2022 EDEN ISS Antarctic Greenhouse Project Report (PDF184)

Authors: Jess Bunchek, Mary Hummerick, Jen Gooden

NASA: Ralph Fritsche, Raymond Wheeler

As we set our sights on returning to the moon and on long-duration spaceflight missions to Mars, developing a sustainable, independent food production system is vital to providing the crew with fresh, nutritious meals that are less reliant on resupply missions from Earth and offer secondary benefits such as improving psychological health and recycling crew consumables. Developing such a system is a long process, especially when considering the holistic nature between the crew, plants, consumables, and hardware.

Collaborative field testing here on Earth is arguably a less costly, more comprehensive, and quicker means of studying this complex relationship than traditional small-scale studies or prototype tests in low-earth orbit. Further, field tests in Antarctica are likely the closest resemblance to a lunar or Martian setting due to the extreme environment, isolation, small crew size, and unique operations and logistics.

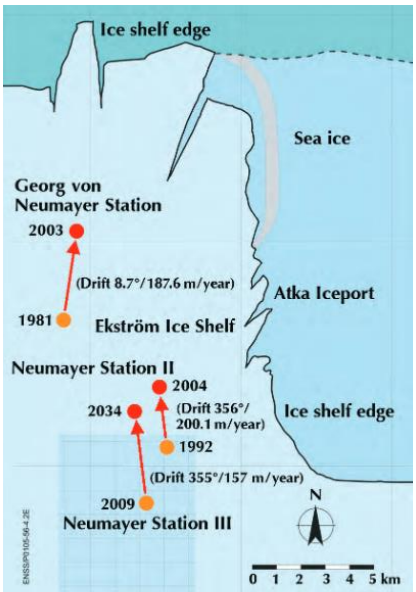
EDEN ISS is a greenhouse container near the German Neumayer Station III in Antarctica. The greenhouse was designed and is operated by the German Aerospace Center (DLR) Institute of Space Systems out of Bremen, Germany. Originally funded by a European Union Horizons 2020 grant, EDEN ISS was installed at Neumayer in early 2018 and was operated for four overwintering seasons until the beginning of 2022. For the 2021 season, NASA Kennedy scientist Jess Bunchek operated EDEN ISS as an overwinterer at Neumayer. While atypical for a scientific project report, the following is written in first-person perspective to better communicate the observations that accompany the operations and collected data.

Due to the vastness of this project and the short amount of time between the end of the 2022 season and submission of this report, the information herein is a first summary of the 2021 season. More in-depth and technical reporting is expected in the coming years that further detail the 2021 season and its findings, plus reports that compare the 2021 season to prior seasons with the EDEN ISS greenhouse, as well as the overall comparison of having EDEN ISS at Neumayer versus prior overwintering seasons without such a facility.

I. INTRODUCTION TO ANTARCTICA & OVERWINTERING

Antarctica and Neumayer Station III

Neumayer Station III ‘‘Neumayer’’ (70°S 8°W) is the third iteration of the German year-round Antarctic research facility. Neumayer was built in 2009 on the 200 m-thick Ekström Ice shelf. The station sits about 6 km to the west of Atka Bay and roughly 20 km from the northern front of the ice shelf, the latter of which is typically used as the ship mooring point for the annual station resupply. With a life expectancy of 25-30 years, this nearly 4500 m^2 station will continue to creep north as the shelf ice migrates, eventually being replaced by a future iteration again farther south.



The Alfred Wegener Institute for Polar and Marine Research (AWI) is the German scientific organization that oversees Neumayer and all associated German polar logistics, the overwintering expeditions, and the overwinterer crew training. Crew selection, the shared overwinterer apartment, and most of the overwintering training take place in the North Sea port city of Bremerhaven, where AWI is located.

Overwintering Crew Preparation Phase

The overwintering crew typically consists of nine specialized individuals: a meteorologist, two geophysicists, an atmospheric chemist, surgeon, cook, IT/radio specialist, station engineer (typically from a mechanical, hydraulic, or marine engineering background), and electrical engineer/electrical technician. For the 2021 season, I was an exceptional tenth overwinterer, specializing in operating the EDEN ISS greenhouse. As part of our selection in March 2020, each team member needed to pass a medical examination that exceeded the requirements of a Class 2 Medical Certificate, which included vision testing, a dental checkup, an abdominal sonogram, and fitness test. Our team training began five months before departure in mid-2020. The ten of us shared an apartment and completed technical trainings such as glacier survival skills and emergency training in the Austrian Alps, confined marine firefighting training conducted by the German Navy, medical emergency and search & rescue training, Antarctic logistics and environmental stewardship training, team conflict and crisis management training, media training, and more specialized courses like familiarization of the various vehicles we would have at the station, harness and fall risk minimalization, and weeks with our respective research groups to prepare for the upcoming season. Shortly before departure, we needed to pass another comprehensive medical examination. At this point, dental work could be required if the dentist suspected problems could arise during the overwintering, such as with wisdom teeth, early cavities, or crowns and fillings. Throughout this process, if a team member failed medical examinations or was deemed unfit, unqualified, or incompatible with the crew, he/she could be removed and replaced by a new member.

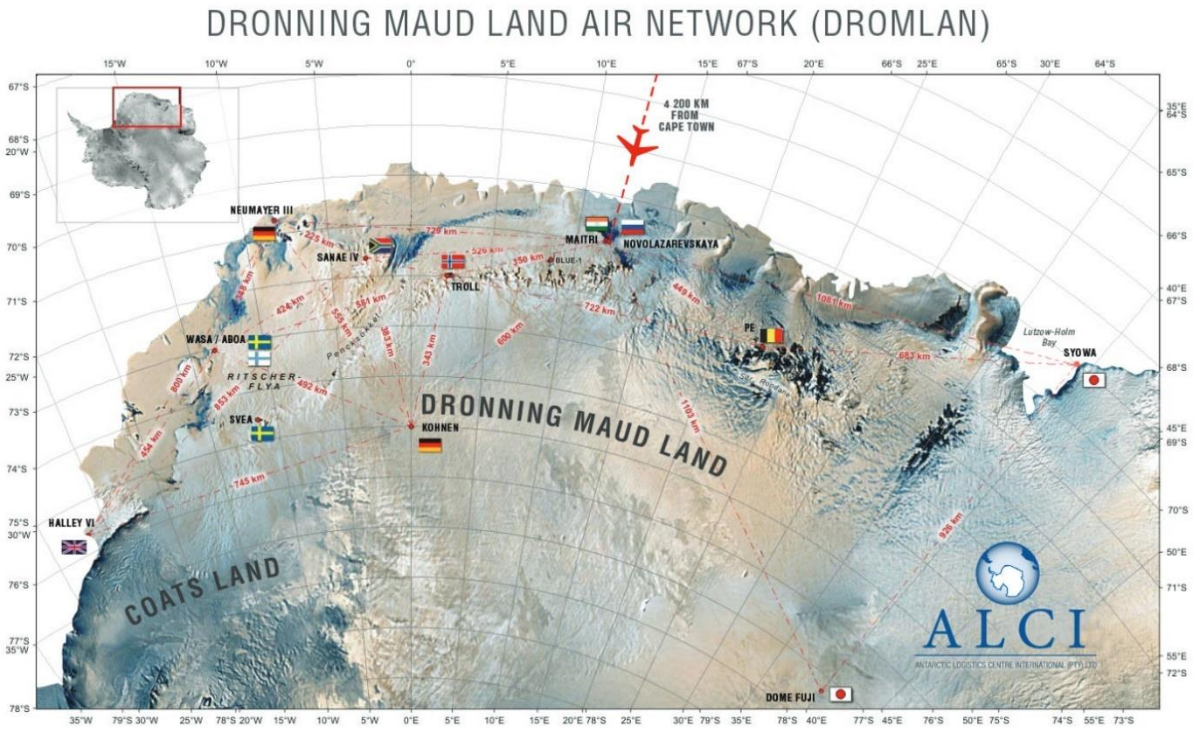
By the time we departed Bremerhaven in December 2020, we were as prepared as possible for the expedition. Because we had already lived and trained together for nearly half a year – particularly, in living quarters ideally for a group smaller than ours – we were cohesive. We had received as much training as we could; the rest would be provided in-transit or once we arrived at the station. Due to our small crew size and isolation, we would need to be our own firefighting and medical team; our fighting team positions were determined based on recommendations from the course instructors. Our station surgeon had trained in new areas such as dentistry, and additional crew members had trained for weeks in a hospital so that they could assist the surgeon at the station if a medical emergency were to arise. We knew what animals we could encounter and how to appropriately act. We knew how to locate and rescue someone if he/she became stranded during a storm. We knew our evacuation plan if the station was ever lost. Mostly, we knew how to prepare for any situation, make smart decisions, and work together.

Effects of the Coronavirus Pandemic

The COVID-19 pandemic affected every step of the expedition. As the only foreigner, I traveled to Bremerhaven early on July 16, 2020, for two-week mandatory quarantining under AWI supervision. Throughout our training, we were required to take extra precautions in public and routinely test for coronavirus. Many of the in-person trainings needed to be conducted online, others featured modified or excluded content, and some trainings were outright cancelled. Traditionally, AWI concludes crew training a couple weeks prior to departure, so overwinterers can return home to spend time with family and friends. At the time of our scheduled break, flight cancellations and traveler restrictions were unstable and continuously changing, so much so that there was growing concern about me being able to return to Germany after my planned break in the US. To avoid missing the departure and expedition entirely, we (I with the support of family, NASA, SURA, Amentum, and AWI) decided the safest option was to cancel the trip home and remain in Germany.

The greatest effect the pandemic had on our logistics was how we would travel to the station. In a normal year, all persons traveling to Neumayer must first convene in Cape Town, South Africa. From there, passengers fly 6 hours to the Russian Novolazarevskaya ‘‘Novo’’ Station (70°S 11°E) on a larger cargo jet. Once on the Antarctic continent, all air travel in our region of Antarctica, the Dronning Maud Land Air Network (DROMLAN), is conducted with a small fleet of Basler Turbo-67 planes, which seasonally reaches Antarctica via Punta Arenas, Chile, and the UK Halley VI Station (75°S 25°W) across the Drake Passage. The planes then disperse across DROMLAN, running supplies and people. From Novo, people typically fly the two hours west in a Basler to reach Neumayer.

From early 2020 until late 2021, the pandemic halted most air traffic in DROMLAN, and AWI did not allow any air traffic through Neumayer to eliminate the risk of coronavirus at the station. To ensure the overwintering campaign could not be compromised, we were notified early in our training that we would instead travel to Neumayer aboard AWI's icebreaker, *RV Polarstern*. The research vessel annually transits the Atlantic Ocean from Bremerhaven to Neumayer to resupply the station, so adding us and a skeleton crew of technical and scientific support for the Antarctic summer season was the best alternative.

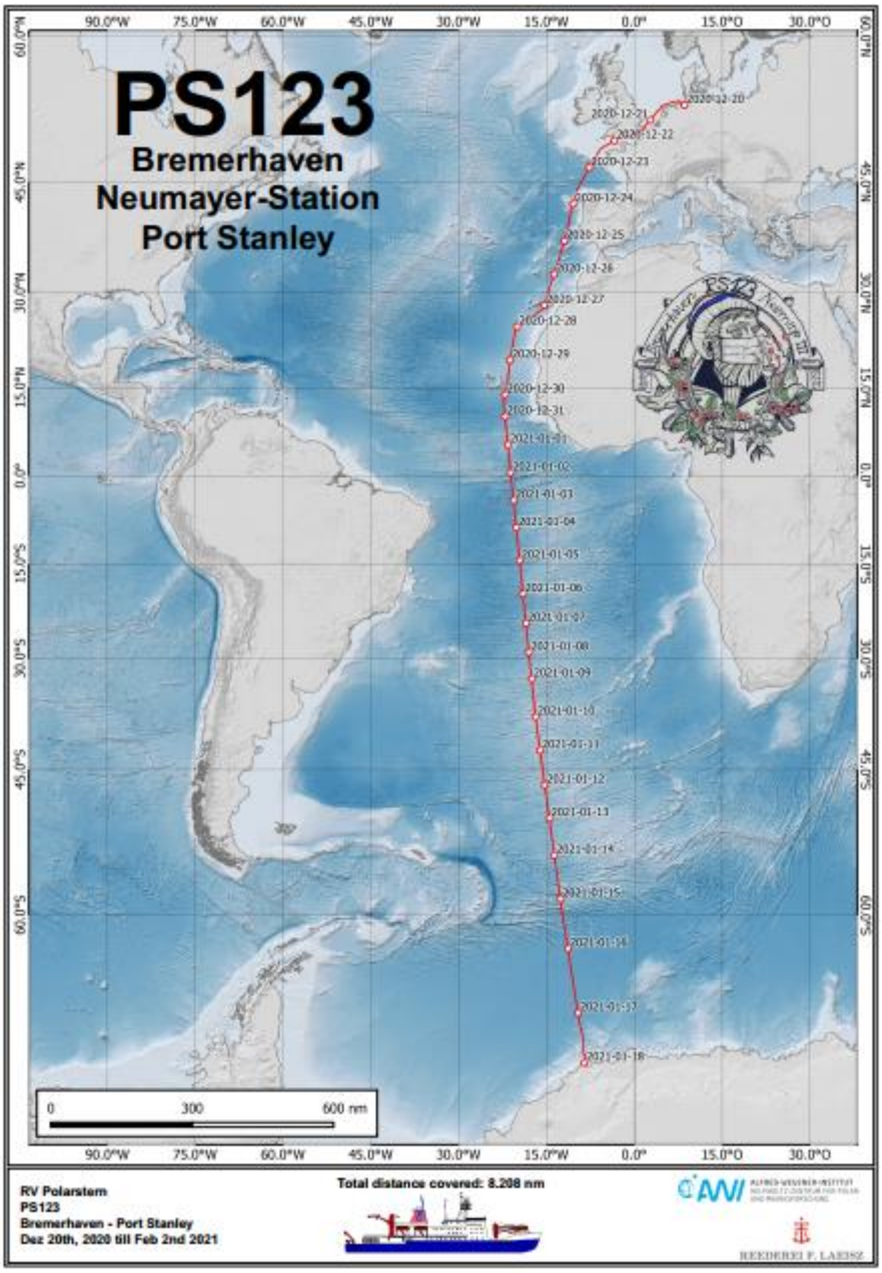


At the beginning of December 2020, we overwinterers started decreasing our contact with the public to minimize the risk of contracting the virus and jeopardizing the expedition. Any trainings at this point were conducted online, and AWI employees brought us mail and groceries. On December 12, 2020, everyone who would be aboard *Polarstern*, crew included, began the pre-departure quarantine. The location and format were the same I had experienced when I first arrived in Germany in July 2020. Each person was in total isolation until December 20, when we left the quarantine hotel and boarded *Polarstern.*

Transit to Antarctica

The four-week transit from Bremerhaven to Neumayer was our first introduction to the incredibly limited internet and contact we would have with the outside world for the next fourteen months. For the first week of the transit, we enjoyed lettuce, apples, and bananas until they were all eaten; we would not have fresh produce again until the first EDEN harvest, four months later in April 2021. During the transit, we overwinterers completed refresher courses and multi-day first aid training. Vincent Vrakking, a member of DLR's EDEN ISS team, was also aboard *Polarstern*. Vincent would be instrumental for training me on how to run EDEN and would help complete the EDEN summer season work. Aboard *Polarstern*, we planned our schedule, studied systems operations documents, and prepared for the upcoming experiment campaign.

We arrived at the mooring point at the northern front of the Ekström Ice Shelf on January 19, 2021. No matter how much training we did prior to our arrival, nothing could fully prepare us for our first time in Antarctica. The environment, terrain, personnel, and logistics are otherworldly and overwhelming. As there is no training version of EDEN ISS in Germany, the summer season was crucial for learning how to operate all of the greenhouse's systems, as well as becoming familiar with the operations, station, and surrounding area.



High-Fidelity Test Environment

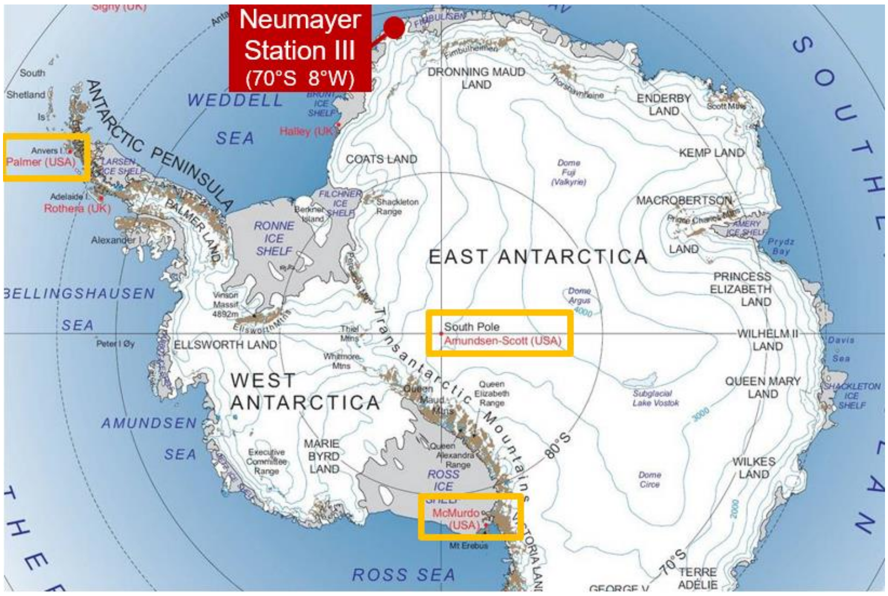
No matter how well spaceflight analogues are constructed and designed, civilization lies just beyond the facility walls or within a few hours' drive. In Antarctica the risks, the isolation, the climate, the type of work being conducted, and the desertedness are real and will kill you, often times simply because of a poor split-second decision. This reality affects your thoughts and actions and is argubly the most valuable asset as to how overwintering expeditions can help us better prepare for long-duration spaceflight missions.

Including the typical 9-person overwintering crew, Neumayer will have 20-60 people at any time during a typical summer season. Because of pandemic restrictions during the 2020/21 and 2021/22 summer seasons, only 20-40 people were at the station at any time during each summer season. Despite ample preparation and crew dynamics testing by AWI, overwintering crews still experience interpersonal disagreements and a lack of privacy, the latter of which is more problematic during the summer seasons. The isolation phase at Neumayer lasts 8-9 months from February/March to

October/November. While we trained for evacuations and emergency situations, once we were in the midst of the winter when the cold temperatures and storms were particularly brutal, we realized that we truly were on our own. There is an emergency base 5 km north of the station with dehydrated food, fuel, and radio equipment, and there is a small container about 300 m from Neumayer with emergency spare clothing. Nonetheless, given the distance from other stations, Neumayer's isolation and lack of an airfield meant that larger jets would not be able to reach us in the event of an emergency. Plus, no Baslers remained on the continent during the winter, and ships could not reach anywhere near us due to the thick sea ice. We had trained that we would have to travel first to the nearest neighboring station, the South African SANAE IV (71°S 2°W), still 225 km away, and then on to another station where a jet evacuation could be possible. However, even polar-grade equipment like the PistenBully plows easily broke hydraulic tubes because the temperatures were simply too cold. If we could not drive 20 km from the station without becoming stranded by a mechanical issue, how could we ever make it to SANAE IV? This is not to mention the preparation time this would require. For a single day trip to nearby Atka Bay, we would prepare 1-3 days in advance. Despite always anticipating an evacuation could be necessary and having contingency plans in place, in a true emergency, we would have a fraction of the time needed to prepare.

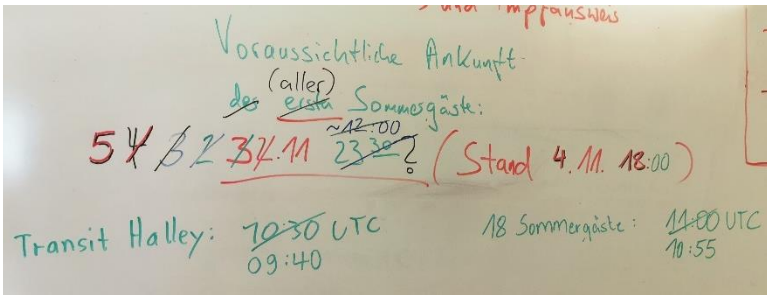
Because we considered ourselves so well trained, trusted one another with our lives, and had enough provisions at the station, we viewed our remoteness as simply part of the job. Nonetheless, compared to the summer seasons, we took fewer risks and were more aware of our actions and surroundings, mainly to prevent injuries. We also carried handheld radios, often even inside the station, to remain in contact with one another. Outside the station or the EDEN container, the only way to reach someone was with a handheld radio. Sea ice, icebergs, and shelf ice could interfere with radio transmissions, and there were often excursions where we lost contact with the station and would need to act with even greater awareness of our surroundings. From inside Neumayer or EDEN, we could call the outside world over satellite phone, which featured a 4-second audio delay, and calls were often dropped or did not go through because of poor satellite range. Internet connection was on par with dial-up capabilities, and if *Polarstern* was out at sea, half of our internet was allocated to the ship. Going outside during storms was normal, so carrying a GPS, radioing the station upon arrival at the container and before leaving, budgeting our energy for the walk back to the station, and planning our schedules according to that particular storm were common practices.

It should be noted that each Antarctic research station features unique environmental conditions and logistics, which can influence the crew, experience, and ultimately the fidelity as a learning environment for long-duration spaceflight missions. The US Antarctic Program operates three stations, two of which are the largest in Antarctica. Compared to Neumayer, McMurdo Station (77°S 166°E) and the Amundsen-Scott South Pole Station (90°S 180°E) experience longer polar night due to their more southernly positions, and the South Pole is colder. However, the crews at most stations are significantly larger; McMurdo has well over 100 people during the winter. Larger stations have more dedicated roles, and while at Neumayer we each had multiple roles and responsibilities, other stations can support dedicated medical support staff, sanitation staff, firefighters and EMTs, aircraft support, among other jobs. The weather at Neumayer is also particularly stormy, and the climate at Neumayer boasts the greatest combined factor of cold temperature and strongest wind speed of all Antarctic research stations. Most stations have shorter isolation periods (McMurdo has only 4 months of isolation), shorter crew deployment lengths, more realistic evacuation plans, and/or less crew training. Even comparing a relatively similar station like the French-Italian Concordia Station (75°S 123°E), whose 13-person crews are also medically tested for spaceflight research, differs greatly from the German program; Concordia's crews undergo just a couple weeks of training, in some cases not even meeting each other until days before deployment.



Finally, conducting field research at Neumayer inherently highlights the complex logistics, demanding schedule, and situational hierarchy that are commonplace in Antarctica and are also factors when considering this environment as a spaceflight testbed. We do not plan to go to Mars because it is an attractive location to grow plants; exploration and scientific activities on Mars will be specific to that location, and crop production will contribute to optimized crew performance and behavioral health. Similarly, polar research is long-established and location-specific. EDEN ISS is an integrated method to help the crew remain as strong and healthy as possible for the entire expedition.

This is especially needed to combat fatigue. During a fourteen-month mission will collectively took only three days holiday (Midwinter on June 21, Christmas, and New Year's Day). The fatigue from this work schedule affected us greater than the isolation, the extreme weather, or the darkness of Polar Night. The responsibilities with the station and the team came before our individual research; we could not survive without the first two. We worked around the clock if necessary, humbly acknowledging that the weather was in charge, and became used to constantly changing situations. And although we were fortunate to be away from civilization for all of 2021 during the pandemic, we still felt the effects. Our first summer season (2020/21) was shortened, which meant that a large amount of annual maintenance could not be completed. The station and external containers were not raised, which required far more shoveling and plowing during the isolation phase than ever anticipated to prevent the structures from being buried by snow. We experienced the two strongest storms ever on record in our area, had the coldest average year in history at Neumayer, and monitored more iceberg activity – including contact damage to our ice shelf – than could be recalled by AWI in decades. We had to be resourceful in solving our own problems, respectful of others' time when asking for help, and equally willing to sacrifice time on our own work to help others. Finally, it is a long and challenging process to reintegrate to society following the expedition, and like astronauts have reported from their missions, overwinterers also often return with a greater sense of responsibility towards planetary stewardship and environmental protection.



All of these considerations, factors, challenges, and events ultimately affected how EDEN ISS was operated for this campaign, and the discussion and conclusions cannot be restricted to the quantitative data alone. When designing a greenhouse for the lunar or Martian surface and planning how it will be operated, all external variables like the crew, habitat, logistics, and environment must also be included in this discussion.

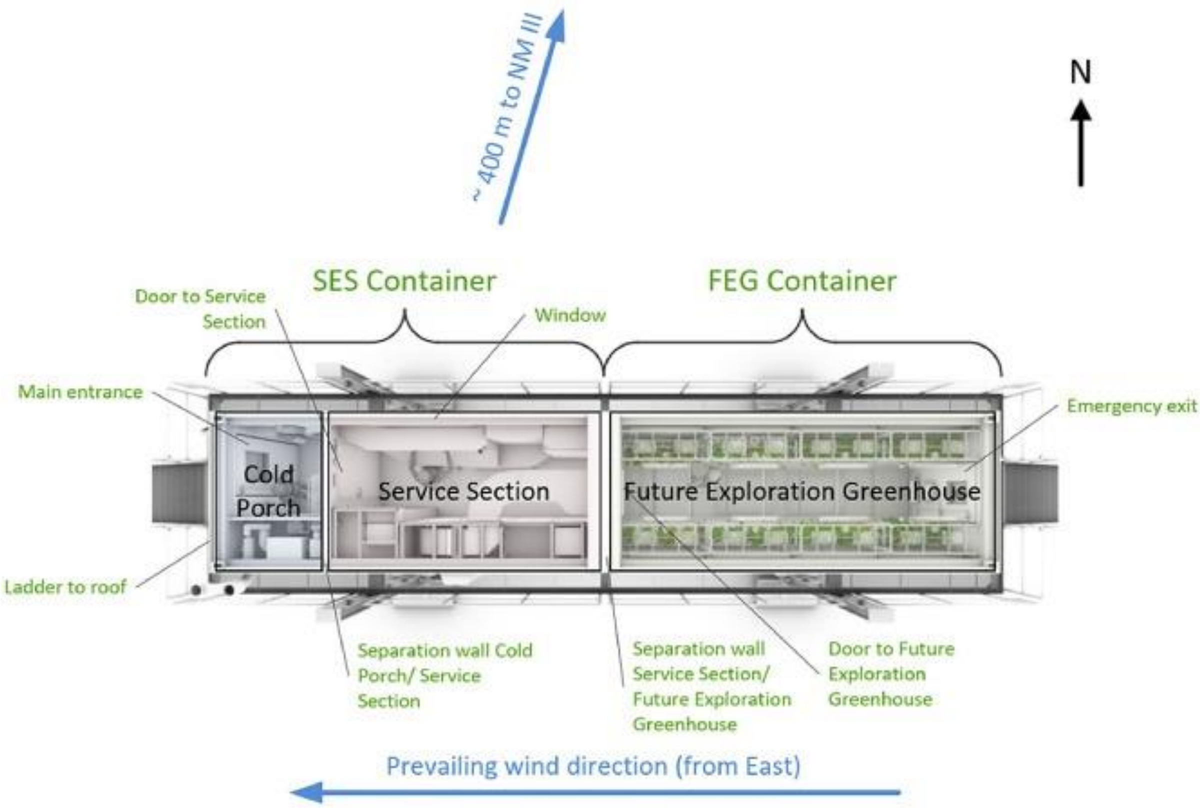
II. 2021: EDEN ISS CAMPAIGN IV

EDEN ISS Background

The EDEN ISS greenhouse consists of two conjoined 20-foot shipping containers. The facility was installed in early 2018 on a dedicated platform 400 m south of the Neumayer Station. The containers are modified with 10 cm insulation. The greenhouse supports 12.5 m^2 cultivation space and is a semi-closed loop system. The containers are directly connected to the station's power supply and IT infrastructure via buried cables, but there is no water connection. All fresh water must be prepared inside the station and manually brought to the greenhouse, and all waste water must be manually removed and returned to and disposed at the station.

The EDEN container is comprised of 3 parts: Cold Porch, Service Section, and Field Exploration Greenhouse (FEG, ''greenhouse''). The Cold Porch served as an airlock of sorts and was used as a storage space for commonly used tools and supplies, as well as a place to remove layers of clothing and hang them to dry. The fresh and wastewater tanks were in the subfloor, and environmental conditions (CO_2 and O_2) could be viewed prior to entering the next sections.

The Service Section housed the supporting subsystems and functioned as a laboratory space. This included the nutrient delivery subsystem (NDS) tanks, nutrient stock solution canisters, and controls; thermal control subsystem (TCS) piping, pumps, and controls; power supply cabinet, Argus greenhouse controls cabinet, air management subsystem (AMS) cabinet with fans, filters, CO_2 injection unit, heating coils, condensation recovery unit, and ducts; dehumidifier for the Service Section, computers and monitors with backup power; work counter, sink, and a large window that faced north towards the station.



The greenhouse had eight plant-growth racks and, with the configuration used for the 2021 season, 38 cultivation spots, each with a tailorable water-cooled Heliospectra LED lamp. Plant-growth trays (40 x 60 cm) were used at 35 of these spots, one was a plant germination tray, and the remaining two spots were used to test the NASA passive porous tube nutrient delivery system (PPTNDS) prototype. Each of the 35 trays was outfitted with aeroponic misters and was connected to the NDS via tubing and piping. A high-pressure pump supplied nutrient solution to each of the four racks on the north (left) side of the greenhouse, and two pumps were used to double-up irrigation to the four racks on the south (right) side of the greenhouse, given the larger plants and subsequent lower number of trays installed on the right side. The pumps were programmed in Argus on five-minute cycles with a 30-second spray period; the pumps operated in rotation to avoid drawing too much nutrient solution from the nutrient tanks and too much power at any given time. Waste nutrient solution drained from the bottom of each tray, down the return piping system, and to filter and sump pump, which would pump the solution back into nutrient tanks.

The crops were supplied with one of two nutrient solution recipes: a lower-concentration mixture intended for crops like mustard greens, lettuce, herbs, beans, and radishes; and a higher-concentration mixture intended typically for longer-growing crops like cucumber, pepper, tomato, and kohlrabi. The greenhouse and NDS hardware in the Service Section featured two complete systems to accommodate both nutrient solution recipes. Within the greenhouse, each rack could be directed to be fed from and return to either nutrient tank via 3-way valves in the greenhouse subfloor. Having two complete irrigation setups created a more complicated hardware arrangement and increased the potential for hardware repairs, maintenance, and cleaning, but two setups also allowed for redundancy, which was particularly helpful when one nutrient tank or sump system needed to be taken offline for repairs, maintenance, or cleaning, or for switching from one nutrient recipe to another to better suit the crops. As each rack's high-pressure pump was located past the 3-way valves, an issue with the rack or pump itself needed to be resolved quickly to get the rack as a whole back online.

The greenhouse subfloor was outfitted with stainless steel, which was ideal for capturing leaks and condensation. The latter posed significant issues throughout the majority of the winter. Despite being insulated, EDEN still had numerous issues related to the extreme external environment. At around and below -30°C external temperature, the surface temperature of the stainless-steel subfloor was low enough that, combined with the high humidity inside the greenhouse, caused condensation to rapidly pool in the subfloor.

III. SCIENTIFIC ACTIVITIES

Having a dedicated on-site operator for the 2021 season allowed for increased operations and significantly more data and biological sample collection than in previous seasons (Table X), particularly compared to the 2019 and 2020 seasons where the overwintering crew split greenhouse responsibilities in addition to their typical tasks. Further, the international collaboration between NASA and DLR created the opportunity to utilize more experts and study the system more collectively than ever before. Data and sample analyses will continue over the next years, and ultimately, we have the novel opportunity to assess the greenhouse from countless angels, to compare across seasons with dedicated greenhouse operators versus analog years that relied on volunteers from the rest of the overwintering crew, and – for a couple parameters – comparing having a greenhouse with seasons prior to EDEN's installation in 2018. The data collected and lessons learned from the 2021 season have already sparked conversations and future project designs that address how to better utilize crew time, design the greenhouse to automate areas of operation that have previously caused crew mental burnout, to design systems that better utilize water, and to design hardware that can accommodate a wider variety of crops.

Repeated areas of testing:

- **Systems Analysis**
  - Power requirement
  - Supplemental CO_2 requirement
- **Hardware**
  - Remote monitoring & data capturing capabilities
  - Remote daily photographs
  - Plant health monitoring cameras
  - Supplies & spare parts
- **Horticulture**
  - Fresh edible and inedible biomass
- **Microbiology**
  - Surface swab & nutrient solution sampling
  - Plant tissue subsampling
- **Crew Time**
- **Crew Surveys**

New or increased areas of testing:

- **Systems Analysis**
  - Weather & climate effects
- **Consumables**
  - Freshwater & nutrient consumption
  - Wastewater production
- **Hardware**
  - NASA passive system prototype
- **Horticulture**
  - Testing new crops
  - Data taken at plant level
  - Nutrition subsamples
- **Crew Time**
  - Collected in greater detail
- **Micro/Molecular Biology**
  - Increased surface, nutrient solution, and plant tissue (sub)sampling
- **Crew Surveys**
  - Revised & aligned with ISS Veggie crew surveys

Systems Analysis

As in previous EDEN campaigns, the power and supplemental CO_2 injection were automatically tracked via the Argus greenhouse controls program. Power requirements for the 2018 season were recently reported at the 2022 ICES conference (citation), and further analyses are expected for the other three seasons, as well as across all seasons. For CO_2 injection, especially for the 2021 season due to the highly detailed crew time recording, we will also be able to better assess the need for artificial CO_2 based on the availability and involvement of the crew in the greenhouse. An area not assessed in previous seasons is the relationship between power requirement, the weather, and climate/weather-related issues in the greenhouse. By integrating meteorological data collected at the station, we can deepen our understanding about how the stark internal-external environmental conditions contributed to challenges in EDEN. A future greenhouse integrated within a habitat structure could require less power and be better buffered against weather-related issues, but as the location of the lunar and Martian greenhouses are not yet determined, we should assume EDEN could be equally exposed. Using EDEN to explore next-generation technologies and designs to better buffer the greenhouse, reduce energy, and hopefully reduce issues caused by the extreme internal-external environmental conditions differential. These developments could further improve greenhouse design and resource-use efficiency here on Earth.

Consumables

This season was the first time fresh and waste water were measured. From this data, we will be able to estimate how much water and fertilizer were needed to grow the 315 kg edible fresh biomass, how much was removed and disposed of as wastewater, and how much was removed as inedible fresh biomass, and how much was estimated to be recirculated in the greenhouse as humidity. EDEN could utilize the station’s wastewater disposal system, and inedible biomass was handled similar to the station's food waste; the Antarctic Treaty dictates that each country must remove all waste. Inedible plant waste was weighed, packed into food-grade plastic buckets, sealed, and stored in a shipping container, which at the end of the season was transported back to Germany. On the moon or Mars, we will need better closed-loop systems which process and reintegrate the waste products of plant production. With the detailed measurements from the 2021 season, we are already preparing to submit intentions for interagency collaboration to integrate the existing efforts of waste processing in space with the greenhouse.

Hardware

Hardware-based and -related data collection performed in previous seasons continued for the 2021 season. EDEN's Data Handling & Management (DHM) subsystem remotely tracked data via the Argus controls program and the power cabinet in the Service Section. The data could be remotely accessed and tracked, even throughout the season. The greenhouse was also equipped with cameras: one overhead at each rack level and eight side-facing cameras throughout the greenhouse. The cameras automatically took a daily photo at midnight, as overwinterers would typically not be in EDEN at that time. These daily photos were automatically sent to DLR in Bremen. Automatically captured daily photographs greatly helped from a crew time and psychological perspective. These cameras also helped with real-time monitoring. The cameras were actually time-lapse capturing cameras with a rate of 1 photograph per second. However, only the daily photographs taken at midnight were transmitted to DLR. From either the Service Section or on the computers in the EDEN laboratory inside the station, I could visually check the plants. This was especially helpful during the strongest storms when going to the greenhouse was not possible and for when an after-hours anomaly was occurring and a quick glance at the photos from inside the station laboratory could provide an initial status check. It cannot be stressed enough how important privacy becomes in a situation like overwintering and with a public project like EDEN, so it was helpful and useful to have the time-lapsing feature strictly as an internal tool.

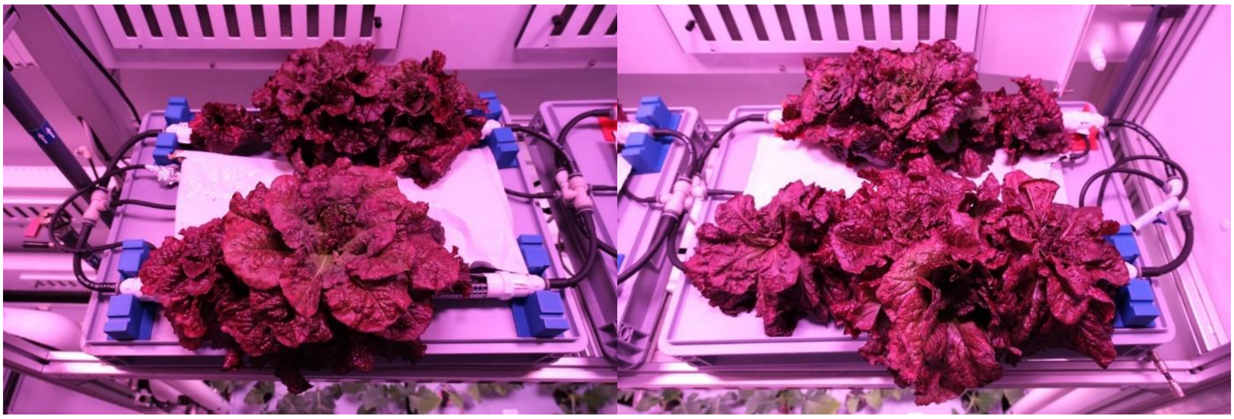
Additional cameras for plant health monitoring (PIs: Paul & Ferl, University of Florida) were installed at select locations in the greenhouse to study early-detection plant stress. The cameras were used in previous seasons but were upgraded on-site during the 2020/21 summer season with new filters.

Inventory was conducted annually, so it will also be possible to track the hardware supplies and spare parts needed to run EDEN, the rate of hardware consumption, and the mass and volume.

Finally, a new plant-growth hardware prototype was integrated and tested for the 2021 season. NASA's passive porous tube nutrient delivery system (PPTNDS). The system was developed at KSC as a new design to (Dreschel date). During hardware development and testing at KSC, this system could be monitored closely. Testing the same system in EDEN, where the PPTNDS was a small component of the facility, aimed to stress-test the system to a greater degree and to provide side-by-side hardware comparisons. The PPTNDS system version we used consisted of two ceramic tubes, which were arranged in parallel. Flexible tubing was connected to the tube ends, and the like ends of the two tubes came together at a T-joint, the third end of which was connected to an ISS water bag. The water bag was filled with 500-1000 mL nutrient solution and positioned in level with the ceramic tubes; the fill amount and positioning helped achieve the right amount of pressure across the system. The seeds were placed directly onto the tubes with a goal of 3 plants/tube. Nutrient solution permeated through the ceramic tube pores to dampen the surface of the tubes and ultimately wet the seeds. The tubes were wrapped with a plastic sheet, which was gently held around the tube, with an opening upwards towards the light source. This plastic covering helped the roots avoid light and to prevent too much evaporation off the tube surface. As this system was a passive version, no pumps were needed; the plants' capillary flow drew nutrient solution from the tubes, and the circuit system design created a closed flow. The system was primed with a syringe and 3-way access valves at the start of the grow-outs and as necessary during the grow-outs to remove air and ensure the kind of flow was not disrupted.

Two PPTNDS units were installed adjacently in EDEN after thorough cleaning with 3% hydrogen peroxide. The first two grow-outs in the PPTNDS were with ‘Outredgeous’ lettuce. For both grow-outs, ‘Outredgeous’ was also grown concurrently in an aeroponic tray for the hardware comparisons testing. For both grow-outs and hardware types, horticulture data, nutrition subsamples, and microbiological & molecular subsamples were collected. A plant health monitoring camera was also fixed above each hardware type. After each grow-out, the hardware was cleaned with the same methodology using 3% hydrogen peroxide. A third grow-out in the PPTNDS with ‘Red Robin’ was conducted towards the end of the 2021 season. Due to the return freight schedule and limited availability of sampling supplies, only horticultural data and plant health imagery were collected on the PPTNDS ‘Red Robin’ plants. Due to the novel nature of this technology, the performance of the PPTNDS and the comparison with the aeroponic system is planned for publication with support from the NASA Technology Transfer Program.

While further analyses are needed, particularly on the nutrition subsamples, anecdotal feedback on the crops is that the PPTNDS ‘Outredgeous’ lettuce was smaller, had tougher-textured leaves that were almost rubbery, were deeply purple with high anthocyanin content, and were not bitter in flavor. Due to their texture, the PPTNDS ‘Outredgeous’ was better enjoyed mixed with other leafy greens grown in the aeroponic trays. The tomato fruit were delicious and flavorful, despite growing on tiny plants. For all grow-outs, the system performed well, and the plants were resilient, especially after wilting events when prime was lost. Because the reservoir was so small, the water bag had to be refilled every 2-3 days, the repetitiveness and frequency of which became annoying. As intended, it was challenging to focus greatly on the PPTNDS with so many other tasks to complete, but having a greenhouse full of other plants and other work was a positive to avoid hyper-fixating on the frustrations of operating this system. One unanticipated benefit of the PPTNDS was that, because it was offline from the nutrient system, it could be used until the very end of the 2022 summer season. By reserving a few liters of nutrient solution, the tomatoes could be harvested and enjoyed while the rest of the greenhouse was emptied, cleaned, and placed into dormancy. This was a great benefit psychologically to continue to have at least something growing in EDEN and at least something to share and eat with others at the station.



Horticulture

Initial seed sowing for the 2021 season was on March 2, 2021, and the final harvest from the aeroponic system was on January 15, 2022, for a season length of 319 days. Across the season, 315 kg fresh edible biomass was harvested. For the first time in EDEN, data were collected at the plant level to allow analysis (to be completed) across the nutrient tray to explore positioning effect. Horticulture data per plant included number of edible leaves and/or fruit, edible fresh biomass, and inedible fresh biomass. Depending on if roots could be definitively separated from neighboring plants, root and rockwool cube biomass (inedible) was quantified either by plant or whole tray. For tomatoes, the number of fruits per plant was counted, and then all fruit were weighed together due to the high number of fruits. For peppers and cucumbers, both of which had lower numbers of fruit per harvest, each fruit was individuals weighed. At the time of fruit plant termination, the remaining unripe fruit were quantified to allow for future analysis of crop lost. Fortunately, unripe peppers and cucumbers were edible and enjoyed by the crew. The crew also attempted to creatively use unripe, green tomatoes, which were stewed and mixed with vegetables.

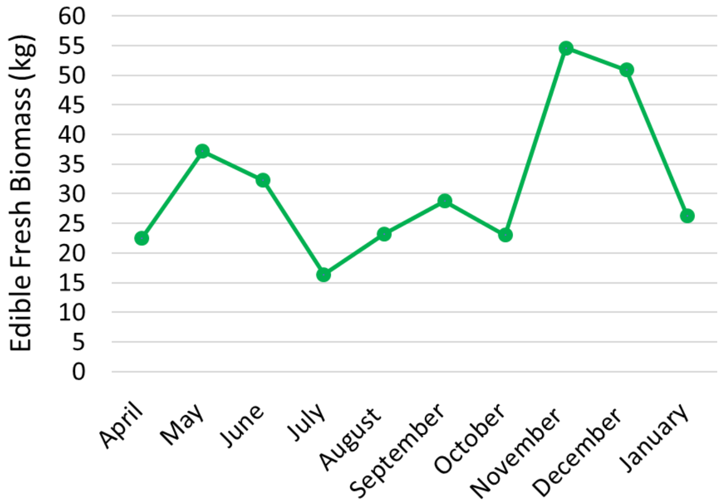
In total, 37 different crop cultivars were grown throughout the 2021 season. This included new cultivars, most of which were in partnership with new crop testing at the Kennedy Space Center, such as broccoli and cauliflower greens; ‘Chimayo,’ ‘Española Improved’ (‘‘Española’’), and ‘Mimi Red’ peppers; beans; peas; and ‘Golden Eye’ spinach (Other spinach cultivars were previously tested in EDEN.). Crops that had been tested in spaceflight included ‘Dragoon,’ ‘Outredgeous,’ and ‘Waldmann’s Green’ lettuce; ‘Amara’ and ‘Mizuna’ mustards, ‘Toscano’ kale, and ‘Española’ pepper, the last of which was growing concurrently in the Advanced Plant Habitat for NASA’s PH-04 study.

‘Red Robin’ tomatoes are being prepared for spaceflight for the VEG-05 study in the Veggie vegetable production system and were also grown in EDEN during the 2021 season.

EDEN ISS provided the chance to test many of these space veterans or candidates in a new environment. By testing the same cultivars in spaceflight, in environmental growth chambers at the Kennedy Space Center (KSC), and now in EDEN, we can gain a better understanding of the systems and microgravity effects on crop production. In addition to the plant growth data, nearly all of the crops grown in EDEN were subsampled for nutrient content. Three plants (50-100 g/plant) were subsampled within each crop. The plants were selected as representatives across the nutrient tray. For each plant, the subsample number of leaves/fruit and fresh biomass were recorded. The edible biomass for each subsampled plant was kept separate and transported back to the station laboratory. Each subsample was placed in a paper bag and dried at 70°C for a minimum of 72 hours, before being brought back to room temperature and weighed for dry mass. The next step was originally planned to be tissue grinding, but the low humidity inside the station created so much static electricity that handling the dried biomass was especially challenging. To reduce the amount of lost dried plant tissue, the biomass was broken into pieces only as small as what was necessary to be stored inside 50-mL centrifuge tubes. The tubes were sealed with Parafilm, stored in vacuum-sealed bags, and stored ambient inside the station laboratory. For return transit, the dried samples were transported inside a 5°C refrigerated shipping container, which prevented the samples from being exposed to temperature extremes as the cargo traveled across the polar and tropical regions. Once the samples reached Bremerhaven, they were transported to DLR in Bremen before ultimately being shipped to KSC. In total, 164 nutrient subsamples were collected during the 2021 season. Funding to analyze these samples is still pending but would be valuable to determine how our diets as overwinterers were supplemented by the crops produced in EDEN. For the crops that have also been sampled at KSC or from spaceflight experiments, we can add EDEN as another test environment. Because so many crops could be grown in EDEN, this testing also allows us the chance to assess the nutrient content of crops in addition to the spaceflight veterans and candidates, which can increase the rate at which we select nutrient-dense crops for future missions. Select crops were also grown and subsampled multiple times throughout the season, which will provide us the chance to assess a time effect, and some cultivars yet were grown as both single-harvest and multiple-harvest crops, which can give us insight as to which harvest method may be more ideal for optimized nutrition.

37 different crops – 315 kg total fresh edible biomass

	Herbs	Lettuce	Mustard greens	Other leafy greens	Stem crops	Tomatoes	Peppers	Other fruit
	Basil (Dolly Genovese)	Dragoon	Amara	*Spinach (Golden Eye)	Kohlrabi (Korist)	Amoroso	Chimayo	Beans
	Chives (Purly)	Expertise	Arugula	Swiss chard (Bright Lights)	Radish (Raxe)	Joy Red	Española	Cucumber (Picowell)
	Parsley (Laura)	Othilie	Broccoli			PAT orange	Mimi Red	Peas
	Oregano (Greek)	Outredgeous	Cauliflower			*Red Robin	Red Skin	
	Rosemary	Waldmann's Green	Frizzy Lizzy					
	Spearmint		Kale (Toscano)					
	Thyme		Mizuna					
			Red Giant					
			Pak choi (Rosie)					
			Watercress					
Edible fresh mass (kg)	**13.7**	**27.1**	**40.9**	**6.9**	**40.0**	**91.1**	**18.5**	**76.4**
Inedible fresh mass (kg)	**6.6**	**15.3**	**18.5**	**2.3**	**10.3**	**55.8**	**25.5**	**11.7**
Edible fresh mass (%)	**68%**	**64%**	**69%**	**75%**	**80%**	**62%**	**42%**	**87%**



Month	Edible Fresh Biomass (kg)
April	22.5
May	37.2
June	32.3
July	16.4
August	23.2
September	28.8
October	23.0
November	54.6
December	50.9
January	26.3
Total	**315.2**

- Challenging weather
- Unanticipated issues
- Summer season prep (Oct)
- Affects whole crew
  - Less help available
  - Operator needed elsewhere

Crew Time

The first crops ready to harvest were leafy greens like 'Mizuna' mustard and 'Bright Lights' Swiss chard. Harvesting began in April and increased into May as more crops were ready to be harvested. Polar Night – the period where the sun does not rise above the horizon – was from mid-May to mid-July. As the external temperature continued to drop across the season, more issues began to arise with operating EDEN. Allowable time for harvesting subsequently decreased during the middle of the season. As the external temperature and conditions improved at the beginning October, more time could be dedicated once again to harvesting. Even as maintenance, cleaning, and repairs were still needed, activities could be diagnosed and completed faster by the latter part of the season. However, with the upcoming summer season, help was needed elsewhere to prepare the station for the arriving guests. Once the summer began, the other overwinterers were unable to help as much in EDEN.

Some of these issues from the 2021 season, which have resulted in many lessons learned, include:

1. **Every component of the greenhouse needs to be designed and installed with the assumption that it will need to be accessed at some point in time.**
   The AMS condensation recovery basin could not be opened or removed, so algae and mineral deposits on the inside of the basin could not be easily cleaned. Days were spent trying to clean this during the summer season, and the cleaning effort was still ineffective.

   The TCS cabinet in the Service Section was poorly organized, and many components could not be reached, even by small hands. The pipes were wrapped in insulation tape, and the sensor numbering in Argus did not properly depict the real-life organization of the system. For this system, which due to the insulation could not be accessed and visually assessed, troubleshooting issues relied heavily on guesswork and trial-and-error, which required literally days of dedicated work.

   The PVC piping of the NDS inside the greenhouse could not always be removed, as detachable joints were located too far apart and would have required shutting down an entire plant rack. Repair efforts in-place were unsuccessful, and the leaks remained for the remainder of the season.

2. **More robust hardware is needed.**
   The high-pressure pumps for the NDS last on average for about a year, but often not. This could cause sudden pump failures, which required immediate mitigation to restore the irrigation and prevent the plants from dying. Given the mass and price of these pumps, it is unacceptable to have hardware with such a short lifespan. For a lunar and Martian surface, it will be even more important to have repairable pumps.

3. **Overdesigning creates more work.**
   Many of the sensors in EDEN failed over time, including the level sensors in the nutrient stock solution tanks, the empty & full sensors in the fresh and wastewater tanks, and the leak sensors on the floors of the Service Section and the greenhouse. These faulty sensor readings and failures ultimately created a false sense of reliance on the technology, which lead to running out of fresh water (Remember: Fresh water needed to first be prepared at the station, at a rate of 1 L/min, and then transported to EDEN.), false positive flood detection shutting down other programs in the greenhouse, and time and frustration needed to investigate and attempt to repair or replace the sensors. This is an example of ''too much of a good thing,'' and visual checks on these components were not only more accurate but also contributed to more broader component checks.

4. **The facility and hardware will inevitably age.**
   With the facility in its fourth year, new hardware issues arose. As these issues were also new for DLR, they were unable on multiple occasions to assist with diagnosis and mitigation. This included a faulty leak sensor in the greenhouse blowing the fuse in the Argus controls box in the Service Section. Identifying the specific cause of issues in EDEN was similar to diagnosing a medical disease and required identifying the (sometimes obscure and misleading symptoms. As the symptoms of this blown fuse were similar to other issues, it took hours – in the middle of the night, nonetheless – to properly identify the cause.

5. **We need better greenhouse controls interfacing.**
   Commercial greenhouse controls companies claim they can address the unique aspects of each greenhouse facility, but Antarctica exceeded the capabilities of the Argus system. We were limited in data tracking, such as various temperature setpoints, and we did not have the permissions to change many parameters or program rules without a representative from Argus. The tracking limitations were especially frustrating when issues with the TCS arose; temperatures had to be manually written down to track changes over time. Additionally, the system should have been better tested for program gaps. The solution pH in the nutrient tanks could be adjusted by adding either base or acid solution. A dosing pump with a solenoid, the latter of which dictated into which nutrient tank the acid solution, for example, were used to lower pH. On one occasion, the pH in nutrient tank #2 was a little high, so the program prompted the dosing pump and solenoid to add acid solution. However, the solenoid stopped working and did not properly switch. This caused pressure to build between the dosing pump and solenoid, and at some point, the tubing ruptured, spraying acid solution around the closed NDS closet in the Service Section. As the pH still remained too high, the program continued to prompt the dosing pump to add acid solution, which was now partially being added to tank #1 and partially out of the ruptured tubing and all over the interior of the NDS cabinet. This started at 2am, and by the time I awoke and checked the Argus computer inside the station around 8am, the pH of nutrient tank #1 was below 2. All plants being irrigated by nutrient tank #1 had been exposed to dangerously acidic solution for hours at this point. After running to the greenhouse, the plants exposed to acid solution first needed to be switched to tank #2. Each rack's pump was then switched on sequentially for at least a minute to thoroughly wash the roots. With all the racks now being irrigated with tank #2, tank #1 was bypassed and could now be addressed. With the proper PPE, the spilled solution inside the NDS cabinet was cleaned up, and the cause was determined. Fortunately, there was a spare solenoid in the EDEN equipment storage in the station gallery, so both tanks were eventually brought back online. However, this issue should have never occurred. The Argus program did not have any commands to stop pH dosing adjustments if such an anomaly occurred. What made this even more frustrating is that we could not add this command to Argus ourselves; we needed to press Argus to add this for us.

   A more ideal greenhouse controls system would have been directly linked with the station controls system, designed by an institution like AWI whose experts understand the environment and isolation in which we were working. Every variable needs to be trackable, accurately mapped to match the physical organization of the greenhouse and its subsystems, and be fully tested and vetted for gaps that could create hazardous conditions for the plants and operator(s).

Other issues and lessons learned were emphasized by the extreme Antarctic environment and/or EDEN's placement as a standalone container away from the station. Documenting these issues, identifying the lessons learned, specifying to what degree the placement of EDEN was exclusively contributed, and ideas for improvement will continue; one such publication on fail safe modes, which will tie in lessons learned from the plant-growth systems on the ISS and testing done at KSC.

Crew time was recorded in previous seasons, albeit in less detail than the 2021 season. In combination with the Veggie on ICE survey, which featured an expanded list of 26 possible tasks, this was the first season during which each crew member's time was individuals recorded for each task. As I was in the greenhouse nearly daily, I used an 8-sided tracking tie from Timeular to record the activities at a broader level: Daily Checks, Harvesting, Cleaning, Maintenance, Repair, Pollinating, Sowing, and Miscellaneous. Like nearly everything else, tracking time was a learning process. The die needed to be connected to my cell phone, as the Timeular app tracked what the die was recording. Thus, my phone needed stable Wi-Fi and Bluetooth connections in the greenhouse, both of which are already challenging enough in Antarctica; these connections were possible via a router in the EDEN container that was linked back to the station. If these connections were disrupted, the die would not detect that it was activated and, therefore, would not record time. If I failed to catch this, activities could be lost or needed to be added in manually retroactively. In all, though, the device was handy, more reliable than unreliable, and it was great to have the data already entered and accessible in the Timeular app and online account for easy access and assessment. In the event that the required connections were disrupted, if I was performing tasks at the station, or whenever other crew members were performing tasks, the times were manually recorded on paper and later integrated into the daily log book.

Like most things in Antarctica and with this project, it took months to find the right rhythm and methods for capturing data. As the season went on, more tasks conducted inside the station were recorded to better show that operating EDEN required far more than the tasks performed solely within the container. While some activities were inevitably not captured, especially early in the season for various tasks inside the station, I can confidently report that the time collected is a fair representation of what is needed to operate such a greenhouse. Any additional points for consideration are anecdotally reported herein.

While a greenhouse may be better suited inside the station, there were originally hesitations from the deciding parties about installing one within the existing infrastructure of the station. As a compromise, the greenhouse could be installed outside but near the station as a sort of trial. The containers were installed onto a platform 400 m south of the station – close enough to still be accessed daily and have direct power and internet infrastructure buried under the ice running back to the station, yet far enough to not be affected and buried by drifting snow as part of the airflow dynamics under and around the station.

In its current iteration, EDEN ISS requires too much crew input. Even with the time committed during the 2021 season, the lack of more personnel simply resulted in a loss of quality. Sacrifices were made to prioritize the most pressing tasks. Examples included falling behind on regular maintenance, sometimes even skipping the maintenance activity for that week or month.

As the operator, the most time was spent on harvesting, hardware/facility cleaning, nutrient solution mixing, hardware/facility routine maintenance, and hardware/facility repair. These were also the five activities with which I was most annoyed. Harvesting is typically a quick procedure, but the data and subsample collection added on a tremendous amount of time. This is a similar time-related issue faced with the Veggie and Advanced Plant Habitat. In EDEN, harvesting was still a rewarding activity because of the delicious fresh produce. However, I felt disincentivized to harvest, knowing that this seemingly simple task could also require data – and often subsample – collection, and if this was the final harvest, the trays would need to be cleaned and refilled with new crops. That meant that new seedlings already needed to be transplanted, which would have required time in the preceding days or weeks to initiate. Ultimately, a procedure like harvesting was surprisingly demanding temporally, mentally, and physically. For a future system, we will need to better weigh the tradeoffs and find the balance between the science component of crop production and the required crew inputs. The amount of time required for cleaning, nutrient solution mixing, maintenance, and repair can be decreased with improved hardware and design and by including automation where most helpful.

Combined across all other crew members who helped in EDEN, their most time-consuming activities were harvesting, hardware/facility maintenance, hardware/facility repair, and other. Activities grouped as ‘’other’’ were specific to EDEN’s external location but were still significant and vital enough to the greenhouse that the time needed to be captured to demonstrate that operating such a facility requires countless hours beyond the direct, expected activities inside the greenhouse. For the crew members, ‘’other’’ activities included plowing around the EDEN container and other exterior maintenance, and to help me move equipment inside the station like the freezers.

	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Total (min)	Total (h)
Daily Operations													
Environment monitoring	140	192	90	245	280	270	315	290	330	220	185	2557	43
Plant health monitoring	1500	192	90	75	200	210	150	105	220	85	90	2917	49
Nutrient solution monitoring, management	140	192	30	75	20	30	20	30	25	30	30	622	10
Irrigating, watering, reservoir filling	40	192	256	20	40	60	200	75	90	90	90	1153	19
Cultivation Operations													
Planting, growth initiation, priming	1000	64	41	0	750	225	350	150	465	220	0	3265	54
Wick opening	30	0	0	0	0	0	0	0	0	0	0	30	1
Plant thinning, pruning, reorganization, training/trellising	130	222	580	610	265	300	640	760	550	395	355	4807	80
Plant pollinating, fertilizing	0	81	300	160	20	20	30	75	100	290	30	1106	18
Diagnostic imaging, mitigation	120	0	0	0	0	30	0	0	0	0	0	150	3
Harvesting	0	271	1751	1935	1280	845	825	1670	1670	1425	3595	15267	254
Routine Operations													
Environment programming	90	0	15	0	60	60	15	20	10	20	60	350	6
Hardware/facility cleaning	3000	768	125	250	350	765	1130	740	695	450	1350	9623	160
Nutrient solution mixing	3000	465	120	120	135	770	450	135	250	420	460	6325	105
Operations training	120	15	30	60	30	0	0	20	0	0	0	275	5
Maintenance													
Hardware/facility routine maintenance	600	855	500	880	70	1340	985	225	280	130	315	6180	103
Hardware/facility repair	600	1607	700	1635	1015	440	745	55	15	130	65	7007	117
Restoring lost prime	60	180	180	0	0	15	0	15	25	20	15	510	9
Utilization													
Plant data collection	240	404	980	465	220	150	635	720	280	165	850	5109	85
System data collection	60	120	120	180	180	55	60	120	450	180	480	2005	33
Data entry, organization, analysis	0	120	120	420	420	480	360	480	600	480	615	4095	68
Produce cleaning	0	0	25	30	45	30	75	60	120	30	120	535	9
Photography	15	5	60	60	30	20	30	60	10	20	45	355	6
Videography, time lapse imaging	0	5	25	0	0	15	20	15	0	7	0	87	1
Voluntary viewing/exposure	0	0	0	0	0	0	30	60	40	30	215	375	6
Outreach	15	0	256	0	0	60	60	180	240	225	300	1336	22
Other	60	210	120	85	90	0	760	120	60	115	180	1800	30
Totals	10960	6160	6514	7305	5500	6190	7885	6180	6525	5177	9445	**77841**	**1297**

	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Total (min)	Total (h)
Daily Operations													
Plant health monitoring	0	124	0	25	0	24	0	0	0	0	0	173	3
Cultivation Operations													
Seed planting	0	0	0	0	220	0	0	0	0	0	0	220	4
Plant thinning, pruning, reorganization, training/trellising	0	45	0	295	130	98	375	0	0	0	0	943	16
Plant pollinating	0	23	44	35	0	0	6	0	0	0	0	108	2
Harvesting	0	341	203	1169	810	404	630	570	315	0	430	4872	81
Routine Operations													
Hardware/facility cleaning	0	0	0	60	20	130	65	0	0	120	0	395	7
Maintenance													
Hardware/facility routine maintenance	0	243	5	235	265	419	280	0	0	145	190	1782	30
Hardware/facility repair	0	779	66	1130	515	78	323	0	0	80	0	2971	50
Utilization													
Plant data collection	0	165	378	145	95	0	0	155	55	0	50	1043	17
Data entry, organization, analysis	0	0	0	0	0	0	0	0	0	0	255	255	4
Produce cleaning	0	0	20	0	0	0	0	0	0	0	0	20	0
Photography	0	166	0	5	15	0	18	0	0	75	0	279	5
Voluntary viewing/exposure	0	63	0	0	0	0	0	0	20	0	0	83	1
Other	0	120	0	355	60	0	180	90	0	410	105	1320	22
Totals	0	2069	716	3454	2130	1153	1877	815	390	830	1030	**14464**	**241**

Microbiological sampling

Due to the massive amount of data collected during the 2021 season, most analyses are still forthcoming. One example of the in-depth analysis already underway is the microbiological sampling. The samples were able to be analyzed shortly after arrival at KSC. Nonetheless, further discussions are planned to assess the relationship between the microbiological data and other areas of the greenhouse operations and production.

Edible plant parts. Like the nutrient subsamples, plant tissue was sampled for microbiological and molecular sampling. Due to limited freezer space, a select list of crops was identified for subsampling, which aligned with crops previously grown in space and/or at KSC, plus a few new crops of interest. Like the nutrition subsampling, select crops were grown and subsampled multiple times throughout the season to study microbial load across the season, and different harvesting schemes were also subsampled. For crops with multiple ''cut-and-come-again'' harvests, only the first and final harvests were subsampled. For each crop tray, five plants were subsampled. Gloves, tools, and surfaces were cleaned with 3% hydrogen peroxide before and between plants. For each plant, 25-50 g was collected for subsampling, which was placed inside a vacuum-sealed bag, taped shut, and placed – weather and daylight permitting – in the Cold Porch or just outside the station to keep the samples cold and preserve the microbes to as close as they were at the time of harvest. Samples were then brought to the station, quickly vacuum sealed using the unit from the kitchen supplies, and placed in the -40°C freezers in the station gallery. The freezers were plugged into the station's backup power system and were periodically checked, ensuring no temperature anomalies. At the end of the season, the freezers were transported with all samples inside back to Germany via ship. Pre-planned logistics allowed the samples to remain at -40°C the entire journey. Once arrived in Bremerhaven, the freezers were transported to DLR in Bremen, repacked in dry ice, and shipped to KSC. The following plant shoots and fruits were sampled (number of samples are in parenthesis):

- Leafy greens
    - 'Outredgeous' lettuce (25)
    - 'Waldmann's Green' lettuce (15)
    - 'Mizuna' mustard (15)
    - 'Amara' mustard (5)
    - 'Toscano' kale (5)
- Herbs

    - ‘Dolly’ basil (5)
    - ‘Purly’ chives (3)
    - Oregano (3)
    - ‘Laura’ parsley (10)
- Peppers
    - ‘Chimayo’ (4)
    - ‘Española’ (4)
    - ‘Mimi Red’ (4)
    - ‘Red Skin’ (4)
- Tomato
    - ‘Joy Red’ (5)
    - ‘Red Robin’ tomato (10)
- Stem + leaf crops
    - ‘Raxe’ radish leaves (5)
    - ‘Raxe’ radish stems (5)

After arriving at KSC, the tissue samples were stored at -80 °C. Plant shoots from leafy greens and herbs, tomato and pepper fruit, and radish shoot and storage root were placed into sterile blender bags and weighed. The tissues were then diluted and plated onto tryptic soy agar (TSA) for aerobic plate count and inhibitory mold agar (IMA) for yeast and mold count. Colony phenotypes from the TSA and IMA plates were selected from each sample and isolated on the respective agars.

***Environmental samples*. Surface swabs and nutrient solution samples were processed using a modification of methods described in Fahrion et. al 2020, Microbial Monitoring in the EDEN ISS Greenhouse. Swab samples were collected using swabs containing 5 mL buffer solution. The swab tubes and liquid nutrient solution samples were removed from -80°C storage and allowed to thaw for two hours at room temperature. After thawing, the swab samples were vortexed for 5 s on the highest setting, followed by sonication in an ultrasonic bath sonicator for 2 min. One mL samples divided into 250 mL aliquots were plated onto four R2A agar and IMA agar plates. One mL of sample was heat-shocked for 15 min in a dry heat block set at 80° C and chilled on ice to room temperature. Four aliquots of 250 mL from the heat shocked sample were plated onto four plates of R2A. The samples were also diluted and plated onto R2A, IMA and TSA to capture any potentially high counts. Samples were collected from surfaces of the future exploration greenhouse (FRG) that had been sampled and analyzed during the 2018 season, including the wall on the right side (FEG 2) and the floor under the cattle grid (FEG 9). A surface swab sample was also collected from the passive porous tube nutrient delivery system along the actual porous tube supporting plant roots (PPTNDS)**

Surface and nutrient solution samples from nutrient tank (NT) one and two were collected at weekly intervals for 10 weeks followed by 7 monthly sample collection. These samples were stored at -40 °C until shipment to KSC where they were stored at -80 °C until processing Thawed liquid samples were vortexed and serially diluted and plated in duplicate on R2A, and IMA. Plates were incubated at 20° C for seven days before enumeration of colonies. Samples were also plated onto TSA and incubated at 30° C as a comparison to previous methods. Individual colony phenotypes were selected after enumeration and reisolated for identification as previously described.

***Roots*. Root samples were thawed and at least a one gram sample was placed into 50 mL centrifuge tube containing 30 mL sterile PBS with sterile 3mm glass beads weighed then shaken for 2 cycles at**

5 m/s for 30 s each on the Omni BeadRuptor (OMNI, Kennesaw, GA, USA). Samples were serially diluted in PBS and plated in duplicate onto TSA and IMA. Plates were incubated up to 7 days at 30° C before enumeration of CFU. Again individual colony phenotypes were isolated, restreaked and identified as described.

***Microbe identification*. Bacterial colonies were identified using the Biolog Micro ID System (Biolog, Hayward, CA, USA). Bacterial colonies that could not be identified using Biolog and all fungal isolates were identified using the MicroSEQ 16S rDNA sequencing kit to identify bacterial isolates while fungal and yeast colonies were identified using the MicroSEQ D2 LSU rDNA kit for fungi o**

(Thermo Fisher, Waltham, MA, USA). DNA was extracted from isolates using the Prepman System for bacteria or the Qiagen Powerlyzer Soil Kit for fungi. All MicroSeq sequencing was completed on the ABI 3500 Genetic Analyzer using the MicroSEQ ID Microbial Identification Software Version 3.1.3 (Thermo Fisher, Waltham, MA, USA). Bacterial and fungal DNA sequences were identified using the MicroSEQ ID Software16s rDNA 500 Microbial Library Version 2019 and the MicroSEQ ID Fungal Gene Library Version 2018 (Thermo Fisher, Waltham, MA, USA). Verification of ID was followed by NCBI Basic Local Alignment Search Tool (BLAST) when necessary. Petrifilms were also used to identify and enumerate *Escherichia coli*/coliform and *Staphylococcus aureus* for microbial food safety screening (3M, St. Paul, MN, USA). Petrifilms were incubated at 35°C for 24 h per manufacturer's guidelines and any colonies positive for *E. coli*/coliform and *S. aureus* were enumerated and re-isolated. Re-isolated colonies were identified using Biolog GEN III plates. To screen for Salmonella, sample extract was incubated at 35°C 35 C for 24 h. A 1 mL aliquot was then transferred into 5 mL of Rappaport-Vassiliadis (RV) broth or Tetrathionate broth (Thermo Fisher,Waltham, MA, USA) and incubated for an additional 24 h at 35°C . The broth cultures were then streaked onto Hektoin Enteric agar selective for Salmonella and incubated at 35°C. Plates were observed for typical Salmonella colonies, reisolated and identification confirmed if necessary.

Results

Bacterial and Fungal Counts on Edible plant parts.

Figure 1 shows the bacterial and fungal colony counts on leafy greens. There were three harvests of Outredgeous lettuce, Mizuna and Waldman's green from April 2021 and December 2021. Bacterial counts trended upward with each harvest of Outredgeous lettuce, however from the five samples from September and the five from December only one from each set exceeded the NASA APC limit of 2 x 10^4 for any one sample or 1x10^4 for two samples out of 5 for non-thermostabilized food. Lettuce grown in the PPTNDS system all remained below the limit. Bacterial counts on Outredgeous lettuce ranged from $\leq$ 50 CFU/g (detection limit) to 3.5 x 10^5 CFU/g. Three out of five samples from the July harvest and two from the December harvest of Mizuna were above the NASA limit ranging from below detection limit to 8.5 x 10^5 CFU/g while Waldman's green bacteria counts were below detection to 1.1 x 10^5 CFU/g. The bacterial counts found on these leafy greens are in the range of similar greens that may be purchased from a market and reported in the literature to range from < 1 x 10^4 to 1 x 10^8 . (Muukherjee 2004, Klapec 2016,Wood 2015, Holvoet 2015, Rastogi 2012, Jackson 2013 Oyiniola 2016 Zhang 2017 Hagenmeaier 1998, Valentin-Bon 2008).

A similar trend in increased fungal counts was seen with the consecutive harvests of Outredgeous lettuce, Mizuna and Waldmans green. The NASA standard for yeast and mold on non-thermostabilized food is 1 x 10^3 CFU/g. Several of the leafy greens and herbs exceeded this standard. Since the bacteria and fungal counts are similar or in some cases lower than market produce, the NASA standards for non-thermostabilzed food may not be applicable expectations for fresh grown crops.

Of the five herbs sampled, Parsley was the only one included in these samples as a “cut and come again” harvest. As with the repeated harvests of the leafy greens, there was an increase in both bacterial and fungal counts after the first harvest.

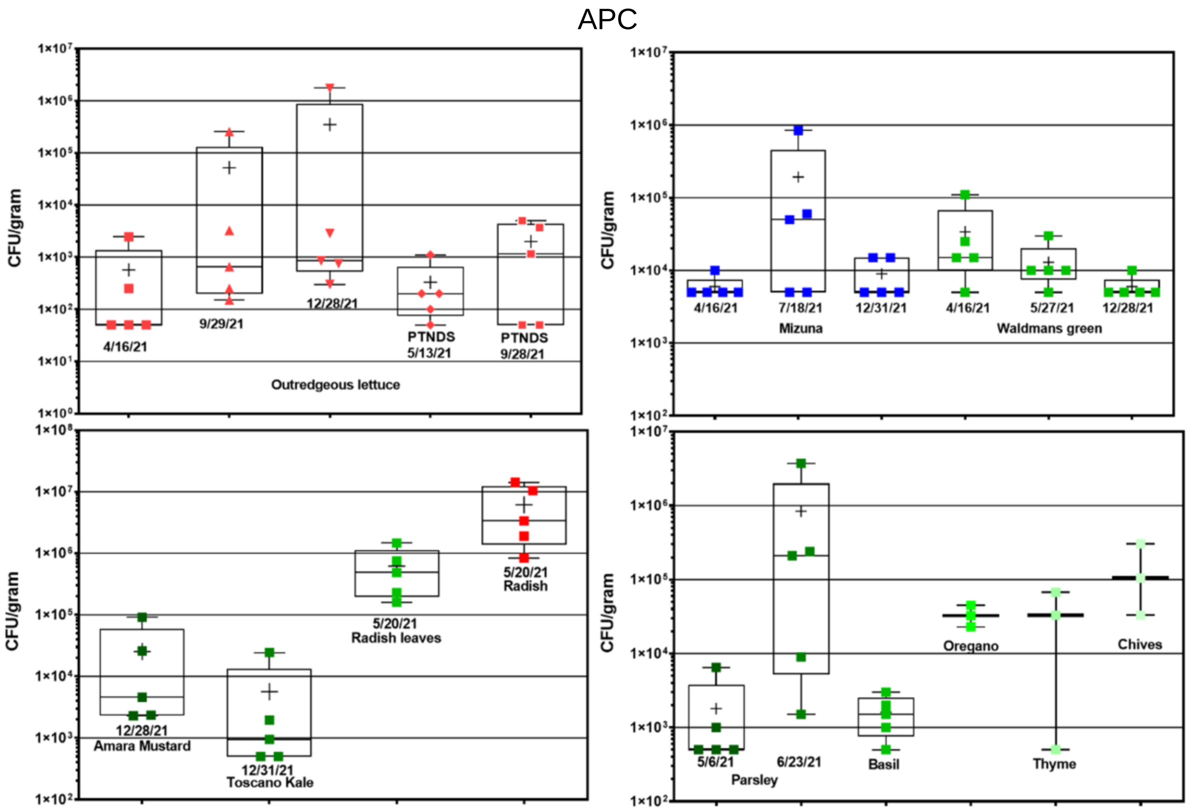


Figure 1. Bacterial counts (APC CFU/g) on leafy greens, herbs and radish. Vertical bars indicate minimum and maximum values, horizontal bars are the median value + indicates the mean.

The four varieties of peppers had low microbial counts overall. The bacterial counts were all below 1,000 CFU/g, Espanola and red skin were below or at detection limit. Red Robin and Joy Red tomatoes had bacterial counts all below 10^4 CFU/g. Fungal counts on Espanola peppers were very low < 50 CFU/g. The highest fungal count on the pepper varieties was 1.7 x 10^3 CFU/g on the Mimi Red pepper.

E. coli, S. aureus, and Salmonella sp**. were not detected in any of the samples.**

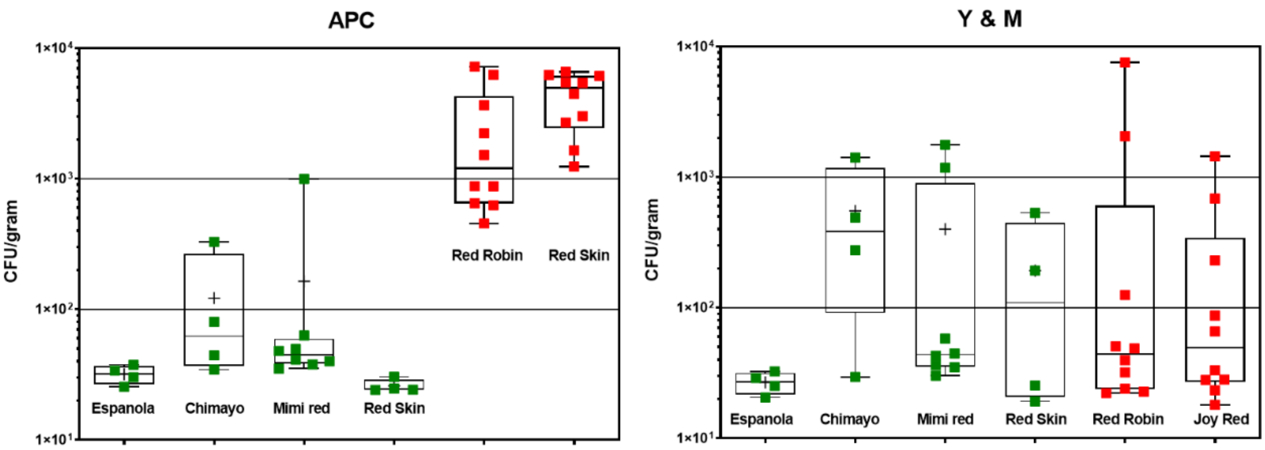


Figure 3. Bacterial (APC) and Fungal (Y & M) counts (CFU/g) on tomato (red symbols) and pepper (green symbols) fruit. Vertical bars indicate minimum and maximum values, horizontal bars are the median value and + indicates the mean.

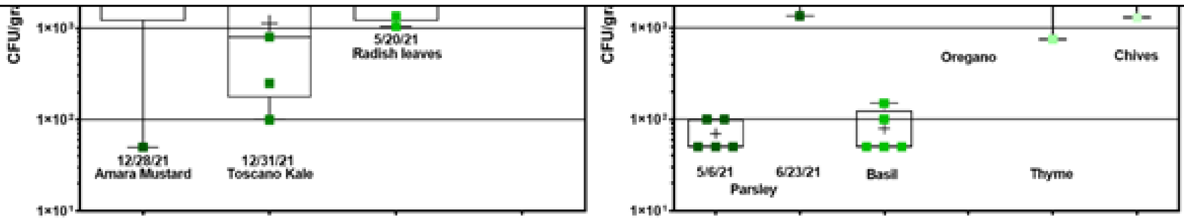


Figure 2. Fungal counts (Y & M CFU/g) on leafy greens, herbs and radish. Vertical bars indicate minimum and maximum values, horizontal bars are the median value and + indicates the mean.

Microbial load on EDEN ISS container surfaces and nutrient solution.

Two areas inside the container were sampled and processed for bacteria and fungi: the wall on the right side (FEG 2) and the floor under the cattle grid (FEG 9). The wall sample was low in bacterial and fungal load, ranging from <u><</u>1 CFU/cm^2 to 385 CFU/cm^2, while the floor sample had the highest bacteria load increasing over the first 10 weeks of sampling from week 6 to week 16 (Figure 4). The nutrient tank levels fluctuated over the 11-month period. Nutrient tank #2 reached a peak of 5.7 x 10^5 bacteria CFU/mL at 11 weeks, but the counts dramatically declined by 3 orders of magnitude after a cleaning event (Figure 4). The surface of the PPTNDS ranged from the minimal detection limit to 2.1 x 10^3 CFU/cm^2. These values are comparable to the range of values found in the 2018 microbial monitoring in the EDEN ISS Greenhouse (Fahrion et. al 2020) except for the high value that we observed at 11 weeks. We also observed low fungal counts over the 11-month sample period. Most samples were below detection limit with the exception of the floor swab sample exhibiting countable plates on the aerobic plate counts ranging from 0 to 2.2 x 10^4 at 11 months.

Bacterial isolates on plants

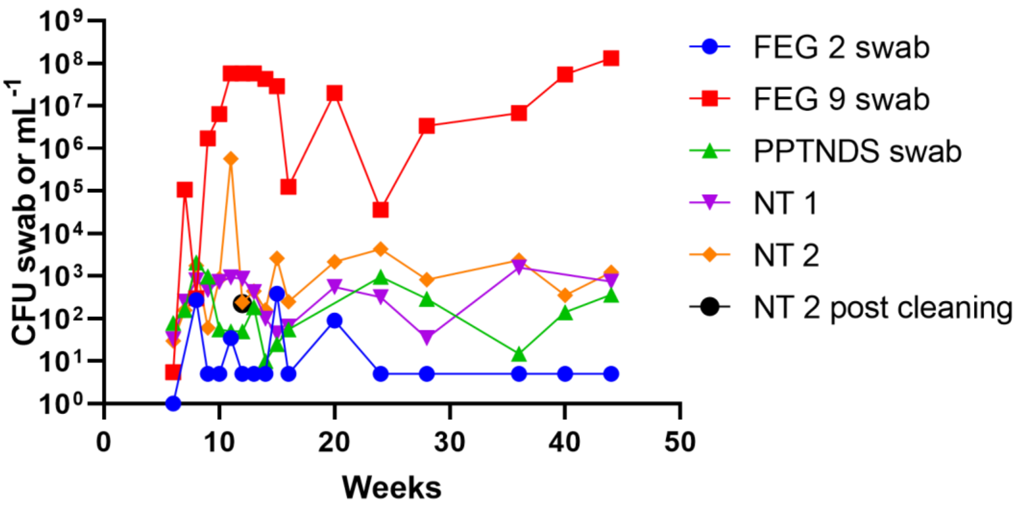


Figure 4. Bacterial counts (CFU on R2A) from surface samples inside the Eden container and nutrient solution tanks. FEG 2 is the wall, FEG 9 is a grated floor, NT1 and NT 2 are nutrient tanks and PPTNDS is the porous tube nutrient delivery system.

Eighteen genera were isolated and identified on the plant samples. Several were identified to the species level for a total of 25 different isolate identifications (Figure 4). Twelve of the bacteria isolated from all the samples where found on the lettuce leaves, while only four where found on the Mizuna and one on Waldmans green lettuce. Of the 18 plant types sampled, *Stenotrophomonas maltophilia* was found on 10 including the leafy greens and herbs, except Waldmans green, basil and thyme. With the exception of the Chimayo pepper it was not identified on any of the tomatoes and peppers. This bacterium is ubiquitous in the environment and commonly associated with plants. It has been report to exhibit plant growth promoting properties (Alexander et. al. 2019). Bacillus was found on all but three plant varities except basil, radish and the Mimi Red pepper. Two isolates were identified to the species level, *B. thuringiensis* and *B. pseudomycoides/cereus.* Interestingly, *B. thuringienis* is well known for the production of biopesticides and is commercially available as a crop spray. Crops known as *Bt* crops have been genetically modified to code for the toxins that are produced by the bacterium

that protect against pests (Wu, 2014). *B. pseudomycoides* is also found in the environment, including soil and the rhizosphere of plants and has been reported to increase the potassium availability in soil (Figueiredo Dos santos, et.al., 2022). Another isolate, *Enterobacter cloacae* found on the tomatoes, peppers, radishes and Outredgeous lettuce is a commensal organism in the human gut but is widely distributed in the environment. It has been identified as both a plant growth promoting bacterium as well as an emerging plant pathogen in chili peppers (Garcia-Gonzalez, et.al. 2018). Six of the eighteen genera found in this study were also isolated from plants sampled from the Eden ISS 2018 season (Fahrion et.al. 2020). The remaining bacteria identified are generally not associated with human disease other than opportunistic infections and are widely distributed in the environment.

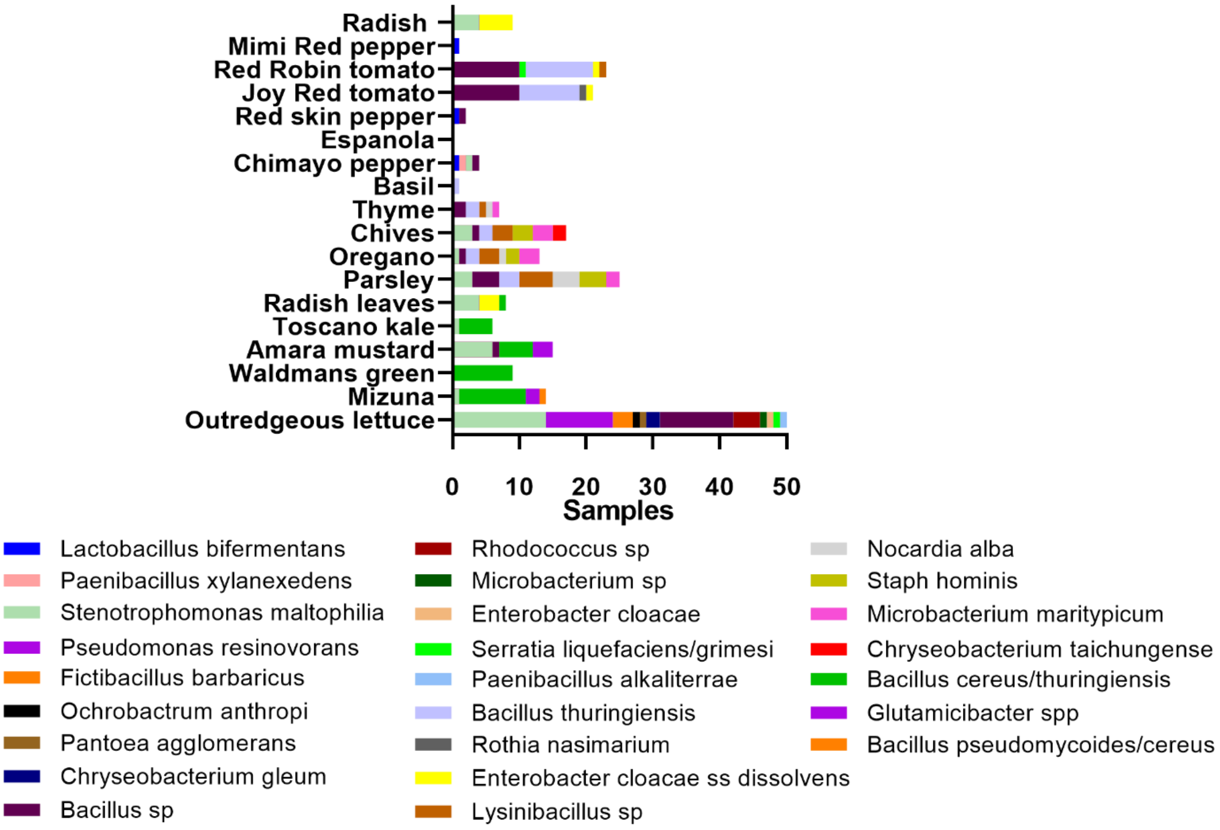


Figure 5. Bacteria isolated from plant samples. Bars indicate the number of samples in which each species was recovered.

Fungal isolates on plants. Sixteen different fungi were isolated and identified from the plants. Two fungi dominated most of the plant samples. *Penicillium spp*. was found on 16 of the 18 sample groups followed by *Fusarium* on 11 of the 18 sample crop types. *Fusarium solani*, isolated from the 'Chimayo' pepper only, is a phytopathogen, its primary hosts being potato, pea, bean, and members of the cucurbit family like cucumber. Some stains may cause infections in humans. Other fungi listed in Figure 5 are saprophytes and common in the environment found in air, water, soil and plant material.

Bacterial isolates from environmental samples. Forty different species in 18 genera were found on surfaces and in nutrient tank solutions mostly from FEG 9, the floor sample and the two nutrient tanks. These were also the sites with the highest bacterial load. Those highlighted in green were also found in the plants. *Clavibacter michiganesis* is a seed born pathogen of tomato and the subspecies found on the floor sample, ‘‘insidiousus,” is a wide spread disease of alfalfa.

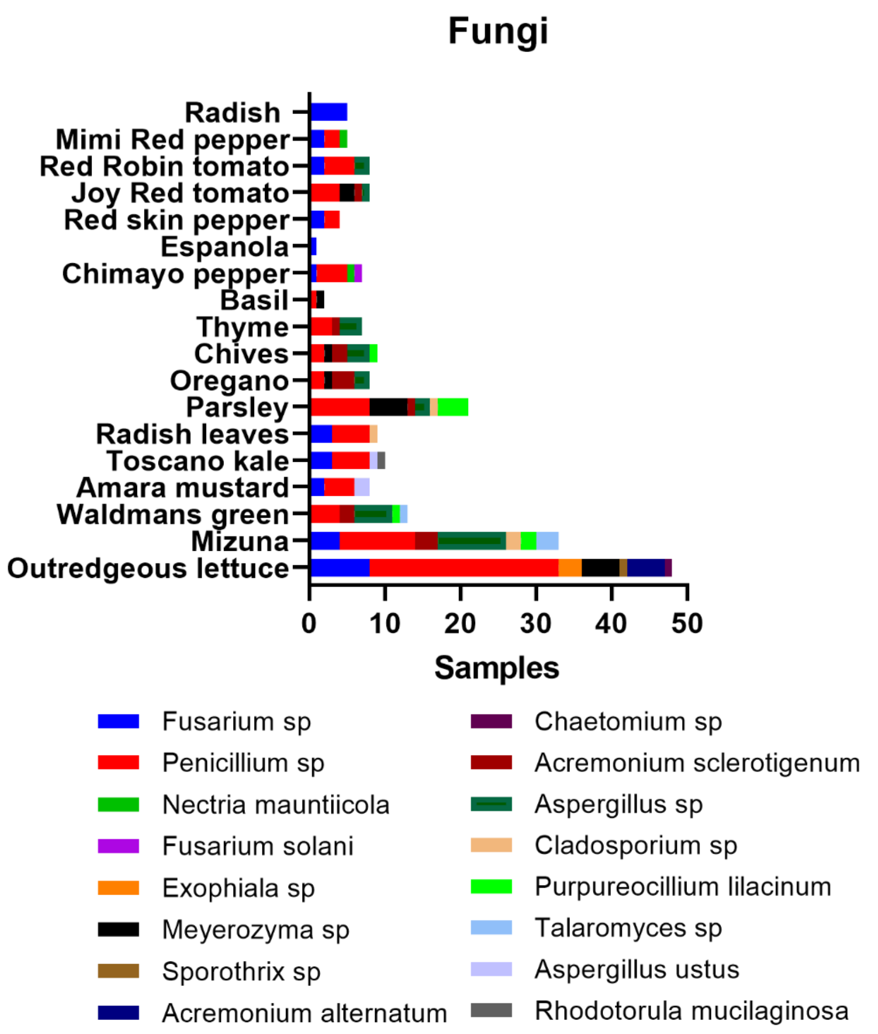


Figure 6. Fungi isolated from plant samples. Bars indicate the number of samples in which each species was recovered

The bacterial and fungal isolation and identification done in this study are based on those microorganisms that can be cultured on the media utilized; therefore, their absence only indicates that on certain samples they may have not grown or were too low to detect. Whole genome community sequencing to identify bacteria and fungi is a method that does not require cultivation and, in the future, could be utilized to expand the list of microbial community members in these samples.

Table 1. Bacterial isolates from environmental samples. Green cells indicate those also found in plant samples.

Bacteria	**FEG 2**	**FEG 9**	**NT1**	**NT2**	**PPTNDS**
Acinetobacter beijerinckii		+			

Bacillus acidicola			**+**		
Bacillus cereus/thuringiensis		**+**	**+**	**+**	
Bacillus decolationis			**+**	**+**	**+**
Bacillus firmus			**+**		**+**
Bacillus macauensis			**+**		
Bacillus marisflavi	**+**		**+**		
Bacillus pseudomycoides/cereus			**+**	**+**	
Bacillus pumilis/safensis		**+**			
Bacillus scohaenanensis			**+**	**+**	
Brevibacterium casei					**+**
Brevundimonas diminuta		**+**		**+**	
Brevundimonas vesicularis		**+**	**+**	**+**	
Chryseobacterium gleum			**+**		
Chryseobacterium taeanense		**+**	**+**		
Clavibacter michiganensis ss insidiousus		**+**			
Curtobacterium herbarum		**+**			
Curtobacterium pusillum		**+**			
Exoguobacterium undae		**+**	**+**		
Fictibacillus arsenicus			**+**	**+**	
Leifsonia aquatica		**+**	**+**	**+**	
Leifsonia poae		**+**			
Microbacterium dextranolytican		**+**			
Microbacterium maritypicum		**+**		**+**	**+**
Micrococcus luteus E				**+**	**+**
Micrococcus yunnariensis					**+**
Mycobacterium smegmatis		**+**			
Paenibacillus agarexedens		**+**	**+**		**+**
Paenibacillus polymyxa			**+**	**+**	
Paenibacillus soli			**+**	**+**	**+**
Paenibacillus tarimensis			**+**	**+**	

Paenibacillus tundrae		+	+	+	
Paenibacillus xylanilyticus			+	+	
Paenibacilus massiliensis			+	+	
Paenibacillus xylanexedens				+	
Rathayibacter rathayi			+	+	
Rhodococcus erythropolis			+		
Sphingomnas paucimobilis		+	+	+	+
Staphylococcus intermedius		+			
Staphylococcus saprophyticus		+	+		+
Stenotrophomonas rhizophilia		+			

Crew Surveys

IV: SUMMARY

Here are the summary calculations completed this far for the 2021 EDEN ISS campaign:

The 2021 season was 319 days long and resulted in 315 kg fresh edible biomass grown within the 12.5 m^2 greenhouse cultivation space. This meant that an average of 0.99 kg was produced each day. While each crew member had personal preferences and consumed varying amounts of the produce, and the type and amount of available produce fluctuated throughout the season, each crew member could enjoy an average of 110 g each day. To produce this amount, an average of 4 h 50 minutes needed to be spent in EDEN each day. This does not account for the majority of the support activities conducted inside the station, and of course, the true amount spent inside the greenhouse fluctuated from day to day. Ultimately, the production rate was 25.3 kg per m^2, which was 790 g per m^2 per day.

Potential for crossover with Sustained Veggie, ISS grow-outs, etc.