



Evaluation of the fluids mixing enclosure system for life science experiments during a commercial *Caenorhabditis elegans* spaceflight experiment

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Abstract

The Student Spaceflight Experiments Program (SSEP) is a United States national science, technology, engineering, and mathematics initiative that aims to increase student interest in science by offering opportunities to perform spaceflight experiments. The experiment detailed here was selected and flown aboard the third SSEP mission and the first SSEP mission to the International Space Station (ISS). *Caenorhabditis elegans* is a small, transparent, self-fertilizing hermaphroditic roundworm that is commonly used in biological experiments both on Earth and in Low Earth Orbit. Past experiments have found decreased expression of mRNA for several genes whose expression can be controlled by the FOXO transcription factor DAF-16. We flew a *daf-16* mutant and control worms to determine if the effects of spaceflight on *C. elegans* are mediated by DAF-16. The experiment used a Type Two Fluids Mixing Enclosure (FME), developed by Nanoracks LLC, and was delivered to the ISS aboard the SpaceX Dragon and returned aboard the Russian Soyuz. The short time interval between experiment selection and the flight rendered preflight experiment verification tests impossible. In addition, published research regarding the viability of the FME in life science experiments was not available. The experiment was therefore structured in such a way as to gather the needed data. Here we report that *C. elegans* can survive relatively short storage and activation in the FME but cannot produce viable populations for post-flight analysis on extended missions. The FME appears to support short-duration life science experiments, potentially on supply or crew exchange missions, but not on longer ISS expeditions. Additionally, the flown FME was not properly activated, reportedly due to a flaw in training procedures. We suggest that a modified transparent FME could prevent similar failures in future flight experiments.

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1. Introduction

The experiment described here was part of the third mission of the Student Spaceflight Experiments Program

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(SSEP). SSEP is a United States national science, technology, engineering, and mathematics (STEM) initiative that aims to increase student interest in science by giving K-12 students the opportunity to propose a scientific spaceflight experiment (<http://ssep.ncesse.org/>). A winning proposal is selected by a review panel of scientists and the winning team is given the opportunity to turn their proposed experiment into reality. Experiment designs can utilize one of three different types of Fluids Mixing Enclosures, a hardware system specifically designed for spaceflight research (<http://nanoracks.com/>). Elapsed time between proposal submission and experiment launch was six months and six days.

1.1. Experiment background: *C. elegans* as a model organism for space life sciences

Caenorhabditis elegans is a small, transparent, self-fertilizing, free-living nematode that is commonly used as a model organism in biological experiments (Riddle et al., 1997). Experimentation with *C. elegans* is relatively easy and inexpensive and the organism has both a short gestation period and a large brood size. Scientists have earned three Nobel Prizes for work conducted in *C. elegans*: in Physiology or Medicine in 2002 (Sydney Brenner, H. Robert Horvitz, and John E. Sulston for “their discoveries concerning genetic regulation of organ development and programmed cell death”); in Physiology or Medicine in 2006 (Andrew Z. Fire and Craig C. Mello for “their discovery of RNA interference – gene silencing by double-stranded RNA”); and in Chemistry in 2008 (Martin Chalfie for “development of the green fluorescent protein, GFP” (two other scientists shared this prize for additional work not conducted in *C. elegans*)). The genome of the organism has been completely sequenced (Consortium, 1998), a development that has led to the emergence of *C. elegans* as a model for increasing our understanding of how the genome functions (see Culetto and Sattelle, 2000; Kamath et al., 2003; Li et al., 2004; Piano et al., 2006). In addition, *C. elegans* and humans both have muscles, a nervous system, integument, a gut, and a reproductive system, and approximately thirty-eight percent of *C. elegans*’ genes have identified human homologues (see Shaye and Greenwald, 2011). All of these characteristics make *C. elegans* an ideal organism to experiment on to further our understanding of both human physiology and biological processes in general.

Eight past *C. elegans* spaceflight experiments have been published to date. On IML-1, male *C. elegans* were found to mate successfully, no major developmental abnormalities were observed after two successfully completed generational cycles, and it was shown that the small amount of increased mutagenic effects of spaceflight can be captured easily using the *eTI* balancer system (Nelson et al., 1994a; Nelson et al., 1994b). On IML-1 and STS-76, cosmic radiation was shown to be directly responsible for increased mutagenic event rates (Hartman et al., 2001; Nelson et al., 1994b). Flown and ground control animals on

STS-95 died of a biocompatibility issue with the hardware (presumptive lack of sufficient oxygen). STS-107 showed *C. elegans* could survive a relatively unprotected atmospheric re-entry and began the validation of the use of a chemically defined liquid growth medium in spaceflight experiments (Szewczyk et al., 2005). ICE-FIRST: (i) validated use of the chemically defined medium in spaceflight experiments (Szewczyk et al., 2008); (ii) confirmed a lack of developmental abnormalities (Szewczyk et al., 2008); (iii) verified that apoptosis occurs normally in spaceflight (Higashitani et al., 2005); (iv) showed that *C. elegans* display many of the same changes in muscle gene expression as mammals (Adachi et al., 2008; Higashibata et al., 2006; Selch et al., 2008); (v) determined that spaceflight partially rescued a muscular defect in a paramyosin mutant strain (Adachi et al., 2008) and intramuscular protein aggregation (Honda et al., 2012); (vi) suggested that altered metabolic (Insulin-like and/or TGF-beta) signaling pathways were responsible for the in-flight changes in gene expression (Selch et al., 2008); (vii) determined that some genes down-regulated during spaceflight are linked to longevity processes in *C. elegans* (Honda et al., 2012); and (viii) confirmed that the *eTI* balancer system can easily capture increased mutagenic effects of spaceflight (Zhao et al., 2006) and postulated that the *eTI* system could be used as a “biological dosimeter” to measure the long-term mutagenic effects of spaceflight (Zhao et al., 2005). Shijian-8 explored some of the potential molecular mechanisms behind spaceflight muscular atrophy (Wang et al., 2008). CERISE showed that RNAi within multiple tissues works as effectively during spaceflight as on Earth (Etheridge et al., 2011a). The most recently published spaceflight experiment, CSI-1, validated automated culturing by growing twelve full generations autonomously and confirmed a lack of any major developmental abnormalities over an extended mission (Oczypok et al., 2012). Together, these experiments show that *C. elegans* is a model organism that can be grown in space relatively easily, imply that physiological responses to spaceflight are similar in *C. elegans* and humans (Hagen, 1989; Oser and Battrick, 1989; Vandenberg et al., 1999), and suggest that *C. elegans* can be used to further our understanding of the effects of spaceflight on human physiology and biological processes.

1.2. Experiment goals

Past experiments that have focused on genomic responses to spaceflight have determined that some changes are observed in genes that are influenced by the insulin signaling pathway or regulated by the transcription factor FOXO (Jamal et al., 2010; Selch et al., 2008) or both. These experiments have concluded that decreased FOXO expression could be used to determine whether or not the observed gene expression changes and consequent physiological changes observed in spaceflight are the result of FOXO action (Etheridge et al., 2011b). In order to test this hypothesis, the experiment utilized a *C. elegans* strain with

a deletion in the gene encoding the FOXO transcription factor (DAF-16). In addition, this experiment was structured in such a way as to obtain needed biocompatibility information with the Fluids Mixing Enclosure hardware.

1.3. Results

Unfortunately, the experiment was not actually activated in flight and only two live worms were obtained after a six-day return shipment period on Earth. The experiment, however, yielded much-needed biocompatibility information and it was determined that the Fluids Mixing Enclosure (FME), the hardware used in this experiment, can most likely sustain only a short-duration (two-to-three weeks) life science experiment, perhaps a resupply or crew exchange mission, but is unlikely to sustain a longer life science experiment aboard future ISS expeditions.

2. Hardware options and selection

2.1. Fluids Mixing Enclosure (FME)

The FME series was designed by Nanoracks LLC specifically for spaceflight research (<http://nanoracks.com/>).

As shown at the top of Fig. 1A, the three different members of the FME series all have the same external enclosure design: an open-ended, opaque, hollow Teflon tube that holds approximately 7.62 ml. At the time of the experiment the FME was sealed using a cylindrical Teflon cap 0.9524 cm in diameter and 0.635 cm thick (Fig. 1C, left). Now, a cap at one end of the FME screws shut and open (Fig. 1A, top right). After the FME is capped, it is sealed with polyolefin heat shrink tubing and then sealed again in a vacuum heat sealed polyethylene bag (Fig. 1B). Before the experiment is returned to the experiment team, these two additional levels of containment are removed.

2.2. Type One FME

The Type One FME holds no other containment devices (Fig. 1A, top). Experimental designs that utilize the Type One FME are essentially passive experiments and, as such, pre-flight and post-flight storage time is extremely crucial.

2.3. Type Two FME

The Type Two FME contains a long glass ampoule that holds approximately 1.85 ml (Fig. 1B). In order to activate

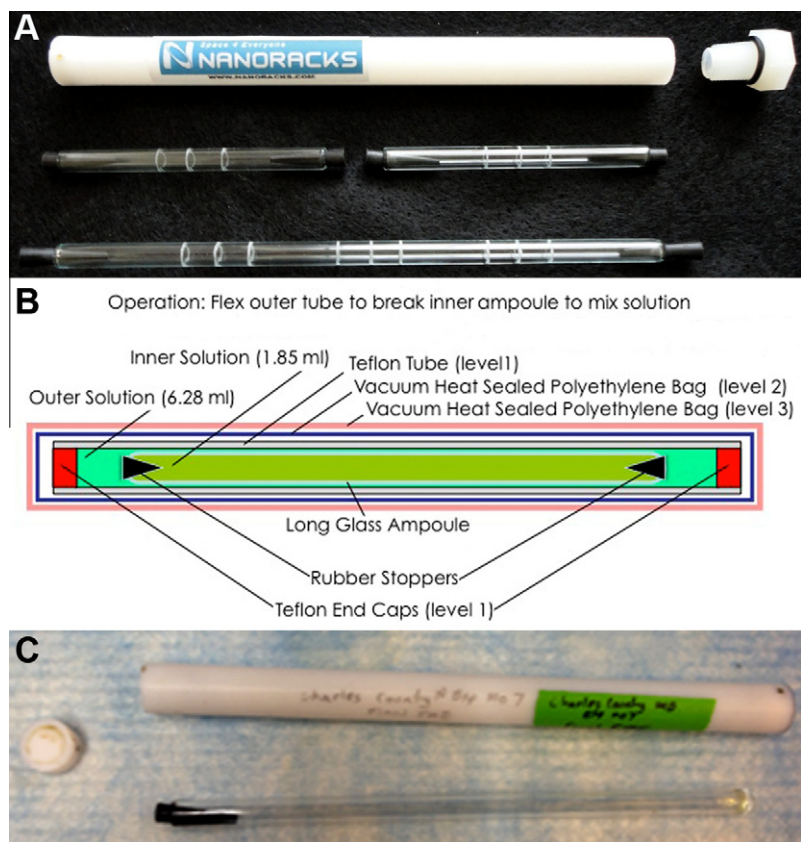


Fig. 1. Fluids Mixing Enclosure (FME). (A) Current FME series. Top: Type One FME. Middle: two short glass ampoules that combine with the Type One FME to make a Type Three FME. Bottom: long glass ampoule that combines with the Type One FME to make a Type Two FME. (B) Schematic of a fully assembled, flight ready, Type Two FME. (C) Post flight photograph of the opened, unactivated, flight experiment Type Two FME. Images in A and B courtesy of the Student Spaceflight Experiments Program.

this type of experiment, the FME must be bent approximately ten degrees in two places, thus breaking the glass ampoule and mixing the materials inside the ampoule and the main compartment of the FME evenly. A simple analogous situation is the activation of a glow stick. The Type Two FME is the easiest type to activate. Experimental designs that utilize the Type Two FME typically return in an activated state.

2.4. Type Three FME

The Type Three FME contains two short glass ampoules that each hold approximately 0.92 ml (Fig. 1A, middle). The activation method is the same as the method for the Type Two FME. Experimental designs that utilize the Type Three FME, however, tend to have experiment materials in one short ampoule, an activating agent in the main compartment, and an inhibitive agent in the second short ampoule. The first short ampoule is activated at any time during flight and the second short ampoule can be activated when the experiment materials have either undergone enough exposure to the environmental effects of spaceflight or the experiment is about to leave Low Earth Orbit. Experimental designs that utilize the Type Three FME typically return in a fixed or deactivated state.

2.5. Selection

As the hardware was to be hermetically sealed, oxygen, which is required to support almost all metazoan life, was the largest limiting factor in the success of the experiment. The use of a Type One FME would maximize the available oxygen. However, the experiment was scheduled to launch aboard Soyuz 30S on 30 March 2012 and return aboard Soyuz 29S on 16 May 2012 and the payload manager, Nanoracks LLC, required the experiment by 24 February 2012. Worms would be in the FME for roughly two and a half months, a period longer than they could survive as a reproductive population without the addition of fresh food (roughly one week when fed bacteria (Byerly et al., 1976) and one month when fed a chemically defined diet (Lu and Goetsch, 1993)). Use of a Type Two or Three FME was required in order to introduce the worms to fresh food and recover reproductive worms post-flight. The long ampoule of the Type Two FME contains almost twice the volume, and therefore oxygen, of each of the short ampoules of the Type Three FME. Because oxygen was the largest concern in the design of the experiment, the Type Two FME was considered the most viable option for success.

3. Experimental design

3.1. Ameliorating concerns: *CeHR*

In the laboratory, *C. elegans* are typically grown using *Escherichia coli* as a food source (Brenner, 1974). Using

E. coli, which also consumes oxygen, as a food source would further strain oxygen in the already limited container (see Section 2.5). In a hermetically sealed experiment, where oxygen is limited, a growth medium that does not require oxygen is optimal. *Caenorhabditis elegans* Maintenance Medium (CeMM) is a chemically defined liquid growth medium (Lu and Goetsch, 1993; Szewczyk et al., 2003) that does not require oxygen and has been used in past spaceflight experiments (Oczypok et al., 2012; Szewczyk et al., 2005; Szewczyk et al., 2008). Although culturing *C. elegans* in CeMM is relatively simple, animals grown in the medium exhibit an altered life history (Szewczyk et al., 2006) and altered growth rates as the animals change the composition of the medium (Szewczyk et al., 2003). Additionally, animals in CeMM are prone to acquire a deletion within the gene responsible for sensing dauer-inducing pheromone (McGrath et al., 2011). Because of the limited pre-flight time, it was necessary to obtain growth medium from a third-party source. A modified version of *Caenorhabditis elegans* Habitation and Reproduction Medium (CeHR) was readily available and used instead of CeMM. CeHR is an axenic liquid growth medium extremely similar to CeMM. The main difference is the addition of milk in CeHR for additional nutrition (Szilagyi et al., 2006). In order to limit potential disparities between data gathered from controls and data from past spaceflight experiments (see Section 1.1), we obtained a modified version of CeHR that did not have milk. Use of this liquid growth medium eliminated the strain bacterial food sources would have placed on oxygen levels within the FME.

3.2. Ameliorating concerns: Multiple ground controls

There were no published papers listed in Pubmed and no technical reports available from NASA (ntrs.nasa.gov) that evaluated or demonstrated the utility of the FME as a viable piece of hardware for space life sciences research. As no past biocompatibility data were available, it was desirable to conduct preflight verification tests, however the relatively short period between proposal acceptance (14 December 2011) and experiment shipment (24 February 2012) made running a full-length test impossible. Multiple ground controls were planned in order to determine the effects of oxygen constraints on the experiment, allow potential modifications to activation and analysis times, and assess the viability of the FME as a piece of hardware to support space life science experiments. Pre-flight controls were run to confirm that the worms could grow and survive in the glass ampoules and also in the glass ampoules within the FME and lastly that worms could survive breakage of the ampoule (activation). One transit control and five ground controls were loaded and scheduled to be opened periodically (see Section 3.5, Table 1). The transit control was sent alongside the experiment to Nanoracks and stored at room temperature, approximately 25 °C. After Nanoracks transferred the experiment payload to

Table 1
Experiment timeline.

Date	Event
April 4	Experiment, Transit Control, Five Ground Truths Loaded; Experiment and Transit Control shipped to Payload Manager in Houston (Nanoracks)
April 5	Experiment and Transit Control arrive at Nanoracks (stored at 25 °C)
May 2	Experiment handover to NASA (stored at 4C); Transit Control shipped to NIH Lab
May 3	Transit Control arrives at NIH Lab (stored at 4C)
May 22	SpaceX Dragon launches with Experiment on board; Transit Control and Ground Truth 1 opened
May 23	Preliminary analysis of Transit Control and Ground Truth 1*
June 13	Experiment reportedly activated at 12:16 CDT
June 15	Ground Truth 2 opened; Ground Truths 3, 4, and 5 activated
June 26	Ground Truth 2* analyzed; Ground Truth 3 opened
July 1	Soyuz 29 landed with Experiment on board
July 2	Experiment shipped to Nanoracks (Houston) and stored at approximately 25 °C
July 5	Experiment shipped to NIH Lab
July 6	Experiment arrives at NIH Lab; Experiment opened; Ground Truth 3 analyzed; Ground Truths 4 and 5 opened
July 9	Experiment analyzed*; Ground Truths 4 and 5* analyzed

*: Contamination Present

NASA for pre-loading storage at 4 °C, the transit control was shipped back to us and stored at 4 °C until analysis on launch day. Analysis of this control showed the effects of pre-flight shipment and storage on the experiment. The other five ground controls were stored at 20 °C, a standard culturing temperature for CeMM (Szewczyk et al., 2006), in order to eliminate the effects of temperature variations and determine the viability of prolonged growth within the FME under optimal storage conditions. The first ground control was scheduled for opening alongside the transit control in order to directly compare the effects of shipment and storage with growth at an optimal temperature within the FME. The second ground control was scheduled for opening shortly after activation in order to determine whether or not the FME could support worms for the length of time required to reach the refeeding stage under optimal temperature conditions. The third ground control was scheduled for opening shortly before landing in order to assess the growth in response to refeeding under controlled conditions. The fourth ground control was scheduled for opening upon experiment return for direct comparison of flight with growth on the ground. Lastly, the final ground control served as a reserve control in case it became desirable to adjust this timeline as the experiment progressed.

3.3. Housing control and experimental worms together

Because worms would not be returned to us directly after landing we decided to use wild-type worms as an internal control. Since we knew what the gene expression changes in response to spaceflight for wild-type *C. elegans* should be (Higashibata et al., 2007; Higashibata et al., 2006; Higashitani et al., 2005; Jamal et al., 2010; Leandro et al., 2007; Selch et al., 2008; Wang et al., 2008), we could determine whether or not these changes had occurred as expected and use that knowledge to filter out changes in gene expression that had resulted from post-flight handling (assuming robust growth in the FME). We could then be

reasonably confident that changes in gene expression not observed in *daf-16* mutants were due to the effect of *daf-16* mutation and not influenced by post-flight handling. The Type Two FME, however, only holds one containment device, the long ampoule. That meant that the wild-type strain and the *daf-16* strain had to be housed together. In order to separate the worms post-flight, a transgenic strain containing a Green Fluorescent Protein (GFP) was selected as the wild-type control TJ356 [*zIs356 (daf-16::gfp; rol-6(su1006)) IV*]. This DAF-16::GFP expressing strain is a sensitive probe of DAF-16 activation because it becomes nuclear localized as part of the stress response (Love et al., 2010). Post-flight control and *daf-16* mutants (Strain PJ1139 (*daf-16 (mgDf50) I; ccIs55 (unc-54::lacZ) V*) (Szewczyk et al., 2007)) could be separated based upon presence or absence of a GFP. Use of a COPAS platform was planned to automate this process (Pulak, 2006), however for small numbers of worms the TJ356 control strain can also be readily identified by the presence of a rolling phenotype that arises from the use of a mutation in *rol-6* (Cox et al., 1980) as a coinjection marker in the construction of the strain.

3.4. Loading procedures

Mixed stage cultures of each strain were diluted to four thousand worms per ml and then combined, creating a culture of the same concentration but housing equal amounts of both strains. Approximately 0.8 ml of CeHR that contained approximately three thousand and two hundred worms (one thousand and six hundred of each strain) was placed in the long ampoule. We loaded mixed-stage animals as mixtures of worms that are in various stages of development are more likely to yield some survivors under the variable ambient conditions experienced during pre-flight storage and shipment than are single-stage cultures (Szewczyk et al., 2005). 4 ml of CeHR was placed in the main compartment of the FME along with the loaded long ampoule. The extra CeHR was for refeeding

starved cultures once in orbit and prompting further reproductive growth. The components of the FME were sterilized at 121 °C for twenty minutes in an autoclave and the rest of the experiment materials were prepared in sterile conditions.

3.5. Experiment timeline

The experiment was originally scheduled to launch on 30 March 2012 onboard Soyuz 30S. An unprecedented delay of the Soyuz launch was announced and a decision was taken by SSEP to launch onboard the maiden flight of the SpaceX Dragon. Accordingly, all initial planned activities required an alteration in the timeline (see Table 1). The experiment and all controls (see Sections 4.2–4.6) were loaded on 4 April 2012 and shipped to Nanoracks the same day. The experiment launched aboard the SpaceX Dragon on 22 May 2012. The transit control and first ground control were opened and analyzed on the same day (see Section 4.2 and Section 4.3). The experiment was reportedly activated at 17:16 Coordinated Universal Time on 13 June 2012. The remaining ground controls were activated two days later and the second ground truth was opened and analyzed (see Section 4.4). The third ground control was opened and analyzed eleven days later (see Section 4.4). The experiment landed aboard Soyuz 29S on 1 July 2012, a Sunday. The Sunday landing resulted in NASA delaying shipment of the experiment to Nanoracks by one day. As Nanoracks received the experiment on 3 July and 4 July is a US federal holiday, shipment of the experiment back to us was delayed until 5 July and the experiment did not arrive until 6 July, at which point the remaining ground controls (see Sections 4.4 and 4.5) and experiment (see Section 4.6) were opened for preliminary analysis.

4. Results

A summary of results is provided in Table 2.

4.1. Pre-flight control experiments

As no published biocompatibility data were available for the FME, we ran several pre-flight tests using mixed stage wild-type and *daf-16* mutant worms. Worms grown in the ampoule alone were largely dead after three weeks.

There was no difference in time until the majority of the population was dead for worms grown at 20 °C with 25%, 50%, or 75% of the volume left for air space or for worms maintained at 4 °C with 25% or 75% of the volume left for air space. These results suggest that three weeks is the maximal time that one can expect to have viable, reproductive populations in the ampoule. Importantly, worms were left in the ampoules after the pre-flight analysis and live worms were observed within ampoules even after three months. These results suggest that it is possible to load worms and restart growth in orbit by refeeding them after a prolonged storage in the ampoule (the scenario we faced given the flight constraints). We next confirmed that both wild-type, DAF-16::GFP, and *daf-16* mutants could survive in the ampoule within the FME. For this test we opened the FME after 2 weeks and, as predicted, the majority of the population was alive. Lastly, we confirmed that worms could survive the activation of the FME (breakage of the ampoule). Taken together, the results from these tests suggest that the FME can be used for short duration (less than three weeks) experiments that utilize *C. elegans*, but more extensive testing is required to both confirm the lack of *C. elegans*-FME biocompatibility issues and to establish the limits of growth of *C. elegans* in CeHR in the FME.

4.2. Transit control

The transit control accompanied the flight experiment during shipment to Nanoracks and was returned to us after Nanoracks turned the flight experiment over to NASA. The transit control was opened on launch day alongside with the first ground control (see Section 4.3). As the pre-flight controls suggested that the majority of animals would be dead (see Section 4.1), we activated the FME prior to opening it in order expose potentially lethargic animals to fresh medium and oxygen. Similarly, rather than attempting to use the COPAS we simply poured the contents of the FME onto fresh NGM agar plates and allowed the contents to settle at room temperature. The next day, the plates were analyzed and it was determined that no worms remained alive. These results were consistent with the results from pre-flight control experiments that suggested that the majority of the worms would be dead after 3 weeks in the ampoule (see Section 4.1) and raised concerns that

Table 2
Summary of Results.

Test No./Type	Results
Pre-flight	Showed worms can survive for 2–3 weeks in the ampoule and can survive FME activation
Transit control	Confirmed a population of worms most likely will not survive past 2–3 weeks
Ground control 1	Showed a few worms from a population can survive past 2–3 weeks in the ampoule but that most survivors cannot reproduce
Ground control 2	Along with above, showed contamination is a issue
Ground control 3	Showed worms from a population are unlikely to survive extended storage periods in the ampoule
Ground control 4	Showed few worms were capable of reproducing following refeeding (activation) in the FME
Ground control 5	No live worms; suggested ability to recover live worms from flown was due to chance
Flown	Not activated, two live worms found, likely due to chance (see above)

under ($<4\text{ }^{\circ}\text{C}$) or over ($>25\text{ }^{\circ}\text{C}$) temperature excursions had occurred, which Nanoracks states did not.

4.3. Ground control 1

The first ground control was opened alongside the transit control (see Section 4.2) on launch day. Like the transit control, we chose to activate the FME prior to opening it and poured the contents onto NGM plates. Forty-five juvenile worms (L2 or L3 stage) survived. All of the worms were lethargic. Contamination was observed in the growth medium, although it is unclear whether the contamination arose in the ampoule or the main compartment of the FME. The animals were moved to fresh plates, when necessary, in groups of two-to-twelve animals and analyzed for movement and progeny production. On five out of seven plates, none of the animals developed past L3. On one plate with two animals, one survived and was fertile. The survivor and all progeny were rollers demonstrating that the survivors on this plate were of the wild-type strain. On the final plate, at least one out of the ten animals survived and produced progeny, and again all progeny were rollers. As with the transit control (see Section 4.2), these results confirmed results from the pre-flight control experiments (see Section 4.1) that suggested that the majority of worms would be dead after 3 weeks in the ampoule. As with the pre-flight control experiments but unlike with the transit control, live worms were recovered. While it may be the case that the transit control worms died due to temperatures conditions experienced during transit, the fact that live worms were recovered from the flight experiment (see Section 4.6), which experienced the same conditions, suggests that the lack of live animals in the transit control was due to chance. The ability to recover worms that were able to reproduce suggests that, again as predicted from the pre-flight control experiments, it is possible to grow animals in the ampoules in the FME and restart growth in orbit by refeeding them. Interestingly, only wild-type animals were found to be reproductive after the extended stay in the FME. Given the low numbers of survivors it is unclear whether or not this was due to chance or if the *daf-16* mutants are more susceptible to adverse conditions in the FME as the result of reduced metabolic capacity (Braeckman and Vanfleteren, 2007; Oh et al., 2006).

4.4. Post-activation ground controls

Ground Controls 2, 3, and 4 were all activated two days after the experiment was reportedly activated. Ground Control 2 was opened directly after activation and revealed contaminated medium and no live worms. Ground Control 3 was opened eleven days after activation and, despite not exhibiting contamination, yielded no live worms. Ground Control 4 was opened alongside the flight experiment. Examination showed no contamination and provided only two living wild-type roller worms, neither of which

produced progeny. As only two worms were present, it is not possible to state if the lack of *daf-16* mutant worms was significant.

4.5. Ground control 5

The fifth ground control was a planned contingency control. The presence of contamination in the first and second ground controls made it optimal to use this control as a second full-length flight control in case the final flight control was contaminated. Thus, the control was activated two days after the experiment was reportedly activated and opened at the same time as the flight experiment. Unlike the fourth ground control (see Section 4.4), no living worms were recovered and contamination was observed.

These results, combined with the previous results from the other ground controls, suggest that the ability to recover live worms after the extended period of time between handover to Nanoracks and activation of the experiment was due to chance alone.

4.6. Flight experiment

When the experiment was opened on Friday, 6 July, it was discovered that activation had not actually occurred. The long ampoule was unbroken and the animals had not been exposed to fresh medium and oxygen during spaceflight. In addition, major contamination was observed in the main compartment of the FME. As the worms had undergone the entire extended experiment timeframe housed only in the main ampoule, few living worms were expected. As such, there was no need for automated sorting in the COPAS, and the contents of the ampoule were placed on fresh NGM plates. Two living animals were identified, both rollers and thus wild-type controls. Neither animal produced progeny.

These results demonstrate that worms can survive a prolonged storage in the ampoule. However, the data from the transit and ground controls suggests that these few surviving animals occur by chance and that following one to two months in the ampoule the odds of finding live worms capable of reproducing after introduction to fresh food are dramatically decreased. The presence of living animals capable of reproduction in the first ground control (see Section 4.3) suggests that if activation had occurred on launch day, some worms may have been able to reproduce in some FMEs, although they would have most likely died long before landing. In contrast, the lack of live animals in the transit control (see Section 4.2) and third ground control (see Section 4.4) suggest that random chance is a large factor in determining whether or not any worms can survive in the ampoule long enough for a viable experiment to be achieved using the timeline for SSEP mission 3. The presence of living animals not capable of reproduction in the fourth ground control (see Section 4.4) suggests that even if the flight FME had been properly activated, no worms would have reproduced in the FME. Another observation

worthy of note is that all surviving worms in the experiment and in all controls were of the wild-type control strain, an observation that may or may be not of significance with regards to the *daf-16* (e.g. *daf-16* may be used to “cope” with growth in the FME). Unfortunately, the extremely small number of surviving worms makes it impossible to determine if the *daf-16* mutants were just dead due to chance.

5. Discussion

5.1. The fluids mixing enclosure

Historically, performing life science experiments in space has been difficult. Space and power constraints are common and experiments are rarely carried out using standard laboratory equipment. Furthermore, even simply determining what equipment is available for in-flight experiments is difficult (Hughes-Fulford, 2004; Space, 2011). In addition, flight safety regulators frequently attempt to eliminate access to ambient oxygen in flight, although it is both entirely safe and entirely possible to perform life science experiments with access to ambient oxygen (Etheridge et al., 2011a; Oczypok et al., 2012; Szewczyk et al., 2005; Szewczyk et al., 2008). As most metazoans require oxygen to sustain life, access to oxygen or lack thereof is a major caveat that needs to be considered when interpreting results from some space life science experiments. Here we have used the hermetically sealed Fluids Mixing Enclosure (FME), designed by Nanoracks LLC, to unsuccessfully conduct an experiment with *C. elegans* in orbit. Because no published biocompatibility data were available and knowing the FME was hermetically sealed, we designed our experiment in such a way as to capture the needed data and determine whether or not concerns with oxygen levels were justified. Results from our preflight tests confirmed that worms can sustain growth for up to two to three weeks in the ampoule regardless of whether or not the ampoule is in the FME. These results suggest that the FME is potentially capable of supporting a life sciences experiment for a two to three week mission, assuming late loading and early retrieval. As we have not closely examined the behavior or life history traits of worms grown in the FME, we cannot definitively say that there are no bioincompatibility issues with the FME or that worm death was due to a combination of exhaustion of nutrients and/or oxygen. Additionally, data from our transit and ground controls suggest that a two to three week delay from loading to launch would render life science experiments not viable. Thus, the FME appears well suited for late load and early retrieval missions that last no longer than two to three weeks, such as ISS resupply or crew exchange missions, but not for longer ISS expeditions.

The FME is currently designed as an opaque containment system. Two problems encountered during our experiment could have been avoided if the FME design was transparent. First, unwanted or contaminating organ-

isms tend to arise at some frequency despite sterile practices (four out of seven loaded FMEs were contaminated) and the use of growth media that, as the name implies, encourages biological organisms to grow, means this contamination can quickly grow and exhaust nutrients meant for experiment samples. Transparent hardware allows for examination of biological experiments for possible contamination on launch day and makes potential swaps of contaminated samples for non-contaminated backup samples possible (Etheridge et al., 2011a; Oczypok et al., 2012; Szewczyk et al., 2005; Szewczyk et al., 2008), thereby ensuring a greater likelihood of a successful spaceflight experiment (NB despite the use of antibiotics in a past flight, pre-flight swaps have still been required (Oczypok et al., 2012)). Second, the astronaut believed he had activated the FME despite not actually having done so. An auditory crack that occurs upon activation can be used as confirmation that activation has occurred, however ambient noise levels aboard the ISS, at approximately 72 dBA (Smith et al., 2003), can make hearing this crack difficult or impossible. A transparent FME would allow for visual confirmation of activation. Additionally, transparent hardware allows direct in-flight visualization of experiments without the need for sample return (Oczypok et al., 2012), an important consideration given the limited amount of mass allowed to return from ISS each mission.

Together, our results suggest that the fluids mixing enclosure can be used to conduct short duration experiments using *C. elegans*, and potentially other organisms, but that careful pre-flight testing should be performed when considering longer duration experiments and missions and that there is room to improve upon the existing FME design.

5.2. A high school student's experience

As an initiative to increase student interest in science, technology, engineering, and mathematics (STEM), Student Spaceflight Experiments Program performs admirably. The first author's experience with this experiment has been nothing short of inspiring: “Everything I've done for the experiment was both something I've never had the chance to do before and something most students don't get to experience until Graduate School, if even then. Although the anti-climactic ending was disappointing to say the least, failure didn't change my aspirations of going into a STEM field. If anything, it strengthened them – with so much to learn, even from a failure of an experiment such as this one, I can barely imagine what we would have learned had this experiment been successful, and what doors such success would have opened. My hope is other potential high school or other student researchers can experience what I've experienced in the future, not only so they can be as inspired as I am, but also because of the potential increase in valuable information in the relatively new field of space research.”

5.3. Commercialization of space life science experiments in the United States

The program that selected and flew this experiment, the Student Spaceflight Experiments Program (<http://ssep.ncesse.org/>), is a US national program that is a partnership between the National Center for Earth and Space Science Education and Nanoracks, LLC. Nanoracks is a US commercial company that provides access to and interface with the US National Lab on International Space Station. The launch of this experiment on SpaceX Dragon: Aquarius, the first commercial spaceflight supply mission to the International Space Station, means that we are quickly approaching the point of using both commercial payload managers and commercial delivery and return from the ISS.

Space life science experiments typically require late loading and early retrieval in order to maximize the health of the flown organism and minimize the post-flight changes in the organism prior to analysis (Hughes-Fulford, 2004; Space, 2011). Our experiment clearly faced both of these challenges. First, we have demonstrated that *C. elegans* can only survive in the FME for two to three weeks, proving that late loading is essential to experiment success. Second, the six-day delay between landing and return of the payload was long enough that an entire generation of *C. elegans* could have grown in CeMM/CeHR (Szewczyk et al., 2006). Any results obtained had the experiment been successful would, at best, be questionable due to the post-flight shipment time. This demonstrates that early retrieval is also essential to experiment success. The need for late access and early retrieval is an unfortunate reality for most space life science experiments. Both of these requirements add substantial cost and complexity to space life science experiment designs. If fully commercial space life science experiments are to become a reality in the US or elsewhere, companies will need to understand and make provisions for these requirements in order to ensure successful scientific return. Additionally, the US must identify how academic scientists will obtain the funds necessary to meet both these commercial costs and other associated costs of the research, most of which has traditionally been borne by NASA (Hughes-Fulford, 2011; Space, 2011). The concerns identified by this experiment must be addressed in order to make commercial space life science experiments a reality.

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