Analyzing the impact of external environmental conditions on Bio-Objects: fungal spores, quail eggs, and mold

Muhsina Musaeva[1], Kamila Temirova[1], Veronika Romanenko[1], Fakhridinova Shirin[1],
Amaliya Dubrovskaya[1], Diyora Kudratillaeva[1], Ezoza Beshimjanova[1], Guzal Madaminova[1],
Diyora Kenjaeva[1], Shakhnoza Khalilova[1], Nodira Tillayeva[2]
[1]UzCosmos, Tech4Impact, UNICEF Uzbekistan, Ministry of Digital Technologies
[2]Nagoya University, Graduate School of Electrical Engineering, Japan
Email: info@unisat.uz

***Abstract*—This paper presents biological response analysis from a CubeSat mission to 27 km stratospheric altitude. Two quail eggs protected by custom-designed shell and foam packaging, along with fungal spore cultures, were exposed to stratospheric conditions and successfully recovered after ground impact. Biochemical analysis via Xanthoproteic and Biuret reactions revealed significant protein structural modifications in both eggs, indicating molecular-level stress responses. Oyster mushroom mycelium exhibited differential viability across four chemical environments: water-based cultures showed active hyphal development and sporulation, boric acid inhibited growth while maintaining sterility, ammonia solution enabled contamination growth, and dry samples demonstrated limited activity. Mold species on nutrient substrates displayed extensive networks and reproduction. Results demonstrate that engineered protection enables biological sample survival through stratospheric stress while preserving measurable biochemical changes, with implications for astrobiology research and spacecraft biosafety protocols.**

***Index Terms*—Stratospheric balloon, nanosatellite, biological experiment, atmospheric data, environmental stress, payload analysis**

I. Introduction

Understanding biological responses to extreme environmental conditions is critical for advancing astrobiology, assessing planetary protection requirements, and developing life support systems for long-duration space missions [1]. The stratosphere, at altitudes between 20-30 km, provides an accessible analog environment for studying biological adaptation to near-space conditions, characterized by extreme temperature fluctuations (56°C to 90°C), reduced atmospheric pressure (3.8

This research was conducted as part of the UniSat 3.0 program in Uzbekistan - a pioneering educational initiative jointly implemented by UNICEF Uzbekistan, Tech4Impact NGO, and the Space Research and Technology Agency under the Ministry of Digital Technologies. Launched in 2021, the UniSat program aims to develop STEM competencies among young women in Uzbekistan while advancing the nation's nanosatellite capabilities. Since its inception, over 700 girls from all regions of Uzbekistan have participated in hands-on training in satellite construction, programming, radio electronics, and space mission operations. The October 2025 mission from Karakalpakstan represents the culmination of intensive training where 20 finalists assembled two three-unit CubeSats equipped with environmental sensors and biological payloads [2].

Uzbekistan's growing nanosatellite ecosystem represents a significant development in Central Asian space research. The UniSat program provides critical infrastructure for conducting stratospheric biology experiments while training the next generation of aerospace engineers. This dual focus on scientific research and capacity building positions Uzbekistan as an emerging contributor to space life sciences research.

A. Importance of Stratospheric Simulations

Stratospheric balloon missions offer several advantages over ground-based space simulation chambers and orbital experiments. Unlike vacuum chambers that can only approximate individual environmental parameters, stratospheric exposure provides authentic combinations of pressure, temperature, radiation, and atmospheric composition [3]. Compared to orbital experiments on the International Space Station, stratospheric missions are significantly more cost-effective, have shorter development cycles, and enable rapid sample recovery. This makes them ideal for educational programs and preliminary investigations before committing to orbital missions.

B. Rationale for Biological Sample Selection

We selected two distinct biological systems that address complementary research questions:

Quail Eggs as Macroscopic Biological Systems: Eggs represent complex, self-contained biological structures with well-characterized biochemistry. Avian eggs have been proposed for closed-loop life support systems in space habitats [**?**]. However, previous stratospheric biology research has focused primarily on microbial samples, leaving gaps in understanding how engineered protection systems can preserve macroscopic organisms while still allowing measurable physiological responses to environmental stress. The choice of quail eggs enables direct assessment of both mechanical protection engineering and protein-level biochemical changes.

Fungal Spores as Model Extremophiles: Fungi represent critical organisms for bioregenerative life support systems and pose contamination risks for planetary protection [4]. Oyster mushroom (Pleurotus spp.) spores were selected due to their potential application in waste recycling systems for space habitats and their documented resilience to environmental stress. By exposing spores to four distinct chemical environments (boric acid, ammonia, aqueous, and dry conditions), we can systematically isolate the effects of stratospheric stress from substrate-specific factors—an approach not previously documented in stratospheric research.

C. Research Gap and Problem Statement

Despite decades of space biology research, three critical gaps remain:

Macroscopic organism protection: While microbial survival in extreme environments is well-documented, systematic studies on protecting larger biological structures during stratospheric missions are limited. No previous research has quantified the effectiveness of custom protective shells for preserving delicate biological samples through launch, exposure, and hard ground impact. **Protein-level stress responses:** Most stratospheric biology studies focus on organism survival rates rather than molecular-level biochemical changes. Understanding how proteins respond to stratospheric stress is essential for assessing food stability and biochemical system functionality in space environments. **Environmental interaction effects:** Previous fungal studies in extreme environments typically examine single growth conditions, making it difficult to separate intrinsic organism resilience from substrate-dependent protection or enhancement effects.

D. Hypotheses and Research Objectives

Based on preliminary ground-based tests and literature review, we formulated three primary hypotheses:

H1 (Egg Protection): Custom-engineered protective shells will enable quail eggs to survive stratospheric exposure and ground impact while exhibiting measurable protein denaturation due to temperature extremes and pressure changes.

H2 (Differential Fungal Response): The post-flight viability and mycelial development of Oyster mushroom spores are expected to be highest in both dry and boric acid environments, as these conditions enforce a state of deep metabolic dormancy, preserving internal resources and integrity against flight stress. Conversely, the aqueous environment will yield substantially lower viability due to partial metabolic activation and increased radiation damage susceptibility. The ammonia environment is predicted to result in the greatest inhibition or complete loss of viability due to its inherent chemical toxicity to fungal spores.

H3 (Contamination Dynamics): Chemical environments (particularly ammonia) will exhibit unexpected contamination patterns due to selective pressure favoring alkaliphilic or extremophile organisms present in the starting material.

This study aims to: (1) validate protective engineering approaches for biological payloads in stratospheric missions; (2) quantify protein structural modifications induced by stratospheric exposure using standard biochemical assays; and (3) characterize fungal resilience mechanisms across varied chemical environments, contributing to understanding of organism-environment interactions under extreme stress conditions.

E. Problem Statement

Most stratospheric biology studies focus on microorganisms, leaving limited understanding of how larger biological systems endure combined stressors such as low pressure, temperature extremes, radiation, and mechanical impact. The performance of engineered protective systems for fragile samples under such conditions also remains largely unexplored. This study investigates how stratospheric exposure affects the structural and biochemical stability of quail eggs and fungal spores carried aboard a CubeSat balloon to 27 km altitude. One egg was enclosed in a 3D-printed shell to test mechanical resilience during descent, while another was exposed to radiation to analyze protein modifications. In parallel, Pleurotus spp. spores were placed in four media - air, water, boric acid, and ammonia - to assess how different chemical environments influence survival and contamination after flight. The core problem addressed is: *How do stratospheric stress factors - temperature, radiation, and impact - affect the integrity and biochemical properties of biological systems, and to what extent can protective engineering and chemical environments mitigate these effects?*

II. Related Work

The Earth's middle stratosphere (20–35 km altitude) provides a natural analog to Martian surface conditions due to its combination of low pressure, intense ultraviolet (UV-B/UV-C) and ionizing radiation, and extremely low temperatures. It has therefore become a valuable environment for testing microbial survival and planetary-protection hypotheses [5], [6], [7].

NASA's E-MIST campaigns, for example, exposed *Bacillus pumilus* SAFR-032 spores at 30–32 km altitude for several hours, revealing rapid loss of viability primarily caused by UV radiation [5]. These findings informed planetary protection models for Mars missions. More recently, missions such as MARSBOx [8] and BIOSEP [9] have extended this approach to various microorganisms under atmospheric pressures similar to those on Mars. Collectively, these experiments demonstrate that UV flux is the dominant limiting factor for microbial survival in near-space environments.

In parallel, aerobiological research has confirmed that viable microorganisms can occasionally be transported into the stratosphere by natural or anthropogenic processes, although long-term survival depends both on the duration of exposure and UV shielding [10], [11]. These results support the view that the stratosphere can serve as both a planetary analog laboratory and a pathway for transient biological transport on Earth.

A. Fungal and Mold Spores Under Stratospheric Stress

Among eukaryotic microorganisms, melanized fungal spores - particularly those of *Aspergillus niger* - display exceptional resilience to radiation and desiccation. Laboratory studies report LD_{90} values exceeding 10^3 J m^{-2} for UV-C, along with strong tolerance to ionizing radiation, which is attributed to the protective role of melanin [12]. Balloon-borne studies such as MARSBOx confirmed this robustness under near-space conditions, revealing that *A. niger* spores retained substantial viability even after cumulative UV doses of approximately 1148 kJ m^{-2} [8].

Further work with extremophilic yeasts isolated from hyperarid environments, such as the Atacama Desert, also demonstrated partial survival after multi-hour exposure to stratospheric UV and pressure cycles [13]. These findings emphasize that shielding, hydration, and pigmentation are key determinants of fungal endurance. However, existing studies have largely compared only dry versus hydrated or UV-exposed versus UV-shielded conditions. No published data systematically examine how chemically distinct media - such as antiseptics or nitrogen-based compounds - affect fungal survival or contamination dynamics under stratospheric stress.

B. Bacterial Survival Under Coupled Stressors

Multiple balloon-borne bacterial studies have shown that survival is primarily UV-limited; even minimal shielding substantially improves post-flight viability [7], [11]. Laboratory follow-ups further reveal that exposure to combined low pressure, radiation, and temperature fluctuations can modify bacterial physiology and antibiotic sensitivity. These observations indicate that coupled environmental stressors, rather than UV radiation alone, can induce adaptive phenotypic shifts - an important consideration for long-term microbial behavior in extraterrestrial environments.

C. Avian Eggs and Protein Stability Under Radiation

Direct exposure of intact avian eggs to the stratosphere has not been previously reported. Nonetheless, controlled laboratory studies on poultry and quail eggs provide relevant mechanistic context. Low-dose pre-incubation gamma irradiation (0.1–1 Gy) has been associated with changes in hatchability, chick mass, and albumen quality [14]. Other investigations show that radiation alters egg-white glycoproteins, such as ovomucoid, and affects color and proximate composition in processed egg powders [15]. While these studies characterize radiation–protein interactions under controlled conditions, they do not replicate the combined effects of UV radiation, low pressure, temperature fluctuation, and typical stratosphere desiccation. Thus, the response of whole eggs to authentic near-space environments remains unexplored.

D. Positioning of the Present Study

Previous microbial missions have generally reached altitudes between 30 and 38 km, using institutional gondolas with specialized instrumentation. The present mission, which achieved a peak altitude of approximately 27 km, operated under comparable environmental parameters but introduced several novel elements.

First, the study adopted a chemistry-aware design by exposing *Pleurotus* spp. fungal spores to four distinct chemical environments - air, water, boric acid, and ammonia - allowing investigation of how local chemical composition affects survival, contamination, and post-flight recovery. Second, it incorporated macroscopic biological samples - quail eggs - to evaluate both mechanical resilience and biochemical alteration under authentic stratospheric conditions. Finally, the experiment was implemented within the UniSat educational CubeSat framework, demonstrating that scientifically robust biological missions can be realized through compact, student-engineered nanosatellite systems.

Together, these features extend existing research by integrating both microbial and macroscopic biological systems in a single low-cost platform, providing new insights into how engineered protection and environmental chemistry influence biological stability in the stratosphere.

E. Research Gaps

Despite advances in high-altitude biological studies, significant gaps persist. Previous work has not addressed the effect of chemically variable environments on fungal resilience, leaving unknown the role of substances such as boric acid or ammonia in modulating survival under radiation and desiccation. Likewise, there are no reports on the exposure of intact avian eggs to the combined environmental stressors of the stratosphere. Finally, while several balloon experiments have been carried out, few document fully integrated CubeSat-scale payloads capable of simultaneously recording environmental parameters and biological responses.

By addressing these gaps, the present study contributes an integrated, multidisciplinary dataset that unites biological, biochemical, and engineering dimensions, advancing the understanding of organismal responses to near-space environments.

III. Methodology

The following section describes the experimental design and technical configuration of the CubeSat used in this study. The methodology covers the mission architecture, biological and material payload preparation, and the environmental data collection process implemented during the near-space flight.

A. Mission Design and Payloads

The CubeSat was divided into three main units, each serving a distinct experimental or instrumental purpose. The first unit contained all the main sensors and electronic components responsible for collecting data throughout the mission. The second unit was designed to host two biological experiments — one involving fungal spores and mold cultures in a custom 3D-printed compartment with six separated chambers, and another featuring a quail egg secured in foam to observe protein stability under stratospheric conditions. The third unit housed an additional quail egg placed in a lightweight 3D-printed holder designed to monitor structural and thermal effects during flight. Together, these modules were assembled into a unified three-unit CubeSat platform, providing both environmental monitoring and biological research capabilities within a compact structure.

Structural and Material Design: The CubeSat's external frame was constructed from metallic rods, forming the main ribs of the structure and providing mechanical strength and rigidity during flight. The side panels were made of transparent plastic, which reduced the overall weight while maintaining structural stability and allowing internal components to be visually inspected.

Most internal components, including the protective casings for biological samples and other support structures, were designed and produced using 3D printing technology. Plastic materials such as PLA were used to fabricate the egg holders, the container for the fungus and spore experiment, and several small compartments inside the CubeSat. Foam material was also utilized in one of the biological setups to provide cushioning and prevent damage to delicate samples during ascent and descent.

Sensors and Measurements: To ensure continuous monitoring of environmental and flight conditions, several sensors were integrated into the CubeSat's system.All sensors were connected to the onboard microcontroller system for real-time data logging during ascent and descent. Two STM temperature sensors were installed: one recorded external stratospheric temperatures, while the other tracked internal temperature fluctuations affecting the biological payloads.

In addition to thermal monitoring, positional and motion data were collected through a GPS module and a three-axis accelerometer. These components provided information about altitude, trajectory, and acceleration throughout the flight.

Environmental composition was assessed using specialized instruments: a UV sensor measured the intensity of ultraviolet radiation, and an ozone sensor detected variations in ozone concentration—particularly relevant for the fungal and spore experiment. Furthermore, a barometer and a hygrometer were included to measure atmospheric pressure and humidity, allowing the correlation of external environmental changes with biological sample behavior.

Fungal and Mold Growth Module: The fungal and mold growth module was entirely designed and produced through 3D modeling and additive manufacturing. The structure was modeled by using CAD OnShape software to ensure precise dimensions compatible with the CubeSat's internal layout. The final model was printed on a 3D printer using durable plastic filament, chosen for its light weight and thermal resistance. The container consisted of six individual compartments: four of them were used for fungal samples exposed to distinct chemical environments (boric acid, ammonia, water, and dry spores), while the remaining two contained mold cultures, one in a moist and one in a dry state. Multiple small openings were distributed along the sides of the printed container to allow radiation and airflow penetration, ensuring realistic stratospheric exposure. The module was then fixed within the CubeSat using double-sided adhesive material to secure it during ascent and descent. This design allowed for controlled observation of fungal and mold responses to extreme temperature, pressure, and radiation variations.

Protein Stability Observation Module: The protein stability module was designed to study how biological material responds to external temperature and pressure changes. A quail egg was selected as the biological subject and wrapped in a protective layer of foam to absorb mechanical shock and thermal fluctuations. The foam was carefully modeled and cut to match the egg's dimensions, then fastened with elastic bands to prevent internal movement during flight. The entire setup was placed in the lower section of the CubeSat's second unit. This module's design emphasized simple yet effective mechanical protection, enabling analysis of the egg's internal protein stability after exposure to the stratosphere.

Quail Egg Design: For the third payload, our team developed a specialized structural setup to investigate how effectively the supporting mechanism could protect the egg from mechanical stress. The 3D modeling team designed a custom holder that precisely matched the shape of a quail egg. The holder was composed of two interlocking halves, allowing the egg to be enclosed securely inside. Its grid-like design ensured both stability and low weight, keeping the egg firmly centered within the structure.

Two small holes were made on each side of the holder to pass a thin yet strong cord through. The cord was selected for its high tensile strength and flexibility, enabling it to absorb part of the impact energy during the mission. Additionally, four holes were placed at the top of the holder so that the cords could be attached to the CubeSat's internal frame. This suspension system held the egg in the middle of the structure, minimizing direct impact and allowing the forces to be distributed evenly.

Prior to integration into the CubeSat, several ground tests were conducted to confirm the setup's stability and damping capability. The results showed that the design successfully maintained the egg's position and provided adequate protection from vibration and shock, confirming its readiness for launch.

B. Data Retrieval and Analysis

All environmental data were stored on the onboard microcontroller in comma-separated format and retrieved immediately after payload recovery. Data integrity was first verified by cross-checking timestamp alignment across all sensors. Minor inconsistencies arising from transient signal loss were corrected using linear interpolation and smoothing algorithms. The GPS altitude channel, which briefly failed near peak ascent, was reconstructed using pressure-derived estimates based on standard atmospheric equations.

Post-flight data processing and visualization were conducted in *Python* using the NumPy [16], Pandas [17], and Matplotlib [18] libraries. The workflow consisted of four main steps: data import, cleaning, synchronization, and graphical analysis. Outlier detection was applied to remove high-frequency electrical noise, particularly in the ultraviolet and acceleration channels. The UV dataset was additionally processed using a moving-average low-pass filter to recover the underlying irradiance trend obscured by analog-to-digital interference.

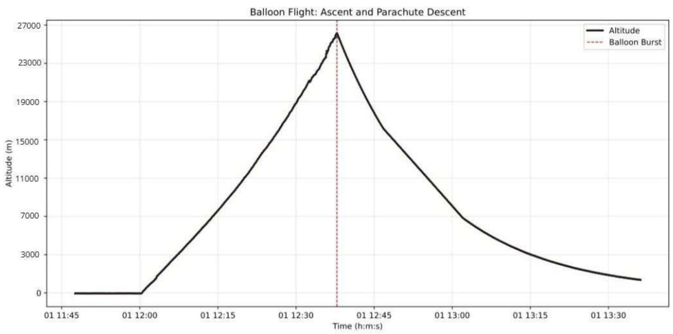

Fig. 1. Altitude vs. mission time. The abrupt drop near 12:45–13:00 is an instrument dropout, not a real loss of altitude.

Environmental variables - temperature, humidity, pressure, acceleration, and UV intensity - were then correlated with key flight phases (ascent, burst, descent, and landing) to characterize the full stratospheric stress profile. Quantitative time-series outputs were combined with qualitative biological observations, including egg protein modification and fungal viability, to interpret how physical conditions influenced biochemical and microbiological outcomes. This integrated analytical approach provided the empirical basis for the evaluation and discussion presented in subsequent sections.

C. Environmental Measurements

During the mission, the CubeSat continuously logged environmental parameters throughout ascent and descent, allowing reconstruction of the stratospheric flight profile. Data were recorded at one-minute intervals and synchronized with payload timestamps.

Altitude and flight duration: The balloon ascended steadily to the stratosphere, reaching a peak altitude on the order of 27 km, and then transitioned into a rapid descent phase under parachute. The altitude profile over time is shown in Fig. 1. The sharp apparent drop to near ground level between approximately 12:45 and 13:00 does not represent an actual loss of altitude; it reflects a temporary failure in the high-altitude positioning chain, during which the GPS solution and derived altitude estimate collapsed and then recovered. Outside of this interval, the curve captures a smooth climb and post-burst descent consistent with expected balloon dynamics.

Internal and external temperature variation: Throughout the flight, the internal payload temperature remained significantly higher than the ambient environment, reflecting the self-heating of onboard electronics and partial thermal insulation of the housing (Fig. 6). During ascent, the external temperature decreased rapidly to below -40 °C, while the internal temperature stabilized around -20 °C. Both sensors recorded a rapid rise in temperature after descent as the payload re-entered denser atmospheric layers. This dual-temperature profile confirms that the biological samples experienced stratospheric cold stress while remaining within a survivable range inside the enclosure.

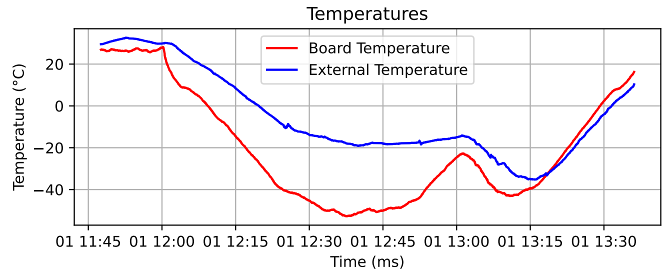

Fig. 2. Internal (red) and external (blue) temperature variation during flight. The internal payload remained warmer due to electronic self-heating and insulation.

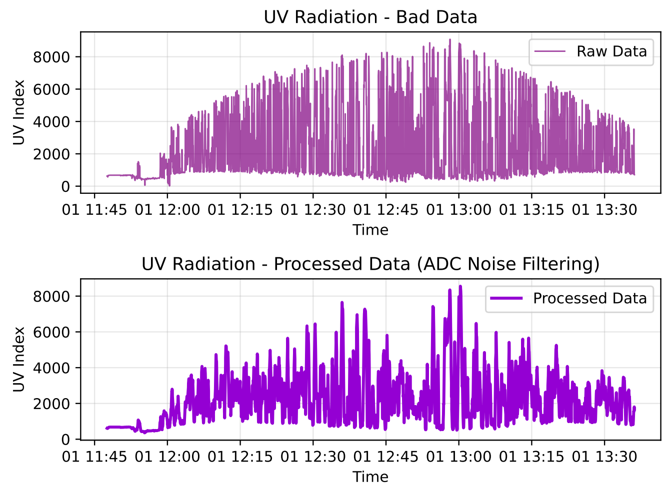

Fig. 3. Ultraviolet radiation intensity vs. time. Top: raw unfiltered signal; bottom: filtered data showing the true UV exposure profile.

Ultraviolet radiation intensity: The UV sensor captured a strong increase in radiation intensity as the payload ascended into the stratosphere (Fig. 3). The upper panel presents the raw data, which contained substantial analog-to-digital conversion noise due to high-frequency signal interference. The lower panel shows the same dataset after digital filtering, revealing a clear irradiance trend that peaks near the flight's maximum altitude and gradually decreases during descent. This processed signal accurately represents the exposure profile experienced by the biological samples, confirming that they were subjected to extended high-UV conditions typical of near-space environments.

Vertical acceleration dynamics: The accelerometer data (Fig. 4) illustrate the dynamic environment experienced by the payload throughout the mission. During the ascent phase, acceleration remained near zero, indicating stable balloon lift with only minor oscillations. A sharp increase occurred shortly before balloon burst, corresponding to the onset of rapid vertical motion. The subsequent large fluctuations and transient negative spikes mark the descent and parachute deployment

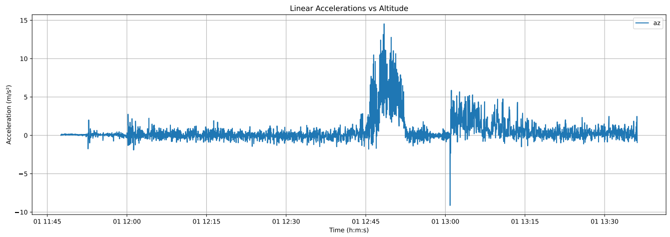

Fig. 4. Vertical acceleration vs. mission time. Spikes correspond to balloon burst, parachute deployment, and ground impact events.

sequence. A short, high-magnitude pulse near the end of the record reflects the final ground impact. These acceleration peaks define the mechanical loads acting on the protective shell enclosing the biological samples.

Overall, the environmental data confirm that the payload experienced authentic stratospheric stressors: low pressure, extreme cold, intense UV radiation, and severe desiccation. These conditions define the context for the biological responses discussed in subsequent sections.

IV. Evaluation

The following section presents and interprets the experimental results obtained from the CubeSat mission. It summarizes the biological and engineering outcomes observed during and after the near-space flight, followed by an analysis of sample integrity, structural behavior, and material responses.

A. Results and Findings

A. **Egg Construction.** The developed engineering solution successfully ensured the mechanical stability of the biological specimen throughout the entire experiment. A system of dynamic shock absorption was designed and implemented to minimize the impact of vibrational and shock loads.

The core principle of protection was the tensorial suspension of the specimen. Using 3D printing, a specialized holder was fabricated that allowed the quail egg to be rigidly fixed in the isometric center of the container's lower section. The specimen was isolated from direct contact with rigid structural elements by means of taut, shock-absorbing filaments.

The system demonstrated high efficacy under flight conditions. During the ascent phase, characterized by significant vibrations, the suspension structure successfully damped the transmission of kinetic energy. The most crucial test was the impact load upon landing, where the shock-absorbing filaments entirely compensated for the energy of the impact. The subsequent analysis confirmed that the quail egg maintained complete external integrity — no macroscopic signs of mechanical damage (cracks or fractures) were detected.

B. Protein Structure. To assess the structural impact of stratospheric factors on the biological material, a comparative analysis was performed on the quail egg protein—one flown to an altitude of 27 km and a control (ground) sample—using qualitative reactions. The results unequivocally demonstrate a substantial structural modification of the protein after the flight.

Fig. 5. Comparative Post-Flight Analysis of Biological Samples. Illustrated here are the three main biological subjects: the quail egg (A) with preserved external structure;Oyster mushroom spores (B) and the highly resilient Aspergillus niger (C) which survived and continued active reproduction after stratospheric exposure

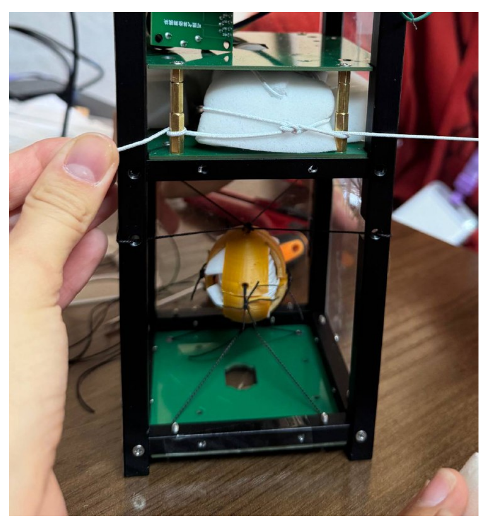

Fig. 6. 3D Model of Egg Cage. The specialized 3D-printed holder rigidly fixes the egg within the container's isometric center, utilizing taut filaments for dynamic shock absorption and complete isolation from rigid structural elements to ensure mechanical integrity during flight and impact.

Fig. 7. Xanthoproteic Reaction Showing Protein Modification After Flight. The immediate yellow coloring of the space-exposed sample, observed without heat activation, suggests that stratospheric stress caused oxidative destruction of the protein's aromatic rings, increasing their chemical accessibility compared to the native control.

Fig. 8. Biuret Reaction Confirming Post-Flight Protein Degradation. The decreased intensity of the violet coloration in the space-exposed sample compared to the control visually confirms the partial hydrolysis of peptide bonds and fragmentation of the protein structure following stratospheric exposure

The **Xanthoproteic reaction**, used to detect aromatic amino acids, revealed noticeable differences in the chemical activity of the samples. The control (ground) protein exhibited yellow coloring exclusively after heating, which is normal for a native protein structure. In contrast, the test (space) specimen reacted **immediately** without additional thermal activation. This phenomenon suggests that the protein likely underwent **oxidative destruction of its aromatic rings**under near-space conditions, making these groups more accessible to interaction with the reagent. The subsequent addition of alkali also highlighted a difference: the ground sample produced a darker orange hue, further indicating deep chemical changes in the state of the flown proteins.

The **Biuret reaction**, aimed at identifying peptide bonds (an indicator of polypeptide chain length), confirmed structural degradation. The control sample showed intense violet coloring, signifying the preservation of long polypeptide chains. Conversely, the space specimen exhibited a **less intense violet coloration**. This decrease in color intensity is direct evidence of the partial destruction (hydrolysis) of peptide bonds. Thus, the protein exposed to the extreme cold and high radiation background of the stratosphere suffered **fragmentation of its polypeptide chains**, a clear sign of degradation and denaturation of its structure.

C. **Aspergillus niger** (black mold). As part of the study, the viability and reproductive activity of the micromycete *Aspergillus niger* (black mold) exposed to extreme stratospheric conditions were assessed. Mold samples, isolated from cherry compote, were prepared in two different states of hydration for comparative analysis: dehydrated (dry) and slightly hydrated (moistened).

Upon return from the stratospheric flight, both samples—the dry and the hydrated—demonstrated **complete survival**. This finding convincingly confirmed the exceptionally high resistance of *Aspergillus niger* conidia to the complex stress factors characteristic of near-space, which include cryogenic temperatures, low atmospheric pressure, and elevated radiation levels.

The most significant result was the confirmation that the reproductive potential of the samples was preserved, manifesting immediately within the container after landing, without the addition of a nutrient medium. Microscopic analysis showed that the fungal structures continued active reproduction, utilizing internal resources or residual nutrients. The formation of new conidiophores and subsequent development of viable spores (conidia) was observed. This data suggests that exposure to the stratosphere did not lead to irreversible damage to the genetic material or mitochondrial functions of the fungus.

D. **Lemon Oyster Mushroom Spores (*Pleurotus citrinopileatus*).** The central element of the biological research component was the assessment of the post-stratospheric viability of **Lemon Oyster mushroom spores** (*Pleurotus cit-*

rinopileatus). Spores, affixed to wooden sticks, were divided into four groups and exposed in the stratosphere within various media to evaluate their protective or destructive effects: dehydrated (dry), in an ammonia solution (NH3), in a boric acid solution (H3BO3), and in distilled water (H2O).

After landing, all samples were retrieved under sterile conditions and inoculated onto a nutrient medium (Potato Dextrose Agar, PDA). Observation during the incubation period revealed significant variability in the spores' ability to germinate.

The **dry sample** demonstrated the best result, with active growth of the mycelium (fungal threads) recorded within 810days. Germination was the most prolific, forming a dense, white, cotton-like mycelium clearly visible to the naked eye. The sample exposed in **water** also showed a high degree of germination, forming a noticeable fluffy mycelium. These results lead to the conclusion that spores best maintain viability in stratospheric conditions either in a state of dormancy (dry environment) or in a hydrated environment, which likely provided minimal protection against desiccation.

A different picture was observed for the samples exposed to chemical agents. The sample flown in the **ammonia solution**showed no signs of mycelial growth, indicating a **complete loss of spore viability**. This suggests that ammonia exerted a strong toxic or destructive effect on the cellular structures, rendering them incapable of germination. The sample contained in the **boric acid solution** showed very weak germination, noticeable only upon detailed inspection. This result indicates that boric acid also had a pronounced **inhibitory (suppressive) effect**, though it did not lead to complete destruction like ammonia. The sample flown in the ammonia solution showed no signs of mycelial growth, indicating a complete loss of spore viability. This suggests that ammonia exerted a strong toxic or destructive effect on the cellular structures, rendering them incapable of germination.

Upon opening the container, a dead, worm-like artifact was discovered. Comparing this with the observation on Earth, where an analogous organism was found alive in the control ammonia sample, it can be concluded that the extreme conditions of the stratosphere, combined with the toxicity of ammonia, led to the complete demise of all life forms within this specific medium.

B. Limitations and Challenges

While the mission successfully reached 27 km altitude and returned intact samples, several limitations affected data continuity and interpretation.

Sensor performance: Not all instruments operated nominally. The altitude record contained a false drop near peak height due to temporary GPS signal loss, and the ozone sensor ceased functioning under extreme cold, confirming inadequate thermal tolerance. The UV detector exhibited high-frequency noise requiring digital filtering, while other parameters occasionally required interpolation. These issues show the need for thermally protected, stratosphere-rated sensors and redundant altitude and radiation channels in future flights.

Thermal exposure and sample variability: External temperatures fell below $-40°$C, inducing strong freezing and desiccation stresses. Internal measurements showed that electronics and insulation maintained warmer conditions (around $-20°$C), meaning different payload compartments experienced unequal thermal histories. The protected quail egg, perforated egg, and fungal cultures in four media (air, water, boric acid, ammonia) therefore underwent distinct combinations of temperature, pressure, and radiation. Future missions should include localized temperature and dose sensors for each biological unit to separate thermal and radiative effects.

Mechanical loading: Acceleration data revealed clear peaks at balloon burst, parachute deployment, and landing. The 3D-printed shell successfully prevented breakage of the enclosed egg, but unprotected compartments likely experienced stronger impacts. Biological outcomes may thus reflect both environmental stress and mechanical shock. Equalized mechanical isolation or quantified shock limits are recommended for comparable future experiments.

Sample size and contamination: The biological sample set was intentionally minimal - two eggs and four fungal environments - so results are qualitative rather than statistical. Observed differences, such as protein denaturation or inhibition of growth in boric acid versus contamination in ammonia, illustrate strong trends but not replicable population-level certainty. Recovery and transport also introduced a small contamination risk before laboratory analysis, especially in nutrient-rich media. Future designs should use sealed yet radiation-permeable containers and include replicate samples per condition.

Educational platform constraints: All payload engineering was performed by students under UniSat's educational framework, which limited mass, volume, redundancy, and pre-flight qualification testing. The ozone sensor failure and GPS dropout both stemmed from components untested under full stratospheric conditions. Ground-based cold-vacuum and vibration testing will be essential to prevent mid-flight data loss in upcoming missions.

Overall assessment: Despite these challenges, the mission demonstrated reliable ascent and recovery, captured multi-parameter environmental data, and returned intact biological payloads. The findings validate the feasibility of low-cost, student-built nanosatellite experiments that integrate macroscopic and microbial samples, while defining the next step—replicated, compartment-monitored, and pre-qualified biological CubeSat missions.

V. Conclusion and Future Work

The novelty of our research Most stratospheric biological experiments have focused on microorganisms, plant spores, or isolated proteins. In contrast, this study used a quail egg as a complete biological system to investigate protein alterations under real stratospheric con ditions. This approach is uncommon because it examines how proteins behave within their natural protective environment, including the eggshell and internal membranes. Unlike traditional in vitro experiments that expose single proteins to controlled conditions, our research analyzed a multilayered biological capsule containing various types of proteins, albumins, yolk lipoproteins, and enzymes, each with distinct sensitivities to temperature, radiation, and pressure changes. This brings the experiment closer to real biological contexts, where natural bar riers play a significant protective role. Furthermore, the mechanical integrity of the egg after stratospheric flight was tested using a grid-like structure with wide rods. The fact that the egg remained intact despite exposure to extreme pres sure and temperature differences demonstrates that biological structures may be more resistant to stratospheric stressors than previously assumed.

Scientific and Practical significance The primary goal of this research was to investigate how stratospheric conditions affect proteins, since proteins are the fundamental building blocks of life and make up a large proportion of the human body. By studying changes in egg proteins after exposure to extreme environmental factors, our objective was to model how biological molecules critical to human health might behave under similar stressors such as radiation, dehydration, or low pressure. The findings contribute to a broader understanding of protein stability and denaturation mechanisms in extreme environments and may offer insights relevant to human health protection during high-altitude and space exposure, as well as to future applications in aerospace biology and biotechnology. 1 1.3 Experimental Insights During the preparation and execution of the experiment, several technical chal lenges provided valuable learning experiences. One of them was related to the 3D modeling and printing of the protective grid designed to hold the quail egg. The initial prototype did not perfectly match the required dimensions, leading us to refine our measurements, adjust the model parameters, and ensure greater precision in data handling and design validation. This process strengthened our understanding of how accurate data management and iterative testing are essential for experimental success. Another important lesson came from the ozone sensor used during the flight. After a period of operation, the sensor stopped functioning due to extremely low stratospheric temperatures- a limitation that was not clearly specified in its documentation. This experience emphasized the importance of analyzing the environmental tolerance of all instruments prior to launch and performing additional ground tests under simulated conditions. It also taught us to ap proach unexpected technical results as opportunities for deeper investigation and improvement in future missions.

Acknowledgment

The authors would like to express their sincere gratitude to UNICEF Uzbekistan (United Nations Children's Fund, Uzbekistan) for its guidance in program organization, and promotion of STEM education for youth. We also acknowledge Tech4Impact (Non-Governmental Organization, Uzbekistan) for coordinating the educational program, providing infrastructure, and organizing hands-on training and mentoring sessions. Special thanks are extended to the Agency of Space Research and Technology of the Republic of Uzbekistan "Uzbekcosmos" for their collaboration in space education. The team further thanks all mentors, educators, and technical advisors whose dedication made this student-led UniSat mission a meaningful scientific and educational experience.

References

[1] S. Furukawa, A. Nagamatsu, M. Nenoi *et al.*, "Space radiation biology for "living in space"," *Life*, vol. 10, no. 9, pp. 1–17, 2020.
[2] UniSat Program Uzbekistan. (2025) About unisat 3.0. Accessed: Oct. 29, 2025. [Online]. Available: https://unisat.uz/en/
[3] NASA Science Editorial Team. (2020) Balloons offer near-space access for space biology researchers. Accessed: Oct. 29, 2025. [Online]. Available: https://science.nasa.gov/science-research/biological-physical-sciences/balloons-offer-near-space-access-for-space-biology-researchers/
[4] M. Dave *et al.*, "A novel egg-in-cube system enables long-term culture," *PMC*, 2022, accessed: Oct. 29, 2025. [Online]. Available: https://pmc.ncbi.nlm.nih.gov/articles/PMC9133561/
[5] C. Khodadad and et al., "Stratosphere conditions inactivate bacterial endospores on aircraft surfaces: E-mist balloon flight," *Astrobiology*, 2017.
[6] NASA Ames Research Center, "E-mist mission briefings," NASA Science Research Reports, 2015. [Online]. Available: https://www.nasa.gov/science-research/nasas-e-mist-experiment-soars-in-earths-atmosphere
[7] Y. Deng and et al., "Adaptive mechanisms of *Bacillus* to near-space extreme environment," *Science of the Total Environment*, 2023.
[8] M. Cortesão, R. Moeller, and et al., "Marsbox: Fungal and bacterial endurance from a balloon-flown analog mission in the stratosphere," *Frontiers in Microbiology*, 2021.
[9] H. Zhao and et al., "Biosep payload description and biological response results from near-space flights," *Earth and Planetary Physics*, 2024.
[10] L. Heitkämper and et al., "Flying microbes—survival in the extreme conditions of the stratosphere," *Microbiology Spectrum*, 2024.
[11] D. Chudobová and et al., "Effects of stratospheric conditions on viability, metabolism, and proteome of prokaryotes," *Atmosphere*, 2015.
[12] M. Cortesão and et al., "Aspergillus niger spores are highly resistant to space radiation," *Frontiers in Microbiology*, 2020.
[13] A. Pulschen and et al., "Survival of extremophilic yeasts in the stratospheric environment," *Applied and Environmental Microbiology*, 2018.
[14] S. Hatab and et al., "Effects of exposing japanese quail eggs to low-dose gamma radiation," *Frontiers in Physiology*, 2023.
[15] D. Lee and et al., "Radiation effects on egg-white ovomucoid carbohydrates," *Food Chemistry*, 1999.
[16] C. R. Harris, K. J. Millman, S. J. van der Walt, and et al., "Array programming with NumPy," *Nature*, vol. 585, pp. 357–362, 2020.
[17] W. McKinney, "Data structures for statistical computing in python," *Proceedings of the 9th Python in Science Conference (SciPy 2010)*, pp. 56–61, 2010.
[18] J. D. Hunter, "Matplotlib: A 2d graphics environment," *Computing in Science & Engineering*, vol. 9, no. 3, pp. 90–95, 2007.