Remote automated multi-generational growth and observation of an animal in low Earth orbit

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Supplementary data

"Data Supplement"


References

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1. INTRODUCTION

Colonization of other planets is deemed realistic [1]. This would mitigate against Earth’s periodic global extinction events [2] and the next ice age; our nearest relative, Neanderthal, went extinct in the last ice age. Promisingly, the space-faring nations are planning long-duration expeditions beyond low Earth orbit (LEO) [3]. The economic cost is vast, estimated at several tens of billions of Euros [4], as technology must be developed to permit human presence on these missions (e.g. advanced life support and integrated sensing systems and superior radiation shielding). Accordingly, we currently know virtually nothing of the (patho)physiologic adaptations associated with habitation beyond LEO and, therefore, little of the long-term prospects for other worldly habitation.

Short-term studies within LEO demonstrate that (patho)physiologic changes occur when living in space [3,5]; radiation exposure and musculoskeletal deterioration are suggested as key obstacles to successful habitation beyond LEO. Animal models are recognized as cost-effective solutions to some problems intrinsic with studying humans (e.g. high cost, low throughput, slow discovery rate and endangerment of human health). The soil nematode Caenorhabditis elegans has been used on the Earth to help understand human biology [6]; its use for the space life sciences has also been demonstrated [7–9].

Briefly, C. elegans: has an evolved neuromuscular system; moves in three dimensions in soil or liquid; senses and responds behaviourally to its environment (detecting and moving towards or away from chemicals, heat, oxygen and ultraviolet radiation [10,11]) and is an accepted model for assaying environmental toxins [12]. Past spacelflight studies established that C. elegans and astronauts show similar alterations in muscle protein synthesis, particularly decreased synthesis of the contractile protein myosin and the transcription factor that controls myosin synthesis [13], that both appear to show alterations in insulin signalling [14], and that C. elegans can be used to detect in-flight radiation exposure [15–17].

While a worm is not a man, many spaceflight-induced molecular changes occur in both. Given the high cost of manned missions, C. elegans could be a cost-effective model for detecting, understanding, and mitigating some of the biological consequences of long-duration exploratory missions.

Here, we report the use of a remotely operated, automated culture system for C. elegans in LEO and demonstrate that it can be used to observe biological measures of animal health such as development, reproduction, behaviour and growth arrest as well as recovery during a long-duration spacelflight. This system could, in the future, be used on interplanetary missions.

2. METHODS AND RESULTS

2.1. Automated culturing and experimentation

Prior to flight, we developed an automated culturing system. We combined liquid C. elegans Maintenance Medium (CeMM), which supported normal growth and development of two generations of C. elegans on-board...
the International Space Station (ISS) [9], with the Opti-
Cell (BioCrystal, Ltd.), which displayed no undesirable
issues with spaceflight hardware [18]. OptiCells were
linked together by infusion pump tubing and payload pro-
gram controllable peristaltic pumps (figure 1); automated
pump activation can be overridden by remote uplink com-
mands. The linked OptiCells were housed within
annodized aluminium fitted with a Plexiglas window.
Visualization of animals through the window used LED
illumination and board mounted miniature cameras
(figure 1); Infinistix lenses (18 mm working distance, 2
primary magnification) magnified the field of view to
approximately 3 \( \times \) 4 mm. We confirmed no problems
with remote operation of the pumps or cameras, or
animal growth over one month on the Earth.

For spaceflight and ground controls, we used the
Commercial Generic Bioprocessing Apparatus
(CGBA) [19] to provide data downlink and temperature
control (22 \( \pm \) 1 \( ^\circ \)C). We relied upon the ISS for oxygen
and power in-flight and the University of Colorado for
both on the Earth. Temperature, oxygen and relative
humidity were monitored twice daily and remained
within established parameters throughout six months
in-flight and on the Earth.

2.2. Normal development and behaviour over 12
generations on-board the ISS

Daily observation of animals (figure 2a) confirmed the
past inference that \( C. \) elegans develop normally in
space [9,20,21]. Identical timings for egg to egg-laying
adult were observed for the ground control and in-
flight populations (figure 2a, 6.5 days at 22 \( \pm \) 1 \( ^\circ \)C
which is consistent with past growth rates in CeMM
on the Earth [18,22]). Lengths of animals measured in
National Institutes of Health IMAGEJ software (n = 40
day per condition, and additional measurements by
grade and high-school students from the USA, Canada
and Malaysia) confirm these developmental stages
(such measurements accurately stage animals [22]).

\( Caenorhabditis \) elegans uses transforming growth
factor-beta and insulin signalling to sense and respond
to adverse environmental conditions such as lack of
food and elevated temperature. These signals control
entry into a developmentally arrested, stress-resistant,
enduring state [23]. Because past gene expression
studies suggested alteration in genes controlled by
these signalling systems in spaceflown
\( C. \) elegans,
\( Drosophila \) and men [14,24], we were concerned that
cultivation of \( C. \) elegans beyond one to two generations
might result in developmental arrest as the result of
continued sensing of an adverse environment. However,
in fed populations, we noted no changes in population
distribution or developmental timing over 12 gener-
ations of growth in-flight (the first three months).
Furthermore, worms were able to sense and respond
appropriately to the presence of food in-flight as starved
animals developmentally arrested and then recovered
when introduced to fresh CeMM (figure 2).

\( Caenorhabditis \) elegans and man both display
depressed synthesis of myosin, and other muscle gene
products, in response to spaceflight and impaired mobi-
licity upon return to the Earth [13]. In-flight movement,
when fed, was identical to that on the Earth (figure 2b),
suggesting that decreases in muscular synthetic capacity are adaptive rather than pathological in-flight. These data suggest, but do not prove, that the same may be true for changes in human cardiac, skeletal and vascular muscles [3,5]. As we detected no movement decline over 12 generations, these results also suggest that the past concerns, that muscular decline may never plateau [3,5], may be false.

We also found decreased movement as food was depleted (figure 2b). This observation further suggests C. elegans remain able to respond appropriately to lack of food in space. Importantly, the movement decline and growth arrest also demonstrate that our system can be used to detect both normal and abnormal growth, development, reproduction and behaviour during spaceflight.

While we were able to recover viable populations after six months, delays with the Space Shuttle program precluded the observation of the full 24 generation experiment.

3. DISCUSSION
We developed a compact automated C. elegans culturing system using off-the-shelf hardware. By combining this system with the established CGBA, we were able to remotely culture and observe C. elegans throughout 12 generations on-board the ISS. Consistent with past experiments in LEO, C. elegans display normal developmental timings when fed, and appropriate alterations when starved and re-fed. Accordingly, we were able to make the first observations of C. elegans behaviour in LEO.

Over 12 generations, animals displayed normal movement rates when fed, and appropriate alterations when starved and re-fed. This demonstrates that it is possible for a multi-cellular animal to live long term in LEO (e.g. more than 10 generations) and that it can be studied, remotely, while in LEO. As C. elegans is an accepted model for assessing environmental toxins [12], including in-flight radiation [15–17], and as behavioural alterations continue to be an earlier indicator of toxic exposure than death [25], we suggest that C. elegans and the culturing system presented here are currently robust enough to consider incorporating biological specimens into future long-distance interplanetary missions. Given the high cost of manned space missions and high failure rate for Mars missions, we suggest that small organisms such as C. elegans, despite not being humans, be used as a cost-effective model
for detecting, and potentially studying, some of the biological effects of long-duration and distance spaceflight. Our system provides a valuable test bed for life support system performance on missions where the risk of component failure is unacceptably high for manned missions.

CeMM was provided by NASA Ames Research Centre. Student participation was facilitated by Orison’s Quest (orisonsquest.org). Thanks to Thomas Drummond and Peter Lawrie and the participating schools for ‘Mission III’ (listed in the electronic supplementary material). N.J.S. was supported by grants from NASA (NNA04CK22A) and NIH NIAMS (AR054342). E.A.O. was supported by a PA Space Foundation Grant. T.E. was supported by MRC (G0801271). D.B. was supported by the Canadian Space Agency. Agensi Angkasa Negara (Malaysia) provided funding for development and in-flight operation of the experiment.

REFERENCES

5 Health NAIoMsCotLSoA (ed.) 2004 astrobiology aspects of Mars and human presence: pros and cons. Hippokratia 12(Suppl. 1), 49–52.