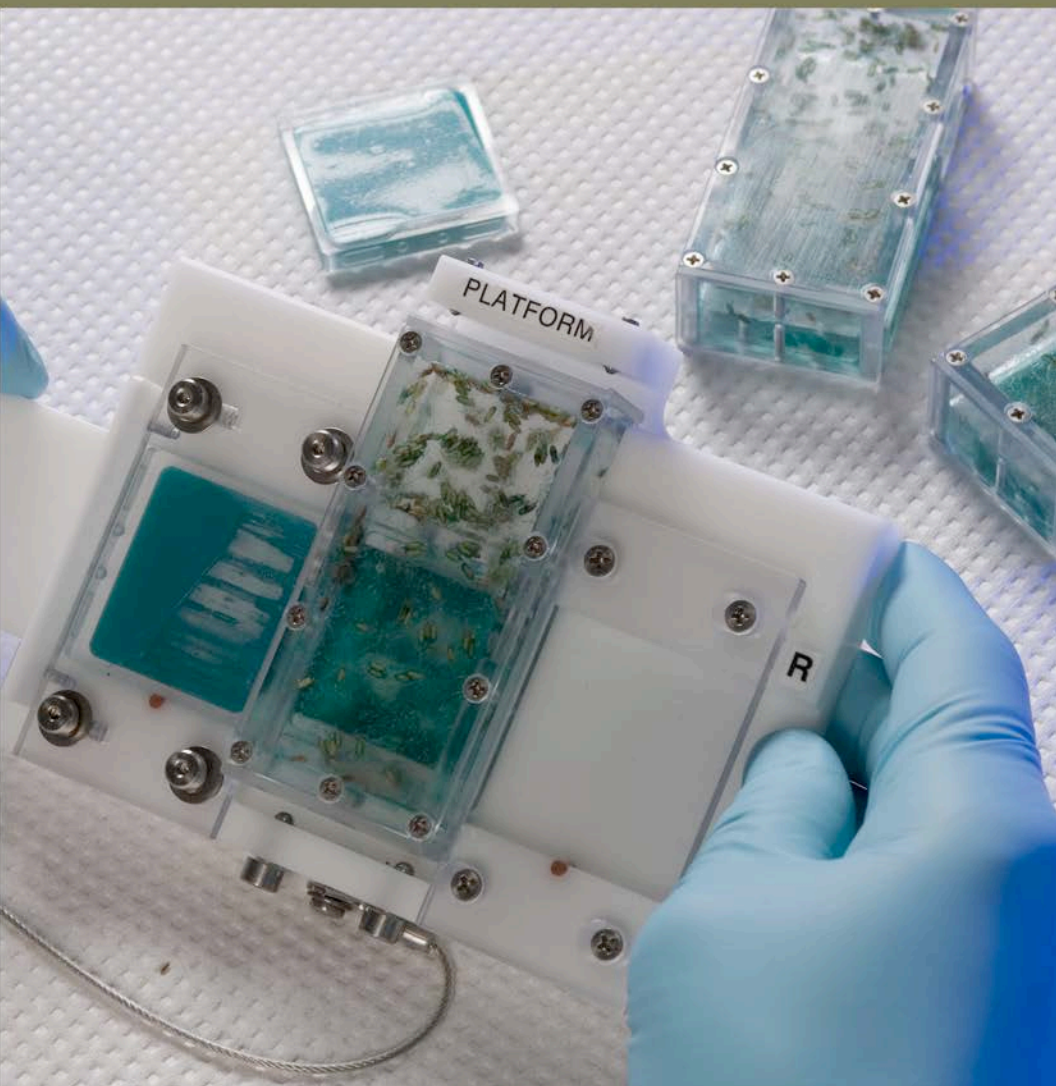
National Aeronautics and Space Administration

A Researcher's Guide to:

INTERNATIONAL SPACE STATION

Fruit Fly Research



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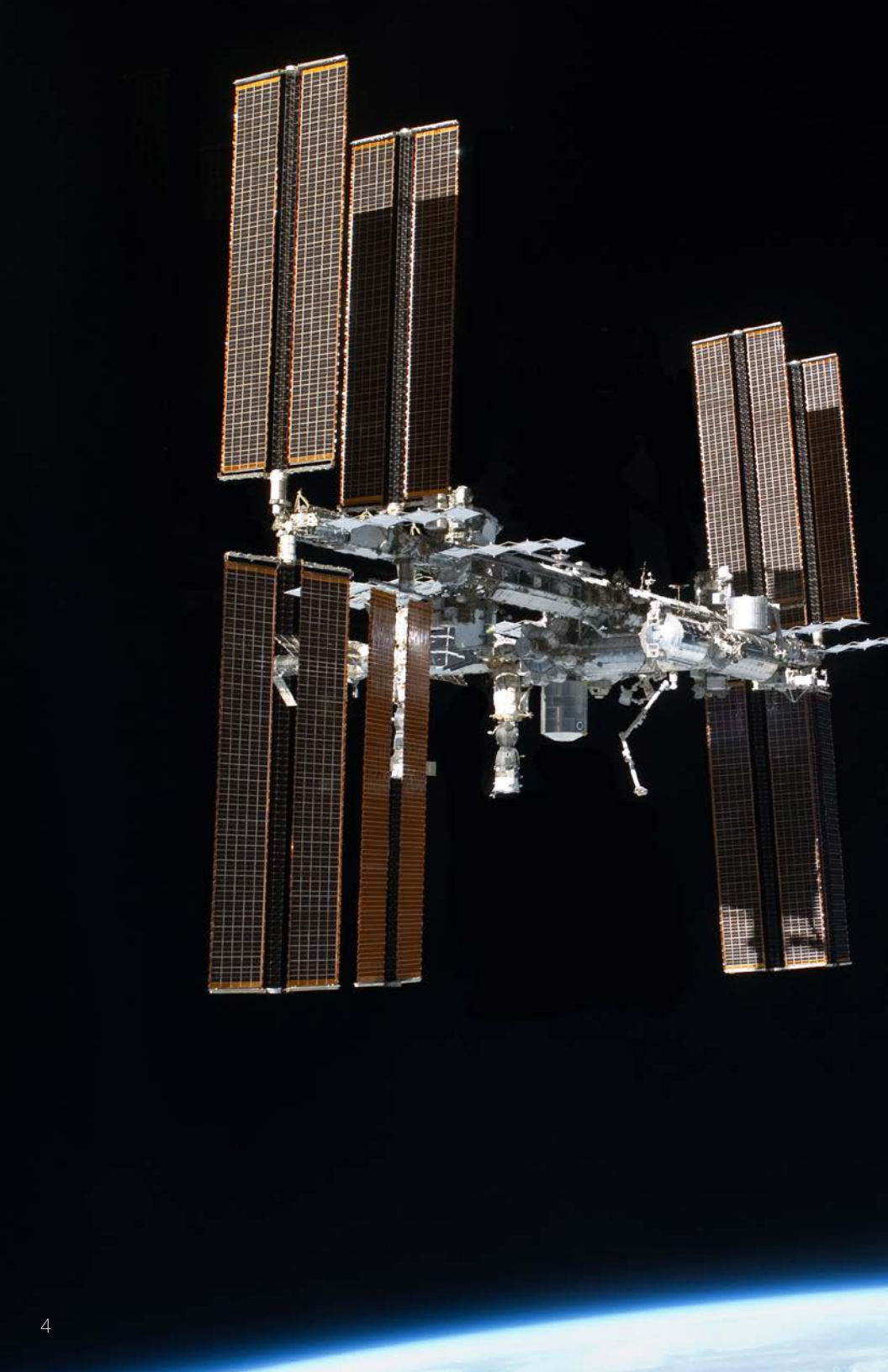
Cover and back cover:

a. View of the food change out platform of the Fruit Fly experiment hardware.
b. Both images are taken during preparation of a student fruit fly experiment that was flown on the International Space Station. At left, Dr. Sharmila Bhattacharya and at right student scientist Chetan Angadi. (Image credit: NASA)

The Lab is Open

The mission of the International Space Station (ISS) Program is to advance science and technology research, expand human knowledge, inspire and educate the next generation, foster the commercial development of space, and demonstrate capabilities to enable future exploration missions beyond low Earth orbit (LEO). This booklet, one of a series of 15 Researcher's Guides to the ISS, has been developed to provide prospective investigators with an introduction to ISS capabilities, characteristics, resources, and processes, as well as the lessons learned, and knowledge gained in the general topic area of fruit fly research.

Project scientist Amy Gresser inspects the physical characteristics of anaesthetized fruit flies. (Image credit: NASA/ Dominic Hart.)



Unique Features of the ISS Research Environment

1. **Microgravity**, or weightlessness, alters many observable phenomena within the physical and life sciences. Systems and processes affected by microgravity include surface wetting and interfacial tension, multiphase flow and heat transfer, multiphase system dynamics, solidification, and fire phenomena and combustion. Microgravity induces a vast array of changes in organisms ranging from bacteria to humans, including global alterations in gene expression and 3-D aggregation of cells into tissue-like architecture.

2. **Extreme conditions** in the ISS space environment include exposure to extreme heat and cold cycling, ultra-vacuum, atomic oxygen, and high-energy radiation. Testing and qualification of materials exposed to these extreme conditions have provided data to enable the manufacturing of long-life reliable components used on Earth as well as in the world's most sophisticated satellite and spacecraft components.

3. **Low-Earth orbit** at 51 degrees inclination and at a 90-minute orbit affords ISS a unique vantage point with an altitude of approximately 240 miles (400 kilometers) and an orbital path over 90 percent of the Earth's population. This can provide improved spatial resolution and variable lighting conditions compared to the sun-synchronous orbits of typical Earth remote-sensing satellites.

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Why Use ISS as a Laboratory for Fruit Fly Research?

In the last 4 billion years, life has evolved and adapted in response to many physical and environmental changes on Earth. However, one key feature, the presence of gravity at its acceleration of 9.8 ms^{-2}, has not changed. Thus gravity's simulated absence (or microgravity) on the ISS, due to its continuous free-fall around Earth, offers a unique opportunity for novel discoveries of molecular, cellular, tissue, and whole-organism adaptation.

The ability to study genetic and molecular biological responses to micro- and fractional-gravity and the effects of altered gravity, in general, on development, immunity, behavior, stress, and reproduction in a small complex organism is a capability that has been lacking, but desired, by all of the international partners for on-orbit space biology research.

The need for utilization of the fruit fly (*Drosophila melanogaster*) research model is specified in both the NRC Decadal Survey, "Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era" (National Research Council, 2011) and the NASA Space Biology Science Plan (NASA, 2015). The NRC report indicates that, "the fruit fly is well-suited to elucidating certain molecular, genetic, cellular and physiological responses to the space environment within and across multiple generations." The fruit fly is identified in the NASA report as furthering the research accommodation goals of NASA Space Biology, the NASA Human Research Program, and the commercial utilization of space.

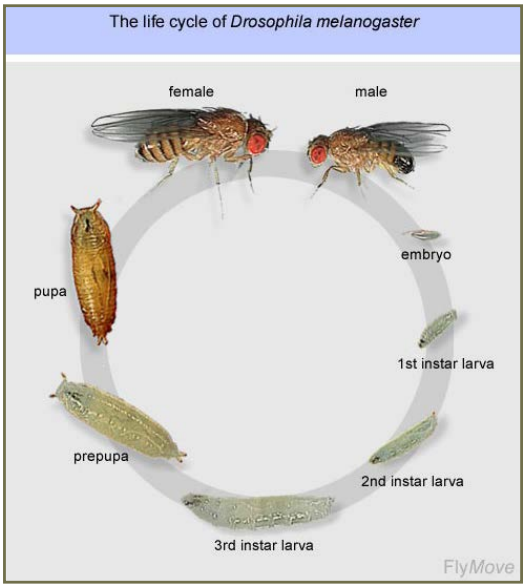


Figure 1: Various developmental stages can be studied in space environments.

The fruit fly *Drosophila melanogaster* provides a well-characterized model organism that is both genetically complex and relatively modest in its habitat and life support requirements. Some of its features are:

- Drosophila have 3 chromosomes, plus X and Y; the entire Drosophila genome has been sequenced
- 77 percent of Drosophila proteins have a match with other organisms (Reiter et al., 2001)
- Adult size is only 3 mm in length; eggs are 0.5 mm in length
- A female can lay up to 100 eggs in one day
- Development requires only about 10 days from fertilization to adult

Research Questions

Subject Area	Potential Research Questions
Immune System Function and Pathogen Interactions in Microgravity	Are the normal defense systems of organisms compromised at micro/fractional/hyper gravity? How is the innate immune system of the fly affected by spaceflight? Can we identify stable molecular markers of the immune response to infection to allow assessment of changes in this response inflight? Can we better understand oxidative stress changes in space at the genetic and molecular levels and test candidate countermeasures?
Cardiovascular System Function in Microgravity	Do changes in gravity and radiation levels affect gene expression related to heart function? Will the muscle cells of the heart lose strength in the microgravity environment? Are arrhythmias more likely to develop after microgravity exposure? Can any observed changes be reversed upon return to 1g, or countered by artificial gravity during spaceflight?
Effects of Long-Term Spaceflight on Development, Aging and Behavior	Do organisms that are raised in altered gravity environments develop normally? Are reproduction, lifespan, and the aging processes affected? What impacts are seen, if any, in multiple generations of flies grown in spaceflight? Is there increased mortality in flies exposed to spaceflight? How does DNA damage and repair, mutational changes and apoptosis occur at a fundamental level? Can the combined effects of radiation and microgravity be defined at the molecular level? What are indices of neurobehavioral effects seen during spaceflight?
Effects of Long-Term Spaceflight on Gene and Protein Expression	Do changes in gravity affect the basic metabolic rate and metabolism of living systems? Are changes in gene expression and protein production in altered gravity environments reversible upon return to 1g?

Table 1: Opportunities for Drosophila Research on the ISS.

Research Areas

Drosophila melanogaster are used extensively in modern laboratories. Scientists have sequenced the complete genome of the fly. Hundreds of strains of mutant flies with known genetic alterations are used to study molecular biology, development, behavior, and other broad areas of research. Many fundamental biological processes, such as embryonic development, skeletal and cardiac muscle function, nervous system function, and circadian rhythms, are highly conserved (very similar) when compared with higher organisms such as rodents and humans, making results from *Drosophila* research highly-relevant to more complex systems.

Drosophila reproduce and mature rapidly, requiring only about 10 days from fertilization to adulthood (Fig. 1). This allows for rapid multigenerational studies with a large, statistically significant number of organisms within a small habitat. *Drosophila* therefore provide a practical model for studying the cellular- and organism-level processes that are altered during spaceflight, and the risk any alterations pose to astronaut health, particularly during long-term missions. Such studies could facilitate the development of future countermeasures. For example, the immune system's cellular and molecular functions are highly conserved and similar between flies and humans. Flies mirror many of the innate immune changes observed in humans post-flight, and serve as a useful and simple model for understanding the underlying molecular mechanisms of space flight-related effects on host innate immunity (Marcu et al., 2011).

Flies and humans also share many of the same genetic and molecular pathways controlling heart development and function. Researchers sent *Drosophila* to the ISS on SpaceX-3 in 2014 in a simple test-tube system to study whether spaceflight increases cardiac dysfunction and cardiac arrhythmias, whether exposure to microgravity alters cardiac cell structure, and whether any genetic and physiologic changes in the heart can be reversed upon return to Earth gravity (Ocorr, 2015).

The rapid maturation and short lifespan of *Drosophila* make them ideal models for studying altered gravity's long term effects on development and aging, as well as its effects on subsequent generations. Long-duration experiments will allow scientists to look for variations in genes and gene expression over time and across generations.

Drosophila share a large part of their genome with humans and approximately three-quarters of genes known to influence disease in humans have homologs in the fruit fly (Reiter, 2001). In addition, in the fly most proteins are encoded by a single gene

whereas mammalian genomes are more redundant, with multiple genes encoding the same or similar proteins, making genetic manipulations more complicated. Whole-genome studies of flies exposed to spaceflight therefore can inform further studies in humans. A current NASA objective, as part of the GeneLab studies is that DNA, RNA, proteins, and metabolites will be sampled from *Drosophila* and other model organisms on the ISS (NASA Ames Research Center, 2014). The raw data will then be uploaded into an open-source life sciences database that contains the integrated gene and biomolecular 'maps' for the tissues and organisms that have flown aboard the ISS (NASA, 2016). The goal is to facilitate access to sets of integrated space-based 'omics' data by the global science community for data mining and comparison with similar data from model organisms and humans generated on Earth. NASA intends to provide grant funds to scientists outside of the agency to work as part of multi-investigator teams in this cutting-edge research that can apply the benefits of space biology research to human exploration in space and human medicine on Earth.

Studying *Drosophila* in space also has the potential to provide insight into normal biological processes on Earth. Microgravity exposure, a unique biological challenge, has unmasked genetic and molecular mechanisms in unicellular and other simpler biological systems, and needs to be studied in more complex whole organisms like fruit flies and vertebrates (NASA, 2015; National Research Council, 2011).

Results from Past Research

Drosophila were the first animals sent into space, and have been used extensively in both Earth and space research in microgravity, hypergravity, and during gravitaxis (movement in response to gravitational force). *Drosophila*-based research has the advantages of small individual size coupled with large population number, powerful genetic tools for manipulating the fly genome, simplicity in rearing, minimal resource requirements for maintenance, and compatibility with space payload constraints on the allocation of power, volume, and mass. The recent development of flight hardware is allowing even more effective science to be conducted in space using *Drosophila* (Marcu et al., 2011).

For decades, researchers have used *Drosophila* to probe the combined effects of microgravity and other conditions of spaceflight with exposure to ionizing radiation, which is significantly higher in space than on Earth. Studies from the 1960s through the 1990s have shown that combined radiation exposure and microgravity causes more genetic changes, often deleterious, than exposure to either variable alone (Oster,1968).

Early Biosatellite research using *Drosophila* suggested that radiation interacts with weightlessness to induce premature aging and chromosome damage in actively growing flies (see Table 2). Multi-generation studies performed on an early Soviet orbital station indicated that flies irradiated before spaceflight had a statistically significant increase in genetic mutations in their first-generation offspring compared to flies exposed to only one of the two variables (Vaulina et al., 1981). Research from the Space Shuttle showed that radiation-sensitive male flies exposed to spaceflight had an increase in lethal mutations among male progeny (Ikenaga et al., 1997).

Drosophila research has also unmasked several of the genes involved in the perception of gravity and gravity's influence on circadian rhythms (Toma et al., 2002; Armstrong et al., 2006). Mapping of the genes and brain regions of the central nervous system that affect gravitaxis (Armstrong et al., 2006) has paved the way for additional experiments examining the effects of microgravity on neural plasticity and learning.

Recent *Drosophila* research on the Space Shuttle focused on how fundamental immune processes are affected by spaceflight, and how microgravity exposure influences host-pathogen responses postflight (Marcu et al., 2011; Taylor et al., 2014). Spaceflight altered cellular and humoral immune responses in both larvae and adult flies, at both the physiological level and at the gene expression level. Maturation of immune cells was delayed, and the ability of plasmatocytes to ingest bacteria was lowered in space-reared larvae. However, adult flies that survived spaceflight were able to clear bacterial infection as efficiently as ground-reared adults.

Date	Response to Microgravity or Simulated Microgravity	Flight	Reference
1967	• Increases in sperm damage and genetic mutations seen in flies exposed to spaceflight and ionizing radiation compared to irradiated ground controls could not be attributed only to weightlessness. Vibration, acceleration, or chemical contamination could also be involved as potential causes	Biosatellite II	(Browning and Altenburg, 1968)
1967	• Higher mortality seen in flown larvae than ground controls, both exposed to radiation • Sex-linked recessive lethality and crossing over when radiation delivered under weightless conditions • Data suggest that radiation interacts with weightlessness to induce premature aging and chromosome damage in actively growing flies compared with ground controls	Biosatellite II	(Oster, 1968)
1975	• Development and aging not affected by microgravity • Mating ability reduced, likely due to wing injuries as a consequence of acceleration or other flight stresses, unrelated to weightlessness	Bion Cosmos (COS 782-10)	(Miquel et al, 1978)
1977	• Mitochondria developed normally in space • Amount of glycogen granules in the wing lower in young flies exposed to microgravity than in ground controls • Developmental processes mostly normal overall • Reduced vitality and shorter life span in flies exposed to microgravity during the first days of life, suggesting accelerated ageing	Bion Cosmos (COS 936-5)	(Miquel et al, 1978)
1981 to 1996	• Flight data suggests that radiation combines with weightlessness to significantly increase chromosome loss and reproductive cell damage	STS-65, Salyut 6 Station; STS-72	(Benguria et al,1996); (Vaulina et al, 1981); (Ikenaga et al, 1997)
1999	• Hardware malfunction in Bioserve incubators leading to indeterminate results for study of neuronal development associated with muscle fibers in embryo and larvae; experiment re-flown on STS-106	STS-93	N/a
2000	• Hardware operated successfully though unexpected temperature drifts occurred in two of the seven specimen containers; data analysis not released	STS-106	N/a
2006	• Changes observed in innate immune function of Drosophila after spaceflight • Capacity of larval blood cells to internalize bacteria reduced postflight • Gene expression changes in response to infection different between space flown adults and ground controls, though female adult flies inoculated with E. coli post flight were able to clear the infection fairly efficiently • Humoral immunity genes down-regulated in larvae postflight • Genes related to Toll pathway affected in adults postflight which may affect fungal pathogenesis	STS-121 (FIT)	(Marcu et. al. 2011; Taylor et. al 2014)

 Table 2: NASA-sponsored Drosophila research flown through 2006.

Opportunities for Research on the ISS:

In 2005 the U.S. Destiny module of the ISS was designated the ISS National Lab (ISSNL) and has become the on-orbit research facility used by NASA-sponsored life sciences investigators. Increasingly, researchers from other Federal entities and self-funded researchers from the private sector have been provided access and have conducted research within the ISSNL. The International partner labs can also be shared and that provides important flexibility and research opportunities. Funding sources for research on the ISSNL are available via NASA, CASIS, other Federal entities, and self-funding by the private sector. NASA will continue to cover the cost of onboard operation and maintenance of research payloads and their transport to and from the ISS. Details on this are provided below.

NASA

NASA's selection of microgravity research projects is guided by recommendations of the National Research Council's 2011 Decadal Survey Report, "Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era" (National Research Council, 2011). The NASA-developed "Space Biology Science Plan" provides an implementation strategy and roadmap based on NRC recommendations and available flight and fiscal resources (NASA, 2015). Extractions of content from research areas and research questions from both of the above sources are included in Table 1 (page 12). The Space Biology Science Plan content extracted was especially from the elements titled, "Animal Biology" and "Developmental, Reproductive and Evolutionary Biology". Additional information on funding sources for this research is provided below.

CASIS

In 2011 NASA chose the non-profit Center for the Advancement of Science in Space (CASIS) to be the sole manager of the ISSNL for non-NASA funded research. Their mission is to maximize innovative use of the ISSNL to pursue national priorities in science, technology, engineering, and mathematics and to expand the U.S. economy. CASIS-related PIs can come from non-NASA government, commercial, and academic entities.

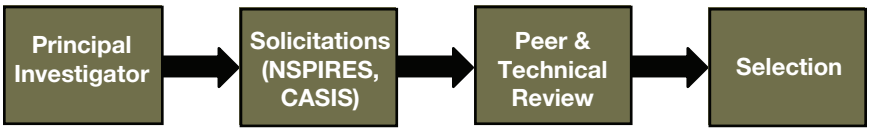
CASIS, in collaboration with NASA ISS staff, can support PIs in all stages of payload development (http://www.spacestationresearch.com/getting-to-space/support-services/) and help to match Principal Investigators with Implementation Partners (http://www.spacestationresearch.com/facilities-hardware/implementation-partners/) who can provide additional support such as access to heritage hardware,

new flight instrumentation, or help to accommodate investigator-provided payloads. Implementation Partners can also collaborate with NASA to help streamline the process of payload development, hardware testing, transport to orbit, and ISSNL integration.

Solicitations, Proposals & Funding

NASA Research Announcements (NRAs) are managed through NSPIRES, the online NASA Solicitation and Proposal Integrated Review and Evaluation System (http://nspires.nasaprs.com/external/index.do). This system supports the NASA research process from the release of solicitation announcements through the peer review and selection processes.

Additional research announcements can be found at the Center for the Advancement of Science in Space (CASIS) http://www.iss-casis.org/Home.aspx. CASIS also encourages unsolicited proposals and sponsors prizes.



Proposals submitted for NASA's research opportunities undergo scientific and technical review. CASIS has a similar process that is streamlined to optimize ease of user access to the ISSNL.

Facilities on ISS and How to Choose Them

In general, U.S.-developed ISS research facilities fall into three availability categories: currently onboard; in development with a targeted launch date; and certified/flown on the STS and undergoing re-certification for ISS. NASA can provide access to hardware in these categories. A fourth category includes facilities or equipment available from a commercial source either through NASA, through CASIS, or provided directly by the developer. The available and soon-to-be available U.S. facilities that support fruit fly research are listed below. At this time there do not appear to be any international hardware facilities available for fruit flies. It is important to recognize that the increasing collaborations between NASA, CASIS, various private sector entities and the ISS international partners, have inevitably resulted in a more dynamic hardware development and utilization cycle. That often includes facility evolution that will be illustrated in this section.

Experiment Facilities

Fruit Fly Laboratory

The Fruit Fly Laboratory (FFL) was developed to support *Drosophila* studies on the ISS. This was done in part by upgrading capabilities of existing NASA hardware used for a successful immunology study ("Fly Immunity and Tumors", or FIT), on STS-121 that returned about 3000 flies for data analysis (Marcu et al., 2011; Taylor et al., 2014). It was also developed in part by commercial entities interested in providing new research facilities for a broader research community.

The goal of the FFL was to provide a set of tools and assays that let Principal Investigators conduct further research on the ISS in areas known to be impacted by spaceflight such as immune response, behavior, development, and genetics. The FFL had three major elements. The first was an assembly of Cassettes that could safely transport fruit flies to the space station (Fig. 2). The second was Food Change-out Platforms used to change the fruit fly food without breaching containment guidelines and allow extraction of the fruit fly larvae for observation and preservation in microgravity (Fig. 3). The third was the NanoRacks, LLC Platform-3 (http://nanoracks.com/products/platform-3/) with Centrifuge and Microgravity Containers (Fig. 5) that housed Cassettes at microgravity and would provide an artificial gravity environment for an equal number as on-orbit 1-g controls or for variable-g studies.

Astrium North America, in the early 1990s, developed a multi-purpose, integrated microgravity research facility named "Biorack" for use in the STS SpaceLab.

One feature of the facility was accommodation of small, standardized aluminum containers that could hold a wide array of biological cassettes that plugged into the various Biorack systems. These systems provided, as either Type I or Type II containers (two sizes), controlled life support conditions, access by the crew to the containers and their cassettes via a small glovebox, and continuous exposure to either microgravity or artificial gravity on a small centrifuge.

NanoRacks, in order to provide variable gravity to its commercial customers conducting research on the ISS, negotiated with Astrium to develop an upgrade of their archived Biorack centrifuge. This NanoRacks centrifuge was integrated with a multipurpose NanoRacks Platform 3 experiment support system that resides on the ISS. Recently, Astrium became part of the Airbus Defense and Space company (Airbus DS; http://airbusdefenceandspace.com/). Airbus DS then, via NASA contract, provided a major upgrade to the Type I container concept described below that integrates with the NanoRacks platform 3 and supports several of the initial research objectives of the NASA Fruit Fly Laboratory (FFL) on ISS.

The FFL was developed under NASA sponsorship and envisioned as having two phases that could possibly have some overlap.

FFL Phase I Systems

Phase I would use fly cassettes like those flown successfully in 2006 on STS-121 in Type I Biorack containers (Marcu et al., 2011; Taylor et al., 2014). But for FFL-01 the cassettes (Fig.2, without Type I container lids) would be inserted in new Airbus DS Observation Units (Fig.4). Phase I would not have active environmental controls (such as temperature, CO_2, O_2, and humidity). Hardware would be kept at ambient ISS conditions, which are typically within a range that supports healthy viable fly cultures. Phase I would allow for multigenerational growth, 1 g in-flight controls (centrifuge), and video observation for health/status checks and behavior observations (Fig. 4, Airbus DS camera observation system).

For Phase I, the on-orbit FFL-01 would:

- Provide 1-g control (NanoRacks centrifuge).
- Support an equal number of habitat cassettes in microgravity as on the centrifuge.

- Monitor atmospheric conditions (temperature and humidity) for specimens and have the capability to store/transmit the data daily to the ground.
- Provide controlled day/night light cycles in both microgravity and centrifuge modes.
- Record high-resolution digital video of *Drosophila* behavior during both day and night cycles for the initial projected 30 day duration flight experiment.
- Allow observation, removal and replacement of all cassettes during the mission to support experiment procedures including food tray change-outs, food tray content observation, biosampling, and preservation.

A ground-based experiment control for FFL-01 would be contained within an environmental simulator facility capable of reproducing on-orbit carbon dioxide and oxygen levels, relative humidity, temperature, and lighting conditions based on actual data logged during the ISS flight mission.

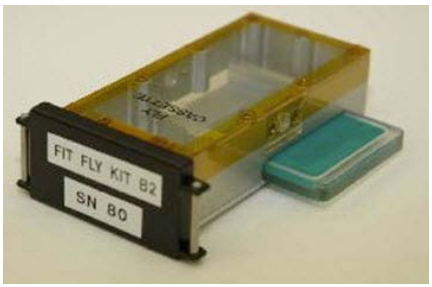


Figure 2: Fly cassette and food tray (partially inserted) shown with a Type I container lid (black).

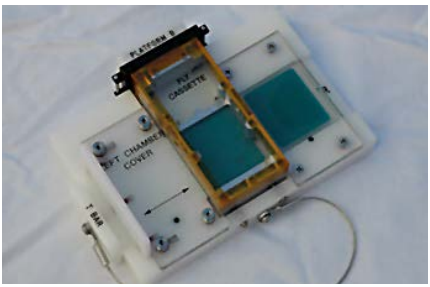


Figure 3: Food tray change-out platform.

Six each of the above fly cassettes, contained within new Observation Units (Fig. 4) would be inserted in the NanoRacks microgravity and centrifuge slots (Fig. 5) at any given time. The NanoRacks microgravity and centrifuge systems would be onboard the ISS for FFL-01. All other FFL elements would be transported to and from the ISS via a SpaceX Dragon capsule.

Successful food tray change-out by crew during flight was demonstrated on STS-121 in order to study multigenerational growth. This same procedure would be carried out on the ISS regularly to support studies over even more generations of flies.

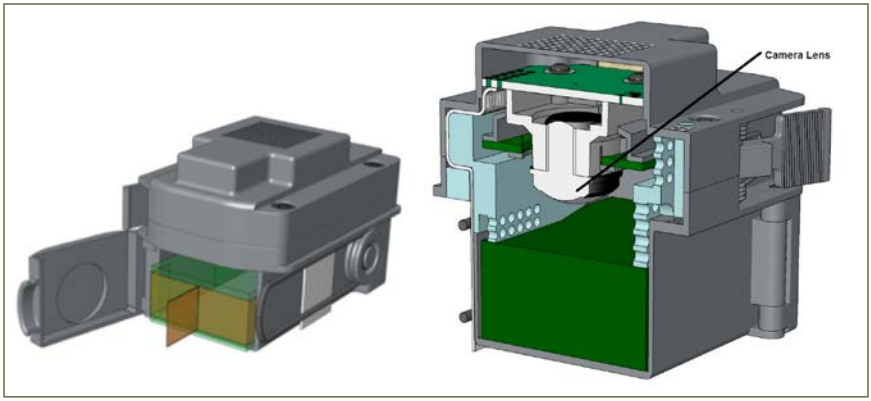


Figure 4: Left: Observation Unit (OU) with fly cassette (tan) installed and door open. Right: Enlarged cut-away back view of OU showing fly cassette (dark green), camera lens and the ventilation exhaust on top.

Airbus DS developed a new video monitoring and light control system within each OU (Fig. 4) compatible with the NanoRacks platform. Previous research validated a methodology to quantify fruit fly behavior and viability data (Fig. 9) from the video footage (Chan et al., 2012; Inan et al., 2009, 2011). The centrifuge and microgravity rack slots (Fig 5) flexibly accommodated the identical OUs.

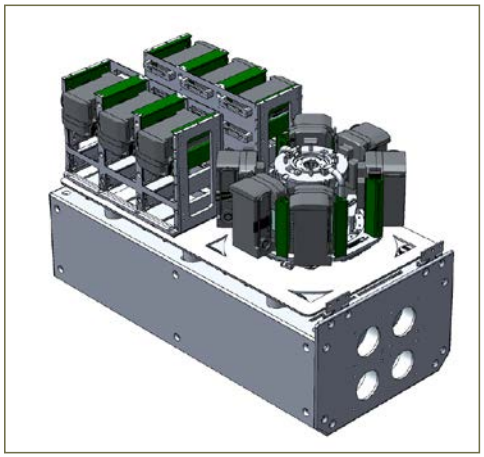


Figure 5: Observation Units (OUs) integrated on NanoRacks Platform 3. Six OUs each are installed in centrifuge and microgravity slots.

New FFL Controller Units (CUs) for these systems were developed by Kayser Italia (http://www.kayser.it). Identical CUs are shown mounted on the central rotor and two empty slots of the centrifuge and similarly within the microgravity racks for data capture (Fig. 6). Two slots in the centrifuge are used to hold system electronics that control the experiment.

Each Controller Unit manages up to six USB 2.0 interfaces for camera

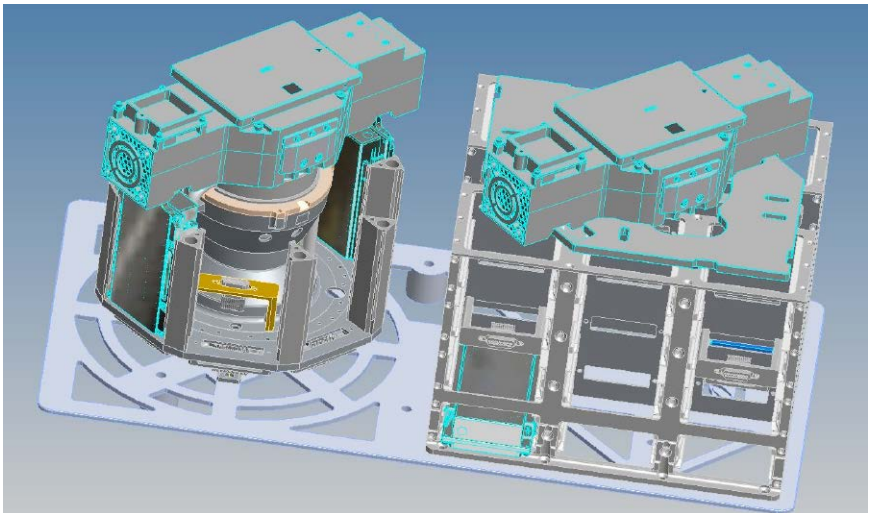


Figure 6: Controller Units (CUs) with removable Solid State Devices (SSDs) installed on microgravity and centrifuge elements of NanoRacks Platform 3.

video and image acquisition from the OUs and provides a configurable set of drivers for visible and infrared illumination, as well as a cooling fan. All data are stored on the removable hard drive (SSD) mounted on top for easy access and replacement by the crew. The design and integration of each CU (Fig. 6) allows independent

Figure 7: The stand-alone CU configuration showing cable connectors and removable SSD on the top.

Figure 8: NanoRacks Platform 3 assembly for insertion in an ExPRESS Rack.

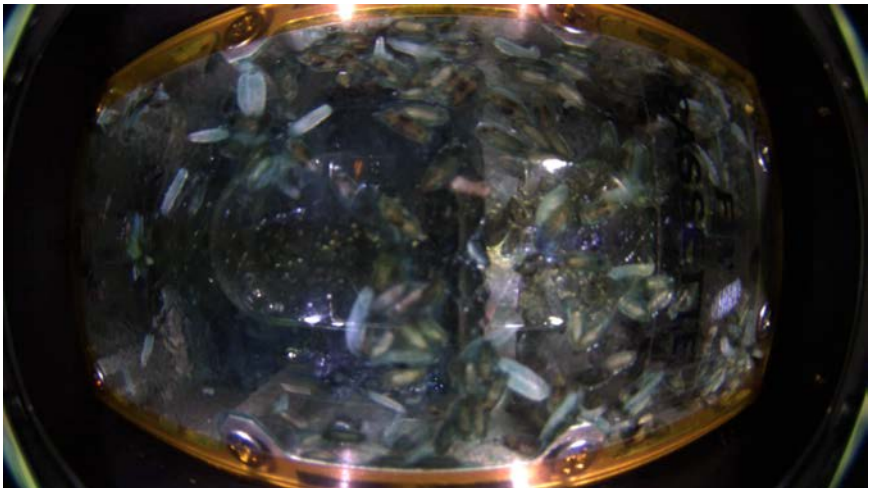


Figure 9: The prototype video camera during development phase showing flies and larvae under white light during ground tests. An infrared light was available, for in-dark imaging.

extraction of OUs, as needed. Intrinsyx Technologies Corporation (http://www.intrinsyx.com) developed the Application Software (ASW) that operates the CUs and is stored in non-volatile memory within each CU. Thus, the removable SSD is used for science data collection only.

Limited fixation of biosamples was available for Phase I of the FFL (Fig. 10) and the first launch to the ISS (FFL-01). A clear flexible plastic Disposable Glove Bag (DSB) was used to contain equipment for many routine onboard operations, including potential future fixation of biosamples. Within a DSB, used food trays could be removed from the cassette using the change-out platform and inserted into an open ended Fixation Bag and then closed with a sealing clamp, as developed by BioServe Space Technologies (http://www.colorado.edu/engineering/BioServe/). After removal of any residual air from the Fixation Bag by use of an Air Evacuation syringe, RNAlater® (an RNA stabilizer) could be injected into the Fixation Bag via a one-way valve using the BioServe Fixation Kit syringe, and the bags labeled and frozen in the Minus Eighty Degree Laboratory Freezer for ISS (MELFI) for return to ground (Fig. 17). The ISS Microgravity Science Glovebox is available onboard as a backup for these operations, should the need arise (Fig 18).

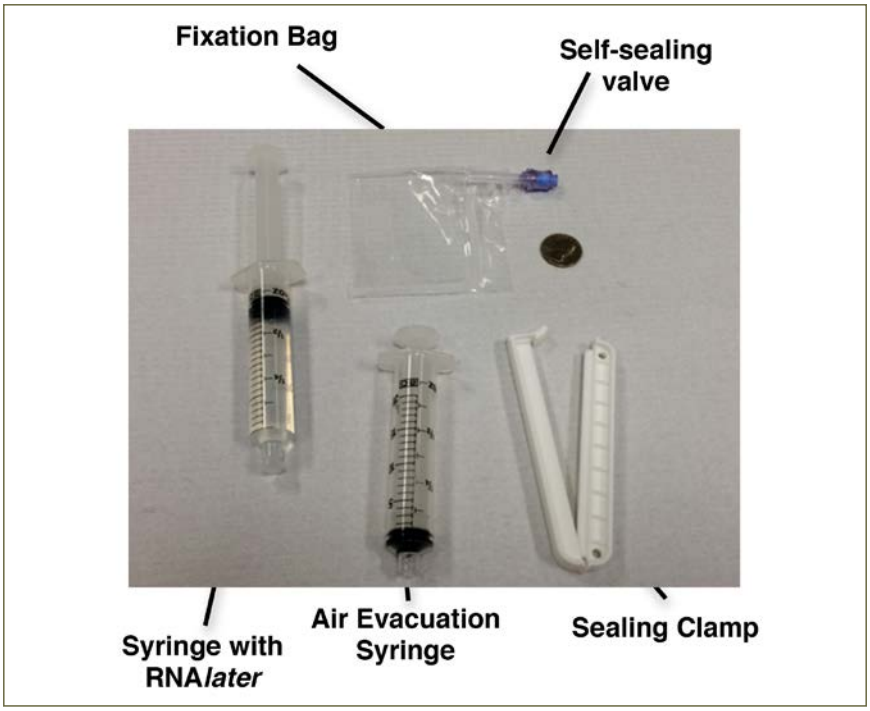


Figure 10: Fixation Tools (BioServe Space Technologies).

FFL Phase II Systems

Phase II hardware was planned to evolve based on the FFL-01 flight hardware validation test results (see Recently Flown section, below) and unmet and/or evolving PI requirements. Several desired additional experiment capabilities for the future are listed here. Some of these capabilities have been or are being provided in additional FF flight hardware systems briefly described in the sections below.

- Environmental controls: Phase I hardware can only be maintained at ISS ambient, with no control of variables such as temperature, relative humidity, and gas composition. Environmental control is important for conducting well-controlled 'omics' type experiments and experiments with isolated stressors (such as heat shock and oxidative stress).

- A larger food volume: Current Phase I hardware only provides 3.4 ml of food, which does not sustain a large population of flies for long. This necessitates frequent food change-outs requiring more crew time and cold stowage volumes. An increased food volume would also ensure that animals remain well-fed in flight.
- A larger chamber volume: Current Type I cassettes have limited space for sample growth, and overcrowding can become an issue. With larger cassettes, larger sample populations could be supported under healthy conditions, yielding improved statistically significant data.
- Separation of life stages to allow inflight fixation of all stages (eggs, young larvae, older larvae, pupae, and adults).
- Real-time or near real-time video data for assessment of fly cultures by investigators on the ground. Current Phase I hardware will permit video collection and storage in flight, with investigator visualization only available postflight.

Recently Flown

The FFL-01 flight was envisioned primarily as a facility and operations validation project as described above (see *Fruit Fly Laboratory*, page 15). That flight was conducted in January 2015 via the SpaceX-5 Dragon capsule to the ISS. The follow-on FFL-02 is anticipated to be the first flight to be driven primarily by PI science requirements. It is now projected to fly on SpaceX-11 but will use NASA-developed hardware described below (see *In Development*, page 26).

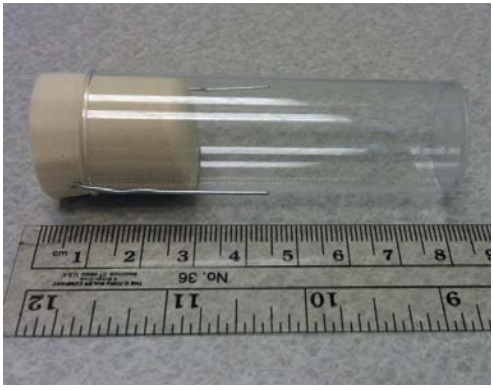


Figure 11: Single vial used to hold male and female flies and food.

The FFL-01 flight validated the function of several of the basic hardware elements in the FFL assembly on the ISS, but two fundamental problems were identified. The thermal load produced by the avionics in the NanoRacks Platform 3 system was

Figure 12: HEART Flies NanoRacks container (left) and the 15 vial assembly ready for insertion.

upstream from the ambient airflow going to the Fly Cassettes inside the Experiment Containers. Since there was no active temperature control in Platform 3 the resulting high temperatures killed the flies. And, compared to the original Biorack centrifuge load (as flown on the STS), the new Observation Units and the Controller Units added significant mass and that apparently prevented proper activation of rotation of the centrifuge motor (crew mechanical assistance was required to start rotation). NanoRacks and Airbus DS reviewed these results postflight with NASA, and the two companies agreed that NanoRacks would no longer be involved and would transfer responsibility for addressing the technical problems identified to Airbus DS. Progress on that is described below (see *In Development*, page 26).

HEART Flies

In early 2014, a fruit fly experiment payload that won a CASIS-sponsored International Space Station Research Competition was flown to the ISS and exposed to microgravity for a month. The goal was to better understand how spaceflight affects the fruit fly cardiovascular system. The HEART Flies payload remained in a Cargo Transfer Bag on Dragon for its berthed phase on the ISS during SpaceX-3.

The HEART Flies payload (http://www.nasa.gov/mission_pages/station/research/experiments/1317.html) was sent to the ISS in a NanoRacks-compatible container

with 15 vials (Fig. 11) and approximately 9ml of standard yeast/molasses fruit fly culture medium in the bottom (not shown). The container box (Fig. 12) had internal dimensions of 94 x 94 x 130mm. The fruit fly vials had open foam plugs so air could passively circulate, and there were air holes in the box as well. There was no control over environmental variables such as humidity, temperature, and light/dark cycles since those were dependent upon the ambient conditions aboard the ISS.

There were 15 females and 5 males per vial; one box containing 15 vials was launched to the ISS for a 30 day mission. These adult flies laid eggs in microgravity that successfully developed, pupated and eclosed producing adult flies that were 1-3 week old on return to 1G. There were approximately 20 to 60 adult progeny per vial and approximately the same number of eggs, larva, and pupae upon return to Earth. The flies included three different wild type lines and two lines of flies that are prone to heart arrhythmias. About 48 hours after splash down the flies were returned to the lab and all adults were analyzed within 8 hours for viability, climbing ability, and heart function.

Post flight, heart and skeletal muscle function was first assessed indirectly using climbing assays in which flies are placed in a vial and knocked down by tapping. Flies are negatively geotactic so they will immediately try to climb up the sides of the vials and the rate at which they climb can be quantified. Heart function was then assessed directly by anesthetizing and dissecting the flies, exposing the heart and using high speed digital cameras to record the beating heart. Analysis of videos provides precise information on heart contraction and relaxation times, as well as rhythmicity and contractility. The researchers also assessed the effects of space flight on morphology by subsequently applying immuno-histochemical techniques to the exposed hearts. Finally a methodology for performing RNA seq on very small tissues samples was developed that allowed researchers to assess gene expression changes in the isolated hearts. Subsequent missions will assess longer term and multi-generational effects on heart function in the fly. In sum, the fruit fly appears to be an excellent model for studying the cardiac effects of prolonged exposure to micro gravity (Ocorr et al, 2015).

The HEART Flies study is viewed as an exploratory precursor to a follow-on effort that could fly on a future FFL.

Ames Fly Experiment (AFEx)

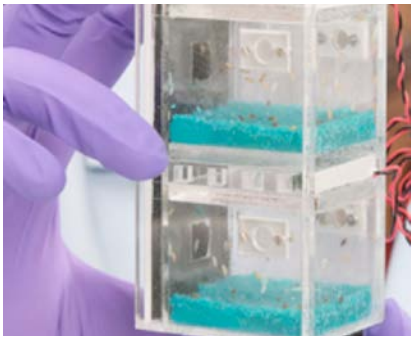


Figure 13a: The two populations of flies were housed in separate transparent chambers. No separation of generations is possible. The fly food (17 - 34 ml each) is colored with blue dye as shown at the bottom of each chamber.

Figure 13b. The integrated AFEx payload was about 10 x 10 x 15 cm in size and utilized external power.

The Ames Fly Experiment (AFEx) payload (Fig. 13a, 13b) flew on SpaceX-4 (2014) and was student-developed in collaboration with the American Society for Gravitational and Space Research (https://www.asgsr.org), NASA ARC and NanoRacks. The payload was housed in a NanoRacks platform within the Japanese Experiment Module on the ISS. Dr. S. Bhattacharya, Director of the Biomodel Performance Lab in the ARC Space Biosciences Division mentored the students in utilization of fruit flies for space research. The overall goal was to provide college students a hands-on opportunity to do an experiment in space from beginning to end. The research goal was to study oxidative stress (OS) during spaceflight by flying normal (wild-type) and OS resistant (mutant) strains of flies and utilize video to monitor fly neurobehavioral responses and OS metabolic pathways analyzed postflight from flight-related biosamples. Biosignatures of OS have been observed in ISS astronauts and the hypothesis was that the mutant strain would show fewer effects of spaceflight than the normal strain. The payload accommodated circadian lighting (day/night video), and monitoring of temperature, relative humidity, O_2 and CO_2 levels. Also telemetry downlink of video images and data was provided. The AFEx hardware itself worked successfully in flight and data collection and telemetry was also successful thereby validating this student developed hardware for future flight missions. However the NanoRacks platform used to power this hardware was not able to maintain the required temperature, therefore confounding the science data and rendering it unpublishable. However, as discussed previously,

the problem with the NanoRacks platform can be circumvented by using other platforms to support this hardware such as the Bioserve SABL (see Vented Fly Box below) or STaARS-1 (page 29). A preflight profile of the AFEx payload provides more detail (http://www.nasa.gov/ames/research/space-biosciences/afex-spacex-4).

In Development

Vented Fly Box (VFB)

This is a NASA-developed upgrade to the HEART FLIES payload (described above) that is undergoing Experiment Verification Tests at ARC, and will be the hardware used for the next two missions in the series, FFL-02 and FFL-03. Use of the VFB requires minimal crew time since no food changes are needed. Temperature control will be provided by installation of two VFBs in a new on-board incubator (SABL - Space Automated Bioproduct Laboratory, BioServe, https://www.nasa.gov/mission_pages/station/research/experiments/1283.html). Temperature is monitored from the ground, and four iButton (Maxim Integrated) data loggers internal to each VFB capture temperature and relative humidity levels incrementally throughout the mission for postflight data retrieval. Fifteen vented vials are provided within each VFB with about 10 ml of food in each (Fig. 14a, 14b).

In the FFL-02 flight configuration six VFBs will be launched to ISS in a Cargo Transfer Bag (CTB) designed to maximize air diffusion. Once on-orbit, the crew will transfer four to the SABL locker held at 18 deg C to slow the development of the samples (flies and eggs) and yield appropriate aged samples postflight. Two

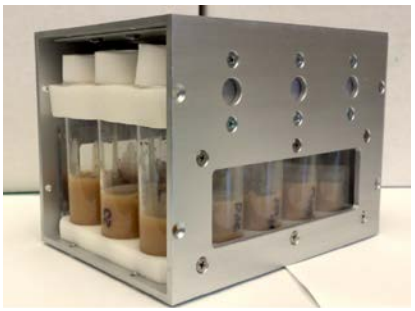


Figure 14a: The vial assembly slides into the ventilated metal shell that allows for even temperature distribution within the SABL unit.

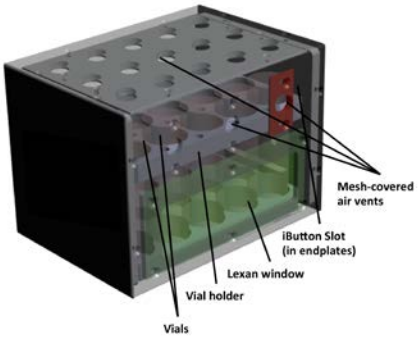


Figure 14b. The VFB is intentionally designed as a simple system. The battery-powered iButton data loggers (located as shown) will capture temperature and relative humidity measurements within each VFB.

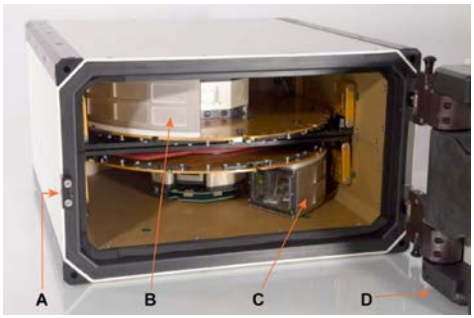


Figure 15a. The MVP locker (A) is shown with its door opened (D) to view its interior. One Experiment Module (B) is shown on the top carousel, one of six that can be accommodated on each carousel (rotating plate). An Experiment Module (C) is also shown on the bottom carousel.

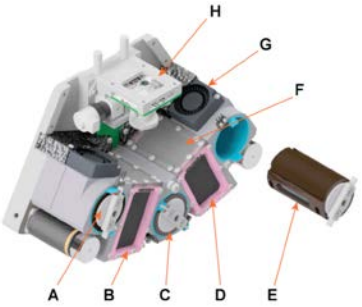


Figure 15b: The Drosophila Experiment Module elements. Food cylinders 1 (A), 2 (C) and 3 (E) are shown. Food cylinder 3 (removed here) shows a covered Food Access Slot on its side that can be opened/closed by turning the handle at the end. Flies have controlled access via the Slots to two Fly Chambers (B & D). The video system (H) monitors the behavior of adult flies via a transparent window (F) in each Fly Chamber. Airflow is provided by a Blower Fan (G) located adjacent to each Fly Chamber.

will remain in the CTB and be held at ISS ambient temperature throughout the flight to yield samples that had nominal growth for postflight analysis. All six VFBs will return in the CTB in which they launched, retrieved shortly after Dragon splashdown, and transferred to the PI for sample analysis. Minimal crew time is required for this experiment scenario.

Multi-use Variable-Gravity Platform (MVP)

This payload is in commercial development by Techshot, Inc. and accommodates various standard biological experiment modules for a range of organisms, including *Drosophila* (http://www.techshot.com/documents/MVP.pdf). The MVP (Fig. 15a) includes twin independent 0 to 2g self-balancing centrifuges allowing a wide range of gravity environments to be studied in spaceflight. The MVP is targeted for launch to the ISS in Summer 2017. It will provide temperature control from 14 to 40°C and humidity control between 50-85%. It will have separate data channels for each experiment module for monitoring and control. Each module will also have its own video system and lighting controls. This facility will actively introduce fresh ISS cabin air to the Drosophila experiment modules thereby maintaining a suitable environment for the flies.

Figure 16: The STaARS-1 Platform. It includes space for eighteen static and six variable gravity experiment containers (EC). The ECs are provided by Airbus DS through their my_bioracks portfolio. Direct communication with the ECs will be maintained terrestrially by STaARS providing researchers updates on experiment progress. Temperature will be controlled and monitored within the platform from ISS ambient to 40˚C.

The *Drosophila* experiment module is shown (Fig. 15b) as a computer graphic with its enclosure removed to view internal elements. Detailed operational capabilities for this module are available (http://www.techshot.com/documents/MVP%20Drosophila.pdf). The module is designed with removable food cylinders and "fly gates" (or slots) to allow controlled access to food and adult fly chambers. This will allow the ability to study multiple generations of flies. The "gates" are manually controlled in the initial design but after validation, the gates could be automated. The MVP is designed to communicate via the ISS data system with the ground-based Techshot Payload Operations Control Center (POCC) where data can be tracked and monitored. Thermal, humidity, light cycle and gravity control can be done autonomously by the MVP. It also monitors on-orbit carbon dioxide and oxygen levels and ambient pressure.

Figure 17: ISS Commander Sunita Williams and Aki Hoshide transferring MELFI samples during ISS Expedition 33.

STaARS-1 Platform

After problems were identified with some hardware during the FFL-01 flight of the FFL, Airbus DS teamed with Space Technology and Advanced Research Systems, Inc. (STaARS), a company with both microgravity science and technology development capabilities (http://www.staars.space/) located in the Houston area. STaARS is developing a replacement for the earlier NanoRacks Platform 3 (see *Recently Flown* section, page 22). All the system elements utilized for FFL-01 will be accommodated. The two companies have a cooperative agreement and STaARS has signed a Space Act Agreement with NASA. STaARS is also an Implementation Partner with CASIS (see *CASIS*, page 13). This commercial team intends to provide this new capability on the ISS by early 2017.

This STaARS-1 Platform (Fig. 16) will support the full Airbus DS "my_bioracks" portfolio (https://www.my-experiment-in.space/assets/my-biorack-rz-web.pdf). The platform will provide access to fully controllable and programmable experiment containers (EC) both for microgravity experiments and via a stronger variable speed centrifuge, for simulated gravity conditions from 0 to 2 g. STaARS-1 will provide an

Figure 18: Microgravity Science Glovebox (MSG).

on-demand temperature controlled environment from cabin ambient to 40°C. Both the static rack and the centrifuge have available space for the Kayser Italia controllers described above (see FFL Phase I System) to control the my_biorack experiment container activation times as required by the researchers. Data transmission from the STaARS-1 to ground will provide confirmation of EC run activation and termination as well as platform temperature conditions. Internal temperature is continuously monitored and recorded. STaARS will maintain redundant systems on the ground providing researchers access to test experimental conditions and run controls if needed.

Multipurpose Facilities

Minus Eighty-Degree Laboratory Freezer for ISS (MELFI)

The MELFI (Fig 17) will support a wide range of life science experiments by preserving biological samples (such as blood, saliva, urine, microbial, insect, or plant samples) collected aboard ISS for return and analysis back on Earth. Although MELFI is technically capable of operation at any set point between +10°C and

-99°C, the three standard operating modes are -95°C, -35°C and +2°C. The Dewar temperature is continuously monitored and recorded real-time. During power-off phases, a battery-powered temperature data recorder operates to continue recording Dewar temperatures. To ensure efficient thermal insulation, the space between the double walled Dewars is pumped to a very high molecular vacuum.

Additional freezers can be used to support *Drosophila* research including the Glacier, and Polar systems. Information for them can be readily found in the ISS Experiment & Facilities Catalog (https://www.nasa.gov/mission_pages/station/research/experiments/facilities_hardware.html#Multipurpose).

Microgravity Science Glovebox

The Microgravity Science Glovebox (MSG; Fig 18) was built by ESA but is operated by NASA and is the largest glovebox flown in space. It is located in the U.S. Destiny module and provides containment for experiments, insuring that small or hazardous materials do not escape or float about in the cabin. Crewmembers use one or more of four glove ports to manipulate materials or experiment systems that are transferred inside via a special opening or through a removable front window. It has a video system and data downlink to allow monitoring of enclosed experiments from the ground.

Lessons Learned

Guidelines and lessons are provided below regarding logistics and operations for conducting research on the ISS, and for fruit fly research, in particular.

General - Payload/Experiment Logistics & Operations

It is important to new experiment proposals and design strategies to understand the lessons learned by others with prior ISS research experience. Some of those are provided below by experienced Implementation Partners, ISS payload support personnel, and/or hardware providers. See the final section for additional information and points of contact. Some general guidelines include:

- Standard laboratory procedures such as pipetting for fluid transfers must be modified for containment purposes. Many payloads use syringes with special cannulae attachments interfaced with needle septa for this purpose.
- Researchers requiring cold stowage need to define their requirements to ensure proper storage of reagents, cell-culture stocks, and biosamples for durations that will prevent the loss of viability. Fixative chemicals and other reagents are assigned hazard classifications that define the required levels of containment.
- The current path for experiment payloads to reach the ISS is by transfer (turnover) to a SpaceX Dragon spacecraft (http://www.spacex.com/dragon) at NASA KSC between 24 and 18 hours before launch. Allowance must be made for the time it takes from launch to docking to transfer your payload to the ISS experiment integration site when designing an experiment.
- Dragon is currently the only payload transportation capsule going to the ISS that also returns them. The need for live biosample return must be carefully considered based on the duration of time the live specimens will be in a 1-g environment on the ground before turnover to the PI.
- There is limited cold and ambient stowage availability for ascent to and descent from the ISS. The GLACIER freezer (https://www.nasa.gov/mission_pages/station/research/experiments/351.html) has a permanent rack location assigned to it within Dragon and the Polar freezer (https://www.nasa.gov/mission_pages/station/research/experiments/1205.html) can also accommodate researchers' cold stowage requirements to and from the ISS.
- The Dragon capsule does not include crew, so manual experiment procedures need to be deferred until experiment integration occurs within the ISS. Return of biosamples to the PI after reentry takes approximately 72 hours.

Fruit Fly Specific - Payload/Experiment Logistics & Operations Scenarios

Preflight Logistics Operations (for Transport via Dragon to ISS of the FFL hardware)

- Initial Fly Population in Cassettes: has typically been about 10 virgin females and 5 virgin males to produce the optimal number of progeny for flight experiments.
- Fly Population Density Optimization: food quantity and fly development volume should not constrain development; larvae need excess food to ensure proper development to the adult stage.
- Fly Type Options: mutants with specific abnormalities (e.g. heart arrhythmias); lab vs. wild types; development time to adult tends to be strain-specific.

Fly Cassette Operations (on-orbit)

- Food Tray Functions: supplies food to adult flies and larvae; provides habitat for egg laying and larval development; provides transfer system for eggs/larvae to secondary development cassette for adult flies; transfers used to separate the generations, prevent overcrowding and maintain healthy cultures throughout flight.
- Treated Food Options: can add microbes to food to study immune response to infection; can add antibiotics or other compounds to study drug effectiveness in spaceflight.

Food Tray Change Out (on-orbit)

- ISS requires full containment of food trays during change-outs from the Fly Cassettes
- The Food Change-out Platform is used inside a transparent Disposable Glove Bag with operations conducted in the area adjacent to the FFL.

Ground Controls

- Learn how to operate flight-type hardware to ensure experiment procedures produce desired results and to streamline crew inflight ops to the maximum.
- Conduct an Integrated Experiment Verification Test using flight procedures to validate operations and the timeline, and to identify unanticipated problems.

- Learn hardware identification system and inflight hardware inventory system so one can oversee crew operations to help minimize errors.
- Takes up to 1 year to develop a flight experiment on ground prior to launch, so allow sufficient time.
- Development duration of flies is often strain-specific and should be tested in flight-type hardware on the ground to clearly define on-orbit crew operational requirements.

Inflight Controls

- The NanoRacks Centrifuge (see section in Recently Flown, above) was designed to provide 1-g and variable-g controls to an equal number of Fly Cassettes (up to 6 each) being held at microgravity to provide high-quality controls and gravity-threshold studies. Note problem found on FFL-01 flight and the STaARS-1 platform proposed redesign as a solution in 2017. Also see MVP solution by Techshot proposed for early 2017.

Experiment Procedure Changes

- Preflight training of crew may be conducted utilizing flight-type hardware and experiment procedures if complicated operations are anticipated. Standard food change-out operations training can be conducted via on-board ISS video.
- Inflight retraining and procedures modification is now possible using ground-to-space video systems that can take advantage of real-time lessons learned by crew and the PI.

Developing and Flying Research on the ISS

Conducting Space Research: The ISS Environmental Conditions

Understanding the key aspects of the ISS research environment is important for developing appropriate experiment designs. Three aspects of the internal environment are profiled below.

Microgravity

Microgravity on the ISS is typically about one millionth of Earth gravity, and is effectively an environment of near-weightlessness. But gravitational force is not the only one that can be relevant to a biological experiment (DeLombard et al., 2004). Disturbances may be caused by vibrations (e.g. pumps, fans, exercise systems) and/or transient operational phenomena (e.g. valve operation). The magnitude of these impacts on the gravitational environment can range from 0.01-g (briefly for a thruster jet) to below one millionth of 1-g (prolonged for atmospheric drag in orbit).

For biological experiments it is important to understand the differences between the direct effects of microgravity, in which the system perceives changes in the gravitational force directly, and indirect effects in which the system responds not to the lack of gravitational force itself, but to changes in the local environment induced by the conditions of microgravity. The reduction in gravitational forces on biosystems results in decreased buoyancy driven flows, rates of sedimentation, and hydrostatic pressure. In general, fluid dynamics are also altered, and there is a near absence of convection (National Research Council, 1998).

Radiation Exposure

NASA's goals for exploration include a significant focus on understanding the effects of space radiation on humans in space and developing strategies to mitigate adverse effects. While there is a large body of existing literature on the effects of low linear energy transfer (LET) radiation such as gamma rays and x-rays on biological samples, including data from long-term animal studies, clinical studies, and others, the information on radiation of the kind encountered in space (e.g., protons and high-LET radiation such as heavy charged ions) is less well-defined (National Research Council (U.S.), 2011).

Crews aboard the space station receive an average of 80 mSv for a six-month stay at solar maximum (the time period with the maximum number of sunspots and a

maximum solar magnetic field to deflect the particles) and an average of 160 mSv for a six-month stay at solar minimum (the opposite condition). Although the type of radiation is different, and therefore biological effects may vary depending on the biological parameter, one mSv of space radiation is approximately equivalent to receiving three chest x-rays. On Earth, we receive an average of two mSv every year from background radiation alone.

Ambient Gas Concentrations and Pressure

The air within the ISS is dynamically controlled to be very close in gas concentrations (nitrogen = 78%, oxygen = 21%, carbon dioxide = 0.5% (significant variability), water vapor = 1%) and total pressure (14.7 PSI) to our atmosphere on Earth. Nitrogen and oxygen stored in tanks is released automatically based on sensor readings and carbon dioxide is chemically adsorbed.

Experiment Accommodation on the ISS

Experiment payloads are all held within International Standard Payload Racks (ISPRs) within the ISS. Each ISPR consists of an outer shell that provides a set of standard interfaces, a support structure, and modular equipment for supporting research hardware. Each can accommodate one or several experiments.

Through the ISPRs, the ISS payload experiments can be provided with the following ISS resources available on the U.S. Laboratory, also known as Destiny:

- Electrical power
- Thermal control
- Command/data/video
- Vacuum exhaust/waste gas
- Gaseous nitrogen

The EXPRESS Rack System is available to support small, sub-rack payloads with power, data and cooling within an ISPR. EXPRESS racks were designed to accommodate payloads originally fitted to shuttle middeck lockers and International Sub-rack Interface Standard drawers, allowing previously flown payloads to easily transition to flight on the ISS (NRC Decadal Study, 2011).

Developing Your Space Flight Experiment

Several milestones along your experiment development path are described below as a NASA-supported process that applies to PIs who are NASA-funded grantees (see *Opportunities for Research on the ISS*, page 13). However, a similar, streamlined process is required for PIs who are not NASA-funded including CASIS grantees, other government grantees or self-funded commercial entities. In this non-NASA funded case, PIs are considered participants on the CASIS-managed ISSNL and support is provided to them by CASIS and one or more Implementation Partners (http://www.spacestationresearch.com/facilities-hardware/implementation-partners/).

After undergoing scientific and technical review, when a proposal is accepted as a space flight candidate, a flight experiment team is formed and the development cycle is initiated and proceeds in phases. The PI will be supported by an assigned Project Scientist who functions as the advocate and liaison for the PI and assists with the experiment development process.

For optimal use of the limited on-orbit resources, experiments may be combined where feasible— for example, those requiring similar biospecimens and hardware. Such teams will work together to achieve individual objectives within the bounds of constrained resources. These teams may be assigned to a flight and their experiments implemented as a group.

Principal Investigator (PI) Role and Responsibilities

The fundamental role and responsibilities of the experiment PI are:

- Defining the basic scientific and operational requirements for the experiment.
- Working with the project team to ensure that research objectives are maintained during design, development and flight.
- Completing and submitting the analyzed data and a final report to NASA, and publishing the results, as appropriate.
- Complying with all safety training, policies, and procedures as required by NASA.

Implementation Team Role and Responsibilities

The space flight Implementation Team is led by a NASA-provided Payload or Project Manager. The team works together to manage and implement the phases of the experiment development cycle. The NASA-provided Project Scientist will work directly with the PI throughout this process.

Definition, Documentation and Testing of a Space Flight Experiment

The following is representative of the documentation, testing, and information that will be required.

To complete a successful experiment in microgravity, a detailed analysis and definition of the proposed experiment must be done. All requirements for the execution of the experiment must be identified and described, and a feasibility analysis conducted.

Additionally, the required hardware and resource requirements must be identified in detail for all phases: preflight, flight, and post flight, including assessment of the maturity of the experiment development and the adequacy of financial resources required for its conduct. If needed, and resources are available, the design, development, and manufacture of experiment-unique hardware will be conducted, and experiment-hardware interfaces and operations will be verified through testing.

The PI will work with the assigned Project Scientist to complete all of the required phases for development and flight readiness. A series of reviews of the experiment will be conducted, including reviews for safety requirements. As per NASA life sciences flight experiment management policy, if satisfactory results are not obtained during testing, a flight experiment may be deselected and perhaps considered for ground research based on peer review, or may need to be cancelled altogether.

Development of Spaceflight Experiment Requirements

The foundation of the space flight experiment is the clear definition and identification of all aspects of the proposed experiment. It is critical that the PI work with the project team in a series of activities that are necessary for developing the space flight experiment.

Science Ground Testing

The Project Scientist and the flight experiment team will support the PI in defining the types of testing which will be required before flight in order to mitigate risks and increase the chance of a successful experiment. These tests would include such things as validation of new hardware and practice runs to optimize and streamline on-orbit operations. The test results will define requirements, procedures, hardware settings, and configurations.

Ground Controls

Proper ground-control experiments are essential for successful and scientifically sound space-flight experiments.

- Synchronous ground-control experiments (housed in flight-type hardware on the ground) will be supported at NASA.
- If appropriate, ground-control experiments may be performed on a time delay to allow for provision of temperature and other parameters that closely simulate actual flight conditions.
- Ground-control experiments will be supported by NASA and CASIS.

Hardware Biocompatibility Tests

Hardware biocompatibility testing is warranted in some cases to ensure that biosamples and/or organisms do not encounter unexpected difficulties while housed in payload system hardware under the prescribed experimental conditions.

Project Integrated Tests

Integrated testing of expected hardware operations and procedures on the ground may be warranted. The appropriate testing will be identified by the experiment team in collaboration with the PI.

Conducting a Spaceflight Experiment: Payload Flight Operations

Launching and delivering a life sciences experiment to the ISS requires extensive preparation in support of the logistics activities. NASA provides laboratories for pre-flight preparation and post-flight experiment activities. All of the laboratory

equipment that is needed for pre- and post-flight experiment processing must be identified, and specialized equipment may have to be provided by the PI. The assigned NASA Project Scientist will assist and work directly with the PI to provide the necessary information and documentation for flight logistics and operations. Contingency planning for launch delays is also part of logistics planning.

Typical pre-flight activities include launch site facility trial runs for pre-flight experiment preparation and processing, training of astronauts for in-flight experiment operations, and activities supporting transfer of experiment payload elements to the launch area. Additionally, trial runs for post-flight experiment activities to be conducted in laboratories on the ground upon return of the experiment to Earth must be considered.

Funding Opportunities/ Points of Contact

NASA research announcements are managed through the NASA Solicitation and Proposal Integrated Review and Evaluation System (NSPIRES). This Web-based system supports NASA research by release of solicitation announcements and proposal peer review and selection processes (http://nspires.nasaprs.com/external/index.do).

Additional research announcements and flight opportunities can be found at the CASIS website: http://www.iss-casis.org/Home.aspx. CASIS also encourages the submission of unsolicited proposals.

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Appendices

International Hardware Facilities

Approval is needed by NASA in order to utilize any non-NASA flight hardware since access to such items is based on international agreements and hardware utilization exchanges.

No international hardware facilities specifically for fruit flies have been identified on the ISS.

Acronyms

ARC	Ames Research Center
CASIS	Center for Science in Space
ESA	European Space Agency
FIT	Fly Immunity and Tumors (Experiment)
FSB	Fundamental Space Biology
ISS	International Space Station
ISSNL	ISS National Lab
KSC	Kennedy Space Center
LEO	Low Earth Orbit
NRC	National Research Council
PI	Principal Investigator
RNA	Ribonucleic Acid
STS	Space Transportation System (Space Shuttle)
USB	Universal Serial Bus

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