Progress is being made on human Lunar and Martian missions by space agencies and private organisations around the world, with the aims of establishing reliable long-duration architectures. Complementing this, research is being carried out under controlled and isolated conditions within simulated space habitats, to gain insights into the effects of such conditions on the research subjects and their impacts on crews’ wellbeing and success. This paper provides an overview of the experiments conducted during two separate 15-day missions—one Martian and one Lunar—conducted in the LunAres Research Base in Piła, Poland, in 2018. Some activities were common between the two crews; others were only carried out by one. Using the same methodology, both collected cognitive function, environmental, physiological, and inventory data, resulting in a larger dataset allowing comparisons between the two missions in terms of varying human factors. Experiments conducted by the Lunar crew included the following: effects of consuming lyophilised food on oral health and saliva production, influence of isolation on hearing capability, feelings on security in the isolated habitat, and research into earthworm growth in different soil compositions. The Mars mission analysed physical performances of the crew and compared them to performances realised during similar activities in Mars Research Desert Station missions and the impact of confinement on their efficiency performing a remote operation of a rover. For each piece of research, an overview of the background, methodology, results, and conclusions is given, referencing the resulting papers. In addition, nonresearch activities are included for completeness and context.

1. Introduction

This paper is a summary of the analog missions ARES-III and LEARN. These missions were conducted in the LunAres Habitat in the summer of 2018, for a duration of 2 weeks, each. For each mission, the experiments and activities conducted by the respective crew are presented here. Where activities were performed by both crews and following the same methodology, they are presented under the “joint activity” part. This summary is an opportunity to present a complete report/example on how analog missions are performed and how they aim at reproducing (at best) Martian or Lunar conditions for an astronaut crew. In this work, the second part is dedicated to material and methods, presenting the LunAres Habitat, the location, and the two different missions. The third part presents all experiments and research and nonresearch activities led by the two crews. This part is divided in three subparts, all started with a summary table: the first one concerns the joint activities, the second one concerns the ARES-III mission activities, and the third one the LEARN mission activities. Finally, the fourth part is a brief discussion and conclusion on the corresponding challenges of such missions.

1.1. Aims of the Paper. This paper provides an overview of the experiments conducted during the ARES-III and LEARN missions. The background, methodology, and, where appropriate, results and conclusions for each study will be given. There is no attempt at further analysis of results in this paper; results are presented, and, where available, additional information is provided in the Appendix for the reader to explore those relevant to their interest and expertise. Nonresearch activities are also outlined, on the basis that if an activity was performed consistently, it might have had some latent impact on the crew.
1.2. Analog Missions: Motivation and Methodology. Current international human space exploration roadmaps envisage month-long duration crew stays on the Moon within the next few decades, with crewed missions to Mars the long-term goal [1]. Such missions present several challenges to survival, even more than those that have been overcome in International Space Station (ISS) operations. The Moon and Mars are barren and hostile environments, on average 961 and 195,850 times as far from the Earth as the ISS, respectively, putting astronauts in a heightened sense of isolation and increasing the criticality and reducing the feasible frequency of cargo resupply missions [2–4]. Specifically, the psychological effects of human spaceflight, especially in the sense of isolation and confinement, need to be explored ahead of deep space crewed missions [5]. In the case of Mars, the distance also means a communication time delay of between 4 and 24 minutes; and there will be an increased dependence on robotic technology both through local and remote operation [6].

The Apollo missions are the only experience to date of living on a different world, but the maximum length of time spent on the lunar surface was a mere 75 hours during Apollo 17 in 1972 [7]. Extended stays on the Moon could be the first step towards a space-based economy, supplementing Earth’s resources with raw materials from the Moon to benefit the global economy here on Earth [8]. To allow astronauts not only to survive but to thrive in these alien environments, practice is needed. Practice involves operations on the ISS, but the ISS cannot simulate all aspects of a Lunar or Martian mission, such as the surface operations or long periods without sunlight. Therefore, Earth-based analogue missions are required to simulate off-world missions to ensure as many aspects of future missions as possible can be prepared for. Similarly, an analogue environment cannot fully replicate that of the Moon or Mars, but a variety of analogue environments that simulate different aspects of the off-world environment can be used in conjunction to prepare for future missions.

Analog missions typically last for a few weeks to a few months and enable the testing and development of hardware and software for future missions, as well as operational concepts, and enable this at a faster rate and lower cost. Among many others, missions exist in deserts to test rover operations and sample collection methods, underwater to trial activities in a low-gravity environment, and in caves to test teamwork and psychological impacts in a challenging environment that is very similar to living off-world [9–11].

2. Materials and Methods

2.1. Project Background

2.1.1. LunAres Habitat. This paper describes the activities carried out in two separate 15-day missions—one Martian and one Lunar—in the LunAres Research Base in Pilis, Poland, in 2018. In this analog space habitat, research is focused on human factors and testing of sustainable technologies. The habitat is fully isolated from the external environment—the complete isolation from the outside world including lack of any windows and thus complete absence of access to daylight allows investigating effects of full isolation on the crew, as well as experiments related to circadian rhythm in humans. The latter is especially useful, when simulating different day times, such as those that would be experienced by humans on Mars. The habitat consists of a domed living area, to which several modules are attached. These were, at the time of the missions, the bathroom, kitchen, dormitory, storage, operations room, biological laboratory, analytical laboratory, and airlock. The layout of the habitat at the time of the missions is shown in Figure 1. Each room is fitted with humidity and temperature sensors and cameras. Adjacent to the habitat is a disused military hangar, which has been converted to a 250 m³ extravehicular activity (EVA) terrain using simulated regolith to model the Lunar/Martian surface. This area can only be accessed through the airlock, in which analog astronauts must wait 15 minutes to simulate decompression before stepping from the airlock to the terrain/recompression before reentering the habitat from the airlock.

Missions are run on Lunar/Martian time, meaning the crew go out of sync with the external mission control crew, which is based away from the habitat and continues to operate on Earth time. The mission control crew (MCC) is responsible for coordinating the mission from the outside and communicating with the crew every day, just like in real crewed missions. The team consists of the flight director, science data officer, capsule communicator, habitatOS engineer, and flight surgeon, with additional roles possible. In Mars simulations, there is approximately a 20-minute communication time delay between the MCC and the analog astronaut crew. The crew consists of the commander, vice commander, communication officer, environmental control and life support system engineer, data officer, and crew medical officer. A group of selected principal investigators (PIs) are also involved in each mission through the provision of experiments for the crew to conduct. Each member of the crew is expected to work 8 hours per day.

2.1.2. ARES-III Mission and LEARN Missions. ARES-III was a 15-day Mars simulation mission, with the crew consisting of six crew members (three males, three females) from four different nationalities, ranging from 23 to 32 in age. As a Mars analog the crew was under a 20-minute time delay in communications with the mission control. The primary method of communication was text communication and with voice communication used in some instances for updates. The length of one mission day mirrored the 24-hour 40-minute Martian sol length. ARES-III crew was constrained to eating only lyophilised food at lunch and dinner. Breakfast was made mainly of almonds and protein shakes. The mission patch of ARES-III is shown in Figure 2(a).

The LEARN mission was conducted by five crew members (three males, two females) from four different nationalities, ranging from 21 to 30 in age. Due to there only being a 1.3-second communication time delay between the Earth and Moon, communications between the crew and MCC were conducted by video and voice methods, as well as by text. The length of one mission day was set to be the
duration between moonrises—a "lunar day"—which is 24 hours and 50 minutes. The LEARN analog astronaut crew was also constrained to eating only lyophilised (freeze-dried) food for the entire duration of the mission. The mission patch of LEARN is shown in Figure 2(b).

3. Summary of Activities and Results

3.1. Joint Activities. The crews agreed prior to the missions to conduct some of the experiments jointly, to generate more data with a larger pool of participants. Some experiments were performed by both crews separately, according to the experiment protocol supplied by the PI. For example, the STRISO and COGISO experiments were originally initiated by the ARES-III crew; however, upon agreement, the LEARN crew was also instructed how to correctly perform the experiment as well. The LEARN crew then performed the experiment as instructed by the PI, and the data was handed over to the PI after the mission. Similarly, the crews of ARES-III and LEARN harmonised the format of the data collected daily, with the aim of generating a larger dataset for future investigation. The joint activities are summarised in Table 1.

3.1.1. STRISO—Stress in Isolation Experiment. The aim was to investigate and monitor stress responses (cortisol and oxidative stress) over the course of isolation. Each crew member had to gather 6 saliva samples: one premission saliva collection, four collections during the mission (third, seventh, tenth, and fourteenth day), and one collection postmission.

Each saliva sample was collected in the morning after waking up (within the first 30 min of waking up). Crew members had to avoid any food or brushing teeth one hour prior to saliva sample collection and rinse mouth twice with water before collecting the saliva sample.

This experiment was led by the University of Antwerp (Antwerp, Belgium) in collaboration with SCKCEN (Mol, Belgium).

PI: Dr. Angélique van Ombergen
Collaborator: Dr. Marjan Moreels
3.1.2. COGISO—Cognition in Isolation Experiment. The aim was to investigate and monitor cognitive performance in several cognitive domains (general, spatial, and nonspatial) over the course of isolation. Each crew member had to perform six full cognitive test batteries: one premission saliva collection, four collections during the mission (third, seventh, tenth, and fourteenth day), and one collection postmission.

The cognitive test-battery consisted of three exercises:

(i) RBANS (for Repeatable Battery for Assessment of Neuropsychological Status)
(ii) vWMM (for Virtual Water Morris Maze)
(iii) Heidi app

The RBANS is a brief, individually administered test measuring attention, language, visuospatial, and constructional abilities and immediate and delayed memory. It consists of 12 subtests, which yield five index scores and a total scale score. The total test takes between 30 and 45 min to administer. For each experimental day, a different form (A, B, C, or D) will be used, in order to avoid that participants still remember words or tasks from a previous experimental day. Therefore, the RBANS will only be administered four times, i.e., prior to the mission, on the third, seventh, tenth, and fourteenth day, and one collection postmission.

The Morris water task is considered the gold standard for testing spatial learning, spatial memory, and navigation in rodents. The virtual version of this test has been validated for determining human spatial learning, spatial memory, and navigation abilities. The basic features of the environment consist of a circular pool located in the center of a room with a square floor plan, partially shown in Figure 3.

The Heidi app is based on a modified version of the “swipe 3-in-a-row” game, where participants move objects in a grid to complete 3-in-a-row and collect points (see Figure 4). This game format is played by millions of people on a daily basis and proven to be highly motivating in the general population. It is easy to learn, and by making minor adjustments in the background, we can capture different aspects of cognition. Heidi can be used on either mobile or tablet devices and uses a touch screen interface.

The vWMM and the Heidi app were used at every cognitive test. The total duration for RBANS, vWMM, and Heidi app was approximately one hour and forty-five minutes.

3.1.3. Food Consumption. The controlled conditions in the habitat allowed the crews to fully monitor their food intake. This was partly in direct support of inventory monitoring, discussed later in this paper.

At every meal, each member of the analog astronaut crew measured the weight of their meal portion for each meal type. For freeze-dried food, the weight would be measured in the dry state, the second weight measurement would take place after adding water, and this water would be logged as water consumed (as stated in 3.1.5). The measured weight for each meal type was then recorded in a custom spreadsheet, calculating the nutrient intake with each meal for each crew member. Together with exercise data (outlined in 3.1.4) and medical data (outlined in 3.1.5), the daily calorie balance and crew member weight change was thus calculated.

These data were published as a part of a physiological data paper combining food consumption, exercise, and medical data gathered by the two crews [12].
3.1.4. Exercise. Every day, the analog astronauts performed 1 hour of scheduled exercise. The most common exercise was running on a treadmill, for a duration decided by the crew member. Any remaining exercise time was used for strength exercises, yoga, or stretching exercises. On completion, distance travelled (in case of running), heart rate, and calories burned were recorded. Each mission had its own additional exercise experiments which are outlined in the subsequent sections.

As stated in Section 3.1.3, these data were published as a part of a physiological data paper combining food consumption, exercise, and medical data gathered by the two crews [12].

3.1.5. Medical Checking. All analog astronauts conducted twice-daily personal medical checks to observe any effects of the mission on basic physiological parameters. Right after waking up and just before going to sleep, commercial noninvasive measuring devices were used to measure body temperature, blood pressure, body weight, heart rate, and pulse oximetry. These measurements (except from body weight) were also taken and recorded prior to and immediately after an analog astronaut had conducted an EVA. In addition, the volume of all consumed and expelled liquids was recorded, as well as the color and transparency of urine.

As stated in Section 3.1.3, these data were published as a part of a physiological data paper combining food consumption, exercise, and medical data gathered by the two crews [12].

3.1.6. Daily Reporting. At the end of every day, there was time scheduled for analog astronauts to complete individual reports and questionnaires on topics including their daily activities, mood, stress levels, and thoughts on fellow crew mates and on isolation. Sleep logs were also completed at the start of every day, where the crew members logged the length of their sleep, whether their sleep was interrupted or not, whether they dreamt or not, and what sensations occurred in the dreams.

3.1.7. Nonresearch Activities. The following sections outline activities performed by both crews without any formalized protocol. These account for the free time of the crew, additional workload such as system maintenance, resource handling, or simulation of operations such as EVAs.

(1) Free Time. The crew were scheduled to have 1 hour of free time every day. Each analog astronaut was allowed to spend their free time as they wished. The free time slot was allocated to accommodate the rest of the crew member’s schedule for the day; therefore, the free hour did not occur at the same time of the day, since the schedule of individual crew members differed based on their individual responsibilities.

(2) Extravehicular Activities. The objectives of each EVA were provided by the MCC, with the crew responsible for
planning their route and actions to achieve the objectives. Each EVA involved three analog astronauts—the EVA crew lead, EVA crew support, and a HabCom (habitat communicator) who communicated with the two analog astronauts conducting the EVA from inside the habitat via a walkie-talkie and ensured they were on track to complete the activities within time. Those going out to the lunar surface wore a multilayer spacesuit, helmet, two pairs of gloves, boots, and a reflective band and carried all equipment they might have needed on their person. An illustrative photo of an EVA is shown in Figure 5.

EVAs were classified into four types: exploratory, experimental, emergency, and operational, and each analog astronaut performed at least three EVAs. Activities conducted during included sample collection, assembly of a mock rover, and a simulation of an injury and rescue operation. EVAs typically took 2 hours from habitat exit to entry, which included a 15-minute de-/recompression countdown in the airlock either side of the activity. Depending on the time of day, it was pitch black or light in the EVA area (shown in Figure 6), meaning analog astronauts had to rely on torches and helmet lights to see. On some occasions, EVAs were performed at 04:00 to simulate an unexpected scenario.

The procedure followed for all EVAs is provided in the Appendix.

(3) Inventory. At the start of their respective missions, the crews created a summary of the inventory available in the habitat. This was primarily done for drinking water bottle supply, food supply, and medical equipment, to ensure that the stored supplies matched the provided inventory data. The ARES-III crew also performed an inventory-based experiment, described in Section 3.2.4.

(4) Cockroaches. Cockroaches are one of the most common laboratory insects. Additionally, they are easy to handle and might be useful in future missions, through producing excrements and nourishing the lunar regolith soil for instance. Light influence on stress was measured by morphological comparison of antenna and legs in a light-deprived group together with a control group of insects. Both groups were in separated boxes, with an equal number of males and females. Cockroaches had to be maintained every 3 days (fed with Tenebrio molitor homogenate and water) and weighed (along with their faeces), and the boxes were cleaned.

(5) Hydroponics. In a hydroponic system, all the ingredients needed for plants to grow are provided by a medium. The plants are arranged in pots in columns and are connected vertically by tubes. The medium is added to the uppermost pot in each column and trickles down through each pot.
before the excess is collected at the bottom. Plants selected for growth in the LunAres hydroponic system, which has eight columns each with five rows of pots, were coriander, rocket, chia, cress, and basil. The temperature of the lab was maintained within 20-30°C and lit for 16 hours per day. General maintenance of the plants was conducted throughout both missions with a medium of pH 6-6.5, consisting of 25 ml of water, 50 ml of kombucha liquid, 0.25 ml of fertiliser, and 25 ml of distilled water. Each plant also had a control in a regular plant pot, nourished with the same medium.

(6) Outreach. Both of the crews maintained a mission diary that was shared on social media. These entries normally captured the activities of the crew for the given day. The crews also took pictures of their activities and prepared outreach videos, such as visits to the individual habitat modules, experiments, and a tour of the habitat.

3.2. ARES-III Experiments. The main objective of this analog mission was to perform neuropsychological research into the effects of living in isolation and confinement and to study a low-resource environment on stress responses, group dynamics, circadian rhythm, cognition, and microbiota. As in most analog missions (such as in the Mars Desert Research Station), the main experiment is the observation of the crew dynamics.

The ARES-III mission simulated the Martian day of 24 hours 39 minutes and 35 seconds. The main advantage of the LunAres station is the absence of sunlight. There were no windows in the station, and the EVAs were inside the hangar. The idea for simulating the Martian day was then to set back the clock 10 minutes in the morning, 10 at noon, 10 during the afternoon, and 10 at the end of the day. Hence, the crew reached midnight after 24 hours and 40 minutes. At the end of the two weeks, the crew had almost a ten-hour time difference between inside and outside the hab. Moreover, the delay between the MCC and the crew was also simulated. The minimum speed for any communication is about 8 minutes, and the maximum is around 18 minutes (when planets are in conjunction). It was decided to simulate the worst-case scenario during the whole mission as most of the studies implied the study of isolation. Hence, MCC had to wait 36 minutes before answering. It corresponds to the maximum delay for obtaining a response ARES-III activities are summarised in Table 2.

3.2.1. Stress and Performance Analysis. The aim of the experiment is to quantify the correlation between the change in length and the reduction of language variation in a written text and the psychological and perceived stress levels in the writer and two cardiovascular parameters (blood pressure and heart rate), which are known in literature to be stress susceptible.

The experiment duration is about an hour and has to be performed during the first, fifth, ninth, and thirteenth Martian day of the simulation. The methodology is the following:

The subject writes a text with a maximum of 500 words in his/her mother tongue. The task should be performed approximately at the same time and should not exceed 30 minutes.

After writing each text, the subject goes through the 3 following questions, answered on a scale from 1 (lowest) to 10 (highest):

(i) How stressed do you feel today?
fore, to study the effects of microgravity conditions in space on the growth and development of plants. Microgravity conditions have been simulated with a random positioning machine (RPM) to quantitatively compare the germination and early growth of cress under blue and red LED in Martian-simulated regolith and Earth fertile soil.

POM—Produce an optimized use of critical resources for human deep space outposts. PI: Patrick Fleith, ARES-III

MICISO—Investigate intestinal microbiota and the gut microbiome composition, based on faeces collection of the crew members and with or without diet-supplemented spirulina. PI: Patrick Fleith, ARES-III

TELEOP—Analysing the evolution of an operator’s learning curve when controlling a rover and the impact of the confinement on the human-robot interaction. PI: Raphaëlle Roy

(ii) How well were you able to perform today?

(iii) How comfortable do you feel today?

Subject finally monitors blood pressure and heart rate with a specific device.

The experiment had to be reproduced one last time seven days after the end of the simulation. The results of this experiment could help interpret vocabulary and changes in language during interactions between analog astronauts and MCC (or even in-between analog astronauts) to identify stress and anxiety.

An experiment proposed by the Italian Mars Society. PI: Simon Bouriat

3.2.2. PING-BE and PING-ME. Gravitropism of plants is considered to be one of the most important responses to adapt to the ground conditions on the Earth. The cultivation of plants as a resource of food and oxygen and for psychological reasons will be one of the essential and fundamental factors for human beings to live in space conditions. Therefore, to study the effects of microgravity conditions in space on the growth and development of plants is considered to be an important subject. Though some studies concerning the effect of microgravity conditions in space on some physiological responses of plants have already been done using spacecrafts, information obtained from those space experiments still seems not to be sufficient to understand the growth and development of plants in space, simulating the microgravity conditions on Earth with a random positioning machine (RPM) [13]. The RPMs used in one of such experiments can be seen in Figure 7. PING-BE (for Plants In Normal and microGravity-Biochemical Experiment) investigated the changes in biochemical pathways associated with gravitropism in plants under simulated microgravity.

Cultivating plants in Martian regolith may be challenging due to a high concentration of perchlorates [14, 15]. PING-ME (for Plants In Normal and microGravity-Martian Experiment) investigated the effects of the perchlorate ions found in Martian soil on plants’ growth under simulated microgravity, thanks to the use of an RPM.

Both experiments PING-BE and PING-ME had to be performed simultaneously. A picture had to be taken every 12 hours. A measurement of plants’ height and weight had to be done at the end of the experiment. After the experiment, the plants have to be rapidly frozen in liquid nitrogen for further biochemical analysis conducted by Dawid Przysuptuski and Agata Górska from the Wroclaw Medical University and University of Wroclaw.

Plant seedlings on the solid medium have been delivered to the habitat in sterile culture bottles ready for the experiment. There were four different bottles. Two of them had to grow in the RPM, while the two others had to be placed in normal conditions. Plants had 12 hours light and 12 hours dark conditions.

PI for this project is Dr. Agata Kolodziejczyk, LunAres.

3.2.3. MARK—Germination and Early Growth of Cress in Martian-Simulated Regolith. The objective of this study is to quantitatively compare the germination and early growth of cress under blue and red LED in Martian-simulated regolith and Earth fertile soil. The responses studied were the germination ratio, the biomass (in mg), the exposed surface via image processing in MATLAB and Python (cm²), and the chlorophyll study via image color analysis in MATLAB.
and Python. An experimental setup for one of the substrates is shown in Figure 8.

The four different substrates were cotton, fertile Earth soil, regular Martian regolith simulant, and salt-rich Martian regolith simulant. Each of these four substrates was fertilised with urine or not. Hence, there were eight possibilities. Sixteen substrates (two of each) were used. Each day had its own protocols.

PI: Patrick Fleith, ECLSS Officer, ARES-III

3.2.4. REMI—Inventory, Modelling, and Recommendations on Resources Utilization on Human Deep Space Habitat. The REMI experiment’s aim was to produce an inventory, model, and recommendations for an optimized use of critical resources for human deep space outposts.

The idea was to use a specific protocol during the whole mission to have an inventory on water utilization, energy utilization, and food consumption.

To keep track of how much water they use and for which task, crew members had to use a dedicated measuring container and report all measurements in a dedicated notepad. Where no measurements were possible, estimation and associated uncertainties were recorded. The power duty cycle of each unit of the habitat was recorded to determine the overall habitat power demand and later propose an optimized power distribution. Finally, crew members had to keep track of the food they consumed.

Data collected are compared with the literature to better help mission planners to assess those needs which are critical from a mass point of view for any mission departing from the Earth. Recommendations will be done to improve practices for deep space habitat and other analog habitats.

PI: Patrick Fleith, ECLSS Officer, ARES-III

3.2.5. MICISO—Microbiota in Isolation Experiment. The aim of this experiment was to investigate intestinal microflora and the gut microbiome composition, based on faeces collection of the crew members and with or without diet-supplemented spirulina.

While keeping an extremely clean spaceship environment (aseptic food, filtered air, etc.) has been an effective preventive measure for protecting astronauts from infections caused by many dangerous pathogens, the lack of microbial intake for long periods of time may have a detrimental effect on the diversity of the crew microbiota and, therefore, on astronaut health, to a sufficient extent that it may not be a viable strategy for long-term space missions. Also, reported dysregulation of the astronauts’ immune system following short and long space flights may be a consequence to inflight alterations of the GI microbiota. It is likely that these negative effects might be potentially controlled or even prevented by diet-based therapies proven to promote a healthier GI microbial composition, such as those with a higher content of fiber including fresh food and vegetables. An alternative approach for introducing a constant input of microbial diversity to astronauts on long-duration missions would be prophylactic use of food supplements including pre- and probiotics. Prebiotics is a generic term used to describe dietary interventions that seek to create a healthy environment for the GI microbiome, while probiotics is a generic term used to describe dietary inputs of beneficial microbes [16].

In that sense, the European Space Agency MELiSSA project appeared to be a very well-suited research platform. In particular, the MELiSSA research performed by the microbiology unit of SCKCEN for the last 15 years put forward a number of interesting features of the MELiSSA bacteria. Rhodospirillum rubrum inhabiting compartment 2 of the MELiSSA loop was successfully studied and tested on mice at SCKCEN for its specific low-density lipoprotein cholesterol-lowering properties as a food supplement. These results gathered in collaboration with MELiSSA spin-off ezCOL and the University of Mons could be valorized into a European patent with reference 17176170.3 – 1466. The same way, research at SCKCEN has demonstrated the high

Figure 7: Two types of random positioning machines: RPM1 and RPM2. After switching on, the machine rotates at regulated manually speed (rad/sek). Each machine consists of two engines (1), experimental platform (2), and board computer (3).
radiation resistance of the cyanobacterium Arthrospira sp. PCC 8005 inhabiting compartment C4a, which is capable of surviving doses up to 6400 Gy of gamma radiation. Current research, in association with the University of Antwerp, includes the study of Arthrospira sp. as a radioprotective agent, i.e., mitigating the adverse effect of irradiation on the gut microbiome, including in-house production of the Arthrospira sp. biomass and preparation for in vivo studies via addition to the chow of laboratory mice. In addition, the previously reported immune modulation function of Arthrospira sp. will be assessed during these experiments.

In parallel, cutting-edge metagenomic profiling of the volunteers intestinal microflora will be performed, based on faeces collection, to assess the gut microbiome composition of the volunteers with or without diet-supplemented spirulina and to study the impact of food supplementation on the parameters listed above.

Each crew member had to gather 6 faeces samples: one premission saliva collection, four collections during the mission (third, seventh, tenth, and fourteenth day), and one collection postmission.

In the first week of the isolation, subjects had a normal diet. During the second week, the crew were split into two random groups of three people. One of the groups was given spirulina, while the other was given a placebo, in order to compare the two.

3.2.6. TELEOP—Teleoperation in Isolation. Analysing the evolution of the learning curve when controlling a rover and the impact of the confinement human-robot interaction was the main point of that experiment. Remotely controlled rovers are widely used in space missions for different purposes: explore the surface and the environment of the specific celestial body, take samples, and carry objects to some place, among other things. It has already been observed that a crew in isolation is getting less efficient around the middle of the mission. For instance, a pilot is less attentive around the middle of his long-haul flight. This experiment gathered data to have a better understanding of this effect and on how to prevent it. An electrocardiogram and an eye-tracker measured and observed physiological data (stress, tiredness, focus, etc.). Moreover, supervisors observed speed and efficiency of the operator during the use of the rover. Some of these elements used in the experiment can be seen in Figure 9. Finally, psychological states had been measured through questionnaires before and after every test.

This experiment has been done and will be done in other analog missions. The idea is also to observe how the context of an analog mission (LunAres, MDRS, and SIRIUS at the IBMP) impacts the data obtained. Is an analog mission really representing what is happening during a mission?

The protocol was the following:

(i) Set up the rover’s track
(ii) Setting up the ECG on the subject and starting the measurement at least 5 min before the beginning of the experiment
(iii) Setup of the eye-tracker system next to the computer and calibration
(iv) Subject does the first psychological questionnaire
(v) Subject operates the rover to complete the track. Supervisor observes the time for completion and the number of mistakes

**Figure 8: Germination in one substrate.**
(vi) Subject does the second psychological questionnaire

The total duration was approximately 20 minutes for the subject and up to 45 for the supervisor. The questionnaires were different at each session, and the track of the rover was changed between each session.

This experiment was conducted by the ISAE-Supaero.

PI: Raphaëlle Roy

3.3. LEARN Experiments. Similar to ARES-III, the objectives of LEARN mission were to perform neuropsychological research into the effects of living in isolation and confinement and to study a low-resource environment on stress responses, group dynamics, circadian rhythm, and cognition, in order to observe crew dynamics. In addition, the mission involved studied the influence of freeze-dried food on the oral health of the crew, sense of security in isolation, and several biology-oriented experiments. LEARN activities are summarised in Table 3.

3.3.1. Earthworm Culture on Meteorite Soil. Earthworms play an important part in soil dynamics, which has implications for the environment as a whole. The earthworm is commonly known for its ability to cycle organic matter and nutrients back into the soil. The burrows and casts of earthworms can increase aeration, increase water infiltration, and stabilize soils. The aim of the experiment was to determine whether earthworms can be cultured within the soil of extraterrestrial origin. The additional goal was to determine if earthworms are capable of transforming the regolith into the fertile soil (vermicompost production).

Test organisms used in this experiment were European nightcrawlers (Eisenia hortensis or Dendrobaena veneta, Annelida, and Lumbricidae). Adult animals were obtained from commercial cultures.

The test was conducted using 3 types of soils: (1) raw, pulverized meteorite soil, (2) pulverized meteorite soil processed for removal of magnetic fraction, and (3) the standard natural fertile soil.

Short-term colonies were maintained at room temperature under the long photoperiod (16L/8D) on one of the three tested soils. Animal food provided were residues of plant origin and cellulose (nonprinted cardboard).
Containers with the colonies were constantly monitored with a thermometer and a hygrometer. One of such colonies is displayed in Figure 10.

The procedure employed by the experiment was as follows:

(i) Label the containers with the following symbols: M, MD, and CTRL (M for meteorite soil, MD for meteorite/soil mixture, and CTRL for regular soil as a control).

(ii) Divide earthworms into three groups of 20 individuals

(iii) Weigh each group and try to align the mass of each group (record the result)

(iv) To the container M, add 50/100 g of meteorite soil; to the container MD, add 50/100 g of meteorite soil without magnetic fraction; and to the container CTRL, add 50/100 g of regular soil obtained from green, commercial containers with earthworms

(v) Adjust the humidity of each habitat at 80% with a clean water sprinkler

(vi) Add one group of weighted animals into each container (record the mass of animals added to each container)

(vii) Add organic residues of vegetable origin (2 g) to each container as well as one piece of cellulose cardboard

(viii) Twice every day (morning and evening), temperature and humidity are to be controlled. The recommended conditions are RT (21°C ± 2) and 80% humidity. The readings should be saved in the log book

(ix) If the humidity drops below 75%, open the container and sprinkle the culture with a clean water sprinkler (note in log book)

(x) Add organic residues of vegetable origin and cellulose cardboard when necessary (weigh residues and note in log book)

(xi) On day 13 or 14, homogenize the soil of each colony and transfer into a glass beaker—photograph. Count the animals and rate their vitality. Weigh the animals (note the results in log book)

This experiment was a follow-up to an experiment performed during a previous mission ICARES-1 in the LunAres habitat. The outcomes of this study can be found in Wasinowski et al. [17].

PI for this project is Dr. Aleksander Wasinowski, Space Garden.

3.3.2. UN/SAFE Sense of Security. A feature of human space missions is that astronauts are isolated from everyday life. As part of a PhD undertaken at the Academy of Fine Arts in Gdańsk, project MATRICE investigated the sense of security...
in space isolation. The premise is that people living in extreme conditions experience a reduced sense of security.

The MATRICE project is intended to be an experimental therapeutic space that gives participants a basic sense of security. The MATRICE space is based on the functioning of human senses, knowledge of conventional medicine, and a holistic approach to the body and human soul.

Within the project, the aim of the UN/SAFE research is to examine the state of the sense of security in the space created for living in isolation.

The UN/SAFE experiment consists of four parts: UN/SAFE_diary, UN/SAFE_space, UN/SAFE_body, and UN/SAFE_scent.

(1) **UN/SAFE_diary.** The UN/SAFE_diary experiment started with the crew completing questionnaires of general questions about the sense of security in space and of their experience with yoga and meditation, three days prior to the start of the mission.

Then, the crew completed a Profile of Mood States (POMS) questionnaire (prepared by Dr. Qing Li and Dr. J.R. Grove) before and after yoga practice and meditation. Additionally, a questionnaire about living in isolation was completed on the 3rd, 7th, 10th, and 13th days of the mission, as well as 7th and 14th days after the end of the mission.

The questionnaire focused on the sensual experience of space in isolation, as well as on the stimuli that build or disturb a sense of security in this space. At each stage, the survey will be relevant to the given stage of the mission, from questions about what is a sense of security to an analog astronaut and how they take care of it in everyday life to questions about how it changed during the isolation and what helped the analog astronaut in a particular situation to regain their balance. A large part of the questions concerned the surrounding space of habitat.

(2) **UN/SAFE_space.** Improving the feeling of safety in isolation also required studying and testing how well the habitat’s usable/residential space served the space isolation. With this, the aim was to propose to redesign or change the living conditions in order to improve the quality of life in places such as LunAres habitat.

(3) **UN/SAFE_body.** The UN/SAFE_body part involved the crew performing the Ashtanga style of yoga and mindfulness meditation with simple breathing exercises using the Headspace app. Yoga was performed for around 30 minutes per day, first thing in the morning, and meditation 15 minutes a day, immediately after the yoga. The feelings resulting from the exercise of mindfulness and body awareness were registered and additionally referred to in the POMS questionnaire.
In creating this experiment, the aim was to develop simple exercises which can be performed in even the most truncated space. Such exercises will assist oxygenation of the body and proper functioning of the brain and thus stabilisation of mood and sense of comfort.

(4) UN/SAFE_scent. The sense of smell is the oldest of senses, having evolved before our ancestors became mammals. In almost all vertebrates, the sense of smell determines survival—it allows oneself to find food, detect an enemy, find a partner, or orient oneself in space. Olfactory stimuli are transmitted to the brain directly—much faster than tactile or visual stimuli.

Currently, the sense of smell is one of the least appreciated by people, but invariably, it is strongly connected with our sense of security. The purpose of UN/SAFE_scent was to create a fragrance which would help the crew to relax their body and mind, based on the research by Dr. Qing Li. This fragrance was used and tested during yoga and meditation and according to the wishes of the team during everyday activities. Fragrance notes and the oils themselves were 100% natural.

PI: Joanna Jurga

3.3.3. The Influence of a Diet Based on Freeze-Dried Products on Selected Parameters of Saliva. A number of physiological changes have been observed in astronauts during space missions, such as muscle atrophy, bone weakness, vision disorders, and changes in the brain. However, little research has been done into the effects of the space environment and the intake of certain types of food on oral health. The purpose of this research was to determine how conditions in the LunAres habitat affected the oral health of the crew. The health and development of the masticatory organs, as well as saliva production, are affected by diet selection and the regularity and frequency of meals, and the functioning of the salivary glands is also affected by the taste, smell, and consistency of food. Throughout the mission, the LEARN crew lived on a diet consisting only of freeze-dried food, eating three meals a day at specified times, and without snacks. The food, prepared according to dietary recommendations especially for the mission, had a soft and mushy consistency. The parameters tested were the pH and buffer capacity of saliva. The pH value specifies the acidity or basicity of an aqueous solution. The value can range from 0 to 14, with 7 being neutral, lower values being more acidic, and higher values being more basic. The buffer capacity is a measure of the efficiency of a buffer in resisting changes in pH, given as the amount of strong acid or base, in gram equivalents, that must be added to 1 liter of the solution, to change its pH by one unit [18].

In the freeze-drying process, food is preserved by drying after freezing using reduced pressure and is later prepared for consumption by mixing it with hot or cold water. This process stops the growth of microorganisms and ensures the products do not lose their nutritional value. Resulting foods are dry and light and have a long shelf life, which makes them particularly suitable for space missions. Freeze-dried foods have been used in space since Skylab and are currently used on the ISS. An example of a freeze-dried meal consumed during the LEARN mission is shown in Figure 11.

On days 1, 7, and 15 of the mission, an examination of the oral cavity was conducted on each crew member, and the quantity of saliva each crew member could produce in five minutes was measured by chewing on a paraffin block and directing saliva into test tubes. The pH and the buffer capacity of the saliva were determined with test kits.

It was found that between the start and end of the mission, the average pH value of the crew’s saliva rose from 7.4 to 7.6, while the average value of the saliva buffer capacity fell from 9.6 to 7.5. The increase in the pH value was significantly influenced by the regularity and specific number of meals, while the decrease in saliva buffer capacity was predicted to be related to the consistency and nature of the meals. Although the meals consumed were nutritionally balanced, their mushy consistency and the low-mineralized water used in their preparation likely caused the decrease in saliva buffer capacity.

For more details, see the papers resulting from this research [19, 20].

PI: Dr. Helena Gronwald, Pomorski Uniwersytet Medyczny

3.3.4. Measurement of Hearing Capability in Isolation. The motivation for this experiment stemmed from the isolated conditions inside of the habitat. With the crew not exposed to noises of everyday life, it was of interest to see, if over the course of the mission the hearing of the crew changes.

On days 1, 7, and 15 of the mission, each of the crew members had their hearing tested using an audiometer. The observing crew member and the subject would use an empty room to conduct the experiment in silence. The observer operated the audiometer, by playing beeping audio cues in a random pattern by pressing a button on the audiometer. The beeps were played at a frequency and loudness set on the audiometer by the observer.

The subject, facing away from the observer and the audiometer, listened to the beeps via headphones and pressed a button on a remote whenever they registered the beep. The observer would then gradually lower the loudness of the beeps, until the subject stopped registering the sound emitted by the audiometer. The last loudness correctly identified for the given frequency was then logged by the observer. The observer would start with any of the frequencies tested and would move to the next frequency, once the lower limit on loudness perception was established. There was no prescribed starting frequency and no strict order of the frequencies, in which they were tested.

On the specified experiment days, every member of the analog astronaut crew was tested. As stated, the experiment was done three times over the course of the mission, to capture the effect of the mission on the hearing capability.

The data for the experiment is currently pending analysis and publication.
Figure 11: One of the freeze-dried meals consumed.

Figure 12: The LunAres hydroponic system.
3.3.5. Nonresearch Activities

(1) LEARN Hydroponic Experiment—Hydroponic Growth with Varied Media. To utilise the hydroponic garden, the LEARN crew designed an experiment during the mission, to explore the effectiveness of growth using various watering media. The hydroponic garden at LunAres consists of 12 columns, of which 8 are “tall” and hold 5 rows of plants. The layout of the hydroponic system is shown in Figure 12. Four of these tall columns were cleared to create room for 20 new plants. In addition, 20 plastic flower pots were prepared as controls, one for each hydroponic pot.

Five candidate plants were selected, based on their sprouting time. The plants selected are coriander, rocket (arugula), chia, cress, and lemon basil. Each of the rows contains the same plant, and for each plant, a flower pot with soil is present with the same plant as a control.

Afterwards, 4 different media were prepared for watering. Medium 1 contained 1.5 liter of technical water and 3 ml of fertiliser (the LEARN attempt of this experiment used “Florovit” fertiliser). Medium 2 contained 400 ml of technical water and 100 ml of kombucha liquid. Medium 3 consisted of 500 ml of technical water and 10 g of powdered spirulina. Medium 4 contained 400 ml of technical water, 100 ml of kombucha, and 10 g liquid of powdered spirulina. Kombucha amounts were selected based on the pH of the resulting solution, with the ratio used having pH of approx. 6. Spirulina was added in low amounts until the solution was saturated, but in such a way that it would dissolve fully in the water used, so that it did not potentially block the hydroponic system.

Each medium was used for one column of the hydroponic garden and the associated controls. As mentioned above, the plants in columns and their controls are identical. There are two sets of hypotheses that can be tested in this setup. First of all, the difference between hydroponic and soil-based setup can be assessed:

H0: there is no difference in growth between hydroponic and soil-based plants
H1: there is observable difference in growth between hydroponic and soil-based plants

The other hypothesis that can be tested is based on the effectiveness of the medium used, as using specific nutrients may impact growth better.

H0: there is no difference in growth between the different media used
H1: one or more media result in different rate of growth

Additional hypotheses may be tested, e.g., effect of a medium on a specific plant type, or comparison of growth of a particular plant in soil vs. in hydroponics.

The experiment was set up during the LEARN mission; however, it was not continued by the subsequent missions. Therefore, only limited amounts of data were generated, not permitting any meaningful analysis to be conducted.

(2) Crickets. Crickets are a possible future food source for astronauts due to their high protein content by weight and that small amount of resources and space they need to survive. Crickets (Gryllodes sigillatus) were maintained every 3 days (fed with Hermetia illucens) and weighed, and the boxes were cleaned.

4. Discussion

From the isolation conditions, experiments, and activities above, it is clear that a lot of work is being done to answer the biggest questions for future human exploration: how do isolation, truncated space, busy schedules, unique members, and personalities carrying their own social, cultural, and emotional background can affect the mission and astronaut health? There are several questions pertaining to what type of crew can be considered ideal: is it a single-gendered crew? Do the crew members come from a variety of cultures and origins? Should their backgrounds be similar, or should they cover as broad a range of skills as possible? Should all of them be of approximately the same age? Analog astronauts are the guinea pigs of these questions. They can safely believe that the technical difficulties to bring humans to Mars or to the Moon will be overcome at some point and is a matter of time. But what about the human psychological aspect? What type of selection should be done on a given population to find the right profile, able to psychologically handle a 2-year mission with high isolation and considerable distance to planet Earth?

This paper, along with many other analog and ground-based endeavours, sets the foundation to identify these profiles, by reproducing exercises, meditation, freeze-dried food supplies, schedules filled with meaningful experiments, or even EVAs in multicultural international crews. Although this paper does not directly answer the above questions, it is an example on how things are being carried out and then new, more detailed investigations to be proposed, in order to take action in solving these issues. Based on the current knowledge, it is difficult to identify working patterns, and a significant amount of work remains to be done.

This work hence only summarised the activities of ARES-III and LEARN analog missions, providing detailed insight into experimental procedures and, where applicable, providing a reference to works published by external investigators. It is not the aim of this paper to analyse the collected data and reach conclusions for the experiments discussed.

Finally, the authors of this paper would like to encourage any interested parties to contact them for any further information and for any advice and point of view or ideas (on station architecture, experiments, etc.) to help improve this field of research. Moreover, we also encourage any interested person to apply to one of the numerous analog research stations around the world to become, as we were, both investigators and subjects of an analog study.

5. Conclusion

A lot of research is being conducted around the world to support off-Earth living, though there is more to be done and understood as this work is ongoing. This paper summarised the activities of ARES-III and LEARN analog missions carried out in the LunAres Research Base in Piła,
Poland. Details of the background, methodology, results, and conclusions of the several pieces of research carried out during the missions have been presented, with references to the resulting papers, as well as an overview of nonresearch activities. As it is not the aim of this paper to analyse the collected data and reach conclusions for the experiments discussed, the authors of this paper would like to encourage any interested parties to contact them for any further information, if necessary.

Appendix

EVA Procedure

(1) Briefing
   (a) Read the EVA scenario
   (b) Create a step-by-step plan for the EVA
   (c) Draw a map of the EVA area on a whiteboard and go over the plan with the two crew members and HabCom operator
   (d) Prepare any ad hoc tools that might be required during the EVA—such as flags and markers, not to waste time in situ. Document new tools and their purpose, and take pictures

(2) Medical—before EVA
   (a) Measure and log blood pressure, heart rate, pulse oximetry, and body temperature of EVA crew leader
   (b) Measure and log blood pressure, heart rate, pulse oximetry, and body temperature of EVA crew support
   (c) Log the measurements in the sheet above, section "Medical before"

(3) Inventory (check out)
   (a) Collect inventory according to the mission statement
   (b) If deemed necessary, choose additional tools
   (c) Place tools in the atrium and decide, which analog astronaut is taking what part of the inventory
   (d) Log the checked out items in the sheet above, including any ad hoc tools from point 1.d

(4) Gearing up
   (a) Each EVA crew member should take the following from the EVA Prep room—a helmet, a suit, under gloves, leather gloves, a reflective band, and boots. Place the items on the floor in the atrium
   (b) Check that lights on the helmet are working. If not, check battery status
   (c) Put on the EVA gear. Once ready, go over the checklist with both crew members

(5) Airlock (out)
   (a) If all previous checklists are complete, the crew can proceed to the airlock
   (b) Once the airlock is closed, HabCom performs radio check individually with EVA crew leader and with EVA support
   (c) The crew can proceed to turn on the UV lights for decontamination
   (d) With airlock closed and UV lights on, HabCom begins the 15-minute decompression countdown
   (e) HabCom then gives the EVA crew a 10/5/1 minute left notice
   (f) After the countdown is finished, HabCom notifies the EVA crew and confirms they are cleared to open the hatch
      (i) Optionally, the EVA crew can turn off the UV lights at this stage
      (ii) EVA crew opens the hatch and proceeds outside of the airlock
      (iii) EVA crew closes the hatch from the outside once all tools and crew members are outside
      (iv) EVA crew notifies HabCom that they are outside and that hatch is closed

(6) EVA
   (a) EVA crew communicated to the HabCom their progress, by announcing start and end times of their activities
      (i) HabCom fills in the "EVA progress" in the sheet above with the reported times
   (b) Primary objectives
      (i) Primary objectives are given by the EVA brief provided by the mission control
      (ii) Primary objectives should be completed first, before proceeding to the secondary objectives
   (c) Secondary objectives
(i) Secondary objectives can be issued either by the mission control, or by the crew themselves, based on tasks they would like to fulfil in the EVA area.

(ii) Secondary objectives should not be critical and should serve as additional activities to fill the 2-hour EVA time, in case primary objectives are completed ahead of schedule.

(d) EVAs normally have a 2-hour allocated slot. HabCom should ensure that EVA crew is back in the airlock 15 minutes ahead of the end of their scheduled EVA time, to allow for airlock time.

(e) Any unscheduled activities and unplanned events should be communicated to HabCom as soon as possible.

(7) Airlock in

(a) After their objectives are finished, or if the oxygen levels become low, the EVA crew proceeds to the airlock.

(b) EVA crew notifies HabCom that they are at the hatch and requests permission to open the hatch.

(i) If the airlock is not closed, HabCom closes the airlock.

(ii) Once the airlock is closed, or if it was closed before, HabCom clears the EVA crew over the radio to open the hatch and enter the airlock.

(c) EVA crew enters the airlock and closes the hatch once all the tools and crew members are inside.

(i) If off, EVA crew turns on the UV lights for decontamination.

(ii) EVA crew notifies HabCom that the hatch is closed and that the UV light is on.

(d) With the hatch closed and UV lights on, HabCom begins the 15-minute pressurisation countdown.

(i) HabCom then gives the EVA crew a 10/5/1 minute left notice.

(e) After the countdown is finished, HabCom notifies the EVA crew and confirms they are cleared to open the airlock.

(i) EVA crew turns off the UV lights at this stage.

(ii) EVA crew or HabCom opens the airlock.

(8) Medical—after EVA

(a) Measure and log blood pressure, heart rate, pulse oximetry, and body temperature of EVA crew leader.

(b) Measure and log blood pressure, heart rate, pulse oximetry, and body temperature of EVA crew support.

(c) Log the measurements in the sheet above, section “Medical After.”

(9) Gear down

(a) EVA crew proceeds to remove their EVA suits.

(b) EVA crew inspects if the suits are in the same condition as before.

(10) Inventory (check in)

(a) EVA crew and HabCom check the inventory upon return.

(b) HabCom reads out the items on the “Inventory Out” list, while the relevant EVA crew member checks whether the item was brought back to the habitat.

(11) Debrief

(a) EVA crew goes over the mission and ensures EVA progress matches the outlined objectives.

(b) EVA crew and HabCom finalise the EVA checklist and submit it as the EVA report.

(c) If no EVA follows immediately after, the EVA crew puts suits and equipment back in the EVA prep room. Please ensure that the radios are in charging stands and are turned off.

Conflicts of Interest

The authors have no competing interests.

Authors’ Contributions

Simon Bouriat, Matej Poliaček, and Jacob Smith contributed equally to this work.

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