a container during flight, and checking for changes subsequent to the balloon's recovery. However, it involved much more consideration



Fig. 2 The interior layout of ATOMIC featured two sets of bacterial cultures inside a foam-core enclosure.

than expected with regards to design. The first design choice of importance was the selection of which bacterial species to use. *E. coli*, as a commonly occurring bacterium, was a logical choice due to its adaptability and pervasive nature in the environment. *Bacillus subtilis* was also chosen due to its hardy nature and ability to sporulate if overly stressed, and its presence in the natural environment. Classroom-grade strains that were certain to be non-pathogenic and completely harmless were selected for both species, and were ordered in freeze-dried form from Carolina Biological Supply.

At this point, the materials with which the box would be constructed had to be chosen. Standard foam-core was opted for given that the material was the least likely to impede ionizing radiation when compared to plastic or metal. The agar growth media with which the bacteria were cultured also had to undergo testing to ensure that the Petri dishes would not rupture, leak, or shatter in any way during the HAB flight. The performance of hand-poured and store-bought agar plates in a vacuum chamber were compared to test if the bubbles present in the store-bought agar would result in rupture. The results of the pressure test were negative for rupturing in both agar types, though the bubbles in the agar did expand into flat disks in vacuum that later collapsed once pressure returned. It was also noted during pressure testing that Petri dishes also remained intact even after being sealed shut with Parafilm, a beneficial observation that allowed for the culture plates to be sealed against contamination. Yet another issue that was encountered was related to the syneresis of agar. As a hydrogel, agar is primarily composed of water, and thermal tests conducted in the weeks prior to launch indicated that the standard formulation of 0.04g/mL LB (lysogenic broth) agar was highly susceptible to freezing and subsequent thawing that damaged the integrity of the agar and the cultures on it. There was a notable lack of literature on the topic of freezing agar from a microbiological perspective; to obtain a viable formulation that experienced minimal to no syneresis after several minutes of exposure to $< -80^{\circ}\text{C}$ temperatures, it was necessary to rely on findings from culinary and food science literature that recommended a 40% glycerol gel.

A thermal test was conducted with standard nutrient agar, LB agar, 40% glycerol LB agar, and Terrific Broth agar (which was based on a more complex media than the LB agar). The results indicated that only the glycerol agar showed no signs of freezing or subsequent syneresis after five minutes at -60°C , with the LB agar performing the second best and demonstrating slightly less syneresis than the others despite freezing. The glycerol was therefore chosen as the primary agar on which the bacteria would be cultured.

Through the course of preparation for ATOMIC's first launch, it became evident that the glycerol agar drastically slowed down the growth of bacteria. Compared to the LB agar, which demonstrated clearly visible growth within 24 hours of inoculation, the glycerol agar cultures required over seven weeks of growth before they were visible as nearly

transparent specks on the media. The obvious disadvantages of the slow growth rate on glycerol agar were outweighed by the fact that the glycerol agar outperformed the LB and nutrient agars in the amount of time it was able to support live cultures before the cultures required transferring. Thus, glycerol remained the culture media of choice during ATOMIC's launch.

ATOMIC was successfully launched during the 2017 total solar eclipse, experiencing a total flight time of 135 minutes and attaining an altitude between 16000 and 17000 meters at the time of totality. A control payload (of the exact same dimensions and construction as the flight payload) was kept on the ground to compare the effects of the radiation dose micro-organisms received in the atmosphere during the eclipse with the effects of the lower dose experienced by terrestrial microbes. As expected, the glycerol plates did not undergo syneresis during the flight, and the bacterial samples were still viable upon recovery. The samples were subsequently successfully cultured in liquid LB media and cryogenic stocks of the bacteria were prepared for storage until the samples could be analysed via genetic sequencing.

IV. Conclusion

PHANTOM and ATOMIC, in their current iterations, are both proof-of-concept prototypes primarily intended to show the feasibility of low cost biological experimentation systems intended for high altitude conditions. The design process through which both payloads evolved is a clear illustration of the methods through which biological experimentation can be adapted to suit the restrictions and requirements of high altitude balloon flights. Both payload designs are stepping stones to the development of experimental systems capable of real-time telemetry and telecommand for the measurement of atmospheric biological and related parameters and data acquisition. The final designs envisages a platform capable of reducing biological contamination to experimentally tolerable levels, with a robust sampling system inherently capable of holding a sterile environment and assuring sample integrity. These experiments represent a crucial step towards the design and planning of future experiments that will widen the scope of aerobiological research, and expand our understanding of the characteristics and migration of aetherophilic populations. Understanding how biological material from the ground may survive, thrive and be transported via the upper atmosphere has national security implications in addition to meteorological and medical significance.

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