

Autonomous Biological System (ABS) Experiments

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Abstract Three space flight experiments have been conducted to test and demonstrate the use of a passively controlled, materially closed, bioregenerative life support system in space. The Autonomous Biological System (ABS) provides an experimental environment for long term growth and breeding of aquatic plants and animals. The ABS is completely materially closed, isolated from human life support systems and cabin atmosphere contaminants, and requires little need for astronaut intervention. Testing of the ABS marked several firsts: the first aquatic angiosperms to be grown in space; the first higher organisms (aquatic invertebrate animals) to complete their life cycles in space; the first completely bioregenerative life support system in space; and, among the first gravitational ecology experiments. As an introduction this paper describes the ABS, its flight performance, advantages and disadvantages.

Key words: animal reproduction, closed systems, aquatic plants, bioregenerative, space systems, gravity, gravitational ecology

Introduction

As the International Space Station (ISS) provides the opportunity for longer flight times to perform gravitational and space biology research, the need for long duration, autonomous life support of experimental organisms will be increased. Little or no material exchange with the cabin atmosphere is desirable for safety concerns and to prevent contaminants from altering experimental parameters. Furthermore, to be of practical use the systems must be small, simple, have low power requirements and require little or no crew time for maintenance. Of added advantage is the ability to withstand periods of no power for hours with no significant detrimental effect. It is also desirable that neither the physical nor biological system adversely affect experimental parameters. For example, a microgravity experiment involving aquatic animals may be compromised if the animals are exposed to a stirred or otherwise moving water environment.

Autonomous biological systems

Responding to these needs, several space flight experiments have been conducted to test and demonstrate the use of a passively controlled, materially closed, bioregenerative life support system in space. The Autonomous Biological System (ABS), developed and patented by Paragon Space Development Corporation (Poynter *et al.*, 1996), provides for long term growth and breeding of aquatic plants, animals, microbes and algae, within a materially-closed system that is isolated from other support systems and atmospheric contaminants. Minimal crew time is needed to check system status when down link telemetry is not available. In May of 1996 two ABS units made their maiden flight on STS-77 (SPACEHAB-04) followed by two 4-month stays onboard the Russian Mir space station during STS-79/81 NASA 3 (September 1996 to January 1997), and STS 86/89 NASA 6 (September 1997 to January 1998). These flights marked several firsts: the first aquatic angiosperms to be grown in space¹, the first higher organisms (aquatic

invertebrate animals) to complete their life cycles in space, the first completely bioregenerative life support system in space, and among the first gravitational ecology experiments. This paper, along with others in this dedicated journal issue, describes the basic design of the ABS as well as objectives and results from research conducted during the three flights. The principle advantage of the ABS being a closed ecosystem is that the flights may also be viewed as an experiment in gravitational ecology, inviting the collaboration of several research teams publishing in this issue.

Material and energy exchange methods

The ABS is entirely contained within a 900ml Lexan® cylinder that admits light for photosynthesis and conducts heat out of the system. The patented passive control of the ABS works by restricting specific nutrients in the system, forcing a balance in the material exchange of those nutrients between autotrophs and heterotrophs. Essentially, the material storage buffer is maintained at the equivalent of empty so there is no excess of select nutrients within the system and input energy (light) is not a limiting factor in photosynthesis. Instead, at the end of a daylight cycle, photosynthesis is restricted by nutrient (including carbon) availability. The passive control system also provides 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 media, circulating materials through diffusion and Maragoni convection during flight.

With nutrient availability—rather than energy—restricting system photosynthesis, organisms in the system respond with increased assimilation in the event of a nutrient release. An energy limited system could not respond as rapidly. Such an otherwise destabilizing perturbation may be caused by events such as a large animal dying and decomposing or plant damage during launch.

A final draw down of nutrients with an increase in O₂ and pH is accomplished by introducing carbonates just before closure. The O₂ is increased to between 26 and 28% in the 100 ml gas headspace, causing a commensurate rise in system pressure. This slightly increases the system C/N ratio and provides enriched O₂ to increase stability during an extended power down. Partially decomposed materials derived from the plants and animals to be used in the ABS are introduced

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as a source of slowly decomposing recalcitrant carbon to makeup for that being deposited in the system by plants and animals.

Selection of organisms

One of the major design decisions involves system complexity, i.e. the number of species. A complex system is more likely to contain species that will survive the perturbations of space flight. A simpler system is more reproducible, making the flight systems more comparable to ground controls. The major disadvantage of a complex system is that as competition for resources between organisms is increased, the system begins to take on chaotic properties where different animal populations may achieve dominance within individual ABS systems. As the effect of space flight on the ABS was not known, we decided to be conservative and fly two complex systems, increasing the chances that at least one ABS would survive the flight.

The flight ABS were modeled on a fresh water aquatic ecosystem. The plants chosen were *Ceratophyllum demersum*, *Lemna sp.* and *Wolffia sp.*. Aquatic animals were introduced as viable populations of two species of gastropods, ostracods, daphnia, amphipods, copepods and planaria. Given the relatively short life spans of some of the invertebrates used, it was necessary for several successive generations to successfully breed in space. The larger number of animal species increased the chance that at least one would survive the 4 month stay on Mir.

A naturally occurring freshwater pond algae and microbial complement were also introduced. The algae served as a backup photosynthesizer in the event that the plants were to die, and as a source of food for select animal species. In a normally functioning system, photosynthesis is dominated by the plants. A risk also existed in trying to exclude a natural mix of algae. Excluding algae is very difficult during the introduction of animals and partly decomposed substrate. The animals would die if an indigestible algae such as chlorella becomes dominant.

ABS hardware and operations

Each ABS is sealed within two separate Lexan® cylinders: a 900ml cylinder containing the aquatic elements and a larger cylinder that provides secondary containment. A light provides adequate Photosynthetically Active Radiation (PAR) and a thermal control system removes excess heat from the light source. Due to the manufacturer's concerns about failure of the light bulb starter mechanism on orbit, continuous illumination was considered. As long duration laboratory tests showed negligible photoperiod stress the light was programmed to run 24 hours a day for the first two flights (STS-77 and NASA 3). A day/night cycle was subsequently used for NASA 6.

For these flights, thermal control and lighting were provided by the BioServe Commercial Generic Bioprocessing Apparatus - Isothermal Containment Module (CGBA-ICM) Payload. A 7 watt fluorescent bulb at 5000K served as the source of PAR and the ICM provided a six sided isothermal system maintained at 18 °C. The internal temperature of the cylinders prior to flight was calculated at 22-23°C when the light was on. However it was expected to deviate from this nominal value as discussed below.

As stated, the use of astronaut time, other than providing

periodic status checks of the ICM, was not required. Paragon, in conjunction with Sony® and utilizing BioServe's integration expertise, developed an autonomous video recording system using a commercial Sony® DCR-PC7™ camera with custom EPROM software that allowed for intermittent recording by simply cycling power to the camera. The digital imaging system facilitated computer analysis of the system structure throughout the four month flight period. The ICM computer was programmed to allow for both long duration (2 minute) filming and "snapshot" (2-4 seconds) filming.

The camera was turned on and off the same time of "day"—after light has been initiated—to allow for relative comparative motility and population tracking throughout the four month period. System states that impact plant growth were of particular interest to capture on video, such as the presence of algal blooms or the leaves being occluded by detritus. Animal population and behavioral dynamics are also of importance to the system. The complete flight system and operational timeline were mimicked in ground control units in a thermal chamber at Paragon's laboratory in Tucson, Arizona. Using preprogrammed flight events and pre-flight expected conditions, the ground units were designed to parallel the flight units in all regards including thermal and camera operations.

Flight experimental protocol

The ABS units were assembled at Paragon's laboratory in Tucson using isolated tanks of plants and animals. For each flight, 10 units were assembled of which 8 were chosen to travel to Cape Canaveral, Florida, for the flight. The units were sealed 4-7 days before flight and pressure tested per NASA requirements. The units were transported in custom modified Igloo® temperature controlled chambers. At the Cape, two units were then selected for flight and the remainder served as ground controls.

The ABS units were then inserted into their outer Lexan® containers and pressure tested once again using a vacuum chamber while immersed in water. Once installed in the ICMs, the units were transported to the Shuttle approximately 24 hours before launch and installed in the SPACEHAB module, incurring approximately 45 minutes of power off time during the transfer. The systems were also occasionally powered down for transfer to, from and within Mir.

The ground controls were returned to the Cape for analysis and sampling, along with the flight units, which were received 5 to 9 hours after landing. Each ABS was extensively filmed and photographed before being broken down in a laminar flow hood using sterile sampling methods specifically developed for the ABS. Water and headspace gas samples were extracted through a septa before breakdown. Representative water samples were immediately filtered, preserved or chilled before transport to labs in the US and Japan.

Results and discussion

As described in the papers contained within this volume, the overall flight experiments of almost 18 experiment-months of accumulated flight testing were largely successful. The headspace gas in all flight and ground ABS had CO₂ concentration levels below 250 ppm and O₂ levels above 22%. Aquatic animals successfully reproduced in all flights, with

successive generations having occurred in the Mir flights. A doubling in the populations of amphipods, daphnia and ostracods in the 10 day STS-77 flight were noted. In the first Mir flight, Algae successfully replaced the plants in the ABS as the primary photosynthesizer, allowing the animals to flourish. The second Mir flight returned with animals, plants, algae and bacteria, all of which successfully grew and reproduced after the flight. The only crew time required was for periodic air intake screen cleaning and ICM computer status checks, and to correct a video recording problem.

Subsequent to the ABS flights, the CEBAS (Closed Equilibrated Biological Aquatic System) Mini Module was flown on the STS-89 Space Shuttle mission in January 1998 for nine days with similar results (Bluem *et al.*, 1998).

Also of interest is that the viable cell count and dissolved organic compound data reported by Ishikawa *et al.* (1998) were predicted by a model that examined the influence of spaceflight conditions on a fresh-water pond ecosystem (Levinskikh *et al.*, 1993).

Deviations from expected thermal performance, especially during flight number 2 (STS 79/81 NASA 3), were attributed to problems associated with the capacity of the ICM to withdraw the heat generated by the light given the higher than expected external temperatures (relative to the ICM) experienced within the Mir. The ICM coldplate temperatures were set at 18°C (64.5°F), and pre-flight calculations using a rudimentary thermal model predicted 22-23°C (71.5-73.5°F) bulk fluid temperatures for the ABSs. Actual external wall temperatures for the ABS were close to 24.3°C (75.7°F) for the early engineering flights. Part of this over-temperature reading is due to the radiative absorption of the temperature sensor itself, that was in the path of light coming from the 7W bulb (though "behind" the Lexan® container). However, this effect only accounts for 0.5-to 1.0°C of the difference between predicted and actual temperatures.

The Mir flights showed a greater deviation from expected thermal performance. This is due to the higher temperatures experienced within the Pyroda and Kvant modules, which averaged 29-30°C, and severely taxed the ability of the ICM thermal system which was restricted to 30 peak watts. During the first Mir flight the lighting system was on a continuous 24 hour mode, resulting in a higher than predicted ABS bulk temperature of 28°C (82.4°F). The loss of the *Ceratophyllum demersum* in the first Mir flight is attributed to a combination of higher than expected temperature and (or) higher than

expected plant stress from the continuous light. The *Ceratophyllum demersum* thrived in the second Mir flight which had a 16 hours on, 8 hours off, light cycle, and therefore thermal cycle, reducing the average bulk temperature to 25-27°C (77-80.6°F).

The range of temperature on Mir was dependent on the status of the Mir station and periodic electrical failures. Table 1 shows events on the 3rd flight (STS 86/89 NASA 6) that may have had a direct effect on the results as detailed in the other papers in this volume.

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Notes: ¹ The USSR reported flying *Azolla pinna* (an aquatic fern) for 6 days in 1982 (Shepelev *et al.*, 1982).

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Table 1 Major Flight Events, ABS, STS 86/89 NASA 6

MET- (days)	Event	Known Effects	Probable Effects on System
0	Launch	Average temp. goes from 23°C to 25.5°C due to loss of convection.	Minor
4.55	Transfer to Mir Pyroda Module	Slight rise in average temp. from 25.5 to 25.75°C	Minor
22.43- 26.49	Transfer to/from Kvant	Mixing of system, no temperature effect	Minor
28-29	Rise in Mir temp. to 29+°C	Average temp. rises to 27°C temporarily (about 3weeks)	Stress on plants and animals.
49.62- 49.98	8 power interruptions on Mir	Temporary reduction in average temp. to 25.75°C (4 days) rising back to 27 by MET55	Reduced stress on system.
55.56- 55.76	5 power interruptions	Very little effect	Minor
80-98	Gradual rise in Mir temp. due to system problems	Average temp. rise to 28.5°C (83.3°F)	High stress on system. Possible animal reduction. Plant stress. Increased microbial growth.
98.44	19 hour power interruptions in station	Average temp. drops to 25.5 then rises gradually to 26.5°C over the next 25 days	Reduced stress on system.
123.75- 127.75	Transfer to Shuttle/Landing	Reduction in average temp. to 24.75°C	Further reduction on stress. Mixing, stratification on landing.