

# The Development and Testing of Visualization and Passively Controlled Life Support Systems for Experimental Organisms During Spaceflight

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## ABSTRACT

This paper describes the development and ground-test results of three passively controlled life support systems for experiments currently onboard ISS, and scheduled for flight on STS-107 as part of the Space Media Inc. (SMI) Science and Technology Research Student (STARS®) global education program.

Two experiments use the Autonomous Biological System (ABS) technology, which has been tested during two 4-month Mir experiments. One ABS will house embryos and fry of the Japanese Medaka Fish, *Oryzias latipes*. A second ABS contains a complex ecosystem experiment. The third system uses a passive life support system based on agar gel for the harvester ant *Pogonomyrmex occidentalis*.

Thermal control, lighting and still and streaming digital imaging during the experiments are provided by the Generic Bioprocessing Apparatus - Isothermal Containment Module (GBA-ICM).

## INTRODUCTION

Opportunities for long-duration space biology and gravitational ecology research will increase as the International Space Station (ISS) becomes operational. Organisms used in the research will need a life support system that will operate reliably in space over periods of months or even years. This paper describes the development of three passively controlled life support systems for experiments currently onboard ISS, and scheduled for flight on STS-107 in April 2002.

One system uses agar gel to provide food, atmospheric moisture, water and disease suppression to a colony of worker ants. The remaining two experiments use hermetically sealed, gnotobiotic, aquatic ecosystems to provide oxygen, food and clean water to fish embryos and fry, and to shrimp. These systems are based on

Paragon Space Development Corporation's patented Autonomous Biological System (ABS) technology (Poynter et al. 1996), which has been previously tested during a 10-day experiment on the US Space Shuttle (Ishikawa et al. 1996), and two 4-month Mir experiments (MacCallum et al. 2000).

The three life support systems are developed for initial use as part of the Space Media Inc.'s STARS® global education program. Participating students perform the experiment in their classrooms during the ISS and STS-107 missions. Along with the ABS experiment on ISS, STARS® is flying seven educational experiments on STS-107, which are sponsored by schools from around the world. The six experiments are: tunneling behavior of Harvester Ants in microgravity, USA; silkworm lifecycle during space flight, China; effect of microgravity on the development of Medaka Fish, Japan; effects of microgravity on crystal growth, Israel; effects on Garden Orb Weaver Spider web production, Australia; adaptation of the Arizona Carpenter Bee to the microgravity environment, Liechtenstein; and, Star Navigation, USA.

## ISS ABS

An ABS was launched to the ISS on a Russian Soyuz rocket from Baikonur Cosmodrome, Kazakhstan, on February 26, 2001. After approximately 72 hours of darkness the ABS was removed from the Progress Module and placed on a dedicated panel in the Zvezda Service Module above the crew dining table. The ABS is currently scheduled for a 3-month experiment on board ISS, with the possibility of a longer duration stay. The ABS contains an aquatic ecosystem with plants and animals, including the small red shrimp *Halocaridina rubra*, snails and several species of small crustacea.

The ABS on ISS is being used for an educational experiment conducted by students as part of Space Media Inc.'s global STARS® program. Over 600 schools

across America, Asia and Australia will participate in the experiment. The objective of the experiment is to study the behavioral adaptation of the animals within the ABS to the microgravity environment, with emphasis on *H. rubra*, and the long term effects of microgravity on the ABS ecosystem. Students participating in the program build their own ground control ABS in their classrooms, to which they compare biweekly observations and video images made by cosmonauts of the ISS ABS available to the STARS students on the Internet. Ground controls are also maintained at Paragon for the duration of the experiment.

## HARDWARE DESIGN AND ON-ORBIT OPERATIONS

The ABS hardware design was based on the previously flown T-GAP configuration designed by BioServe Space Technologies, Boulder, Colorado. The T-GAP was used for three previous ABS flights on the Shuttle and Mir Space Station, and consists of a hermetically sealed 840ml Lexan® polycarbonate cylinder, sealed within an outer Lexan® cylinder. Both the inner and outer cylinders were sealed and tested for leakage in a vacuum chamber prior to being placed within a third layer of containment provided by a heat sealed Aclar® envelope. Gortex® endcaps were applied to either end of the cylinders with attached Velcro® strips for attachment to the ISS Zvezda Service Module dedicated panel. The third layer of containment was designed and manufactured by SPACEHAB Inc. Four ABS were manufactured and approved for flight, one of which was chosen randomly to fly, and the remainder were kept in Paragon's laboratory as ground control units.



Figure 1 shows the flight configuration of the ISS ABS, with Velcro® strips for attaching to the dedicated panel in Zvezda Service Module.

Two Active Thermal Carriers were used for transporting the four ABS to Houston and Moscow for Flight Acceptance Testing, and to the launch site in Kazakhstan. The Active Thermal Carriers are Igloo® Coolers, modified by Paragon to provide thermal control and lighting to the ABS, and operate in 12V, 110V and 220V power systems.

For launch of the ABS and transport to ISS within the Progress Module, the entire flight configured unit was

placed within a foam transport storage assembly, or Insulating Launch Container, designed and manufactured by SPACEHAB, Inc. The assembly is made of two layers of 0.5" thick LF200 2pcf Munciel® foam, covered with a layer of Gortex®. The assembly helps prevent the ABS from experiencing fluctuations in temperature and protects against impacts during the Progress Vehicle loading, launch, orbit and rendezvous operations. It was removed from the ABS immediately upon destow from the Progress Vehicle and discarded.



Fig 2. Shows the Insulating Launch Container.

Once on orbit and installed within the Zvezda module, lighting and temperature control are provided by the ambient conditions within the Service Module. The location of the ABS was chosen to place the unit close to two lighting fixtures to ensure proper lighting conditions of between 300 – 2000 LUX, for a 16-hour light cycle in a 24 hour period. The temperature within the module is maintained at 18 – 22C.

The ABS is passively controlled, with no astronaut or cosmonaut time required for the operation or maintenance of the system. However, in order to meet the STARS® program experiment requirements, the cosmonauts provided biweekly observations of the animal behavior and ecosystem changes, recorded on video. Every two weeks, three minutes of video is recorded of the ecosystem with observations made by the cosmonaut according to a set list of quantifiable parameters, such as number of species, number of individuals within the species, water clarity etc., repeated at each observation session. Each observation session is downlinked to RSC-Energia in Moscow who provides the data to SMI for dissemination via its web site.

## WETWARE DESIGN

The ABS ecosystem design was derived from a system flown previously on one 10-day Shuttle and two 4-month Mir experiments, containing primary producers, herbivores, detritivores, and decomposers through which

materials would flow and ecosystem structure would develop (MacCallum et al., 2000). A method for passive control of the ABS was developed by Paragon to increase the ecosystem's resilience—the ability to return to prior conditions after a perturbation occurs. Perturbations may be the death and decomposition of a large animal or plant damage during launch, both resulting in nutrient release. The system works by limiting specific nutrients in the system, forcing a balance in the material exchange of those nutrients between autotrophs and heterotrophs. With nutrient availability rather than light restricting growth in the system, organisms can respond with increased assimilation in the event of a nutrient release. An energy-limited system could not respond as rapidly. In essence, the material storage buffer in the ABS is maintained at the equivalent of empty so there is no excess of certain nutrients within a system where light is not a limiting factor.

The passive control system also requires a means of material transfer between producers, consumers and decomposers. In its simplest form, the ABS uses aquatic plants and animals in a modified hydroponics nutrient medium. Materials circulate by means of diffusion and, during flight, through Marangoni convection.

A final draw-down of nutrients with an increase in O<sub>2</sub> and pH was accomplished by introducing carbonates immediately before closure of the ABS. During the Mir experiment the use of the carbonates in photosynthesis after closure increased the concentration of O<sub>2</sub> to between 26 and 28% in the 100ml gas headspace, causing a commensurate rise in system pressure, slightly increasing the system C/N ratio. (ref). This step provides extra metabolic oxygen and pH buffering in anticipation of extended periods with no electrical power, when no light can be provided for photosynthesis, such during launch and transport to ISS where the systems are in complete darkness for approximately 72 hours.

Partially decomposed materials derived from the plants and animals to be used in the ABS were introduced as a source of slowly decomposing recalcitrant carbon to make up for that being deposited in the system by plants and animals. The amount of recalcitrant carbon in an ABS is a function of its decomposition rate and the rate at which it is created through the death of animals and plants in the normal course of ecosystem function. Adding this slowly decomposing carbon source is necessary for the long term stability of the ABS. Double the amount of detritus per ecosystem volume was used in the ISS ABS compared to the previously flown ABS to allow for increased populations of animals to develop within the ecosystem.

There is one species of vascular plants in the ABS onboard ISS: the rootless, tiny floating aquatics, *Wolffia* sp. The aquatic invertebrate animals were introduced as populations with the exception of one, *Halocaridina rubra*, and also included: one species of gastropod, *Helisoma planorbis*.; ostracods; *Daphnia* sp.; the amphipod *Hyaella azteca* and cyclopoid copepods.

Eight individuals of *H. rubra*, three individuals of the snail species, 5-6 ostracods and copepods, 3 *Daphnia* individuals, and 10 *Hyaella* were introduced into each ABS. The ABS was inoculated with nitrifying bacteria populations of the species *Nitrobacter wynogradskyi* and *Nitrosomonas europea* along with a ubiquitous species of filamentous algae found in aquarium tanks. While the algae were a source of food for some of the animal species, they were the primary autotrophic component. A dried piece of Gorgonia sea fan was placed in the ABS to serve as surface area for the bacteria and algae to inhabit. Given the relatively short life spans of some of the invertebrates, it is necessary for several successive generations to successfully breed in space for the species to survive the 3-month flight.

Of the species flown, all have previously been in the microgravity environment for long duration experiments with the exception of the shrimp *H. rubra*. Results and further descriptions of the ABS system were reported at the ICES 2000 (MacCallum, et al., 2000).

The ecosystems were assembled and sealed in each of the four flight cylinders six weeks prior to launch, four weeks earlier than for previously flown ABS experiments. Two weeks are required to allow the ecosystem to develop and a state of dynamic equilibrium prior to the perturbation of launch. The reason for the extended period was due to the flight approval of the hardware having to be performed on flight ready articles, which included the ecosystem, and a two week launch slip.

#### ISS ABS EXPERIMENT UPDATE

First downlinked images from the ISS ABS showed that the ecosystem is healthy and the animals active. No further data is available as of the writing of this paper.

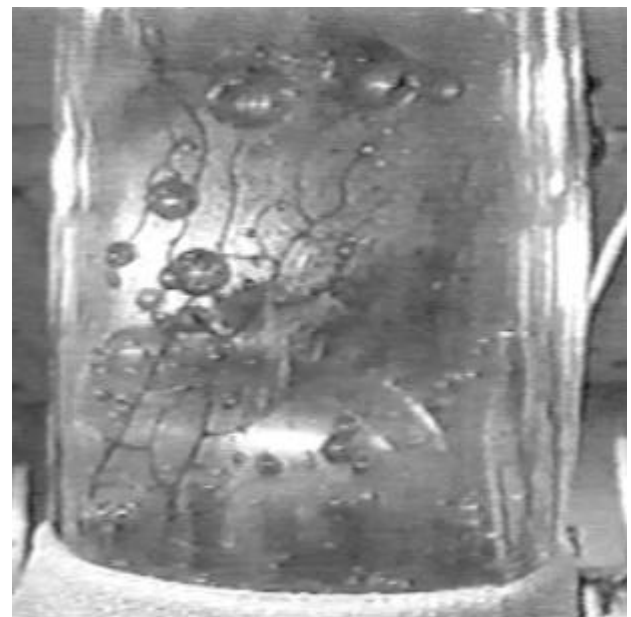


Figure 3 shows an image of the ABS 1 day after delivery to ISS. Shrimp can be seen behind the air bubble near the top of the photograph.

## STS-107 EXPERIMENTS

The U.S. Space Shuttle STS-107 is a 16-day science mission, currently scheduled for launch in April of 2002. Seven STARS® experiments are under development for STS-107, of which six will be housed within BioServe's Generic Bioprocessing Apparatus - Isothermal Containment Module (GBA-ICM). Two of these experiments are described herein. The first habitat houses embryos and fry of the Japanese Medaka Fish, *Oryzias latipes*. The experiment is designed in collaboration with Ochanomizu University, Japan, and is a study of the effects of microgravity on the development and behavior of the Medaka Fish.

The second system uses a passive life support system based on agar gel for the harvester ant *Pogonomyrmex occidentalis*. The agar gel simultaneously serves as the ants' source of food, atmospheric moisture, water, disease suppression and tunneling medium. The experiment is supported by the University of Syracuse and an associated high school, and focuses on the tunneling behavior of a worker colony of harvester ants in microgravity.

### HARDWARE OVERVIEW: ICM, AUTONOMOUS IMAGERY

The ICM is a middeck locker insert (Figure 4) which houses biological experiments. It interfaces with the Shuttle and ISS. The ICM provides its science specific components with a uniform thermal environment. It also provides data acquisition and control as well as analog and digital imaging capabilities. The ICM has previously flown on STS-95 (2 units) and STS-93 (2 of 3 units).

The STS-107 habitats are shown on the prototype insert in Figure 5. The STARS® experiments rely heavily on video observation of the habitats on orbit, video downlink of these images, and mission-parallel ground control experiment at the student's home sites. Flight images are provided through the Internet to each school.



Figure 4. ICM incubator facility inside the program-provided middeck locker (composite middeck locker shown; uses metal Spacehab locker on STS-107). The insert will be removed from the locker and opened on orbit to allow manual sample processing and activations once power is turned off and cables have been disconnected.

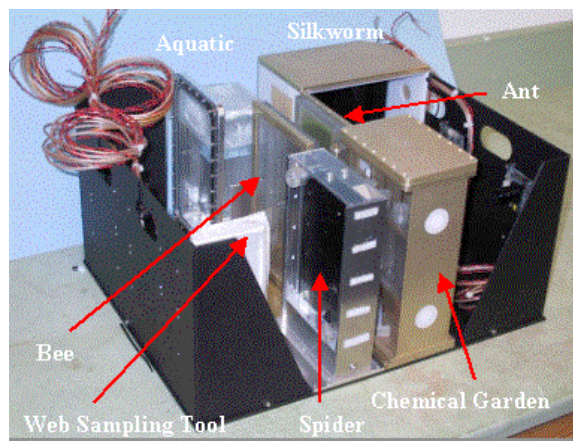


Figure 5. STARS® STS-107 habitats in prototype insert.

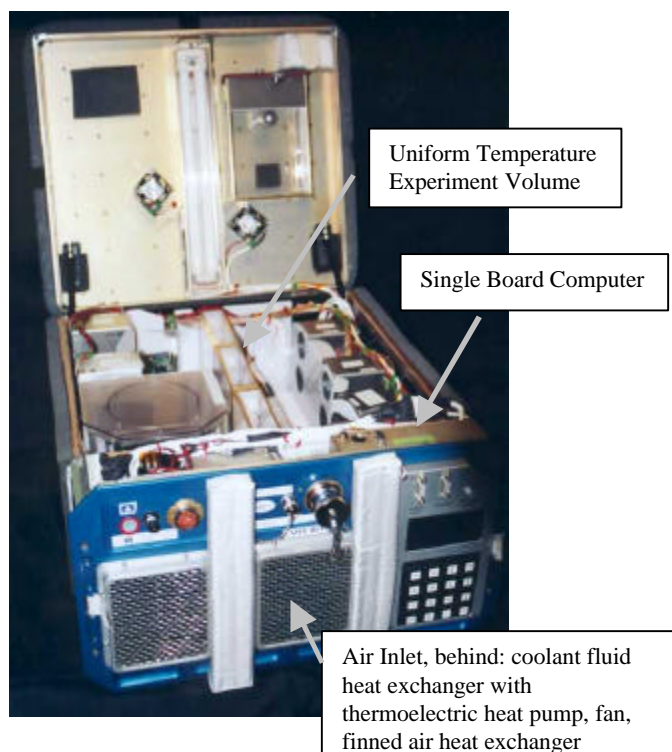


Figure 6. ICM in the STARS® configuration as flown aboard STS-93 as CGBA-2 (Ladybug - left). Shown in center row: Two Ladybug habitats (one with and one without aphids), one sweet potato rooting experiment. The Butterfly habitat is on front left. The spare Ladybug habitat shown on lid served as replacement for ladybug-loaded habitat in case ladybugs in primary habitat did not survive launch.

### MEDAKA FISH EMBRYO EXPERIMENT

While reproduction of the Japanese Medaka fish, *Oryzias latipes*, has been demonstrated in microgravity, only three to four days of post-hatching embryo development has been observed in space (Ijiri, 1994). The proposed experiment will examine the development and behavior of Medaka embryos and larval fry once they hatch in microgravity for the 16-day duration of STS-107. Three, five and seven-day old Medaka eggs will be flown in an aquatic animal habitat with a biologically based life support system. Two video cameras will record the development and behavior of the embryos and fish during flight. A biologically balanced

ecosystem, or ABS, maintains water quality for the embryos and fry, and provides food and oxygen for the duration of the experiment.

### Habitat Design

The habitat housing the Medaka fish experiment is a single-walled vacuum molded polycarbonate body with a single layer lid. Its outer dimensions are approximately 19.812cm (7.8 inches) high, 8.014cm (3.155 inches) deep, and 11.113cm (4.375 inches) in width. The inner dimensions give the habitat an internal volume of approximately 842ml. The body is molded out of a solid piece of polycarbonate to eliminate seams that might leak and to eliminate any materials incompatibility with the samples contained within the habitat. The lid is made of polycarbonate and is secured to the main body with a series of screws about its perimeter. It is sealed via an o-ring and a gasket. The lid is clear to allow for video images to be taken of the activity within the habitat. A mechanical drawing of the habitat is shown in Figure 7.

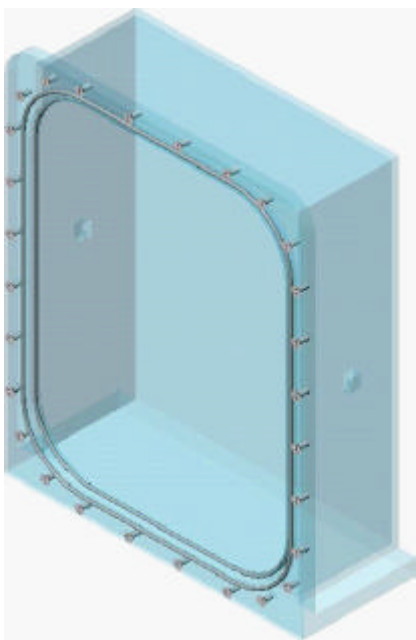


Figure 7. Mechanical drawing of aquatic habitat.

### Wetware

To prevent cabin atmosphere contaminants from altering experimental parameters or causing stress in the flight animals, the aquatic habitat is a completely sealed container. The aquatic habitat will contain a bioregenerative life support system for experimental aquatic plants and animals, similar to the ABS used for ISS described above, and uses similar methods of passive control of water quality and ecosystem stability mechanisms. It can readily maintain all the fry once they have hatched and continue to develop through the duration of the flight.

The biological life support system is made up of the vascular plant *Ceratophyllum demersum*, used for

primary O<sub>2</sub> production and maintenance of water quality, along with several species of microbes and algae, including the nitrifying bacteria *Nitrobacter wynogradskyi* and *Nitrosomonas eruopea*. Detritus, plant material, algae and snail feces from *Helisoma planorbis* are used within the system to support a mixed culture of infusoria and rotifers, which is the principal food source for the Medaka fry. An infusoria refugium is used to prevent the fry from reducing the population of infusoria below a level capable of reproducing fast enough to sustain the fish. The refugium houses boiled wheat kernels as a food source for the microfauna on which the infusoria thrive. The wheat kernels are inoculated in a culture of infusoria prior to insertion into the ABS. A population of *Daphnia pulex* provides additional food for the fry, as the one week old fry will consume the newly hatched daphnia. The adult daphnia are too large for the fry to consume which prevents the species from being eradicated by the fry.

Prior to the Medaka embryos being inserted into the system, the ecosystem is assembled and allowed to equilibrate for two weeks under similar temperature and lighting conditions to that provided on orbit (23C, 16 hours of light/day).

Within the habitat, the Medaka embryos are placed singly into a netted enclosure, which maintains the eggs in one clustered location for ease of visualization. This enclosure also allows the different ages of eggs to develop and hatch separately, making recording the development of individual embryos practicable. The mesh of the enclosure prevents the eggs from passing through, but allows the fry to escape the enclosure once hatched. The mesh also excludes most of the animals from reaching the eggs, which is particularly important in the case of snails, which might eat the eggs if they had access to them.

Test experiments in the Paragon laboratory have succeeded in realizing 100% hatch rate of all fertilized eggs (in two experiments one unfertilized egg was mistakenly placed into one flask in each experiment) with 100% survival of fry for the duration of the experiment scenario. The full test experiment time is 22 days to allow for integration, load, launch scrub and weather delays plus 16 days of on-orbit mission time. Two to six eggs have been placed into the test ABS and successfully supported under flight-like environmental parameters. The carrying capacity of the ABS is not yet known, but it is anticipated that the ABS will eventually support eight – twelve fry of the appropriate ages on orbit. The aim is to maintain twelve fry for the duration of the experiment: four of each age group of 3, 5 and 7 day-old embryos at the time of launch.

### Species and strain selection of fish

Medaka fish were selected as the fish species for this experiment because they have been successfully flown in space, including a flight on IML-2, where adults successfully bred. An extensive body of knowledge

exists with regard to the embryology, developmental biology and laboratory care and maintenance of these animals. In addition, they are a very hardy species; the eggs and fry are robust and are tolerant of varied O<sub>2</sub>, pH and temperature regimes. Two strains are under consideration for the experiment. One is a strain that has been developed to adapt very well to the microgravity environment, ccT strain (Ijiri, 1994). A second wild-type strain of Medaka is being tested that is commercially available in the U.S.

Experiments have been conducted during a parabolic flight campaign onboard a Novespace Airbus 300 airplane in December 2000, which gave a total of 77 parabolas. Each parabola produces 22 seconds of microgravity. Adults of 3 strains of Medaka were flown (including the commercially available wild-type, but excluding the ccT strain), and 75 larval fry were flown of the 3 strains. As anticipated, most of the adult fish demonstrated some level of disorientation in the microgravity environment, somersaulting and corkscrewing, as has been previously observed on orbit. However, not one larval fry was observed swimming abnormally during insertion into the parabola or during microgravity. These recent microgravity trials demonstrate that for the purposes of behavioral adaptation of larval fry to the space environment, the strain of Medaka fish is not important. Further laboratory trials are underway to determine which strain is the hardiest and thrives best within the flight configuration.

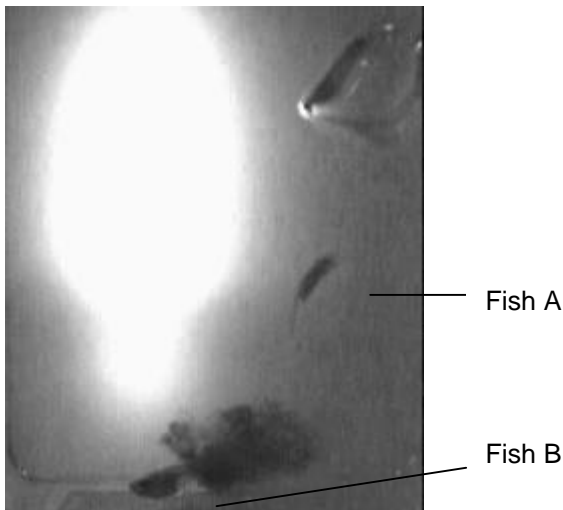


Figure 8 shows swimming behavior in 2 adult Medaka fish during parabola induced microgravity. Fish A is somersaulting rapidly, while fish B is swimming normally.

### Experiment Methods

Several wild-type Medaka fish breeding pairs, along with the space adapted Medaka strain ccT will be induced to mate using the standard regime of a photoperiod of 16 hours of light and temperature at 78F. The fish generally complete the mating behavior within two hours after onset of light in the daily light cycle. After mating, eggs are laid by the female and form a cluster attached to the fish's abdomen. Within one hour of mating the eggs are

harvested and maintained in the standard embryo rearing salt solution for freshwater teleosts with added mold inhibitor. At the appropriate day of development (one, three and five days, or approximately development stages 17, 28, and 31) the eggs will be examined for proper initial development and selected for flight and ground controls. This daily procedure will be started far enough in advance of the flight such that embryos at the appropriate stages of development will be available on the day of payload integration and later in the event of a launch delay or scrub.

Shortly before final integration of the experiment package, ten to fifteen selected eggs of the appropriate age will be placed into the egg restraint. Each egg is visually inspected using a dissecting microscope to verify that the egg has been successfully fertilized. The number of eggs has yet to be determined based on laboratory experiments to verify and maximize the carrying capacity of the ABS. The egg restraint will be placed into the operating aquatic habitat. The habitat will be sealed and vacuum tested, whereupon it will be transferred to the GBA-ICM for closeout and loading on the Shuttle.

The eggs are expected to hatch on days 3, 5 and 7 of the flight under normal launch and operational scenarios. During the flight, video and still photographs will be taken each day to record the development of eggs and fry, and the behavior of the fry. Upon retrieval after landing, observations will be taken and recorded of the flight and ground control animals' gross morphological attributes as well as the swimming and feeding behavior of the fry. The young fish will then be transported to Ochanomizu University, Japan, for grow out to maturity whereupon their behavior, reproduction and morphology will be observed.

The control vessel will be maintained within the ground control GBA-ICM at the SPACEHAB Astrotech Facility at Cape Canaveral for the duration of the flight.

Three to four months prior to the launch date, a full ground-based experiment will be performed under flight like conditions within the GBA-ICM at BioServe to verify proper functioning of the hardware, wetware and protocols.

### ANTS IN SPACE EXPERIMENT

Although the harvester ant, *Pogonomyrmex occidentalis* has been subjected to the microgravity environment during a series of parabolas on board the Novespace Airbus 300 in December 2000, no experiments have been conducted with these animals on orbit. The objective of this experiment is to observe and characterize the effects of space flight on the tunneling behavior of harvester ants during a 16-day long space shuttle flight. Particular attention will be focused on the activity level of the ants and their social interactions. Upon their return, the ants and their tunnels will be

examined and compared to an equivalent colony kept under similar environmental conditions on the ground.

Laboratory experiments using the life support system design and methodology described below have verified that queenless ant colonies can be maintained for extended periods with no addition of food or water, and minimal gaseous exchange. Colonies have been maintained for over eight months in Paragon laboratories and are still living at the time of this article. Upon completion of a successful flight test as provided by the STS-107 shuttle mission, a system will be complete that allows for long-duration maintenance of ants on orbit for months on ISS, with the possibility of use with other insect species. Experimentation with alternate insect species has not yet been done.

### Hardware Design

The containment hardware for the ant experiment has external dimensions of approximately 11.113cm wide (4.375 inches), 1.27cm deep (0.5 inch), and 18.415cm in height (7.25 inches). The volume is composed of a larger rectangular area containing the agar/food gel, and a smaller side passageway providing an area for depositing tunnel material. A small space in the wall separating the two sections allows the ants to access the gel for tunneling. The vent slots on the outer sidewalls have a membrane cover, which will keep the ants inside while allowing air to enter the habitat and thus controlling the humidity. A small chamber or nest attached to the outer passageway keeps the ants from tunneling until they are released on-orbit by a crew-activated plunger mechanism.

### Wetware and gel

Workers of the Harvester ant species *Pogonomyrmex occidentalis* will be placed into a single experiment enclosure. *P. occidentalis* was selected, as it is a very hardy species, thriving in broad temperature and humidity ranges, and typically lives up to one year in the wild and have been maintained for eight months under laboratory conditions in the Paragon facilities. Additionally, these ants are advantageous because they are large, allowing for good visualization of the ants, their behavior, and their large tunnels.

The tunneling medium is an agar-based gel. Numerous tests were performed with several types of standard captive ant tunneling media, such as sand, soil, pumice and vermiculite. With all of these media the tunnels risk collapse due to the vibration of landing, and tend to be prone to fungal infection if an ant dies within the habitat, or from food molding. The agar gel was chosen as the tunneling medium as it is firm enough to maintain integrity during launch and landing vibrations, and provides fungus and mold suppression through inhibitors in the gel. The gel is provided by Plant Technologies, Inc., New Jersey.

The ants tunnel through the gel in a similar manner to the way they would tunnel through sand, soil or other standard medium. The workers bite off pieces of medium and carry it out of the tunnel, placing it outside the tunneling medium area, as they would with pieces of sand. The gel is colored to provide contrast with the ants for easier visualization with the video. A starter tunnel of approximately 1cm in depth is provided in the gel to stimulate the ants to commence tunneling once inserted into the tunneling area of the habitat.



Figure 9. shows worker ants within tunnels they have made through the agar gel.

The agar gel contains sucrose to stimulate the ants to eat it. Amino acids, vitamins and minerals are added to the gel to provide an appropriate diet for the ants. As the agar gel is largely made up of water, the ants also receive all their water from the gel as they eat it. Both the metabolizing of the gel by the ants, and the evaporation of water from the gel provides humidity within the tunnels. Adequate ventilation in the habitat ensures that no water collects within the tunnels or habitat area.

Unlike the sealed systems based on the ABS design, the ant system depends on limited gas exchange with the cabin atmosphere for removal of CO<sub>2</sub>, supply of Oxygen and removal of water vapor. Gas exchange occurs through Millipore brand 0.5-micron filter paper. Six openings of 6mm diameter each with a filter are distributed across the headspace of the system. Distributed openings reduce the chance that gas exchange will be precluded by the ants depositing gel over the openings.

The gel provides disease control by suppressing fungal and mold growth – the primary cause of death in captive colonies after desiccation. As the animals tunnel through the medium and eat it, the mold inhibitor contained in the gel eradicates mold and fungal spore, which also prevents the ants from being a source of infection in the case of the death of one of the workers.

## Experiment Samples and Materials

Ten days prior to integration of the experiment into the GBA-ICM, the ants are placed onto a diet of agar gel, similar to the gel used for the flight medium. This ensures that the exterior of the animals' exoskeletons and their digestive tract, are free of fungal spore that could later contaminate the experiment on orbit.

Several hours prior to the final integration of the experiment payload for the shuttle launch, the harvester ants will be placed into an activation chamber, located within the experiment habitat, to maintain them separated from their tunneling material. A small amount of the agar gel is placed in the activation chamber to provide food and water for the time the ants remain within the activation chamber, which is expected to be approximately 43 hours under a nominal launch scenario.

Once on orbit the activation chamber will be opened by a crewmember, thus activating the experiment and allowing the ants to have access to the experiment volume.

Video and still images are recorded daily of the ant habitat. Students at the participating schools maintain ground control habitats in their classrooms, and compare the ant tunneling and activity to that of images of the on-orbit ant habitat. High fidelity ground control habitat is maintained within a GBA-ICM at the SPACEHAB Astrotech Facility at Cape Canaveral for the duration of the nominally 16-day Shuttle flight.

Three to four months prior to the launch date, a full ground-based experiment will be performed under flight like conditions within the GBA-ICM at BioServe to verify proper functioning of the hardware, wetware and protocols.

## **ACKNOWLEDGEMENTS**

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