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The ABS (Autonomous Biological System): Spaceflight Results from a Bioregenerative Closed Life Support System

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ABSTRACT

Materially-closed aquatic life support systems containing vascular plants, invertebrate animals, algae and microbes were tested in three space flight experiments with ground controls. Termed Autonomous Biological Systems (ABS), the 0.9 liter systems were completely isolated from spacecraft life support systems and cabin atmosphere contaminants, and needed minimal intervention from astronauts. The first experiment, aboard the Space Shuttle in 1996 for 10 days, was the first time that aquatic angiosperms were successfully grown in space. The second and third experiments aboard the Mir space station had 4-month durations, in 1996-97 and 1997-98, and were the first time that higher organisms (aquatic invertebrate animals) completed their life cycles in space. Compared to the ground control ABS, the flight units showed clearer water and slightly higher total organic carbon and soluble free amino acids. ABS units from all 3 flights returned as diverse and complex ecosystems. The ABS are the first completely bioregenerative, closed ecological life support systems to thrive in space, demonstrating their efficacy for research in space biology and gravitational ecology.

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. Since exposure of research organisms to the varying composition and contaminant burden of the space craft cabin atmosphere alters experimental parameters in a way that is difficult to control, the life support system must have no material exchange with the cabin atmosphere.

To be of practical use the systems must be small, simple, have low power requirements and need little or no crew time for maintenance. Additionally, the ability to withstand periods of hours without power could be critical. Neither the physical nor biological system should 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.

While prior studies have documented effects of microgravity on growth and development of individual species, the study of species' interactions in space and the effect of microgravity on ecosystem and species structure—the field of gravitational ecology—is new. Difficulty with reproducing small, contained complex life systems has been cited as one of the limitations to furthering gravitational ecology research. Reproducibility is essential to comparisons with ground controls for the determination of the effects of gravity on structure and function.

The capability of making reliable, high resolution images of the ecological systems during the flight helps to extend space biology research to the study of life cycles, behavior, and ecosystem structure through time. Though sensors have been developed for use in space, high image quality is available in commercial-level video products. Such products do not yet have a history of reliable, long-term operation for space applications.

The Autonomous Biological System (ABS) developed by Paragon Space Development Corporation is a complex, passively controlled, bioregenerative ecological system designed for long duration space flight. Providing for long-term growth and reproduction of aquatic plants, animals, algae, and microbes, the ABS operates within complete material closure, isolated from the spacecraft life support system and cabin atmosphere contaminants. Minimal crew time is needed to check system status when down link telemetry is not available.

The ABS was tested in 3 space flights with two ABS in each flight. In May of 1996 two ABS units made their 10-day maiden flight on STS-77 (SPACEHAB-04). This flight was principally a short duration test flight in preparation for long duration testing on Mir. Successful completion of the test flight led to the first Mir flight four months later.

The other 2 space flight experiments, each of 4 months, were flown on board the Russian Mir space station during the STS-79/81 NASA 3 mission (September 1996 through January 1997) and the STS-86/89 NASA 6 mission (September 1997 through January 1998). The purpose of the 4 month flights were to test the design of the ABS for long duration exposure to microgravity, to characterize both biological and ecological phenomena observed to occur as a result of space flight, and to test resilience and reproducibility of the complex ecosystem. The potential of ABS for future experiments with fish was also considered.

After the systems were retrieved on the ground, the ABSs were opened and measurements were made on both the flight and ground control units. The health of the ecosystems was diagnosed through water and sediment analysis. Video imagery was taken during the second Mir flight in order to observe animal behavior and record system function through the four-month stay on Mir.

Tested under the adverse electrical and thermal space flight conditions on Mir, the ABS proved to be reliable and qualitatively reproducible for both space biology and gravitational ecology applications. The ABS was the first completely bioregenerative life support system flown in space. The ABS ecosystems contained the first aquatic angiosperms to be grown in space and the first higher organism (aquatic invertebrate) to complete its life cycle in space—from reproducing adult through reproducing adult. Video images of the ecological system were successfully recorded and returned for animal behavior research and evaluation of ecosystem performance, changes and adaptation over time. This places these experiments among the first in the field of gravitational ecology.

This paper reports the physical and ecological design of the patented ABS (Poynter 1996), and reviews its advantages and disadvantages. It briefly summarizes results of the 3 space flight experiments that tested its efficacy for long-duration gravitational ecology and space biology applications. Animal behavior, water chemistry, plant gross morphology and fine cell structure, and ecosystem dynamics in microgravity are evaluated with reference to the ground control units. Performance of the video system is summarized together with technical problems encountered. Our understanding of both space biology and gravitational ecology can move forward as more experiments using complex, materially closed ecological systems are flown in space.

Two ABS are scheduled to fly on STS 107 in 2001 supporting three species of fish. The ABS are also being modified to improve video image quality for behavior and developmental biology research.

SYSTEM DESIGN

ABS HARDWARE

Each of the ABS flight experiments had 2 replications of a fresh water, complex ecological system. Each ecosystem was doubly contained in concentric Lexan® cylinders that admitted light and conducted heat out of the system. The light source for both ABS was a single 7 Watt fluorescent bulb at 5000K, also within a separate Lexan® cylinder placed adjacent to the 2 contained ecosystems (Figure 1).

Figure 1 The small cylinder contains the light. The two larger cylinders marked T-GAP each contain an ABS. The video camera is shown at left.

Space flight accommodations for the ABS units were provided by the Generic Bioprocessing Apparatus - Isothermal Containment Module (GBA-ICM) shown in Figure 2. The GBA-ICM was designed for use in the space shuttle middeck and is also compatible with lockers on the Russian Space Station Mir. Each GBA-ICM contained a total of 8 experiment housings called Group Activation Packs (or GAPs). Each GAP can contain up to 8 different test-tube like experiments, or in this case, 2 GAPs were modified to house the 2 ABSs. The GBA-ICM provided a six-sided isothermal system maintained at 18 C that removed excess heat from the light source and ABS units.

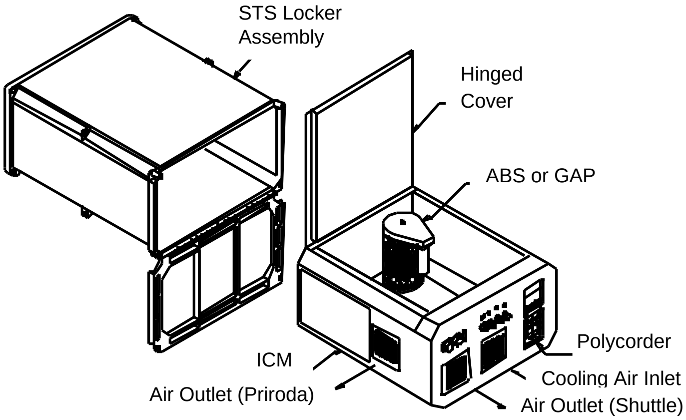

Figure 2. The GBA- ICM and locker assembly.

ABS WETWARE

The ABS design was modeled after a fresh water pond, containing primary producers, herbivores, detritivores, and decomposers through which materials would flow and ecosystem structure would develop. Though the components of the systems were not an exact duplication of any given pond, the same functions were present.

A method for passive control of the ABS was developed 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 Maragoni convection.

A final draw-down of nutrients with an increase in O_2 and pH was accomplished by introducing carbonates immediately before closure of the ABS. Use of the carbonates in photosynthesis after closure increased the concentration of O_2 to between 26 and 28% in the 100 mL gas headspace, causing a commensurate rise in system pressure, slightly increasing the system C/N ratio. 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 as may occur on Mir.

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.

There were 3 species of vascular plants in the ABS: the rootless, submerged aquatic macrophyte,*Ceratophyllum demersum*, and the tiny floating aquatics,*Lemna minor* and *Wolffia* sp. The aquatic invertebrate animals were introduced as populations, and included: two species of gastropods, including *Physa* sp.; ostracods; *Daphnia* sp.; the amphipod *Hyalella azteca*, cyclopoid copepods and *Planaria* sp. Three individuals of both snail species, 5-6 ostracods and copepods, 3 *Daphnia* individuals, 8 *Hyalella*, and 2 *Planaria* were introduced into each ABS. A naturally-occurring pond algal and microbial component was used to inoculate the systems. Inoculum is maintained in tanks in Paragon's laboratory thar were started using water and sediment samples taken from fresh water ecosystems in Arizona, California and Florida. The ABS were inoculated from the same tank for all three flights. While the algae were a source of food for some of the animal species, they were also a back-up autotrophic component in the event that the vascular plants died.

Given the relatively short life spans of some of the invertebrates, it was necessary for several successive generations to successfully breed in space for the species to survive the 4 month flight. We used a strategy of species-packing for these experiments, anticipating that a larger number of animal species would increase the chance that at least one would survive the 4 month stay on Mir. One of the major design decisions involved species diversity, or the number of species within the system, and the complexity that emerges due to the interactions and connections that form between the species. Though a diverse system was considered more likely to contain species that will survive the perturbations of space flight simply because there are more choices, a simpler system may be more reproducible. Reproducibility allows predictable results to follow from a smaller number of experimental units. The major disadvantage of a complex system is that as competition is increased, emergent properties of each system tend to diverge, with different animal populations gaining dominance in individual systems. For example, we observe that either gastropods or amphipods tend to dominate in a given ABS.

As the effect of space flight on the ABS was not known, we decided to be conservative and fly a more complex system to learn which species had the highest survivorship, and to increase the chances that the system would not collapse into a simple microbial ecosystem. Quantitative measurement of the reproducibility of the ABS ecosystem as flown have not been made. Qualitatively the systems are reproducible. All of the animal species survived and reproduced in the ABS, and plant biomass as well as air and water composition remained relatively stable at levels near those obtained one week after closure.

IMAGING SYSTEM

Paragon, in conjunction with Sony® and BioServe, developed an autonomous video recording system using a commercial Sony® DCR-PC7™ camera with custom EPROM software that allowed for camera on/off recording by simply cycling power to the camera. Other minor modifications were made to the body of the camcorder, including the removal of a cover on connectors, and a rubber piece on the viewfinder. In order to start and stop recording automatically, the control software stored in an electronically programmable, read-only memory chip was replaced by a modified program. This modification of the software enabled selection between several default settings that were different from those of the commercial model. The default, optimized for the experiment, was loaded into the camcorder when power was supplied to it. The miniDV tape loaded on the camcorder was essentially the same as a commercially available one, except slightly longer than that of the nominal 60 minutes and extensively tested for defects in the tape.

In order to calibrate the color of the recorded image during post flight analysis, a chart of standard color tiles was placed between the two cylinders. All the time sequences of filming were registered in the memory of the control unit and executed at its time mark. In case an electric power outage happened at a planned time for recording, an alternate filming session was initiated as soon as the power was recovered. Since the control of the ABS could be overridden by a manual command made by a crew member, additional imaging sessions could be made in orbit.

The ICM computer was programmed to allow for both continuous (2 minute) filming and "snapshot" (2-4 seconds) filming, and the camera was turned on when each light cycle was initiated. The digital imaging system was used throughout the second 4-month flight experiment, allowing subsequent analysis of changes in the ecosystem. Total length of time of recording in orbit was designed to fill a full length of the tape, 64 minutes. States impacting plant growth, such as the presence of algal blooms or the leaves being occluded by detritus, were of particular interest. Organism motility, animal population changes and behavioral dynamics were additional aspects of the research that were made possible by the imaging system.

METHODS

PRE-FLIGHT ASSEMBLY AND FLIGHT PROTOCOL

Two weeks prior to each flight 10 ABS units were assembled at Paragon's laboratory in Tucson, Arizona, as described in the sections above. The photosynthesis restricting nutrients selected were principally CO_2 and secondarily nitrogen and phosphate. From the 10 systems 8 were chosen to travel to the Cape for the flight and pressure tested per NASA requirements. The units were transported in custom modified Igloo® temperature controlled chambers. For the Mir flights two units were randomly selected for flight and the remaining 6 served as ground control units. The ABS units were then inserted into their outer Lexan® containers and pressure tested using a vacuum chamber while immersed in water. After installation in the ICMs, the units were transported to the Shuttle approximately 24 hours before launch and installed in the SPACEHAB module, with approximately 45 minutes of power-off time during the transfer. The systems were also powered down for transfers between the shuttle and Mir.

GROUND CONTROL UNITS

The ABS used for ground control were returned to Paragon's lab. The complete flight system was mimicked for the ground units within a thermal chamber. Using preprogrammed flight events and expected conditions, the ground units were designed to parallel the flight units in all regards including thermal, light, and camera operations.

POST-FLIGHT HANDLING OF ABS UNITS FOR ANALYSIS

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 videoed and photographed, and water and headspace gas samples were extracted through a septum. For the Mir flights, the systems were broken down into research components under a laminar flow hood, using sterile sampling methods developed for the ABS, (Ishikawa et al. 1998). Representative water samples were immediately filtered, preserved, or chilled before transport to laboratories in the US and Japan. Live specimens were extracted with care to maintain the integrity of representative samples.

WATER CHEMISTRY

Carbon, nitrogen and other ions

Shuttle-1996 (STS-77)

Ion and carbon analyses were made of the water that was retrieved from the ABS units. CO_2 and O_2 contents in gas phase and DO and pH of the water were measured just after landing. Measurements of Na^+ , Ca^{2+} , Mg^{2+} , and K^+ were made using an atomic absorption analyzer. NH_4^+ , NO_2^- , NO_3^- , and PO_4^{3-} measurements were carried out with an autoanalyzer, and SO_4^{2-} was analyzed by ion chromatography. Total carbon, inorganic carbon, and total organic carbon were measured by the combustion-IR method.

Mir-1997 (NASA 3)

After the ABS was shaken so that all the materials in liquid phase were well mixed, it was left still for several hours. The upper portion of the liquid part was sampled as Fraction 1. The lower portion of the liquid part was sampled as Fraction 2. The layer of detritus was sampled as Fraction 3. Certain portions of the Fractions 1 and 2 were filtered through 0.2 µm membrane filter and the solids were separated from the filtrate. The material on the inner surface of the ABS container was scraped. Analytical methods were the same as the Shuttle mission, under sterile conditions.

Mir-1998 (NASA 6)

The sampling methods were the same as the Mir-1997 mission. NH_4^+ , NO_2^-, PO_4^{3-}, SO_4^{2-}, Na^+, and K^+ were analyzed by ion chromatography. Ca^{2+} and Mg^{2+} were analyzed by ICP-mass spectroscopy. The rest of the analytical methods were the same as described for the Shuttle mission.

Assay of endopeptidase activity

Samples were filtered through 1.2 micron membrane filters. Endopeptidase activity was determined after the method by Chien (1978) with a modification as follows. 0.1 mL of a supernatant of each sample was mixed with a substrate solution (0.5 mM 4 phenylazo-benzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg in 0.1 M imidazole buffer, pH 7.5, 2 mL). Then dioxane solution of 1 mM fluoresamine (1 mL) was added to the mixed solution above, and the solution was incubated at 303K. Fluorescence (F) of the solution was measured with excitation wavelength of 390 nm and emission wavelength of 475 nm. Peptidase activity was calculated from dF/dt, where glycine derivative with fluoresamine was used as a standard. For this analysis the unit of measure (1 U) is that amount of enzyme that catalyzes the hydrolysis of 1 micromole of peptide in one minute, under fixed conditions.

In the Mir-98 experiment, alkaline phosphatase activity was assayed instead of endopeptidase activity since alkaline phosphatase is more stable than endopeptidase, and more fundamental data on alkaline phosphatase in the biosphere have been reported than that on endopeptidase. Concentration of phosphorous compounds, amino acids and TOC were also determined. All the samples were taken from the upper part (Fraction 1) of each flight and ground samples.

Assay of alkaline phosphatase activity

Alkaline phosphatase activity was determined for both filtered and unfiltered samples using 1mM p-nitrophenyl phosphate dissolved in 0.6 M Tris buffer (pH 8.0) as a substrate. Three mL of the substrate solution and 4 mL of the sample solution were mixed and incubated at 30°C for 24 h. Activity was calculated from the formation rate of p-nitrophenol by measuring absorbance change at 410 nm. Activity of the filtered (0.2 microns) sample is referred to as dissolved phosphatase activity, while activity of the unfiltered sample is referred to as total phosphatase activity.

Determination of total amino acids

Amino acids in the same sample were analyzed with high performance cation-exchange chromatography (Shimadzu LC-6A HPLC) after hydrolysis with 6 M hydrochloric acid at 110°C for 24 hours. Amino acids were detected fluorometrically with post-column derivatization with orthophthalaldehyde and N-acetyl-L-cysteine.

Amino acid concentration of filtered and unfiltered samples is referred to as total dissolved amino acids (TDAA) and total amino acids (TAA), respectively. Total organic carbon (TOC) concentration was determined with a TOC analyzer based on non dispersive infrared absorption, where the filtered samples were used.

Carbon isotopic ratio

The samples were vacuum-dried and combusted to form carbon dioxide as described elsewhere (Mizutani and Wada 1985). The amount of gas thus generated was measured manometrically after purification. Organic carbon content was calculated from its volume, and the gas was later used for carbon isotopic measurement. To determine the carbon isotope abundance, the gaseous carbon dioxide was introduced to a Hitachi RMU-6R mass spectrometer. The carbon isotope data were corrected for ^{17}O (Craig, 1957). In accordance with convention, the carbon isotope ratio was expressed in ‰ deviation from the PDB carbonate standard, a Cretaceous belemnite (*Belemnitella americana*) from the Peedee Formation of South Carolina, U.S.A. Working standards of carbon were calibrated against U.S. National Bureau of Standards isotope reference material No. 20 and No. 21. The $\delta\,^{13}$C values for the two working standards were -19.4‰ and -12.0‰. Standard deviations of the measurements were less than 0.1‰.

Further details of water chemistry methods used in these experiments are found in Ishikawa et al. (1998).

PLANTS, ANIMALS, AND MICROORGANISMS

Vertical distribution of microorganisms and fine structure of *Ceratophyllum demersum*

Material processing including fractionation is described elsewhere (Ishikawa et al. 1998). Aliquots of the fractioned sample solution were filtered with 0.2 μm membrane filters. Trapped cells were washed with 3 mL of sodium phosphate buffer (pH 7.0, 20 mM). The washed filters were placed on glass slides and stained. Measurements were carried out from 20 min through 50 min after staining.

Distribution of microorganisms and fine structure of the water plant *Ceratophyllum demersum* were measured by a fluorescence method using a fluorescence microscope (Zeiss Axiovert 135M) equipped with a cooled CCD camera (Photometrics CH250). Viable cells were detected by 5-CF and algae cells were detected by chlorophyll fluorescence. Fine structure of the plant was measured by differential contrast method. The images recorded with the cooled CCD were stored in magneto-optical disks. Cell density was determined by an image analysis software, IPLab (Signal Analytics).

Colony-forming units of bacteria were measured by spreading a fraction of specimens on agar plates (90 mm diameter) supplemented with nutrient broth and incubated for 3 weeks at 37°C, and the number of colonies was counted with the unaided eye. Colony-forming units of fungi were measured similarly except that standard potato dextrose plates supplemented with Chloramphenicol (15 mg/plate), Streptomycin (1 mg/plate) and Penicillin (50,000 units/plate) were used and incubated at 27°C.

Behavior and reproduction of invertebrate animals

After taking off the lid of the ABS units and sampling a small amount of water, animals were collected before separating the whole water into 3 fractions. Some of the invertebrate animals were kept alive while others were preserved for later examination. The onboard video images of the ABS units were obtained for the second Mir experiment. Behavior of the animals during their 4 month residence in microgravity was analyzed from these images.

See Ijiri et al. (1998) for further details.

VIDEO CAMCORDER TECHNICAL ANALYSIS

Even though the function of the retrieved camcorder was in the range of normal, two technical issues were found in the video image taken in orbit and the camcorder. One was a defect of pixels that were found in the retrieved camcorder. It is the so-called "white spot" that is caused by an increase in the dark level of the pixel. This defect was originated by the exposure of the imaging device to the space environment, and it was irreversibly damaged. The other issue was the white balance function of the camcorder. It was disturbed during a period in orbit, but recovered afterwards. In the following sections, those two technical items are described in detail.

Distribution of dark level and pixel defects due to the space environment

After the 4 month flight on Mir, the 2 Sony DCR-PC7 video camcorders were tested, one from the space flight and one from the ground control. The dark level of the video signal at each pixel was measured with the lens closed. Measurement of the dark level and data processing were executed by Video Measurement Set (Tektronix, VM 700A). The signal analyzed was Y signal from S-video output of the camcorder. The video signal was averaged over 32 frames as a preconditioning for the data reduction. The ambient temperature during the test was 25ºC. Since the dark level of the CCD pixel is quite sensitive to the thermal properties of the associated semiconductor, temperature of the outer casing of the camcorder was measured and logged during the test.

The electronic gain of the CCD signal was confirmed to automatically increase to +18dB when the image was taken with the lens closed. The picture taken and recorded on the miniDV tape by the camcorders was transferred to a personal computer. To convert an image file into the format of film strip, a personal computer, Power Macintosh G3 MT (Apple) with a DV capture card, FireMax-2 (ProMax), was configured to transfer the digital video data directly through the IEEE 1394 interface. The image file acquired was processed and analyzed quantitatively using Adobe's Premiere and Photoshop software.

Auto white balance

In order to evaluate the normal range of the shift in color data for adjusting white balance, values of red, green and blue components of the video signal were determined quantitatively. Photoshop software was used to analyze the image data at the standard color chart placed in the middle of the viewing field. Two successive frames from January 2nd and 3rd, 1998, in which the image got greenish, were subjected to this analysis.

See Yamashita et al. (1998) for further details.

RESULTS

WATER CHEMISTRY

Carbon, nitrogen and other ions

Shuttle-96

Both organic and inorganic carbon were found to be more abundant in the flight compared to the ground sample for both the filtered and unfiltered samples. High oxygen percentages in the gas phase were measured in both the flight (29.66%, 26.27%) and ground control (28.78%, 22.28%) samples. Since pH measurements were high (flight = 9.61, 6.57; ground = 9.48, 9.42), this is considered to be similar to the phenomena in natural pond water at the end of the day.

Mir-97

Organic carbon in filtered water samples from the flight units was higher than that in the ground units, and higher than that in the Shuttle-96 flight units. This increase of organic carbons in the flight sample may be explained by the deterioration of the *Ceratophyllum* in space, dissolving some of the carbons into the water. The ground control plants did not show deterioration. The carbon of the unfiltered water showed similar results. Inorganic carbons were more abundant in the fraction 3 samples compared with the samples from fractions 1 and 2. The increased portions might come from the deteriorated shells of snails.

The range of C/N ratios of the detritus was approximately 9-10. The flight C/N ratios were somewhat higher than the ground ratios, possibly due to the deterioration of the plant in the flight units. Ammonia, nitrites, and nitrates were basically low in all cases, suggesting that the water quality was maintained over the course of the experiments. The other ions that were analyzed showed no significant difference between flight and ground ABS.

Mir-98

The amount of inorganic and total organic carbon measured in the filtered samples were on the same level as the first Shuttle mission. In all of the samples (from fractions 1 and 2) the flight sample had higher total organic carbon than the ground, similar to the Mir-97 samples. Although there were not enough filtered water samples from fraction 3 to run the analysis, the unfiltered water sample showed higher inorganic carbon for fraction 3. This phenomenon was observed commonly between Mir-97 and Mir-98. The C/N ratio in the flight samples were in the same range as the ground samples for this mission. The *Ceratophyllum* was not deteriorated, explaining why the C/N ratio had not changed in the water samples. Nitrogen levels and concentrations of the other ions were similar to the previous 2 experiments.

Assay of endopeptidase and alkaline phosphatase activity, and determination of total amino acids

Mir-97

The following measurements were made for ABS fraction 1, the upper portion of the water. Among amino acids, glycine was predominant, followed by alanine, ß-alanine and γ-aminobutyric acid. Total carbon in the form of the eleven major amino acids were 0.26- 0.91 ppm, which was 0.7-1.6 % of TOC. There was a positive correlation between total dissolved glycine (the predominant amino acid) concentration and TOC.

Endopeptidase activity was of the order of 10 mU/L. There was no significant difference between endopeptidase activity in flight vs ground samples, since the variation of activity within the same group was quite large. There was a negative correlation between dissolved total glycine concentration and endopeptidase activity. It is recognized that the synthesis of an enzyme in vivo is suppressed by the presence of the final product of its catalytic reaction in the media or in lake water (Kobayashi et al., 1987). When the concentration of amino acid-related compounds dissolved in aqueous media (TDAA) was high, amino acids tended to be obtained easily by organisms, which may have suppressed the peptidase synthesis in the system. One of the major problems in the assay of endopeptidase activity is that peptidases are quite unstable due to self-digestion. Preservation of the samples before the assay of peptidase activity should be considered in the future.

Mir-98

Similar to the Mir-97 results, a strong correlation between TOC and the concentration of total dissolved amino acids (TDAA) was found. The flight sample measurements showed higher TOC and TDAA than the ground samples. The decomposition of plants may have raised the TOC and TDAA levels. Dissolved alkaline phosphatase activity (DAP) ranged from 0 to 3.3 mU L^{-1}, which was approximately 10% of total alkaline phosphatase activity (TAP). The flight samples showed less DAP than the ground samples, and negative a correlation was observed between TOC and DAP. Orthophosphate concentration of each sample was less than 100 ppb. In laboratory incubation experiments of bacteria or algae, depression of phosphatase activity in vivo could be observed when orthophosphate concentration was over 10 ppm level (Torriani, 1960; Fitzgerald and Nelson, 1966), which was much higher than the level in the present systems. Variations in phosphatase activity and orthophosphate have been observed in a microcosm (Sugiura, 1998). It was suggested that alkaline phosphatase supplied orthophosphate when the orthophosphate concentration in the system was low (Seki, 1999).

In the present Mir-98 experiments, more organic compounds (including phosphorous compounds) seemed to be dissolved from plants in the flight units than in the ground units. Then less alkaline phosphatase was required in space than on the ground to keep the ecosystem active.

Carbon isotopic ratio

Shuttle-96

For the Shuttle-96 experiments, dissolved organic matter (DOM), particulate organic matter (POM), and hornwort (HRT) were fractionated from each of the four microcosms (pre-flight fresh, pre-flight 1-year old, ground control, and post-flight), and their carbon isotope ratios were examined. DOM was obtained by collecting the filtrate of the liquid medium through a Whatman glass filter. POM was the particulates that remained on the filter. HRN was simply picked up out of the medium and rinsed with distilled water. All of the samples were then vacuum-dried. The difference in masses of the filter before filtration and after the drying was the mass of POM. The mass of DOM was calculated in the same manner.

Mir-97

Three fractions of the medium were examined for the Mir-97 experiment. The results of the isotopic for the Shuttle-96 experiment and from the Mir-97 experiment are given in Ishikawa et al. (1998). The ^{13}C values were variable.

PLANTS, ANIMALS, AND MICROORGANISMS

Vertical distribution of microorganisms

Fraction 3 from both ground and flight samples of the Mir-98 experiment showed 10 to 50 times the density of viable microorganisms and cells containing chlorophyll compared to fractions 1 and 2. There was no significant difference between flight and ground samples. In Mir-97, similar results were obtained.

The cell densities from the Mir-98 experiment were several hundred times larger than those obtained by the classical colony counting method; similar discrepancies have been reported previously in the literature (Tsuji et al. 1995). No fungi were detected in the samples from the Mir missions.

See Kawasaki et al. (1998) for further details.

Fine structure of *Ceratophyllum demersum*

After the 10 day flight on the shuttle, *Ceratophyllum* from both flight and ground samples looked healthy. Apart from the gross morphological similarity between samples, a significant decrease in the number of chloroplasts, and disordering of chloroplasts of the flight plants was observed. The *Ceratophyllum* plants in the flight samples from Mir-97 were completely decomposed.

In the Mir-98 experiment, the plants were reduced in volume and looked partially deteriorated as shown in Figure 3. Over-all decomposition in the flight samples was not observed. Within plant cells both a decrease in the number of chloroplasts and disordering of chloroplasts were observed in both the ground and flight samples.

See Kawasaki et al. (1998) for further details.

Behavior and reproduction of invertebrate animals

Shuttle-96

No distinct differences were noted between the flight and the ground samples. In both, the water plant appeared healthy and the animals were alive. *Daphnia* and ostracods seemed to have produced offspring in all samples, evidenced by their number and the presence of small-sized animals. Some *Daphnia* were observed with embryos in their egg sac.

Mir-97

In both ABS units flown in space, the water plant had disappeared completely. In both flight and ground units there were many ostracods, pond snails, and amphipods. There were no *Daphnia* in the flight units, though they were observed in the ground units. The flight units differed in the densities of amphipods and snails: one ABS had many amphipods and only a few snails, and the other had few amphipods with more snails. This apparently inverse relationship between snail and amphipod density was also observed in the ground units. The 2 flight units also differed in clarity of water, which seems to be intrinsic to each unit rather than a result of disturbance.

The amphipod behavior of dropping straight downward, head first, to the bottom of the unit was observed in both the ground and flight units, though far more frequently in the flight units. In addition, the dropping direction of the amphipods was straight downward in the flight units.

Figure 3. Flight and ground control ABS from the second Mir experiment. The flight cylinders are the first and third (F1 and F3), ground cylinders are second and fourth (G2 and G4). Note the difference in branching between the flight and ground control plants.

The range of body size from large to very small suggests that amphipods produced offspring during the 4 month space flight. In addition, some individuals were carrying eggs. Several amphipods had 1 or 2 of their legs bent abnormally, as shown in Figure 4. Judging from their morphology, this was probably due to a physiological abnormality rather than landing shock.

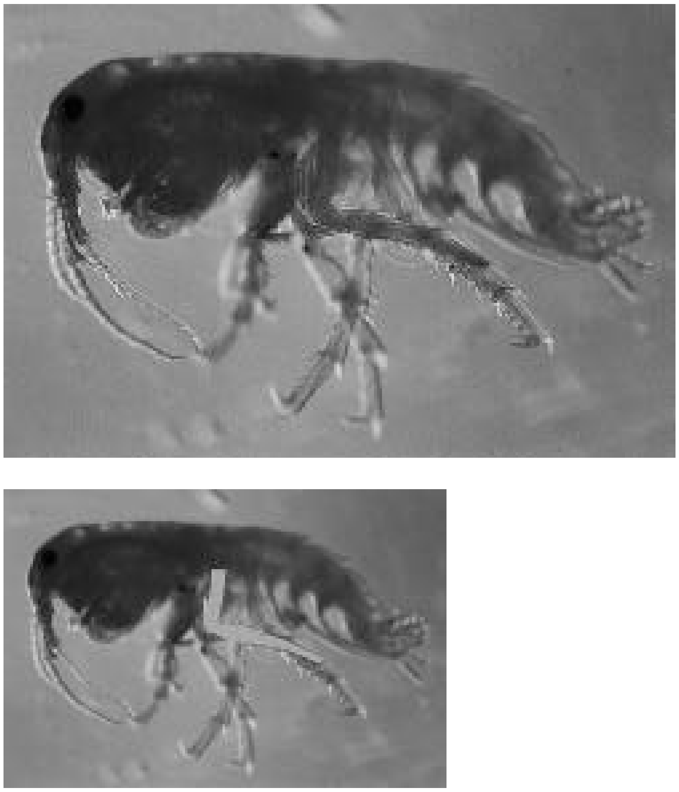

Figure 4. Legs bent abnormally as observed in amphipods in flight units when recovered on the ground after the first Mir experiment. The small panel shows the bent leg printed in grey.

Ostracods seemed to have thrived in both flight and ground units. In one of the flight units, a lack of short shell-size may indicate a lapse in production of offspring at a certain point during the 4 month flight.

Mir-98

Video images of both flight and ground units were obtained during this experiment. The images cover approximately 2/3 of the area of each ABS unit. Detailed analysis of the video tape was carried out, especially the detection of animals and their movements in space. Ostracod counts just after landing and during the space flight showed rapid fluctuation in their population. This probably reflects the minimal amount of movement exhibited by ostracods in microgravity. Of those that moved, about 50% moved in a looping fashion, quite different from the ground controls, as shown in Figure 5. Immediately after return to gravity, there were many ostracods moving in the flight units in a similar pattern to that of ostracods in the ground units.

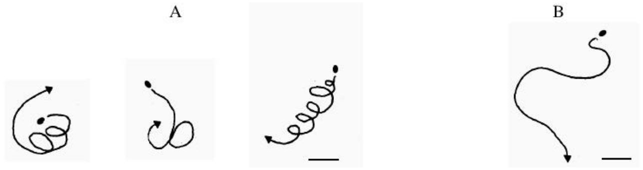

Figure 5. Ostracod movement under microgravity (A) and 1 G (B, the right-most figure) in the second Mir experiment. (A) Looping movement of ostracods under microgravity, traced on video scenes. The date and duration (in sec for each movement traced) of the three patterns are, from left, October 16 (4 sec), October 22 (3 sec), and October 28, 1997 (7 sec). (B) A typical movement of a ground-kept ostracod. The duration of the movement traced is 4 sec. Scale bars indicate 10 mm.

Several *Daphnia* were detected on the video images from the Mir-98 experiment, and some were constantly looping under microgravity, shown in Figure 6. *Daphnia* in the ground units moved in a continuous repetition of up and down movements within a very short distance. The movement of both amphipods (Figure 7) and pond snails in microgravity was similar to that observed in the ground control units.

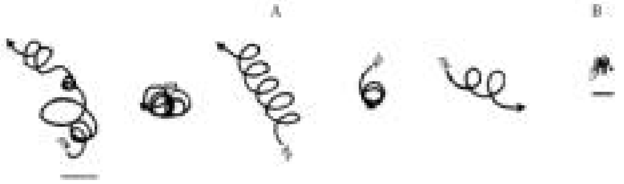

Figure 6. *Daphnia* movement under microgravity (A) and 1 G (B), the right-most figure) in the second Mir experiment. (A) Looping movement of *Daphnia* under microgravity, traced on video scenes. The date and duration (in sec for each movement traced) of the five patterns are, from left, October 10 (7 sec), October 27 (5 sec), November 9 (5 sec), November 22 (4 sec) and December 14, 1997 (3 sec). (B) A typical movement of a ground-kept *Daphnia.* The duration of the movement traced is 3 sec. Scale bars indicate 1 mm.

See Ijiri et al. (1998) for further details.

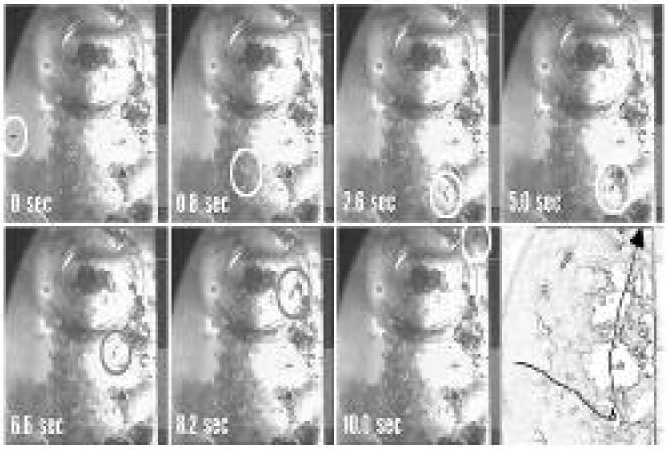

Figure 7. Movement of an amphipod under microgravity in the second Mir experiment. In each panel, the position of the amphipod is circled, and the time from the upper left-most panel (0 sec) is given. This video scene was taken on December 5, 1997. At the upper half of each panel, a large air bubble existed, which was not observed at early times in space. In the lower-right panel, the total movement traced during 10 sec is shown. From 2.6 sec to 5.0 sec, the amphipod slowly walked turning its direction, then swam upward.

VIDEO CAMCORDER TECHNICAL ANALYSIS

Distribution of dark level and pixel defects due to the space environment

General Performance

The quality of the images taken and recorded by the flight camcorder was satisfactory for the purpose of scientific observation of ABS. A patterned dark stripe either in the horizontal or vertical axis, and a dark spot area at the left end zone are seen in the captured pictures when the lens of the camera unit was closed. However, these patterns did not deviate from the normal range, and were found to be acceptable for the analysis.

Distinctively damaged pixels were surveyed among the video image taken in orbit. It was hard to find such defects in the recorded image, even though the location of the defects are known by the picture of the plain black image. The process of image compression might sweep such dot-sized image of the damaged pixel away, in case the image has rather complex features. Several points in the images are defects of CCD pixel caused by its exposure to the space environment for four months.

The functions of taking a picture, recording on tape and any other features activated at the post-flight test were found to be normal, and none deviated from the stated function of the camcorder. The characteristics of the video signal, such as the voltage level of sync pulse, was found to meet the specification of NTSC video signal. Chroma sync waveform was not in its specified range.

Damaged pixels

The ground control unit gave a plain black image when a cap closed its lens. The dark image from the flight camcorder showed many dots at single pixel size with color of white, red or blue. No adjacent two pixels showed such defect. The distribution of dark level was analyzed after image data were digitized and averaged over a definite number of frames. The ground camcorder had 5 pixels that exceed 3 IRE. The brightest pixel was at a level of 2.6 IRE. Distribution of the IRE number among the pixels had a normal statistical distribution. Zero IRE and 100 IRE correspond to black and white at each extreme, respectively, as defined by the Institute of Radio Engineers. The flight camcorder showed 363 pixels that exceeded 3 IRE just after the power was turned on. The biggest number, i.e. the brightest pixel, was 40.8 IRE. The IRE number for the flight camcorder was dispersed widely for damaged pixels, overlapping to a narrow peak distribution for normal pixels.

Effects of data compression for image of damaged pixels

Image data taken by CCD was compressed before it was recorded on a miniDV tape. The amount of degradation from compression was evaluated. The number of damaged pixels in the processed image was compared to that in the original image without compression or recording. Four hundred and eighty-seven pixels that exceeded 8 IRE were found n the real time video output. This number was reduced to 415 in the video image replayed from the recorded tape. The dot image was kept the same in the replayed frame. The compressed image at this test was essentially plain black. MiniDV image compression might give less degradation for the image of fine dots embedded in a plain black background.

Temperature dependence of black level

Temperature at the outer casing of the flight camcorder equilibrated after 90 minutes of operation. The equilibrated temperature on the surface was 10.2°C higher than ambient. There were 43 pixels higher than 16 IRE at the beginning, which increased to 206 after 150 minutes from the start. The brightest IRE value also increased from 40.8 IRE to a level more than 100 IRE, i.e. the saturated value. In ordinary camera units, the temperature dependence of the black level is compensated by the signal from a part of the CCD chip where the pixel is masked against light. The level of signal from that part has the same temperature dependence with other active pixels. Because of this capability to compensate for thermal effects, the black level of the processed video signal is not affected by temperature much. However, the signal level of the damaged pixels shifted brighter when the CCD chip was warmed up.

As a subsidiary effect of the thermal properties, the following tendency was found in the image taken in this test. The signal level was 8 to 9 % lower at the left end of the frame when the camcorder was warmed up by its internal power dissipation. The dark level was lower by a similar degree along the vertical axis of the image at the left end. It was suspected that the right hand side of the CCD chip is located either next to a cold part in the camcorder unit, or is close to a heat conducting component around the chip.

Auto white balance

In a part of the image where reflected light from the lamp dominates, red, green and blue values were found to be saturated. The color component showed a 20% increase in green, and 20% decrease in red.

The function of auto white balance in the camcorder was tested by taking an image of a white paper and a plain paper colored to 20% greenish. Both images appeared white when they were processed by auto white balance. The camcorder was found to be capable of adjusting white at such a degree of deviation in color. It can be concluded that the greenish color recorded in orbit was not in the normal range of the auto white balance capability of Sony DCR-PC7 camcorder.

See Yamashita et al. (1998) for further details.

DISCUSSION

With almost 9 months of accumulated flight testing, the flight experiments with Autonomous Biological Systems have shown very promising results. 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 was observed in the 10 day Shuttle flight. In the first Mir flight, microalgae successfully replaced the autotrophic function of the plants, allowing the animals to flourish even though the plants had perished. The second Mir flight returned with animals, plants and algae all of which successfully grew and reproduced after the flight. The headspace gas in all flight and ground ABS had CO_2 concentration levels below 250 ppm and O_2 levels above 22%. The only astronaut or cosmonaut time required during space flight was to clean the air intake screen used for heat exchange and to correct a video recording problem.

PHYSICAL SYSTEM

The Mir flights showed a greater deviation from expected thermal performance. This is due to the higher temperatures experienced within the adjacent modules that averaged 29-30ºC, and severely taxed the ability of the ICM thermal system that 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 28ºC. The loss of *Ceratophyllum* 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* 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 25-27ºC.

The range of temperature on Mir-98 was dependent on the status of the Mir station and periodic electrical failures. There were a number of events on the 3rd flight (STS 86/89 NASA 6) that may have had a direct effect on the ABS performance.

Shuttle-96

The first space experiment using ABS was 10 day's duration on board the Space Shuttle. The ABS units flown showed only slight deviation from the ground control units. The organic carbon compounds suspended or dissolved in the in the water were more abundant in the flight samples. Gross morphology of the water plant *Ceratophyllum* appeared normal in both flight and ground units. However, only the plants from the flight units showed a significant decrease in the number of chloroplasts, and had spatially disordered chloroplasts in its cells. Since both ground and flight units were under continuous illumination for the 10 days, the condition leading to chloroplast changes seems to originate in microgravity rather than from illumination.

All of the invertebrate species survived, and some species reproduced. Space flight clearly does not inhibit reproduction of invertebrate animals in the ABS.

The increase of organic carbon contents in the water suspension of the flight samples could be attributed to uniform distribution of materials in the system under microgravity. Biological processes produce organic materials, and digest it to inorganic carbon, CO_2. The rate of the flows in the system depends on the availability of nutrients and the number and kinds of organisms present. In space, all members of the system disperse uniformly in water. The material flow and its balance in such a homogeneous mixture are different from the ones under normal gravity where stratified patterns and structure are formed. On the ground, detritus sinks to the bottom and the water stays relatively clear. There may be structure-dependent efficiency in material flows in microbial ecology.

The increase of dissolved organic carbon in the water may also be attributed to diffusion. Where diffusion is presumed to be the dominant process of dissolved material flow between organisms in the flight ABS, convective mixing of the water is presumed to be the dominant process in the ground control ABS. Assuming that the organisms producing and consuming the dissolved organic carbon are separate and material must be moved between them in the water, the equilibrium concentration of dissolved organics in the water would be higher with the diffusion rather than the convection process. Equilibrium production and consumption rates would be the same.

Mir-97

The flight units from the second experiment showed signs of stress in several aspects. Both *Ceratophyllum* and *Daphnia* did not survive the mission. The other animal species maintained their populations. Oxygen required to sustain those animals was effectively supplied by microalgae after the water plants died. Prominent abnormalities were found in the morphology of the legs of amphipods. Physiological stress from microgravity or from continuous light may have contributed to the leg abnormality. However, since this did not appear in the ground control animals, it probably was not due to continuous light alone.

The invertebrates reproduced successfully over the 4 months, with a range of size classes present for each species. In many species, stressful environments induce their life cycle to deviate from normal. One strategy is to go into dormancy, but there was no indication of this in the ABS. Another strategy is to increase the reproductive effort. Therefore, an increase of the animal population in the ABS flight units can not be directly accepted as an index of health of the ecosystem.

The content of total organic carbon and concentration of soluble amino acid in the water was higher in the flight samples. Free amino acid in water indicates that the rate of its release at cell death and decomposition exceeds the rate of its consumption that is mainly by bacteria.

In summary, the ecological systems of the ABS showed signs of extensive stress during the 4 months in space during the second mission. Continuous lighting and the resulting heat load were found to be a negative factor for them in microgravity, and is a good example of synergetic effects in ecology. However, even though stressed, an intact and diverse ecosystem of animals and microorganisms survived.

Mir-98

The third flight, with a cycle of 16 hours of light and 8 hours of dark, was a clear contrast to the previous mission in terms of the health of water plants and animal populations. The gross morphology of the *Ceratophyllum* was normal, though there was a slight reduction in the volume of the plant and decomposition of some parts. The branches of the flight plant were growing directly away from the stem (perpendicular to the stem) whereas the ground control plant stems were growing away from the gravity vector at angles to the central stem.

There were no significant differences between the flight and ground control units. A slight increase of organic carbon and amino acid content in the water was evident in the flight units, as in the other 2 experiments. Plants from both showed the same degree of decrease in the number of chloroplasts in the cells, and the same degree of disorder of chloroplasts. All of the invertebrate species survived after the 4 month flight. These findings suggest that the ecological system used in these ABS can be sustained in space, if environmental factors are properly controlled.

The video imagery that was taken during this experiment allowed the study of animal behavior. *Daphnia* and ostracods often showed looping behavior under microgravity. Once the ostracods returned to the ground, they returned to their normal swimming pattern. It is not clear that loop swimming directly affects the ecology and material flow in the system, though it may gradually influence the health of the animals.

The detritus and air bubble in the flight units could be clearly seen on the video images. The brown colored detritus was found uniformly distributed in the ABS. The driving force for making these normally 'sediment' materials disperse was the mechanical acceleration or shock applied at the transfer of the ABS units from the Space Shuttle to the Mir Space Station at the start of the mission. The dispersed brown materials seemed to coagulate with each other in time. The video images also allowed the location of the bubble in the ABS to be observed over the 4 month Mir –98 experiment, showing a constant acceleration that is presumably due to the gravity gradient in Mir. Acceleration events were also noted and attributed to thruster firings.

VIDEO CAMCORDER

Damages in the CCD chip were found in the flight camcorder, though the effects did not hinder the use of the images for the study of animal behavior and population changes. The damaged pixels were visibly present in the image that was taken with the lens closed. These damages are related to the exposure of CCD chip to space radiation environment in low earth orbit for a period of four months. In contrast to the flight camcorder, no defect was found on the image taken by the ground control camcorder. The dark level of pixel output is highly sensitive to temperature. It should be noted that even at a Space Shuttle mission of one week, there might be about 20 pixels damaged exceeding a threshold level of 3 IRE. This could be a quite distinguishable number compared to 5 found in the ground control camcorder. It is unknown whether it was linearly accumulated with the dosage of space radiation. The number of damaged pixels increases when the camcorder is warmed up.

An evaluation of damaged pixels is feasible with the replayed video signal that is compressed before its recording on miniDV tape. This makes in-orbit-examination feasible to study propagation of damages without real time data collection. At the same time, these features, together with knowledge on sensitivity of the element against space radiation, opens the way to utilize CCD camcorder for a convenient radiation monitor system for space biology experiment.

CONCLUSIONS

- The ABS has been established as a useful model for studying ecology, and the influence of gravity on the dynamics of ecological systems. For all 3 space missions, ABS proved to be robust, and also sensitive to gravity. Environmental parameters, such as the light and thermal cycles, were found to be critical under the microgravity environment. Further work is needed to establish the quantitative reproducibility of the ABS. A large enough number of replications should be flown so that statistically significant comparisons of the flight and ground control systems can be made.
- Organic carbon and free amino acid contents in the water were higher for the ABS flown in space. These indices can be a measure of microbial ecology, though the reason for the increased organic carbon in all the flight systems is not understood.
- The importance of gravity at the level of ecological system was clearly presented by the drastic difference in populations and composition of species between space and ground control. In addition to those findings in ecology, effects of gravity at the organismal, organ and cellular level were found simultaneously. Further research is needed to understand the effect of gravity on an ecosystem.
- The ABS is now a flight tested means of long term life support for experimental aquatic animals. The ABS will be flown with fish on STS 107.

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