

Worms in Space for Outreach on Earth: Space Life Science Activities for the Classroom

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ABSTRACT

Long term spaceflight is associated with the loss of skeletal muscle mass and function. The Molecular Muscle Experiment (MME) seeks to identify the causes of muscle decline in space and test potential therapies to attenuate this in the microscopic worm, *Caenorhabditis elegans*. This is the first UK-led experiment in the almost two-decade history of the International Space Station. We therefore intend to complete significant and widespread educational outreach activities to promote interest in science, technology, engineering, and math (STEM), and to increase engagement with our space life science experiment. This paper describes three education outreach activities relating to MME that are suitable for use in the classroom: (i) observing normal and mutant worms; (ii) observing the effect of unloading (simulation of

microgravity); and (iii) handling spaceflight hardware. Activity packs are provided at a starter and advanced level to support these activities. This paper also provides three posters that may be used as learning resources for educators. These posters provide information on: (i) why worms are used for research; (ii) spaceflight human physiology; and (iii) the specifics of the MME. Details of further planned engagement activities are outlined to increase the awareness of the MME.

INTRODUCTION

Long term spaceflight is associated with the loss of skeletal muscle mass and function (Fitts et al., 2010), even with participation in exercise training, a known stimulus for gains in muscle mass and function on Earth (Trappe et al., 2009). These observed declines are sufficient to threaten both the health of astronauts and mission success (Buckey, 1999) and therefore hinder our ability for interplanetary spaceflight such as to Mars (Gaffney et al., 2017). Despite the potential severity of these losses, the exact molecular cause(s) of spaceflight-induced muscle decline have not yet been identified, hindering efforts to devise effective countermeasures.

Our current spaceflight experiment, the Molecular Muscle Experiment (MME) builds on previous work showing that *C. elegans* can

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grow and reproduce in space with no major health problems (Nelson et al., 1994; Oczypok et al., 2012). Previous work has also shown that these worms exhibit similar changes to humans during spaceflight, including changes in muscle properties and energy metabolism (Higashibata et al., 2006; Higashibata et al., 2016). Thus *C. elegans* have been established as an excellent model to study the mechanisms of muscle atrophy during spaceflight. Based on this, our current study (MME) will utilize *C. elegans* to investigate: (i) the mechanisms underlying spaceflight-induced muscle decline and (ii) whether drug-based countermeasures can alleviate spaceflight-induced muscle decline.

MME is the first UK-led experiment in the history of the International Space Station (ISS). We want to capitalize on the novelty of this experiment, and recent media and public interest in spaceflight (stimulated through Timothy Peake's Principia mission as the first British European Space Agency astronaut (ESA, 2017)), by organizing significant and widespread educational outreach activities. These activities include visits to schools, science fairs, and museums across the UK using the activities described in this paper. More broadly, we are increasing our outreach through the use of social media (Twitter: @worms_space) and a website (<https://www.mme-spaceworms.com>).

Specifically, this paper outlines three space life science activities based on *C. elegans* that are suitable for use by educators in a classroom-type setting. The overall learning objective of these activities is to increase understanding of spaceflight-induced changes in muscle.

LIFE SCIENCE ACTIVITIES USING THE WORM

C. elegans As a Useful Educational Resource

Our educational outreach activities use the microscopic roundworm, *C. elegans*. The use of *C. elegans* in education has proved valuable and attractive for similar reasons to the research lab environment, including its short life-cycle, small size, transparency, ease of cultivating, and similarities to the human genome (Kaletta and Hengartner, 2006; WormClassroom, 2018). In addition, *C. elegans* can also taste, smell, and respond to light and temperature (WormClassroom, 2018).

Education using *C. elegans* is well supported with large online resources including:

WormClassroom (www.wormclassroom.org), a specific *C. elegans* education portal; WormBook (www.wormbook.org), which contains peer-reviewed information on the biology of *C. elegans* and other nematodes; and WormBase (www.wormbase.org), a resource detailing the genetics and biology of *C. elegans*. For educators wishing to develop practical activities with *C. elegans*, strains can be obtained from the Caenorhabditis Genetics Center (CGC: <https://cgc.umn.edu>).

Spaceflight Related Outreach Activities

In addition to the utility of *C. elegans* for practical education sessions in biology, genetics, and neuroscience, they also act as an excellent resource to investigate changes that occur in physiology during spaceflight, which is the purpose of the MME. For those learning environments where practical handling of *C. elegans* is not possible, *C. elegans* can still be utilized to facilitate learning. To exemplify this, we have designed three novel classroom-based activities. Based on MME outreach activities that we have taken to museums and science fairs, these activity packs have been designed to explore spaceflight physiology using *C. elegans*. We have chosen practical activities since active learning has been shown to increase engagement of learners in STEM subjects and even improve performance in subsequent exams (Freeman et al., 2014). Involving the learner in engaging activities facilitates higher order thinking that is more difficult to foster when listening passively to an expert (Freeman et al., 2014). Moreover, active learning also promotes group work, which is highly translational to life beyond education.

Activity 1: Observing Wild-Type and Muscle Mutant Worms

The first MME outreach activity involves the set-up of two bright field microscope stations for the visualization of either wild-type (i.e., normal, healthy worms) or mutant (i.e., worms with muscle dysfunction) worms. As spaceflight causes the loss of muscle mass and strength in worms (and rodents and humans), this outreach activity provides an overt example of muscle dysfunction. A schematic of the experimental set-up and anticipated observations is shown in Figure 1.

The main learning objectives of the experiment are to assess the differences between worms grown on Earth (wild-type) and mutant

worms, which simulate the effect of spaceflight. The experiment aims to inform learners about the importance of maintaining muscle health and the challenges associated with spaceflight. To replicate the learning objectives in the classroom, we have developed two activity packs for learners. These packs contain images

and videos from the outreach activity that show how wild-type worms are healthy and represent “before spaceflight,” while the mutant worms (*unc-105*) show loss of muscle size and movement ability and are somewhat representative of changes that would be seen “after spaceflight.”

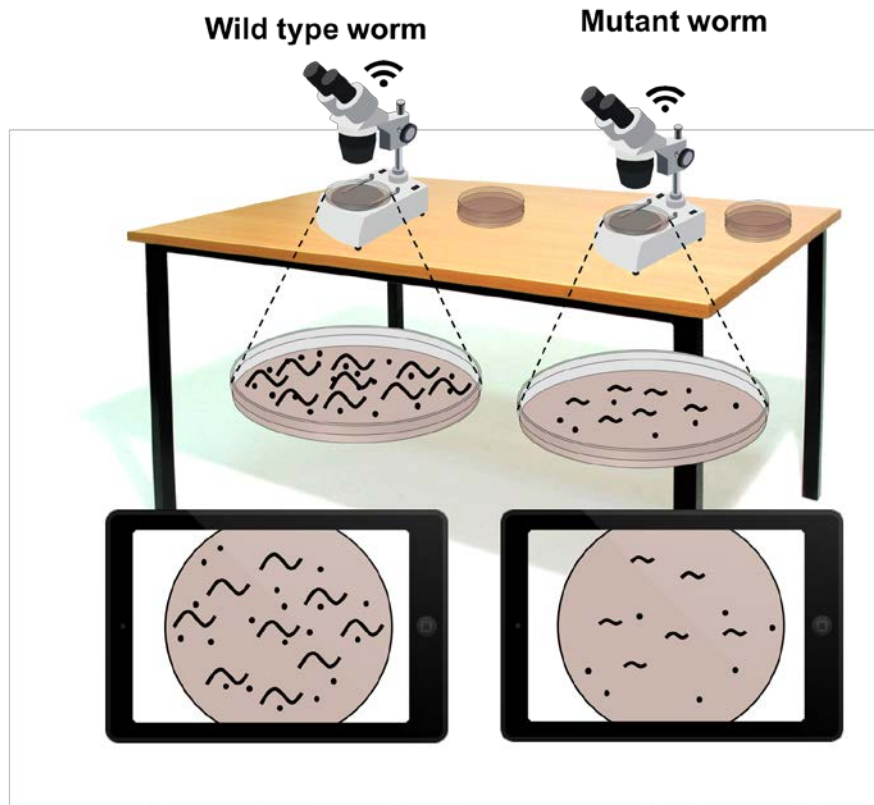


Figure 1. Activity 1 setup for outreach events: observing wild-type (i.e., healthy/ normal) and mutant (i.e., dysfunctional/ diseased) worms using a microscope and wireless tablet setup. Activity pack 1 provides data derived from this experimental setup.

In the starter pack, learners are asked to measure worm size and convert movement units. Learners are also asked to determine whether movement is better in worms on Earth or those in space. Finally, learners are asked to determine whether green fluorescent protein (GFP) images of muscle are from wild-type or mutant worms. Learners should be able to identify that wild-type worms are longer, show good sinusoidal movement (S-type shape of crawling), and show good reproductive capacity by laying a large number of eggs. In contrast, the mutant worms are smaller and hypercontracted, displaying little or no movement so they appear paralyzed. The mutant worms also lay very few eggs.

In the advanced pack, learners are challenged to analyze the movement rates of swimming worms from the videos, as described previously (Gaffney et al., 2014). Learners are then instructed to plot these data and provide an explanation as to why movement rates are different between strains. To equip learners with the knowledge for this explanation, the learners are sign-posted to WormBase (www.wormbase.org) for independent research. Finally, in this pack, learners are challenged to identify the differences in muscle architecture that they would expect with *unc-105* and then identify which images are taken from wild-type or mutant worms. Of note, the *unc-105* mutant strain was first isolated by Bob Horvitz (Park and Horvitz, 1986) who shared the 2002 Nobel

Prize in Medicine for his discovery of programmed cell death using other *C. elegans* mutants. This provides a link for educators wishing to further explore/explain the use of *C. elegans* as a model having led to transformations of biology (e.g., discovery of programmed cell death, the advent of whole genome sequencing, and the use of GFP). Although the *unc-105* mutant strain is used for this outreach activity (a mutant that contains a hyperactive mechanosensory membrane channel) (Gaffney et al., 2016), this activity could be expanded using other (muscle) mutant strains. For example, *unc-112* that contains a defective muscle attachment complex (Etheridge et al., 2015); *cha-1* that contains abnormal choline acetyltransferase (Rand and Russell, 1984); or *dys-1* a model of Duchenne muscular dystrophy (DMD) (Gaud et al., 2004). Each of these additional mutants have mutations in genes that have previously been reported to change with spaceflight (Wang et al., 2008; Higashibata et al., 2016). Thus, educators can

also guide more advanced learners toward looking at molecular changes associated with spaceflight and/or illustrate the use of *C. elegans* to understand human disease (further discussed in Activity 2).

Activity 2: The Effect of Unloading on Duchenne Muscular Dystrophy Worms

The second activity we use for MME outreach is exploring the visible differences in worms that are on solid nematode growth media (NGM) or in liquid media. The premise of this experiment is to mimic the effects of microgravity (with liquid) and determine the effect of unloading. To best demonstrate the effect of unloading, we use a DMD worm (the *dys-1* mutant strain BZ33). On solid media, this mutant displays abnormal movement patterns, whereas in liquid media, the *dys-1* mutant stops displaying movement patterns and instead coils, demonstrating how microgravity can impact the motility of an organism. This activity can be run in one of two ways as illustrated in Figure 2.

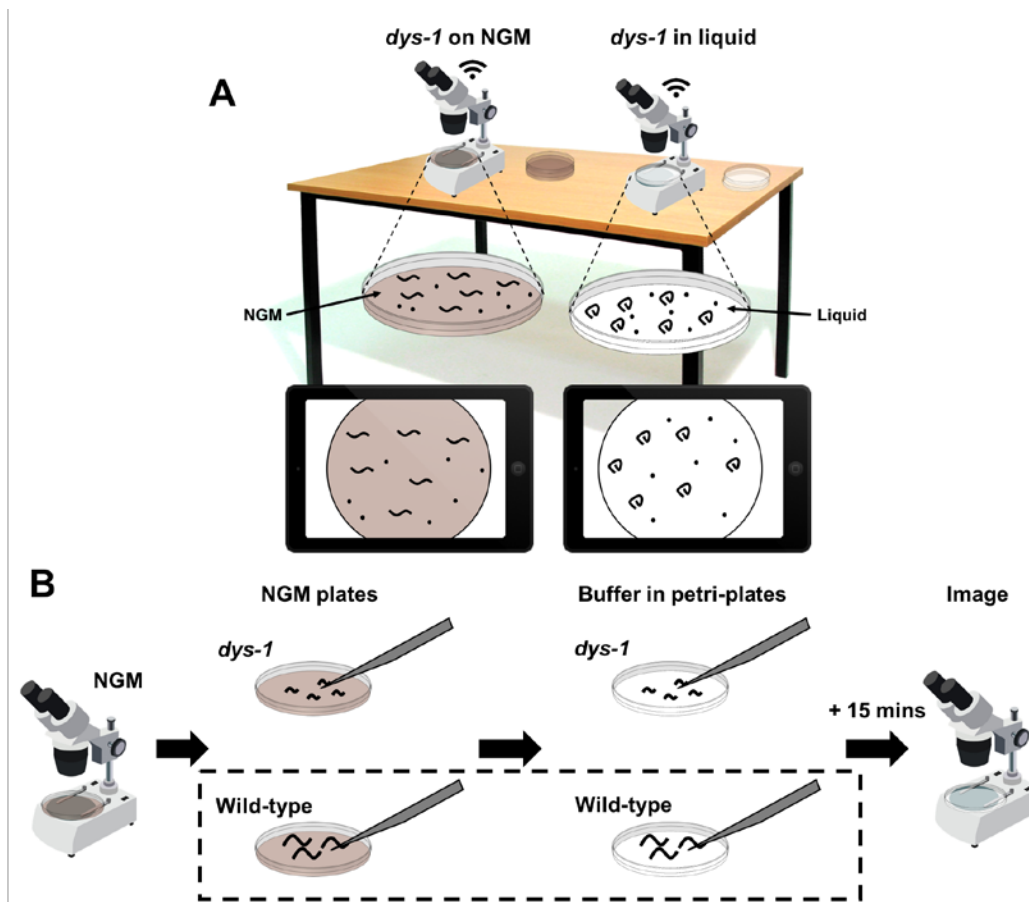


Figure 2. Activity 2 setup for outreach events: observing the effect of unloading on wild-type and mutant worms. (A) Mutant worms (e.g., *dys-1*) are observed in parallel while on solid nematode growth media (NGM) and in liquid within petri plates. **(B)** For an acute treatment, worms are transferred from NGM plates to buffer in petri plates and are left for 15 minutes before imaging. This can also be done with wild-type worms as an optional extra activity.

In Figure 2A, *dys-1* worms are prepared on solid media and in liquid buffer the night before or the morning of the activity and learners can observe the difference in the worm movement. Alternatively, *dys-1* worms (and if time allows, wild-type) are transferred from solid media to buffer during the activity so that learners can watch individual worms losing movement with time (Figure 2B). Note that for the latter setup, lack of movement starts to occur 15-30 minutes after transfer with more and more individual worms showing loss of movement over the next 24 hours.

The learning objective of this experiment is for learners to explore how certain diseases can alter muscle physiology. To replicate the learning objective of this experiment in the classroom, the starter pack for this activity provides images of unloading the *dys-1* mutant worms for 1, 2, and 4 hours. The *dys-1* mutants coil as a result of unloading, so learners are challenged to quantify this in wild-type and *dys-1* mutants over the multiple time-points using the images provided. Learners then plot these data, conclude which worm coils the most, and provide an explanation as to why this might happen.

For the advanced pack, learners start by explaining what muscular dystrophy is, before proceeding to measure movement rates of wild-type and *dys-1* mutants from videos supplied. Learners then plot the movement data before answering questions on why movement is different in DMD, and how understanding DMD relates to spaceflight. Similar to the starter pack, learners then score the number of coiled worms following unloading, plot these data, and answer a final question on the causes of coiling in *dys-1* worms. For higher level learners, statistical analyses can be completed on the movement coiling data to further increase the complexity of, and transferable skills achieved by, completion of the activity. Similarly, for higher level learners the choice of *dys-1* worms enables further learning around the use of *C. elegans* as a disease model and for drug discovery (Gaud et al., 2004).

Activity 3: Spaceflight Hardware

For the third activity, we have a physical collection of hardware from the current spaceflight experiment (MME) and from previous spaceflight experiments (International *Caenorhabditis elegans* Experiment First Flight (ICE-FIRST); Commercial Generic

Bioprocessing Apparatus Science Insert – 01 (CSI-01); *C. elegans* RNA Interference in Space Experiment (CERISE)). Part of this collection is the experiment cassette, cage, and bags that were developed by Kayser Italia under a European Space Agency (ESA) contract. These hardware components, depicted in Figure 3, will be used to house the worms during the MME in 2018. During education outreach, different aspects of the hardware collection are introduced, including the purpose and history (or future purpose) of each item. Outreach attendees are then allowed to handle the hardware and, where possible, construct items that are made from multiple components.

The learning objectives of this experiment are to encourage learners to think about the logistics of conducting an experiment in space by looking at space hardware, and to also develop their own ideas for spaceflight experiments. To replicate the learning objectives in the classroom, we have provided an activity pack with pictures of the spaceflight hardware to be used for the MME. This comprises three major components: (i) the experiment cassette that encases the scientific experiment and protects it during launch and landing; (ii) the cage that is made up of four pieces that hold the bags between them and fit together in the experiment cassette; and (iii) the bags that contain the worms and the liquid food that they live in.

For the starter pack, learners are given photographs of all of the components required for the MME hardware, with a task to identify how to assemble the components in the correct order so that the worm bags are secure and protected, and that they fit within the experiment cassette.

For the advanced pack, learners are challenged to explain some of the design features of the experiment cassette and the cage. This challenges learners to think about the interaction of surface area, membranes, and gaseous exchange. Advanced learners can also be encouraged to look at the past flight hardware used and consider how similar and different the design features were. Past hardware used for comparison includes ICE-FIRST (Szewczyk et al., 2008), CSI-01 (Oczypok et al., 2012), and CERISE (Higashitani et al., 2009). As a further activity in the advanced pack, learners are asked to design their own experiment using the MME hardware based on research about what other

student experiments have been done in space. Learners are encouraged to do an independent web search to identify groups that allow students to fly spaceflight experiments. To facilitate this activity, learners can be directed to the Student Spaceflight Experiment Program, which provides details of past and ongoing experiments using NanoRacks hardware that has

been used on-board the ISS (<http://ssep.ncesse.org/about-ssep/>). This also allows them to consider flying an actual experiment, for example, as done by a US High School student (Warren et al., 2013) and by Canadian Secondary School students (Graveley et al., 2018).

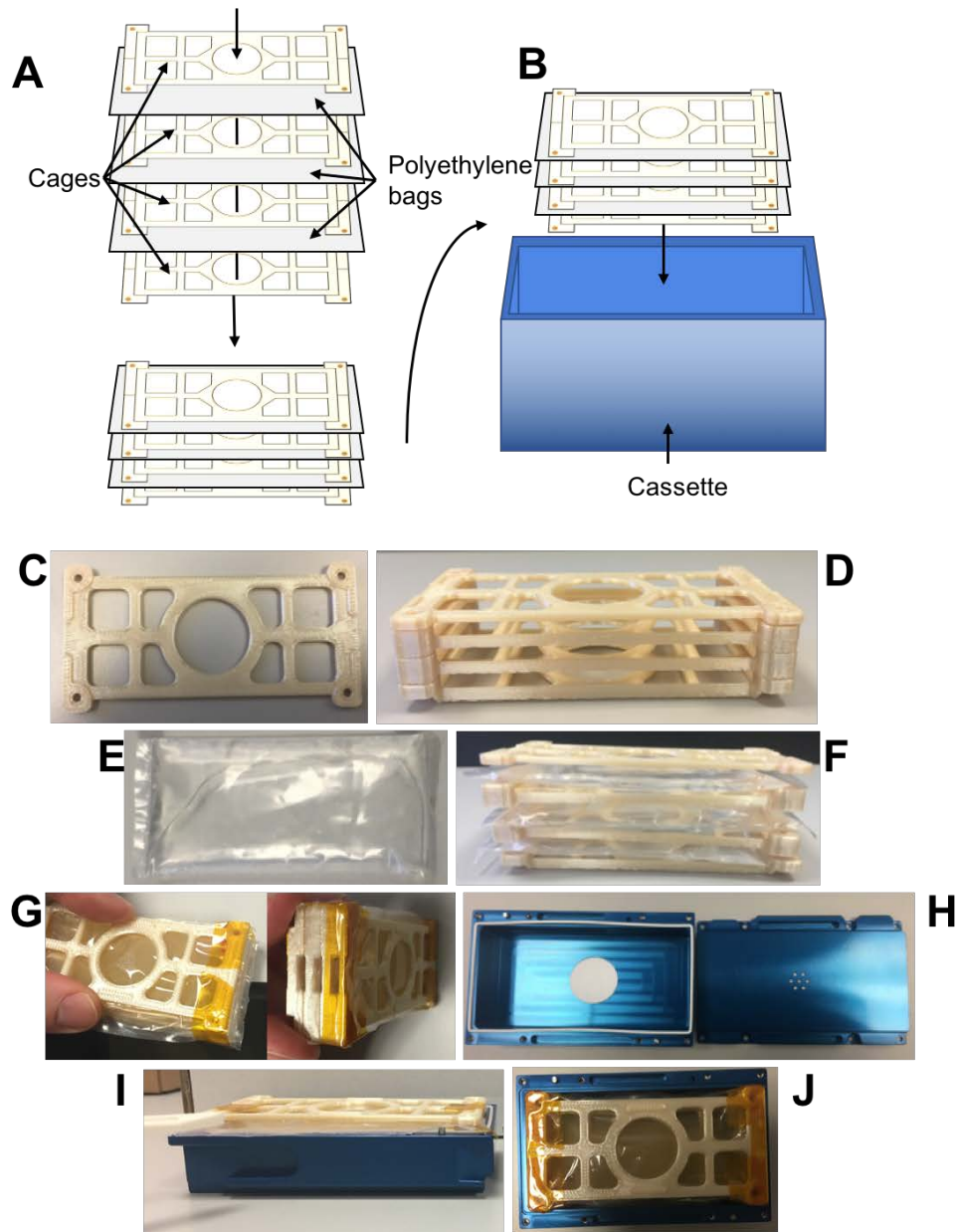


Figure 3. Spaceflight hardware. (A) Diagram of how cages and polyethylene bags come together and are then (B) inserted into the cassette. Photographs of *Molecular Muscle Experiment (MME)* spaceflight hardware components: (C) a single cage; (D) four cage compartments connected without bags; (E) polyethylene bags; (F) cages with three polyethylene bags enclosed; (G) corners of polyethylene bags taped to ensure a good seal within the cassette; (H) a single cassette; (I) lateral view of cassette with cage and bags *in situ*; (J) aerial view of cassette with cage and bags *in situ*. Activity pack 3 provides questions based on these images and photographs. Cassette dimensions are (height x length x depth as depicted in (A)) 24 x 91 x 56 mm. Hardware was developed by Kayser Italia under a European Space Agency (ESA) contract.

DISCUSSION

In both the UK (Department of Education, 2018) and the US (US Department of Education, 2016), strategies are in place to increase the uptake of STEM with a view to foster growth and innovation in our future economies. Spaceflight offers an area of appeal to those with a broad interest in STEM areas with the success of the MME flight dependent upon successes linked to each of the STEM areas. The activities described in this paper engage learners in all STEM areas, with a particular focus on the science aspect of STEM. The activity packs included in this paper provide an opportunity to engage learners in spaceflight-based life sciences at a very minimal cost. Beyond these activities, the *C. elegans* model makes it easy for educators to obtain and culture strains, and access online educational resources to support further activities in the classroom with relatively low-cost implications.

To assess the impact of our activities we are using Researchfish (<https://www.researchfish.net/>), which is a platform used by UK research funders to record and assess research impact. Researchfish currently captures the size and composition of the audience of engagement activities as well as the most significant impact of each activity from the following categories: (i) requests for further information; (ii) audience reports of changed views/opinions; (iii) plans for future engagement activities; (iv) own/colleague reports of changed views/opinions; (v) decisions made or influenced; and (vi) not aware of any impact. Since the beginning of 2017, we have taken part in 22 engagement opportunities ranging from science fairs to broadcast communications. Our audiences have included, and are not limited to, the general public, undergraduates, postgraduates, and school students. The majority of our engagement activities have resulted in requests to bring our activity to other engagement events and so fall into the 'plans for future activities' impact category. Based upon feedback from events, we have also occasionally reported changes to our audience's views with learners becoming more interested in STEM subjects. However, it remains to be seen whether these are long-term changes and we are not measuring if they are.

While we are required to report the impact of our own engagement activities, it may also be useful for those using our resources to measure

the impact of the activities on learners. The nature of how this assessment would take place will clearly depend upon both the country and the setting in which our activities are used. At a basic level, this might involve using surveys to ask students before and after carrying out the activities what their interest in STEM subjects is. These surveys could also evaluate if the resources have influenced the learner's opinions and views. At more advanced levels, the assessments might include prospectively following STEM uptake among participants, use of various active learning assessment tools (e.g., Brame, 2016), and/or integration of these activities into local curricular based teaching.

The MME flight is currently scheduled for late 2018 and the majority of educational outreach activity will take place in close proximity to the scheduled launch, to capitalize upon media attention surrounding the flight. In addition to activities at science fairs and museums, and the use of online resources such as the MME website and Twitter, a video documentary will be produced to report on preparation for and execution of the flight. Promotion using mass media conduits (i.e., television) will hopefully engage new audiences that may not otherwise engage in the experiment through outreach visits or targeted online materials. These additional resources can also be used by educators to increase the interest and knowledge of learners around space life sciences.

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