Growth, development and behavior of *Caenorhabditis elegans* through ten generations of autonomous culturing onboard the International Space Station (ISS)

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Abstract

Multi-generational growth will be essential for any hope of long term human colonization of the cosmos. However, there is a lack of information about any species in space beyond three generations. In addition, trips to the Moon or Mars will result in greater exposure to space radiation, although little is known about cumulative biological effects. *C. elegans* provides a simple model system in which to study multi-generational growth and radiation exposure in space. Cultures of *C. elegans* (wt CC1 and balancer eT1 strains) were maintained on-board the ISS for periods well in excess of 3 months. Worms were grown through 10+ generations on the ISS using an automated culturing system employing defined liquid medium, commercial growth chambers, peristaltic pumps to passage worms and control instrumentation. The culturing system, the *C. elegans* Habitat, was housed in a temperature controlled incubator located in the ISS module Destiny. Integrated video cameras with micro lenses, combined with data downlink, were utilized to image worms for real-time assessment of larval stages, population density and movement behavior. Data analysis was performed by students at 35+ middle and high schools across the United States, Canada and Malaysia, allowing students to learn about the benefits of *C. elegans* research while gaining experience in the scientific method. While preliminary, data confirm what was inferred from past shorter duration spaceflight missions: growth, development, and behavior of worms are grossly unaltered during spaceflight. Changes in worm muscle that were previously observed (decreased myosin heavy chain and MyoD expression, movement defect) may reflect adaptive changes in muscle in space or may simply be artifacts of past culturing techniques. Planned post-flight analyses should distinguish these two possibilities. Post flight analysis of eT1 worms will determine if increased rates of genetic mutation occur with long-term exposure to low-Earth orbit radiation. This should lead to further insights into radiation concerns for future planned interplanetary human exploration missions. We have demonstrated for the first time that there is no major gravity-dependent process associated with spaceflight that precludes essentially normal animal growth and development for at least ten full generations in *C. elegans*.

C. elegans as a model for space life sciences

Spaceflight induces biological changes across phyla, some are a medical concern (muscle loss, etc.)

Spaceflight is also associated with increased exposure to radiation (also of medical concern)

***C. elegans*: established model system; researchers studying relevant topics (radiation, muscle, etc.)**

5 Past flights involving live *C. elegans* tell us:

- **Males mate**
- **Animals undergo two generations in-flight without gross defects**
  - **Normal population growth**
  - **Normal apoptosis (radiation damaged cells can be cleared)**
  - **Normal lengths at molt (L1 *may* be smaller)**
- **Animals experience increased rate of mutation**
  - **Due to radiation alone (no 'microgravity' component, shielding protects)**
  - **Animals *may* have shorter telomeres**
- **No difference in growth on solid or in liquid medium (no surface tension 'issues')**
- **Altered gene expression**
  - **As much as 10% of genome shows changes**
    - **Changes are somewhat culture/population dependent**
    - **Changing genes are enriched for Insulin and TGF-β targets**
  - **Decreased expression of Myosin Heavy Chain and MyoD**
    - **In both body wall and pharyngial muscle (probably not 'activity')**
    - **Some returning animals have a movement defect**

Automating *C. elegans* culturing

Liquid culture allows automation

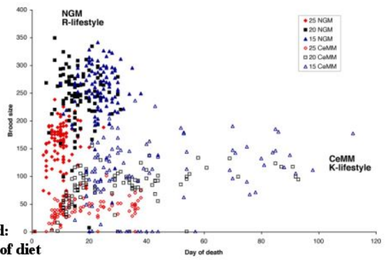
***C. elegans* Maintenance Medium (CeMM) is a chemically defined liquid (or solid) medium**

Animals in CeMM are health and 'normal' but 'different' from NGM grown animals:

Apparent R to K lifestyle 'switch'

Different:

- **Developmental timing**
- **Broodsize**
- **Reproductive period**
- **Lifespan**
- **Metabolic stores**
- **Stress sensitivity**
- **Gene expression**



Most/all changes appear due to increased levels of DAF-16

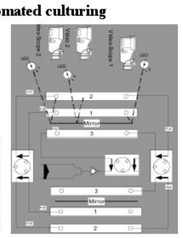
Developmental timing, Reproductive period: 'Fixed' percentage of Lifespan regardless of diet

Animals grow well in a variety of standard laboratory hardware (flasks, cell culture dishes, tubes, etc)

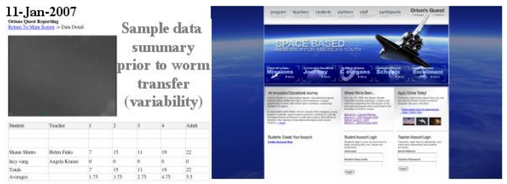
OptiCells®, infusion pump tubing, and peristaltic pumps allow automated culturing







Orion's Quest: Involving 4-12 students in actual spaceflight experiments



Orion's Quest (OQ) is a non-profit educational organization that works with middle and high school students and teachers across the country. OQ educators use real world research being conducted by NASA scientists to develop classroom activities and curriculum for student "missions". Each mission is designed to have students participate in supporting some aspect of real-world research. The OQ staff is responsible for the development, delivery, and coordination of each mission. Programs are supported and managed via the Internet. At the completion of each mission, OQ compiles information and data gathered by students and provides this information to NASA for review and, if applicable, included in research databanks.

Key concept: Leverage kids enthusiasm for spaceflight to encourage education in science and math

Past *C. elegans* "missions" have included:

- **Tracking population growth and progression through the four larval stages over a four week period in CeMM**
- **High altitude balloon launch, operations, and recovery with post "drop" analysis of *C. elegans* behavior**

Current mission (Mission 3):

- **Learn about the Space Shuttle program (specifically STS-116)**
- **Learn about the International Space Station (ISS) and its crew**
- **Count animals in still images from ISS or ground controls**
- **Measure animal lengths using Image J software**

Schools participating in Mission 3:
Arcadia High School, Phoenix, AZ
Cesar Chavez Academy High School, Detroit, MI
Cherryland Middle School, Elk Rapid, MI
Chippewa Hills High School Remus, MI
Churchill High School, Livonia, MI
Chippewa Valley High School, Remus, MI
Cocoa High School, Cocoa, FL
Copper Ridge Math/Science Academy, Scottsdale, AZ
Crestwood High School, Dearborn, MI
da Vinci Middle School Jackson, MI
Darcel Ave Senior Public School Mississauga, Ont
Desert Mountain High School, Scottsdale, AZ
Detroit Academy for Math/Science/Tech., Detroit, MI
Elk Rapids High School, Elk Rapids, MI
John Glenn High School, Westland, MI
Geisler Middle School, Walled Lake, MI
Harrison High School, Farmington, MI
Hastings High School, Hastings, MI
Holmes Middle School, Detroit, MI
James Logan High School, Union City, CA
Malysia Schools, Kuala Lumpur, Malaysia
Menomonie High School, Menomonie, WI
Mesa High School, Mesa, AZ
Middle School at Parkside, Jackson, MI
Milan High School, Milan, MI
North High School, Phoenix, AZ
OW Holmes School, Detroit, MI
Pershing High School, Detroit, MI
Seabrook Intermediate School, Seabrook, TX
Smith Middle School, Troy, MI
Southwestern High School, Detroit, MI
Space Exploration Academy Oakland, CA
Summit Middle School, Boulder, CO
University School Shaker Heights, OH

Remote culturing

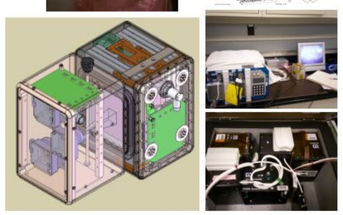
Remote culturing, imaging, environmental monitoring and temperature control we achieved by placing worm "habitats" within the Commercial Generic Bioprocessing Apparatus (CGBA)

The CGBA has a rich heritage, having previously flown on 18 missions (13 STS, 2 Mir, 3 ISS)

For this mission we utilized the CGBA "freezer" which provides:

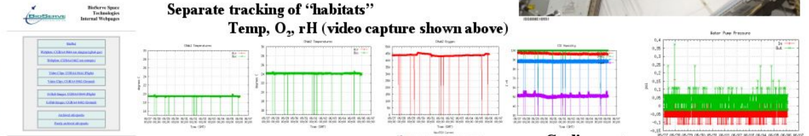
- **Stable (+/- ??°C) temperature within a -16°C to 37°C range**
  - **Capable of temperature maintenance during long duration spaceflight**
- **Real-time data/video downlink**
- **Real-time payload command**
- **Autonomous control of payload**
- **Astronaut user interfaces**
- **A grid pattern for hard-mounting of experiments**
- **Adjustable restraint system for retaining experiments in "micro-gravity"**



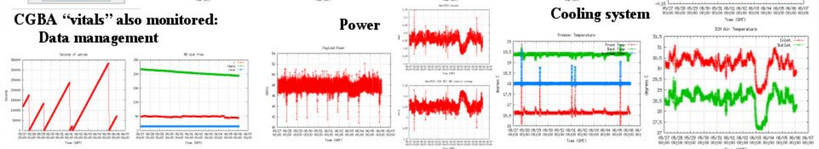


Web-based storage allows remote access to data downlinks:

Separate tracking of "habitats"
Temp, O_2, rH (video capture shown above)



CGBA "vitals" also monitored:
Data management
Power
Cooling system



Data analysis

Multi-pronged approach to data analysis:

Real-time data downlink to mitigate against inability to successfully return worms and to also provide:
- **The first in-flight observation of *C. elegans* development and behavior in space**
- **Answer to the hypothesis that worms will "dauer up" following more than three generations**

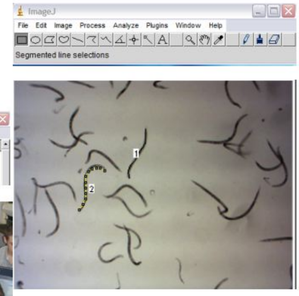
Standard post-flight analysis to maximize "science return"
- **"Read out" of *C. elegans* "biological radiation dosimeter"**
- **Micro-array analysis of gene expression for comparison to shorter durations flight(s)**
- **Observation of any "adaptations" or "selected mutations" in response to spaceflight**
- **Assay of status of "innate immune system"**

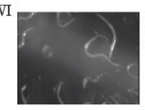
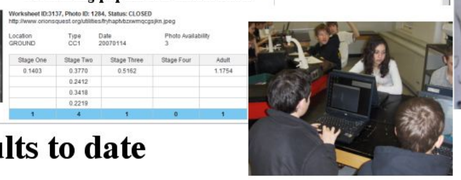
Distributed data analysis
- **Responsibility for in-flight and post-flight analysis assigned to most appropriately experienced**
- **Allows "real" participation for students**

Student use of Image J

Software selected based upon ease of use and being "real" science tool

In previous "missions" students used Image J to gain experience in:
- **Counting worms**
- **Measuring animal lengths**
- **"Staging animals" based upon length**
- **Measuring population distribution**



Results to date

Visually confirmed normal growth and development inferred from past post flight analyses

Determined growth and development is grossly normal through at least ten generations in flight
- **5 times longer than previously demonstrated**
- **Suggests there is not a continued "deterioration" of *daf-2* signaling resulting in "dauering up"**
- **Suggests lack of detrimental effects of multi-generational animal development in space**

Determined movement rates are normal in flight through at least ten generations
- **Suggests past post flight observation of movement defects result from "adaptation" to microgravity**

No obvious behavioral alterations in response to growth and development in space

Future plans and possibilities

Finish planned post flight analysis

Perform more in depth analysis of Student data
- **Roughly 300 images per day analyzed**
- **Roughly 500 students performed the analysis**

Upgrade video to accommodate DsRed
- **Drive DsRed from "stress", "radiation", "spaceflight" induced promoters?**

Fly on autonomous missions?
- **Small satellites (gene stat, etc)**
- **Moon/Mars/other celestial body probes**

Adapt for drug/toxin response screening?



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Acknowledgements

CeMM generously provided by NASA's now defunct Space Station Biological Research Program as well as NASA Ames' Space Life Sciences Operation's Branch. Past work on OQ "missions" by Dr. Catharine A. Conley provided the basis for the Image J protocol(s) used. Funding provided by NASA (NJS), CSA (DB), and Angkasa (LS). The experiment was transported to and from the ISS by STS-116 and STS-117 respectively and maintained by Expeditions 14 & 15.