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MSc Research Thesis

CENTER OF ASTROPHYSICS AND PLANETARY SCIENCE

Can tardigrades survive Hypervelocity Impacts?

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Abstract

There are many objects in space. It may be unlikely that life evolved there, but if material was removed from a planetary surface as impact ejecta it could contain biological materials and then distribute it across space along with the rocks themselves (e.g. Martian meteorites found on Earth). For this idea, Lithopanspermia, to work, the biological materials or even life itself, needs to be able to survive the shock pressures associated with impacts. So, could tardigrades survive this?

This thesis therefore aims to show whether or not microorganisms like tardigrades can survive hypervelocity impacts. This could demonstrate that not only organic compounds, sugars and spores could survive the phenomenon known as panspermia, but also, complex organisms like tardigrades.

To achieve this, we used the Light Gas Gun at University of Kent. A shot program was developed and followed to shoot frozen projectiles containing tardigrades. This was done to simulate a possible asteroid or comet which impacts a planetary body, and contains tardigrades frozen in its ice. Optical microscopy was used to detect any survival and to perform different controls on the tardigrade's development throughout this project.

The results are discussed in Chapter 4. They show a survival rate of 100% up to ~1GPa. It seems 825 ms^{-1} is the threshold for tardigrades survival to these high speed impacts. The targets used were sand, and a new target type of water was tested, with preliminary experiments also reported. A set of

experiments should be carried out to confirm these results in the future (this is explained in Chapter 5 as future work).

Keywords: tardigrades, hypervelocity impacts, survival, Light Gas Gun, Centrifuge, Optical microscopy, panspermia, frozen projectiles.

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A big thank you also goes to all my office and lab colleages, for all those nice and random chats to make the days a bit nicer. In specific, I would like to thank Aishling and Vassi. Those Skype sessions during lockdown and the cat's pictures really helped to keep my head sane and focus on my thesis. Great tips that I will keep with me forever!

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1 Chapter 1: Introduction

Tardigrades were discovered around 250 years ago [1]. (Figure 1). They are small (typically < 1 mm) multi-segmented, water dwelling creatures. The survival of tardigrades against many severe environmental stimuli has long been reported (including vacuum and UV). In the article *"Actions différentielles des rayons x et ultraviolets sur le tardigrade* Macrobiotus areolatus*",* in 1964, for the first time it was suggested that tardigrades, due to their high resistance to radiation, 2.5–20 kJm^{-2} (10-100 times higher than radiation on Earth [2]), could be model animals for space research [3]. Almost 40 decades after, in the article *"Tardigrades as a potential model organism in space research",* a similar concept was suggested by Bertolani et al. [4].

What could be the explanation for their survival? Guidetti et al. [5] suggested, that they can lower their metabolic activity when entering into dormant states (anhydrobiosis induced by dehydration and cryobiosis induced by freezing); this could explain how they are able to withstand chemical and physical extremes. However, a large tolerance is shown also by active animals; they can be stored in dry state for many years without loss of viability [6]. It is this survival against environmental stress that suggested them as a potential model for survival in space, and, accordingly, samples of tardigrades were eventually exposed to space, in the Biopan 6 experiment on board the FOTON M-3 mission in 2007 [7]. Biopan 6 was a self-contained capsule, that was placed on the outside of the ISS for 10 days [7] and then brought back to Earth. Some survival of tardigrades was found, around 68% of the specimens revived within half an hour, despite high UV fluxes (the full

solar UV flux at 1 AU, unattenuated by the Earth's atmosphere) and the high vacuum. More details are given in Chapter 2, but it shows that in general they can survive in space where exposed to vacuum of around 10^{-8} torr and two different radiation spectral ranges: UV-A and UV-B ($UV_{A,B}$, 280–400 nm), and the full UV range from vacuum-UV to UV-A (UV_{ALL}, 116.5–400 nm). Samples were also exposed to ionizing solar (7000kJ/m^2, 1000 times higher than radiation on Earth's surface) and galactic cosmic radiation [7]. Since then several similar experiments featuring space exposure have been conducted (See Chapter 2 for a summary).

Figure 1-1: Tardigrade in Moss by NASA (scale size modified, only augmenting the number making reference to the size). https://science.nasa.gov/tardigrade-moss

There are many objects in space. It may be unlikely that life evolved there, but if material was removed from a planetary surface as impact ejecta it

could contain biological materials and then distribute it across space just like the rocks in Fig 1-2. It has been suggested that a potential mechanism for panspermia is for rocks to be launched into space as a result of a nearby giant planetary impact throwing up high speed ejecta, achieving escape velocity. These rocks then orbit the Sun until they hit a new body – this process was labelled Lithopanspermia [8]. The initial launch into space would involve a shock, rather than static, high-pressure loading of the sample. Laboratory experiments have shown that such impact ejecta can carry viable micro-organisms despite the shock they experience. [9][11]

Similarly, on arrival at a new home, the rock will experience another, even greater shock, due to the sudden deceleration from the high speeds (typically km s^{-1}) involved in space travel. The resistance of various micro-organisms to such shocks has been reported. For example, in [10] it was shown that spores could survive impacts at 5 kms^{-1} (albeit with low survival rates). This was followed in [14], where the previous results were confirmed for impacts at 5 kms^{-1}. Similarly, shock experiments showed that microbial survival could occur at 38 GPa [12]. Survival rates were then found vs. peak shock pressure, e.g. [13], showing a steep fall off above a few GPa.

Figure 1-2: (a) Asteroid impact. https://astrobiology.nasa.gov (b) Artist's rendering of asteroids and space dust. In a similar way would ejecta from an impact travel from one body to another. (Credit: NASA/JPL-Caltech)

It was suggested [15] that tardigrades could be vehicles for panspermia, migrating through space on meteorites. Also, lithopanspermia needs to be consider in this scenario. It will involve a shock and consequent high pressures, when launched into space, and again, when back into their new home [15]. In laboratory experiments, it has been shown that tardigrades can survive high static pressures, up to 7.5 GPa [16]. Survival depends on the duration of the exposure to the high static pressure, with 100% survival rates for exposures up to 6 hrs, and a calculated 0% at 13 hrs. But it is not clear what dynamic loading, i.e. a shock event will do.

Interest in testing of Panspermia as a viable mechanism, has triggered a variety of experiments. A whole range of spores, microbes and seeds have been tested against different stresses in the laboratory and exposed in space. As an example, Figure 1-3 shows neatly how larger number of survivors are recovered following shock compression compared with static pressurization for both wild and pressure adapted samples [17].

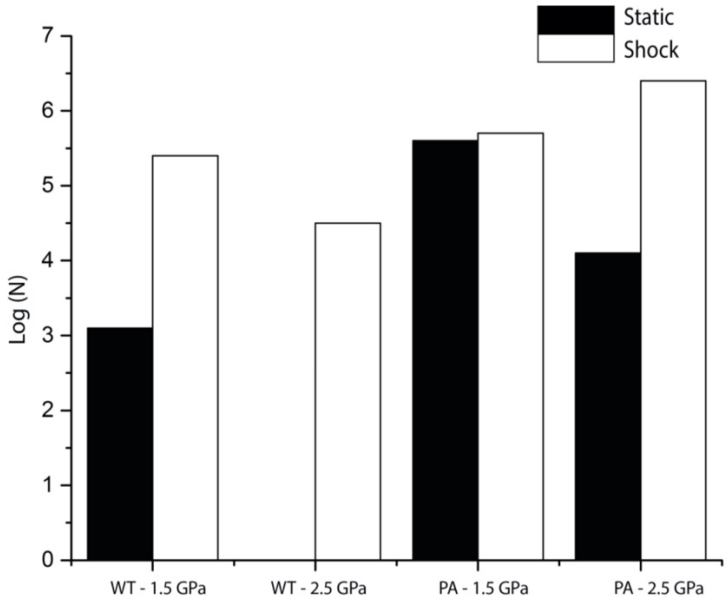

Figure 1-3: Bacterial survival on a logarithmic scale using N as the no. of colony forming units per ml. WT stands for wild type and PA, for pressure adapted samples. The graph shows static vs. shock compression experiments. Source: Figure 3. [17]

For microbial samples survival against shock pressures decreases sharply above a threshold of a few GPa (see Fig 1-4 for an example). It has also been shown, that passage of the shock wave through microbial samples causes delamination of cell walls, leading to decreased survival, indicating how damage occurs in shocks [18]. Several other types of material have also been subject to shock experiments, including seeds [19], which fail to germinate after shocks of around 1 GPa scale, due to several internal damage. Thus, the larger, and more complex an organism, the greater the ability of passage of a shock wave to cause damage.

This brief review of lithopanspermia has shown that, simple microorganisms may be able to survive the extreme shocks required, but more complicated mm scale objects like seeds do not. It is thus unclear what would happen to tardigrades. This has been followed up by testing tardigrades in centrifuges, simulating up to 16,000 rpm [15]. Survival was again observed, with decreasing rates at higher g. This method of centrifuging will be carried out in the lab as part of the master's project.

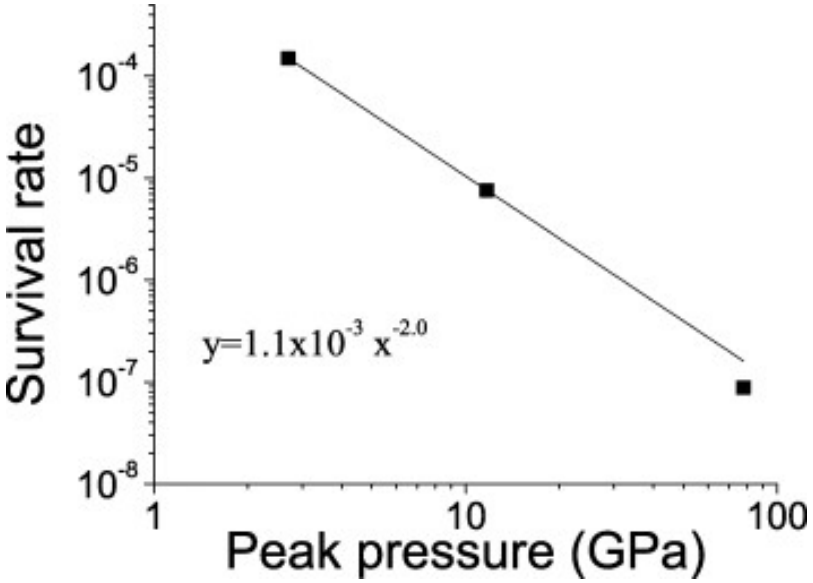

Figure 1-4: Survival rate (percentage) for impacts of Rhodococcus erythropolis on agar targets [13]

Although so far, we have considered Panspermia as a natural event, driven by large impacts throwing up ejecta, human activity in space involves space vehicles that can also carry bioloads. This is something we are responsible for, and therefore we have to consider whether we are spreading biological materials through space, to potential new habitats. Planetary protection

protocols [20] are a means to consider this and evaluate and control the risks involved. To quote: ''Planetary Protection is an agreed international practice that is defined by the United Nations; promulgated by the Committee on Space Research; and practiced by space-faring agencies such as NASA, the European Space Agency, Japanese Aerospace Exploration Agency, and others'' [20].

To accomplish these goals, the NASA's Office of Planetary Protection [21] contributes with recommendations and activities such as:

- the construction of sterile (or low biological burden) spacecraft
- developing flight plans that protect planetary bodies of interest
- the development of plans to protect the Earth from returned extra-terrestrial samples
- application and formulation of space policy as it applies to Planetary Protection

Although other planets may, or may not, harbour life, the Earth certainly does. As pointed out in [22], ejecta from giant impacts on the Earth may impact the Moon at speeds of just 2 – 3 kms^{-1}. In [23] it has been noted that this corresponds to peak shock pressures of some 15 – 20 GPa, within the range where micro-organisms can survive with low survival rates. But, again, what about more complex life forms like tardigrades? Indeed, in April 2019, an Israeli Spacecraft crash-landed on the Moon (the Beresheet Mission). According to some reports, its payload included tardigrades to test survival under lunar conditions [24]. Whilst the crash was unintentional, any containment systems for the tardigrade experiments may have been compromised, and, if they survived the impact they may still be there.

This mishap leads to new questions, for instance, how does this accord with the Planetary Protection Protocols if they are able to survive, and, more broadly, what can we learn about Panspermia? The key question here is: Can they survive the high-pressure shocks involved? And indeed, if tardigrades are to be considered a vehicle for lithopanspermia in general, this question requires an answer.

2 Chapter 2: Origin of Life

2.1 Introduction

This chapter gives an introduction to two topics that appear distinct but are actually very closely related. These are firstly the formation of life, and secondly how it is spread through the Universe. Many different types of organisms can be found on Earth, only a very small fraction of which can survive in outer space. One of these latter organisms are tardigrades, which are the focus of study of this work.

The ability of tardigrades to survive in space suggests these creatures tolerate extreme environments involving radiation, impacts, and radical temperatures. Also, the concept of panspermia arises when exploring this topic. If small molecules or simple organisms can travel frozen on the surface of an asteroid, and survive the shock into their new home, what about more complex organisms? What about tardigrades? Can they survive these extreme processes too?

In order to answer these questions, we first need to discuss what life is, where does it come from and how did some organisms like tardigrades evolve to be able to live in two such extremely different environments like space and the Earth?

This journey begins by exploring what life is made of, what is the 'Michelin star' recipe for it. Following this, is a discussion of how life appeared on

Earth, followed by a further discussion on a topic that is currently very popular: Is there life anywhere else in our Solar System?

In the latter part of this chapter, panspermia will be explored, discussing the possibility of complex organisms travelling from one planet to another. A brief taste of the different types of extremophiles will be given, and a more in depth investigation will be done on tardigrades. Specifically, the species *Hypsibius dujardini*, which is the one involved in this project.

Finally, a brief description will be given of some experiments that have been carried out in the ISS (International Space Station) with tardigrades, and in particular how they survive vacuum conditions.

2.2 What is the recipe for life? Life on the Early Earth

Over 3 billion years ago, life began on Earth. Over time, it evolved from the most primitive of microbes into an astounding array of complexity. But how did the first organisms develop from the primordial soup, if that was indeed their point of origin?

For many years, scientists thought that life might have had arisen on Earth from non-living predecessors in some ideal environment, such as the 'warm little pond' envisioned by Charles Darwin [25]. Deep sea hydrothermal vents and fissures in the Earth's crust and ocean floors have also been considered as sites for the possible origin of life on Earth, e.g. [26]. These sites are highly studied nowadays, and evidence for them has also been found on Europa and

Enceladus [27]. Laboratory experiments have shown that complex organic molecules (COMs) like amino acids and other prebiotic molecules can be created, in the presence of an energy source, from simple precursors suchas H_2O, NH_3 and CH_4. [27] Understanding how and under what conditions life arose on Earth would, in turn, lead to a better assessment of the probability that it exists elsewhere, within or beyond our solar system.

In the 1920s, Aleksandr Oparin and J. B. S. Haldane proposed what is now called the Oparin-Haldane hypothesis: that life on Earth could have arisen step-by-step from non-living matter through a process of "gradual chemical evolution" [28]. They thought that the early Earth had an oxygen-poor atmosphere (reducing atmosphere) in which molecules tend to donate electrons.

In 1953, an experiment was carried out to simulate a primitive atmosphere, trying to prove the Oparin and Haldane hypothesis. This is known as the Miller-Urey experiment [29,30]. Miller and Urey built a closed system that contained a mixture of gases that were, at that time, thought to have been abundant in the atmosphere of the early earth (H_2O, H_2, NH_3 and CH_4) and a heated pool of water (Fig 2-1). Miller and Urey sent sparks of electricity through their experimental system, to simulate the lightning that might have provided energy for chemical reactions in Earth's early atmosphere [29,30].

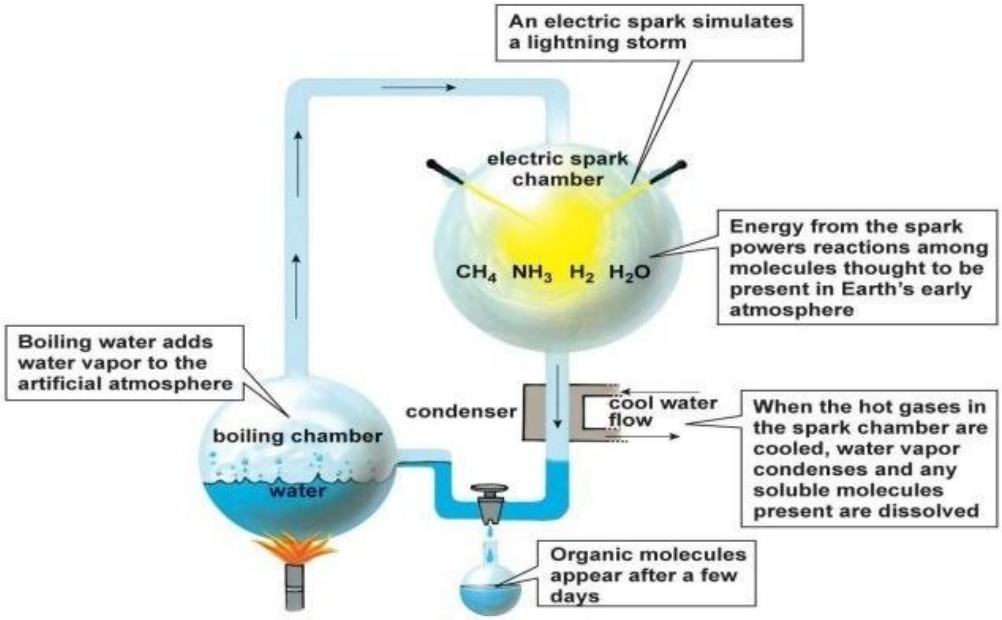

Figure 2-1: Apparatus and description of Miller-Urey's Experiment [2011 Pearson Education, lnc]. The liquid (initially pure water) in the boiling chamber was heated, and as shown adds vapour into the atmosphere above it. This then flows into the spark chamber where energy is added to the mixture. The resulting mixture of gases and water vapour was then cooled and condensed and flows back into the original liquid chamber. The presence of a tap allowed some liquid to be sampled during the process and revealed the presence of amino acids which had appeared [29].

It is now thought that early Earth's atmosphere was different than in Miller-Urey's experiment setup (not reducing, not rich in ammonia or methane) [29][30]. Therefore, it is questionable as to whether Miller and Urey did an accurate simulation of the early Earth. However, they did show that complex molecules, amino acids, could be formed from raw ingredients. Further, experiments done over the years since, have shown that organic

molecules (mostly amino acids) can form from inorganic predecessors under a fairly wide range of conditions [31].

It seems reasonable to think that, after these experiments, at least some of life's building blocks could have formed abiotically on early Earth. However, exactly how and under what conditions, remains an open question.

Another possibility could be, that these organic molecules came from outer space. Many scientists are studying the possibility of organic molecules travelling through space on meteorites. It has been found that organic molecules can be produced from simple chemical precursors present in space, under conditions such as high UV irradiation and low temperatures or via impacts onto mixtures of ices on the surfaces of comets or icy satellites [32]. We also know that some organic compounds are found in space and in other star systems. [33] There is also evidence of amino acids and complex organic molecules on comets, with glycine reported on 81P/Wild-2 by the Stardust mission [34] and a variety of compounds reported on 67P/Churyumov-Gerasimenko by the Rosetta mission (see [36] for a recent review).

Various meteorites have also turned out to contain extra-terrestrial organic compounds, i.e. not originated on Earth. One early example of such a meteorite, the Murchison meteorite, was observed to fall in Australia in 1969 and was immediately recovered, thus reducing terrestrial contamination. It was found to have carried nitrogenous bases (like those found in DNA and RNA), as well as a wide variety of amino acids, some of which have no terrestrial counterpart [37]. Another well known example is ALH84001 (Fig

2-2), which came from Mars and contained organic molecules with multiple ring structures. [38]

Figure 2-2: The Martian meteorite ALH84001 meteorite, on display at Smithsonian Museum of Natural History. Photo by J. L. Stuby, CC BY-SA 3.0.

Martian meteorite rocks were exposed to the extreme conditions of space during their (lengthy) transit to Earth, and a final hypervelocity impact when reaching the destination planet, in this case, Earth. As mentioned above, some molecular bases were nevertheless detected on them. So, in the case of a Martian meteorite, if these simple organic structures survived this process of transfer from one neighbuoring planet to another, could they survive the transfer to a wider range of other planets in our Solar System or beyond? [39] Indeed, the question if more complex organics can survive this process too arises. And what about life? Tardigrades for example, we could study the possibility of finding them somewhere else in our planetary system, and apply this to future space exploration missions.

These questions are considered in this thesis. In particular I aim to explore if extraordinary creatures like tardigrades could potentially spread through space via panspermia.

2.3 Life on the Solar System

Where is the first place everyone has in mind when thinking about extra-terrestrial life in the Solar System? The answer to this is often Mars, as it had an Earth-like past with water on its surface. It is in many ways an ideal candidate to have developed life.

However, there might be a bigger chance on other Solar System bodies further away. Today, the modern mantra for locations where life may originate, is to say you need liquid water, an energy input (external or from chemical disequilibrium), plus some organic molecules. This is not just limited to Mars. Other bodies, particularly those with possible surface oceans match these criteria [40]. If we ever discover life outside our planet, it will most likely to be in the form of extremophile microorganisms. To quote NASA's 2015 Astrobiology Strategy, "*Life on other worlds is most likely to include microbes, and any complex living system elsewhere is likely to have arisen from and be founded upon microbial life. Important insights on the limits of microbial life can be gleaned from studies of microbes on modern Earth, as well as their ubiquity and ancestral characteristics*" [41]. The assumption of other planets and their moons being habitable, is increasingly seen as very reasonable, especially after it has been found here on Earth that organisms can perfectly function using energy provided directly from the rocks deep underground and far away from sunlight. [42]

Even if Mars may have harboured microbial life at some point in its history [43] it may not be active today and any evidence of life may only be found as fossil traces. Accordingly, the scientific attention these days is increasingly focused on icy moons (see Fig 2-3) and some other small bodies in the Solar System, because the ideal conditions for life may still be present today, unlike modern Mars. If we were to find life in any of these places, how would have it appeared on these planet/moons? Did it emerge on them, or it travel from somewhere else? Has panspermia played a role in this situation? This is discussed in the following section.

Figure 2-3: Saturn's ice moon Enceladus. One of the possible life harbouring moons of Jupiter and Saturn. https://phys.org/news/2015-10-saturn-icy-moon-enceladus.html

2.3.1 Panspermia

The idea of Panspermia as a concept, started being discussed ~2400 years ago . Meaning literally 'seeds everywhere', this hypothesis suggests that life came to Earth from outer space. It has been one of the central topics between scientists when discussing the origin of life.

The first allusions to this idea seem to have originated in the 510-428 BC (Pre-Socratic Era), with the philosopher Anaxagoras of Clazomenae [44]. He thought that:

'Living creatures arose from the moist being evaporated by the Sun. In the beginning man was very similar to a different kind of animal, namely a fish' [45]

In the Pre-Socratic Era, intellectuals were after rational explanations to their surrounding world. Anaxagoras used to mention 'seeds' (spermata in Greek) when deliberating about the universe. His disciples took this as life had to come from elsewhere. [46] This was considered as an avant-garde idea for the time and was not followed up.

After this period, the concept of life travelling through the universe was mostly abandoned, probably with a lot of other "anti-theological" thought. With the Age of Enlightenment came Europe's reignited interest in Science. In the 18th century, Benoit de Maillet, a French government official and natural historian, wrote a book called Telliamed (De Maillet backwards). In

it, he theorized that the cosmos was filled with seeds of anything that can survive in space. [47] Also, he claimed that these seeds were 'more numerous around the opaque globes in thick airs and in waters than in the immense spaces, in these oceans of void by which the globes are separated.' [47]

Even though De Maillet proposed his theory in the 18th century, the concept of Panspermia was not discussed more seriously until the 19th century. Three scientific hypothesis and theories helped make this a serious and important topic. The first one was the Kant-Laplace Hypothesis.

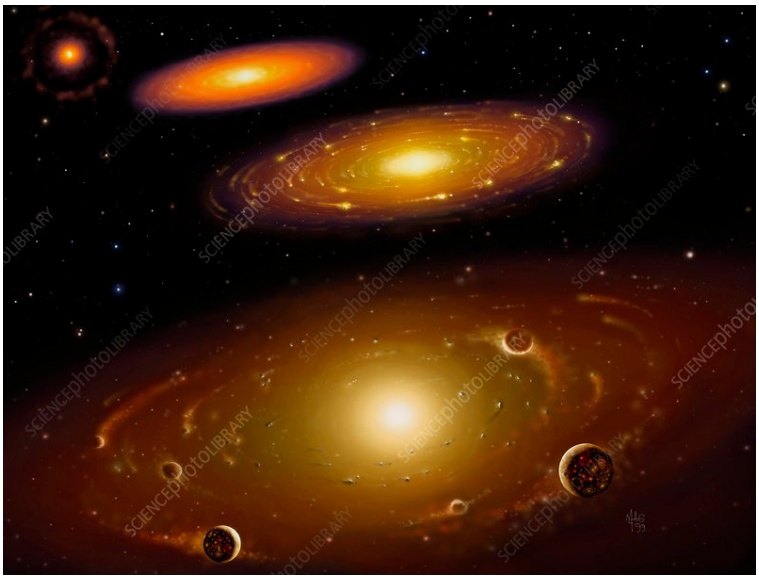

Figure 2-4: Solar system formation. Artwork of stages in the formation of the solar system. By Mark Garlick, in /media/324277/view/artwork-of-stages-in-the-solar-system-s-formation. The sequence starts top left when a stellar core ignites, it then heats a surrounding clod of material which forms a disk. Clumps of material form in this disk and then coalesce into asteroids and planets (bottom centre).

This is also known as the Nebular Hypothesis. Kant's key idea was that the solar system started as a cloud of distributed particles. Pierre-Simon Laplace, 40 years later, made a major contribution to Kant's hypothesis. [48] His model represents a Sun already formed and rotating. Its atmosphere extended beyond the distance at which the farthest planet would be created, implying that the early Earth was too hot to harbour any life in the beginning. [48]

The theory of Laplace incorporates Kant's idea of planets 'coalescing' from dispersed material (see Fig. 2-4). This is why both approaches are combined into this single model known the Kant-Laplace hypothesis. This model for solar system formation was widely accepted for about 100 years. [48][49]

In parallel to this, in 1859, Darwin finalised his theory of Evolution. This was published in his book 'On the Origin of Species', still available today [50]. Whilst it seemingly was concerned with evolution, it removed a theological basis from the discussion, and leaves open the origin of life itself - this latter point then became a subject of debate amongst other scientists.

Finally, the third contribution was made by Louis Pasteur. He discredited the idea of 'spontaneous generation', the idea that life arose from non-living matter. He showed that life comes from life, with his famous flask experiment. [51] This approach of life coming from life, still left open the question of the original origin of life itself however.

These three historical events inspired, amongst others Hermann Richter, Lord Kelvin and Hermann Helmhotz to come up with 'Cosmozoa'. [52] This

theory stated that, through meteorite impacts, life could have arrived on Earth from space in the form of spores from outer space. Once on Earth, the life flourished and evolved as predicted by Darwin, to provide the varied lifeforms we see today. [52]

The 1903 Chemistry Nobel prize winner, Svante Arrhenius, developed the hypothesis of panspermia into a more detailed scientific proposal. Naming his theory as "Panspermia", he was sceptical of the idea that life was brought to Earth via meteorite impacts; suggesting that life, as seeds, was directly transported through space via solar radiation pressure (see his 1906 book, Worlds in the Making, available as an English translation in 1908 [53]. It was later realised there were problems with this theory, mostly concerning the damage that radiation would do to seeds in space.

The idea of seed based Panspermia was revived later in the 20th century by Fred Hoyle and Chandra Wickramasinghe. They noted that dust travelling through space had a high organic content. They also speculated that light extinction curves suggested organic materials such as cellulose were present in space. [54] This made them think that these dust particles could enter Earth's atmosphere, bringing new diseases and viruses, even life itself to this planet, along with the genetic uniqueness necessary for macroevolution. [54] This idea ended up languishing. However, by the 1990s, the idea had arisen of lithopanspermia [8] with rocks being exchanged from Mars to Earth, (maybe containing life?). This idea allowed people to focus on a specific question, if life was on one planet (Mars) how could it get to Earth? Some people broke down the transfer into a series of steps and looked at the

hazard at each step (shock, pressure/vacuum, radiation, temperature.) [55] Whilst most hazards were tested in lab experiments, the issue of shock was relatively neglected, because it was known [56] that the shocks were at the GPa scale, but it was believed that even MPa shocks killed life – however this had never been convincingly shown in experiments.

After this, a new breakthrough was achieved by Burchell et al. [10,11] They focussed on the issue of survival of organisms against shocks in high speed impacts typical of those rocks from space would undergo. Their experiments showed that after impacts up to ~30GPa (5.4 km s^{-1}), bacteria could survive. [10,11] Other experiments have followed by a variety of groups, showing survival of a range of spores and microorganisms in a variety of shock experiments e.g. [9, 11, 12, 13, 14, 57]. This suggests that, despite the previous belief, microorganisms can survive the shock pressures impact needed in the panspermia process.

The other hazards involved in panspermia and lithopanspermia have also been directly tested in space. For example, three relevant series of experiments have been carried out in the outside of the International Space Station between 2008 and 2015. [58] These experiments look at the survival of microorganisms, spores and biomolecules in space vacuum and solar flux for extended periods of time (~1.5 years). [58] In brief, survival can occur under certain conditions. One of these experiments is discussed in detail later on in this chapter.

Recently, in November 2019, for the first time, sugar molecules have been detected in meteorites. [59] This goes further than the earlier work of Elisha et al., who found evidence for amino acids on comet 81P/Wild-2 [34], and subsequently [35] showed in the laboratory a mechanism for making such amino acids on comets. Taken together, this suggests that basic biochemistry can be produced on small bodies such as asteroids or comets. This supports previous theories about the 'RNA world prior to a DNA based origin of life' on Earth. [60]

Unfortunately, panspermia can neither be proved nor disproved – not unless we observe it in action. Yet, nonetheless, life has to have had at least one beginning, and in Earths' specific case, the origin remains unknown. [61] Panspermia is a still unsolved enigma.

2.3.2 Extremophiles

Extremophiles are organisms that survive in environments that were once thought not to be able to sustain life. These, what to us are extreme environments include, amongst others, extremes of temperature, highly acidic environments, extreme pressure and extreme cold. Yet it is now being realised that diverse organisms have evolved in different ways to adapt to these environments. [62]

The organisms that survive extreme heat are called thermophiles and prosper in temperatures between 45°C - 100°C. For example, in 1960, at the Yellowstone National park, a thermophile bacteria (*Thermus Aquaticus*) was

discovered which could survive the high temperature of the hot springs in this park (50°C - 80°C). [63] Also, in hydrothermal vents (Fig 2-5), another bacterium was discovered that could live in even more extreme conditions. [64] In this case it did not just survive extreme temperatures (~340°C), it was also subject to high pressures (250 atmospheres). [64] Organisms with the same abilities as these bacteria, surviving temperatures above 100°C, are called hyperthermophiles. [65]

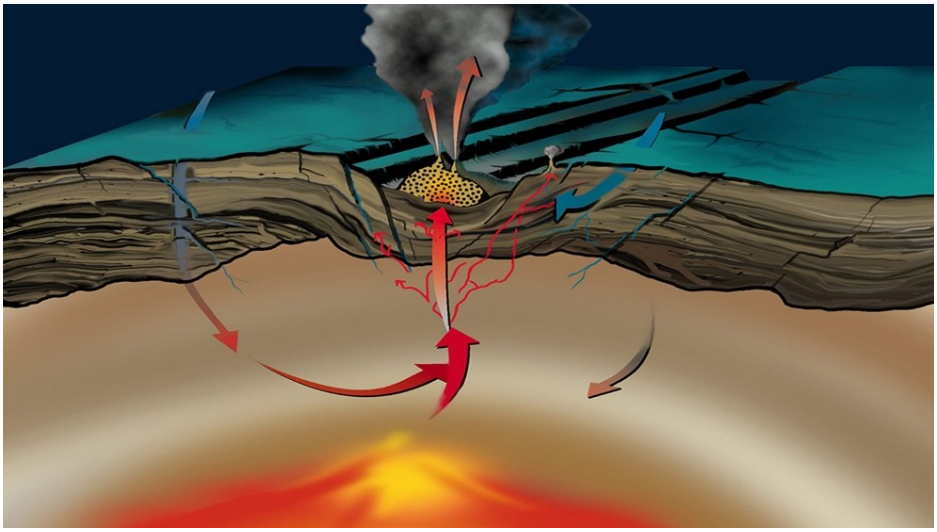

Figure 2-5: *Diagram showing how hydrothermal vents are formed in volcanic areas undersea. A deep hot plume rises towards the surface from the interior. Meanwhile, water penetrates downwards through fractures. The resulting heated material forces its way to the ocean floor.* https://www.whoi.edu/oceanus/feature/the-discovery-of-hydrothermal-vents/

The additional surprise that came with this discovery was that, a few centimetres away from these vents where the water was cooler, a whole ecosystem living of bacteria was found. [66] The organisms found in these

areas do not carry out photosynthesis, instead they capture energy and CO_2 from the vents. This is because the sunlight doesn't reach these deep ocean locations, so an alternate energy source is needed. [66]

A common hypothesis that circulates nowadays is that life on Earth could have had emerged in these vents. [67] The main logic behind this is that oxygen was present in these vents before it was in the atmosphere, or at least in sufficient quantities for life to develop. However, this is disputed by some scientists with the following argument:

The chemistry used by the organisms found near the vents is based on sulphates. Because photosynthesis is the process that gave the ocean its oxygen saturation, they could not have been developed until this occurred. [68]

By contrast, some organisms have adapted to survive the complete opposite circumstances, namely extreme cold. These extremophiles receive the name of psychrophiles/cryophiles. They can survive at temperature below -15°C. [69]

If we go to the deepest part of the ocean floor, the Mariana Trench (about 10km deep), organisms can be found there. They are named piezophiles/barophiles, as they can survive and prosper in high pressure environments. [70] Not many studies are done on these creatures as is still very hard for humans to access these regions. [70]

In addition to this, extremophiles can also be found in acidic environments, low/non oxygen habitats, high radiation mediums, etc. The organisms that survive these conditions are called:

- Acidophiles → optimal germination with pH levels lower than 3 [71]
- Alkaliphiles → optimal germination with pH levels above 9 [72]
- Anaerobes → they do not require oxygen to grow [73]
- Radioresistant → they survive to high levels of ionising radiation exposure [74]

Knowing that there are so many types of organisms that can survive all such extreme conditions, the possibility of finding life elsewhere outside Earth is more probable than possibly thought before. This can be sustained with the existence of polyextremophiles. These are organisms that can survive and prosper in more than one of the situations described above. [75] A good example for this would be tardigrades. Organisms such as this have shown that they can survive vacuum conditions [76], high radiation (both of these will be discussed at the end of this chapter) [77], extreme temperatures [78], etc. In the experiments carried out for this MSc, tardigrade survivability was tested against hypervelocity impacts. These are speeds (in excess of a kms^{-1}) are typical of impact events in space and generate shock pressures at the GPa scale. This will be discussed in Chapter 4.

Here we next discuss tardigrades. Being able to survive and prosper in all these extreme environments, what does it take to be such incredible creatures?

2.3.3 Tardigrades

Tardigrades are also known as water bears or moss piglets. [79] They were first discovered in 1773, by Johann August Ephraim Goeze. He named them Tardigrada, which literally means slow stepper. [79] They are organisms which size range between 0.05mm to 1.2mm, with 4 pairs of legs with 4 to 8 claws in each (See Figure 2-6). Tardigrades belong to the Phylum category, while humans belong to the Chordate Phylum (spinal cord), tardigrades do not , as they do not have a spine, just a nerve cord. [80]

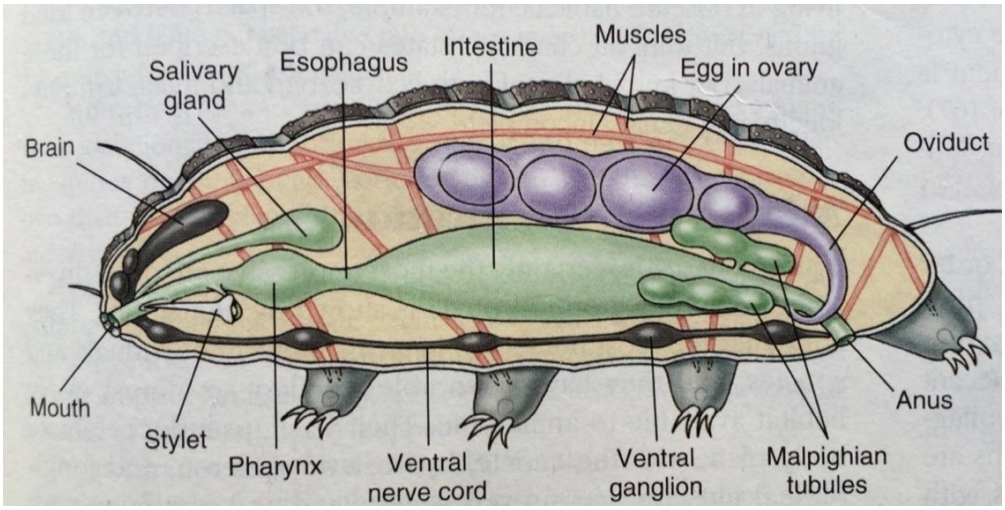

Figure 2-6: A typical tardigrade's internal structure including nervous system, digestive system, muscular system and ovary [82] Typical length is 1 mm.

There are over 1000 different types of species known according to ITIS (Integrated Taxonomic Information System). Tardigrades have been around for over 530 million years. [81] As mentioned before, these hardy organisms have shown resistance to exposures of high ionising radiation (>4500 Gy). [82] Their ability to tolerate these, and other extreme conditions, makes

them the perfect candidates to study the likelihood of panspermia with pluricellular complex organisms.

Their ideal habitat is usually a humid environment. When that humidity decreases, they feel threatened and they are able to shut down their metabolism to the point where they evacuate 97% of their body water. This is how they enter in the state known as tun (See Figure 2-7). [83]

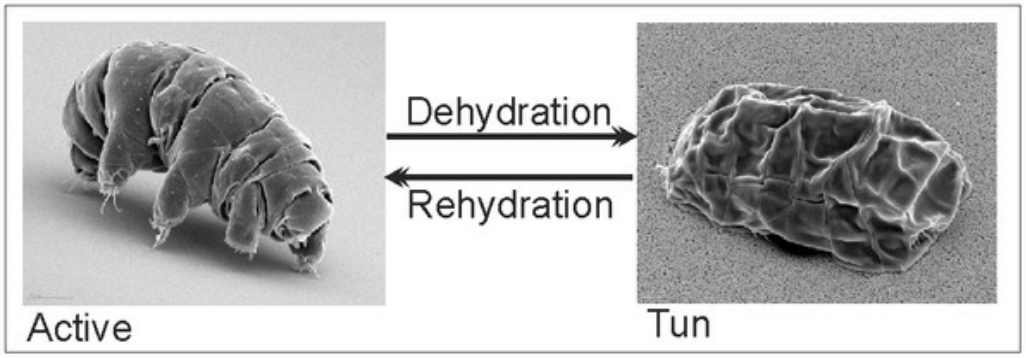

Figure 2-7: Tardigrade with full metabolic activity on the left. Tardigrade in the tun state on the right. In the tun state the metabolism is shut down almost entirely. When active, the normal size for a tardigrade is about 1 mm, whereas in the tun state it is smaller than 0.1 mm. [83]
https://journals.plos.org/plosone/article/figures?id=10.1371/journal.pone.0009502

When they find themselves in the "tun" state, tardigrades can survive temperatures up to 200°C and as low as -250°C [83], as well as both space vacuum and X-rays. [84] Once in this state, when they get back in contact with water their body absorbs it and it restarts their metabolism. [84] Recent

studies found that even after 120 years frozen, a tardigrade was reanimated, and stayed alive. [85]

For the study here, the tardigrades used were *Hypsibius dujardini* (Figure 2-8). This is the most commonly found tardigrade species. [86] They feed on unicellular algae (A68 chlorococcum). [87] Other tardigrades have been known to eat rotifers and nematodes, but this is not the case for *H. Dujardini.* [88] They are estimated to live an average of 8 years in the wild. [89] By contrast, their lifespan is only 3 to 4 weeks in captivity. This species reproduces via meiotic parthenogenesis. A haploid egg returns to diploid by mirroring the number of chromosomes. When they moult, the shed becomes the new envelope where the eggs are deposited (see Fig. 2-8). [90] Hermaphroditism is not as common as reproduction by parthenogenesis, but it has been seen in some studies in 2001. Also, sexual reproduction occurs, as there are both female and male specimens in the colony. [90]

It has been observed that the gestation period in *Hypsibius dujardini* lasts about 4 days (this has also been seen in the lab while carrying out the experiments in this work), with an average offspring number of 3-4. There is no parental involvement in this process, not even after hatching. [90]

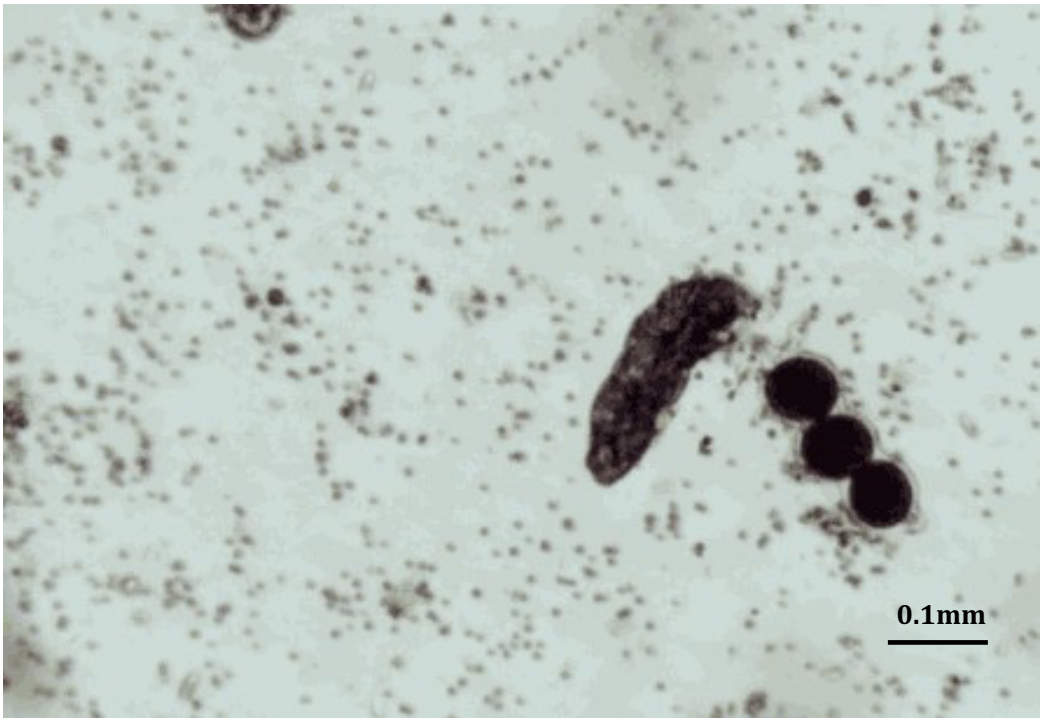

Figure 2-8: Active tardigrade moulting three eggs. Captured by Noah Feehan. Private communication.

There are many other interesting details available in the literature about tardigrades, and a thorough review is available at [82].

2.4 FOTON M-3

Foton-M3 was a Russian automatic mission launched onboard the Soyuz-U rocket on the 14 September 2007, from Kazakhstan. Scientists from NASA's Ames Research Centre and Montana State University collaborated with Russian investigators to manage the experiments planned for this mission. [91] Foton-M3 spent 12 days in low-Earth orbit (258-281 km above sea

level), exposing the experiments to microgravity and also, part of the experimental package was exposed to the harsh conditions of space vacuum and radiation. It re-entered the atmosphere on the 26 September 2017, landing on the Russian-Kazakh border. [91] The spacecraft (Figure 2-9), carried a 400kg payload, including experiments in fluid physics, exobiology, radiation exposure, biology and crystal growth which were carried out during the 12 day mission. [92] After the landing, the experiments were taken to ESA's facilities and data analysis was performed.

Figure 2-9: Foton-M3 spacecraft that carried out a 12 day mission on low Earth Orbit The rear is a battery pack, the sphere is the re-entry capsule containing experiments. The white disk just visible on this sphere is where an experiment like Biopan is located. The front bit is the service module. Total length about 6.5 m. https://phys.org/news/2007-09-foton-m3-earth.html

Why is this mission important for this MSc study?

During this mission, 3000 tardigrades were part of one of the experiments. Their survivability to extreme dehydration, space vacuum and high ionising radiation was tested. [93] The results (discussed below) showed that they do survive these extreme conditions. [93] All this was conducted experimentally in a facility located on the outside of Foton-M3 called BIOPAN. [94]

Knowing this, and thinking about other space travelling circumstances like impacts, the question of 'Would they survive if they were to travel on an asteroid and impacted on a new planet?' arose. We will discuss the answer to this question in Chapter 4.

2.4.1 BIOPAN- 6

BIOPAN was a research program developed by ESA. It was designed to study the effect of the space conditions on biological material. These were to be exposed to solar and cosmic radiation, space vacuum and weightlessness. BIOPAN thus presented radiobiology, astrobiology and material science experiments. [95]

BIOPAN was installed on the external surface of every Foton's mission descent capsule. It had an electrical lid, which displayed (that is, opened and generated) a plane of 180° when located in Earth's orbit (see Fig 2-9). It exposed the samples placed for each experiment to space conditions. When

returning to Earth, the facility closes, and it is protected with an 'Ablative heat shield'. [94]

For Foton-M3 spacecraft, the BIOPAN facility used was called BIOPAN-6. [95] The layout of it can be seen in Figure 2-10. The display and operations worked exactly the same way as the other BIOPAN facilities.

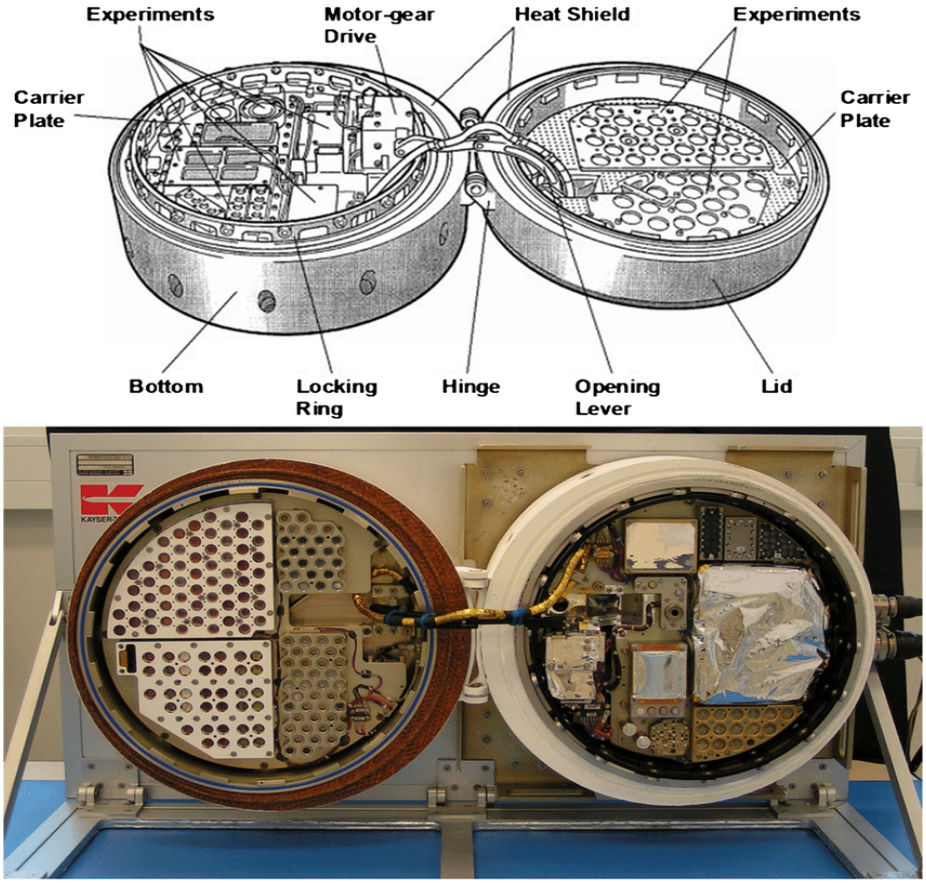

Figure 2-10: BIOPAN-6 facility. Image courtesy of the European Space Agency (ESA)

The experiment was carried out for 10 days. The facility was equipped with UV and pressure sensors, radiation dosimeters and thermometers. The

electronics consisted of: signal acquisition board, microcontroller board with its flight software and a memory board. [95]

The tardigrades used for this experiment were *Richtersius coronifer* and *Milnesium tardigradum*, from the eutardigrades family. [93] They were dehydrated and located in small metallic chambers as seen in Figure 2-11. Some chambers were exposed directly to space vacuum, and some had various solar filters to reduce the solar UV flux. It was found that specimens were able to survive the vacuum of LEO. Some specimens even recovered after combined exposure to space vacuum and solar radiation (details are in the next paragraph). [96] Their surviving these conditions makes them very special, as they become part of the small list of organisms that can survive these space conditions.

Figure 2-11: Chamber used to place the tardigrades used during Foton-M3 mission in the BIOPAN-6 facility (left hand image – size ~ 7 cm). Some of the spaces in the metallic chamber carried a sun filter. The tardigrades shown on the right image are the Richtersius coronifer specimens. Image by K. Ingemar Jonsson. [6]

During the 12 days mission (10 days with the Biopan-6 facility open), the tardigrades were exposed to two different UV radiation spectral ranges: UV-

A and UV-B (280–400 nm respectively), and the full UV range from vacuum-UV to UV-A (also known as UVALL, 116.5–400 nm). [97] The experiment was divided into three different set ups. Samples just exposed to space vacuum, samples exposed to space vacuum and UV-A, UV-B, and samples exposed to space vacuum and UVALL. [97] Ionising solar and galactic cosmic radiation were also constantly hitting the samples during the entire experiment.

Controlled samples were kept and compared with the samples exposed. The survival to the first set up (only space vacuum exposure) was high for both tardigrade species (~80%). However, when studying the set ups with combined exposures, the survival rate dropped drastically. [97] To be specific, samples exposed to solar radiation showed a strong negative effect on survivability with survival after 10 days exposure dropping to ~20% – 30%. [97] By contrast, neither survival nor reproduction were affected by just the space vacuum, supporting the theory that that tardigrade cells, including eggs, can tolerate not only the most extreme dehydration but as a consequence other extreme environment as well (e.g. as needed to survive space conditions such as vacuum and cosmic rays). [98]

Once back on Earth, various tardigrades survived revival into an active state, not just those exposed to the vacuum, but also after being exposed to a radiation dose of more than 7000 kJm^{-2}. How they did this is still a mystery. [99] It would be interesting if their DNA was studied and the different mutations it suffers after the exposure was noted, this would help us understand what makes them survive.

2.5 Summary

In this chapter the concept of how life originated on our planet was tackled. It was understood that we roughly know when it did start but how is still a very well-kept secret. The uncertainty of not knowing how, drove scientists to develop different experiments that could explore this mystery. As an example, Miller and Urey were mentioned with their experiment trying to recreate the primitive Earth's atmosphere and show how it was conducive to forming amino acids, key building blocks for life.

The idea of panspermia as a vehicle for live to arrive to Earth has been also discussed, and it is seen that is still a hypothesis that can neither be proved or disproved. This helped drive the development of experiments on space missions to low Earth orbit, like Foton-M3, trying to check survivability of different samples in space, including tardigrades.

All this provides motivation for this work. If we think of panspermia as a vehicle for life to arrive on Earth, could organisms survive the high-speed impact (and associated shock pressures) that this process involves? In particular, after learning (via Biopan) that they can survive some of the extreme conditions in outer space, could they survive this as well? That is what is explored and discussed in the rest of this thesis.

3 Chapter 3: Equipment

3.1 Introduction

This chapter introduces the different types of apparatus and equipment that were used during the study carried out for this MSc project. This will help to understand how the full experiment was developed. The selected materials used as targets will also be described, as well as the apparatus used to image the tardigrades throughout the entire experiment. Finally, the method followed to calculate the impact shock pressure will be discussed in detail.

3.2 Two-Stage Light Gas Gun

By the end of the 19th century, smokeless powder and high strength steel were invented. This facilitated the increase to supersonic speeds in gun velocities. With this, the age of supersonic flight of bodies (projectiles) began. [100] The problem appeared when the understanding needed for this type of flight was not available. To try and solve this problem, the forces of the different bodies during the flight were measured, but poor instrumentation delayed real progress. [101]

Accordingly, in the 1940s, the 'Aerodynamic Range' was developed. The purpose of this apparatus was to precisely record a projectile's motion in flight, and hence all the forces involved. [101] Aerodynamic ranges were used during WWII to keep track of the aerodynamic tendencies of free-falling

bombs, projectiles fired from guns, missiles, rockets, etc. During this period and the 1950s, space travel and potential moon landings started to be planned, and one hazard considered was the effect of micrometeorite impacts on vehicles in space. Tests to check the survival of materials to the high speed impacts in space required methods to fire projectiles at speeds above a few km s^{-1}, i.e. in excess of what traditional guns could achieve. To do this, a gun capable of generate speeds a few kms^{-1} was needed. [102] The resulting gun designs are what is now known as the 'Two-stage light gas gun' (Figure 3-1). The method to propel the projectile is separated into two stages, hence the name. This is to achieve that acceleration needed for shots above 1-2 km s^{-1}. The light gas gun part of name comes from the use of hydrogen as a typical propellant of the second stage. [102] This is discussed in more detail below.

Figure 3-1: R. The left hand panel shows the gun in its original configuration, firing horizontally from right to left. The right hand panel shows a new vertical structure allowing a vertical gun barrel to be mounted to fire into the target chamber at the extreme left of the image. Hibbert et al. The Hypervelocity Impact Facility at the University of Kent: Recent Upgrades and Specialized Capabilities. 2017 [104]

For the development of this MSc project, the Two-Stage Light Gas Gun at the University of Kent's Impact Laboratory was used (Figure 3-1). Here I discuss how this apparatus works and in what way it was used for this experiment. First, the firing process is explained. It occurs from left to right if we follow the diagram on Figure 3-2. Everything starts with burning a powder charge, which gives the initial acceleration of the piston. The powder is a typical gunpowder and is used in a cartridge from a normal shotgun. Once in motion, the piston compresses a gas of low relative atomic mass. [103] The gas is suddenly released when the diaphragm (known as the rupture disk and usually made of aluminium with a thickness of 50 microns) containing the gas in the pump tube breaks (all this after the gas has been compressed). Finally, the sabot which carries the projectile is accelerated in the vacuum of the launch tube. [103] Before a shot, the launch tube and range of the gun are evacuated to a vacuum of typically 50 mbar in order to avoid generating a shock wave at launch, and the low pressure avoids even minor deceleration of small projectiles in flight.

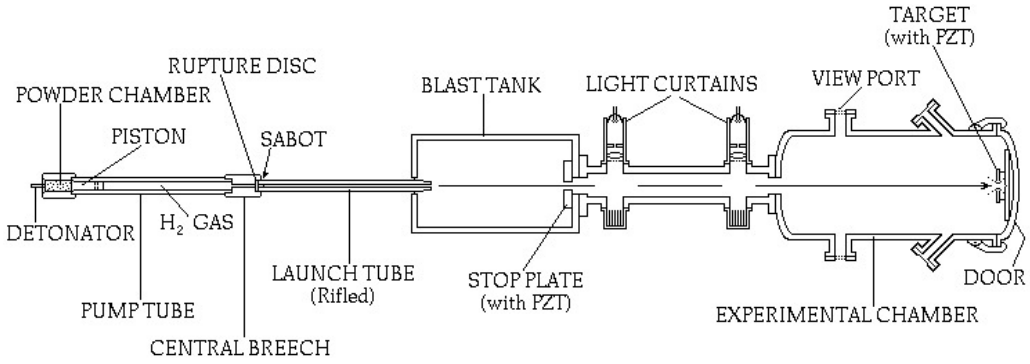

Figure 3-2: Diagram of the LGG at UKC.

The energy (KE) of the light gas is given by the molecular kinetic energy of a gas (Joules, J):

$$KE = \frac{3}{2}kT \qquad \text{Eq. 3.1}$$

k stands for the Boltzmann constant (JK^{-1}), and T refers to temperature (K). When this gas is free to expand, this energy will be almost the same as mechanical kinetic energy:

$$KE = \frac{1}{2}mv^2 \qquad \text{Eq. 3.2}$$

and thus

$$\frac{1}{2}\ mv^2 = \frac{3}{2}kT \qquad \text{Eq. 3.3}$$

where m stands for mass (kg) and v refers to expansion velocity (ms^{-1}). If temperature is kept constant, it can be seen that this velocity is proportional to the inverse of the square root of the molecular mass of the gas:

$$v \propto 1/\sqrt{m} \qquad \text{Eq. 3.4}$$

This simple derivation helps demonstrate that the lower m, the higher the expansion velocity will be. Using this and the results of the test shots, the shot velocities can be roughly predicted by varying the gas used (see Table 3.1).

Table 3.1: Variables needed to reach certain shot velocities.

Shot Velocity (km s^{-1})	Gas	Pressure plus gunpowder
1.2 – 2.2	Nitrogen	40 - 70 bar and 8 - 10 grams
3 – 4.3	Helium	45 – 70 bar and 10 grams
4.4 – 5.7	Hydrogen	35 – 70 bar and 8 – 10 grams

To measure these speeds accurately, the light curtains shown in Figure 3-2 are used. As the particle passes through the light curtain, the signals from both photodiodes illuminated by the light curtains (lasers) are interrupted. Knowing the separation distance of the two lasers and the time it took the particle to go through (this can be seen in the photodiode signal spikes on a fast digital oscilloscope), the speed of the shot can be calculated to within ±1% or better. [103] The best resolution is achieved with larger projectiles. In the work here, the whole sabot passes the laser stations, producing a large and clear signal on the oscilloscope reading out the photodiodes. This gives velocities in this work accurate to ±0.5%.

For this study, velocities below 1 km s^{-1} were required. To do this, the same facility at University of Kent was used. Instead of using the original two-stage gun, we used an upgrade in the gun which was installed in 2017 [104]. It allowed the gun to be converted into a single stage gun and shoot at speeds from ~100ms^{-1} to 1.2kms^{-1}. To enable this, a change in the rupture disk was required, using a 12V battery to power (and melt on demand) the new disk (known as Electronic burst disk) used instead of the traditional unpowered and thicker one. [104]

The way this works is:

First, the gas is fed into the pump tube at a preselected pressure from an external flask. The battery then passes current through the disk (made of aluminium), it heats the disk up almost immediately, melting it like a fuse. What was trapping the gas has now melted, so it is free to expand through the launch tube and accelerate the projectile, as happens in the two-stage light gas gun, but at a lower pressure. [104]

3.2.1 The Cold Gun

Another important feature of the gun at the Univ. of Kent, and that was used during this study case, is the cold gun. The cold gun is used to enable firing of frozen projectiles in the gun with the use of a brass holder. This holder is kept in a freezer (-26°C to -30°C) before use. The launch tube is held in a CO_2 freezer (-180°C) before use. The gun is then set up as fast as possible by the experimental officer (Mr. Cole), keeping track of the temperature. Some of the gun mountings have coolant fluid pumped through them to help keep a low temperature whilst the rest of the parts are being set up. This process takes roughly 10-15min, and the temperature is usually held at -13°C /-20°C at the breech (measured externally) The interior of the breech warms up slower than the exterior so is even colder. This means that the projectile positioned next to the breech is still frozen when fired (see Figure 3-2 for reference).

This was the process followed during tardigrade shots. The tardigrades were frozen in a nylon sabot and fired onto a target. This will carefully be described in Chapter 4.

3.3 Projectile Material and Design

To fire the tardigrades through the light gas gun, a solid cylindrical nylon sabot with a central cavity (Figure 3.3) was used for each shot. Tardigrades were removed from the colony, usually 3-4 at a time, and located in the cavity of the sabot (containing water and unicellular algae). As stated, a more detailed description of this process will be given in Chapter 4. The sabot was placed in the freezer for ~ 48 hours at around -28°C prior to the shot.

Several hours before the shot, the sabot containing the tardigrades was placed in a brass holder (which was pre-cooled and used to carry the sabot from the freezer to the gun, this also helped with quick insertion of the frozen sabot into the launch tube) and returned to the freezer. This allows the projectile to stay frozen during the entire setting-up of the gun.

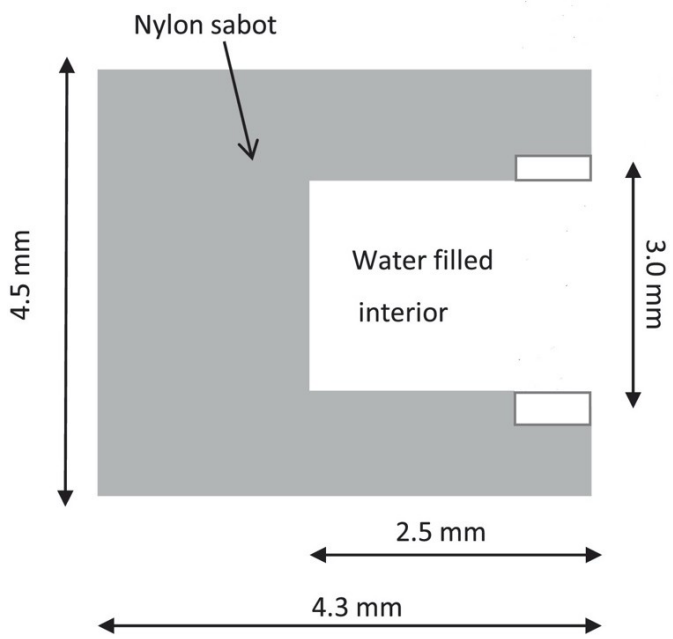

Figure 3-3: Diagram of the solid cylindrical nylon sabot used during the tardigrade shots. This shows a cross section through the sabot.

3.4 Target Material and Design

Two different targets were used during this study, a sand target and a water target.

SAND TARGET

The sand used was called "Kiln Dried Sand". It was very fine grained (0.2-0.7 mm grain size) and contained minimal moisture. It was 95% silica. The sand was poured into a water rich plastic bag (~50 μm wall thickness thick). The bags were 6 by 6 cm squares and in each shot were filled with roughly the same amount of sand (~280 grams), and, once filled were about 2 cm thick (the bags were filled until they had the dimensions indicated). To seal the bag, Sellotape was used, making sure the sand stayed inside the bag even

after exerting some pressure with our hands to produce an even regular shape to the bag.

Figure 3-4: a) (Left) Ocean target holder with water rich bag filled with sand. b) (Right) Cover box for the target holder. Dimensions: 17.5cm x 17.5cm and 17cm height

When the bag is ready, it is placed in the Ocean target holder (Figure 3-4-a). This target holder had originally been designed to hold bags of water to be used as targets, hence the name. However, the sand had the same problem that it is non-solid and flows, so the design was also suitable for the current work. A back plate (aluminium, 5 mm thick) was located behind the sandbag to capture any projectile fragments that penetrated the target. This was in case the thickness of our bag filled with sand was not enough for the shock pressure exerted after the impact. The front plate has different size rectangular openings and a square hole in the centre, as can be seen in Figure 3-4-a. The target is positioned in the gun during an experiment such that it will be impacted by the projectile as it passes through that central square. A metallic box with a hole in the centre of one of its sides (Figure 3-4-b) and a

tray was used to cover the target. This was useful because after the impact, most of the ejected sand was then captured inside the box and the analysis was made easier, as the contamination was controlled inside this box. All the equipment was cleaned with IPA (Isopropyl Alcohol) before introducing the target.

The final step was to locate the target with its cover inside the gun's target chamber as shown in Figure 3-5. The target was clamped to the chamber, making sure the hole in the cover was aligned with the expected projectile trajectory. This was done by using a laser (aimed down the centre of the launch tube) and moving the target until both were lined up. Measurements were taken of this position, so following shots were faster to align.

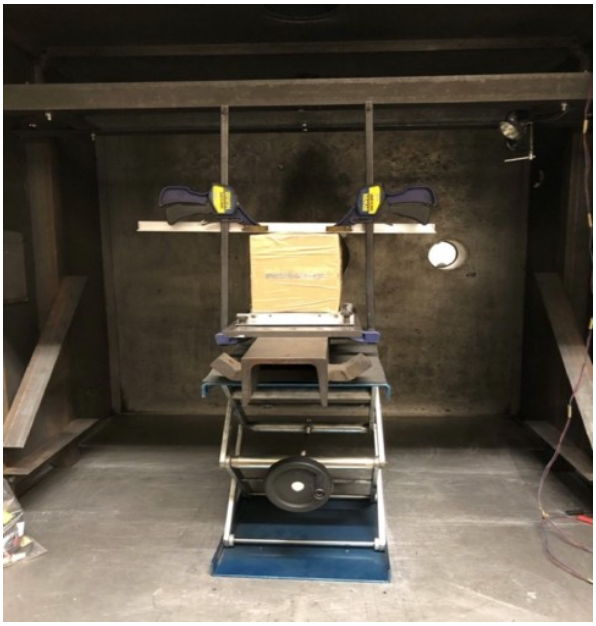

Figure 3-5: The target with its cover clamped into the gun's target chamber. The hole in the cover (which is in the centre of the front of the cover) is aligned with the projectile's trajectory by raising the height of the adjustable table it stands on and moving it left or right as required. The yellow target cover was 17 cm by 18 cm in size.

WATER TARGET

Due to COVID-19 the planned full set of experiments with this target was not finished. But it is fully described so it can be followed up as possible future work.

The water used is Volvic water, as this is the one that has been used for the tardigrade's culture and is the type of water they need to prosper correctly. This water was introduced into a water rich bag (~50 μm thick). A blank shot was done to test the contamination that we will obtain after the shot, and also to see if the set up was properly laid out. The bags were filled with roughly the same amount of water (~250ml), and about 1cm thick each. (see Figure 3-6 for reference). Taking these measurements allowed us to calculate the volume of water easily. To seal the bag, Sellotape was again used, making sure the water stayed inside the bag. However, a small hole was left in the top so the bags did not explode after the impact due to the pressure exerted by the expanding shock wave. Instead, some water could flow out of the top if necessary.

In the test shot, the projectile penetrated the water and impacted the backplate with great force. Since we want to contain the real impacts in the water, in the real experiments the thickness of water was doubled by increasing the number of bags to four.

Figure 3-6: Water target set up. a) Image on the left. On the first attempt two water bags were used, this was changed to 4 after the blank shot. This is because the projectile impacted the metal backplate, whereas we wanted the impact event fully contained in the water. b) Image on the right. Water target set up inside the chamber. It can be seen that the only difference with respect to the sand target (Fig. 3-5) is the baking tray underneath the target itself. This was used to collect the water spilt after the impact.

3.5 Optical Microscopy

To keep track of the Tardigrade's development, conduct control experiments, and look for survivability of these creatures after impacts, an optical microscope was used as the main analysis instrument.

The microscope used in the Impact Lab is a research-grade Leica MZ16 Stereo Microscope, see Figure 3-7. It provides high contrast and high resolution with wide sample overview, which is helpful when looking for

alive tardigrades. Its resolution is of 1150 Lp/mm (where Lp stands for line pairs), allowing observation of structures measuring of ~ 6 microns. Objects can be viewed in a magnification range from 1.0x up to 230x.

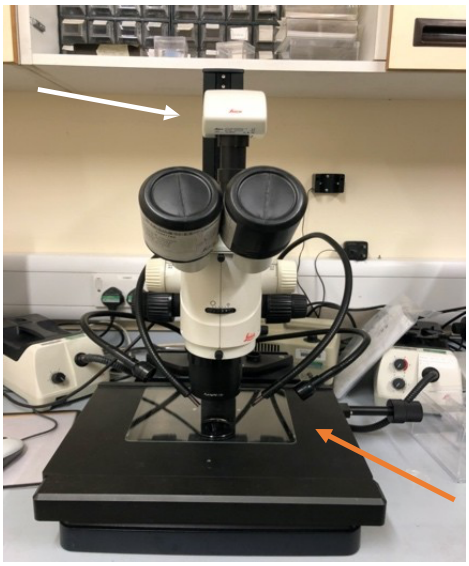

Figure 3-7: Leica MZ16 stereo microscope used during the experiments to check the tardigrades before and after the impact experiments. The camera is shown by an arrow (white) . The black cylinders covering the eyepieces are to block entrance of light when the camera is in use. The sample is mounted on a 2 axis movable stage in the location shown arrowed (orange), and illuminated from beneath or via the black light wands.

A camera is included in the microscope set up, permitting the taking of images during the analysis process. The camera was a Nikon Digital Sight DS-5Mc-U1 cooled colour camera. An example of a picture taken with the microscope can be seen in Figure 3-8.

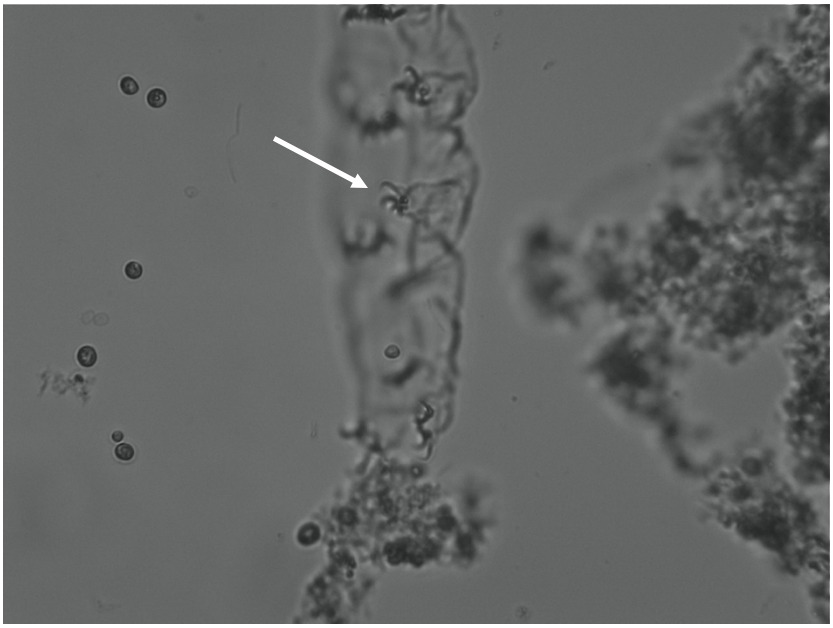

Figure 3-8: Image showing most of a tardigrade body. The claws at the end of several limbs are clearly visible (shown arrowed). The image can be compared to the textbook example shown in Fig 2-6. Algae is also present in the picture (bottom centre and right edge).

3.6 Centrifuging Tardigrades

This part of the work was done in collaboration with the School of Bioscience at University of Kent. The desire was to be able to readily locate and manipulate individual animals, even though they were mobile.

The problem was that I found that the original method used to pick tardigrades from their "home" petri dishes (to place them inside the nylon sabots for firing), used a glass pipet but was not as accurate as desired. This

was because of the movement of the tardigrades during the process. It therefore proved difficult to see exactly how many were introduced in the sabot in this fashion so was very time consuming to get right. So, although this method was used to prepare the filled sabots for the first set of experiments with the sand target, I also investigated a new method to be used in future work.

The thought of having the tardigrades immobile and being able to pick them one by one, was a desirable one. Therefore, research was done on how tardigrades are affected by centrifuging forces, as this will allow us to have a still mass of tardigrades where we could select them individually.

As mentioned in Chapter 1, it has previously been shown that tardigrades can survive up to 16,000 g when centrifuged, [15]. Knowing this, and wanting the tardigrades to suffer the least load possible, they were centrifuged at 4,400rpm for 5 minutes at 4°C. To do this, a 10ml plastic tube was filled with tardigrades, algae and water (see Figure 3-8). It was then placed in an ice filled container for 5 minutes before being placed in the centrifuge.

Figure 3-9: Centrifuge used on the right and plastic tube with tardigrades on the left.

The centrifuge used was a 5702R (Eppendorf), shown in Figure 3-9. A control test was done before taking the tardigrades back to the lab, to see if they could survive the stress generated by the centrifuge. As it can be seen in Figure 3-10 with an example imaged after centrifuging, the tardigrade was physically intact, and its subsequent motion showed it was alive. In general, it took them less than a minute to go back to normal behaviour.

After the test was carried out, the tardigrades were centrifuged again and the obtained 1ml concentration mass was taken back to the lab and stored in the fridge (4°C - 6°C) to keep them immobile. This was used then when carrying out the water target experiment. As the centrifuging concentrates them in one part of the tube, and the low temp then keeps them immobile, we now know where to find them.

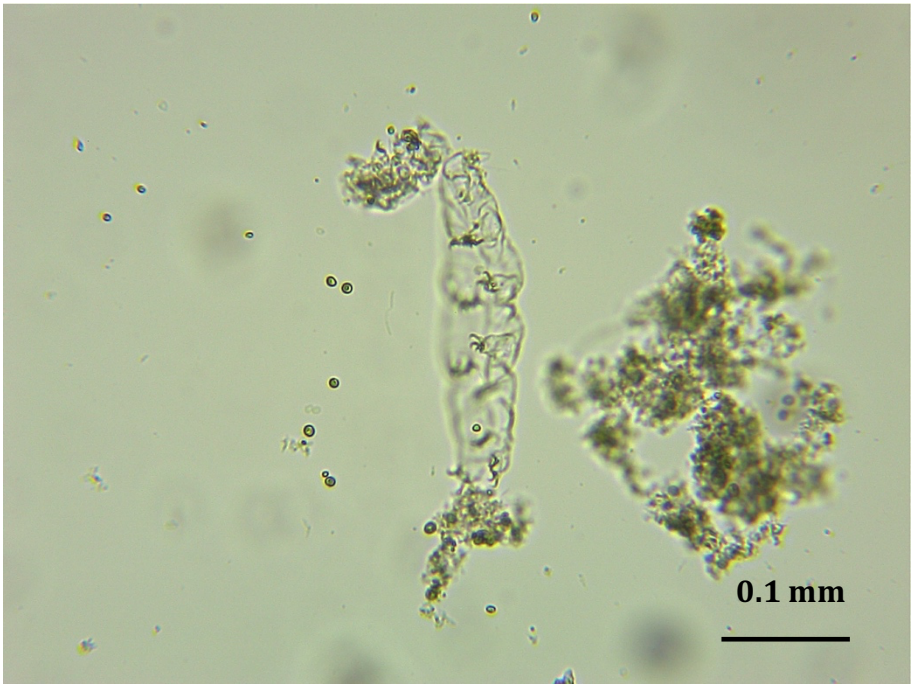

Figure 3-10: Tardigrade after being centrifuged and rested. As before, great detail can be seen of the claws and the animal is still intact. It began to move normally after about 1 minute of observation, indicating it was alive (Same image as 3-8 but without zoom in on).

3.7 Planar Impact Approximation (PIA)

To compute the maximum pressure, particle velocity and shock velocity in an impact, the Hugoniot equations with an equation of state are used [105].

The quantities such as peak pressure, are then obtained using the Planar Impact Approximation (PIA). This is considered a valid method as long as the length of the projectile is smaller than the shock propagation distance. Thus, this is a valid calculation through most of the 'contact and compression stage' of an impact when the peak pressures occur. [105]

The PIA assumes that two infinitely wide plates of the projectile material and the target material (this avoids taking into account shapes, leaving a 1D calculation of the impact process), impact each other at a speed equal to the impact velocity. In other words, the pressure in the target and the projectile will be equal. [105]. The Hugoniot equations are as follows:

$$\rho(U - \mathrm{u_p}) = \rho_o U \quad \text{Eq. 3.5}$$

$$P - P_o = \rho_o u_o U \quad \text{Eq. 3.6}$$

$$E - E_o = \frac{1}{2}(P + P_o)\left(\frac{1}{\rho_o} - \frac{1}{\rho}\right) \quad \text{Eq. 3.7}$$

where P is pressure, ρ is density, u_p is particle velocity, U is the shock velocity and E is the internal energy per unit mass. These equations are equivalent to the conservations of mass, momentum and energy across the shock front respectively. [105] They work for all materials, but do not give enough information about the impact. To know more about this, we need a fourth equation – relating the shock speed and particle velocity:

$$U = c + Su_p \quad \text{Eq. 3.8}$$

where c and S are empirical constants.

Table 3-2 lists the values of c and S obtained from Table AII.2 in ref [105].
When the target and projectile materials possess a linear wave speed

relation, an expression for the particle velocity in the target (u_t) is given by the standard solution to the following quadratic:

$$u_t = \frac{-B \pm \sqrt{B^2 - 4AC}}{2A} \qquad \text{Eq. 3.10}$$

where

$$A = \rho_{ot} S_t - \rho_{op} S_p \qquad \text{Eq. 3.10}$$

$$B = \rho_{ot} c_t + \rho_{op} c_p + 2\rho_{op} S_p v_i \qquad \text{Eq. 3.11}$$

$$C = -\rho_{op} v_i (c_t - v_i S_p) \qquad \text{Eq. 3.12}$$

v_i stands for impact speed, p and t refer to projectile and target respectively. If all these equations are combined, a final expression for the shock pressure can be obtained.

$$P = \rho_{ot} u_t (c_t + S_t u_t) \qquad \text{Eq. 3.13}$$

$$or$$

$$P = \rho_{ot} u_t U_t \qquad \text{Eq. 3.14}$$

The pressure in the target and in the projectile are the same by construction of the solution.

Table 3.2: Empirical constants used in the linear shock wave speed relation and the PIA to calculate the shock pressure in an impact. The initial density is also given. The values are shown for a wide range of possible materials used as the projectile or target. The ones highlighted are the materials used during this MSc project. (Melosh, 1989)

Material	ρ_o **(kgm^{-3})**	**c (kms^{-1})**	**S**
Aluminium	2750	5.30	1.37
Basalt	2860	2.6	1.62
Calcite	2670	3.80	1.42
Cocomino Sandstone	2000	1.5	1.43
Diabase	3000	4.48	1.19
Dry Sand	1600	1.7	1.31
Granite	2630	3.68	1.24
Iron	7680	3.80	1.58
Permafrost	1960	2.51	1.29
Serpentinite	2800	2.73	1.76
Water	9979	2.393	1.333
Water ice	915	1.317	1.526

Using formulas 3.10, 3.11, 3.12, 3.13 and the empirical constants *c* and *S* for the target and projectile materials, the peak shock pressures can be calculated (see Table 3.3 and Figure 3-11 for an example for ice impacting dry sand). These pressures correspond to the peak ones the tardigrades were exposed to during the impact.

The problem here is that the PIA is an approximation. There are several possible sources of error when applied here. The first is a systematic inaccuracy, that is, does it give correct values? There are more sophisticated methods of calculating shock pressures in impact events (hydrocodes). These can provide full 3D simulations using equations of state for all the materials involved. In past work [106] where use of similar loaded sabots was simulated in both the PIA and a hydrocode (Autodyn), the value given by the PIA was some 16% lower than the mean peak impact pressure in the sabot. This suggests there may be an overall uncertainty in all the shock pressures here of that magnitude (16%).

There is also the question of whether inside a single event, the peak pressures is the same across the whole of the contents of the sabot. The use of a filled cavity in the sabot was designed to try to ensure that the contents were shocked to a similar peak pressure. However, in the hydrocode simulations mentioned above, the maximum differences from the mean calculated pressure were estimated to be of order 11 – 14%. However, these occurred in small regions of the interior, with the bulk lying much closer to the mean peak pressure. Given the size of the tardigrades, it seems reasonable to assume that even though we don't know exactly where in the sabot's interior they are located in any shot, they will experience a peak shock pressure close to the mean value, to within less than ±10%.

Table 3.3: Shock pressures calculated using formulas 3.10, 3.11, 3.12 and 3.13 showed above and the empirical constants c and S for each of the target and projectile materials. These specific values correspond to the dry sand target and the frozen sabot (see Table 3.2). For these calculations it was assumed the projectile was made of just water ice to simplify the calculations. The speeds are those obtained in the experimental programme.

Speed (kms^{-1})	Shock Pressure (GPa)
0.556	0.62
0.695	0.812
0.728	0.86
0.825	1.01
0.901	1.136
1.000	1.305

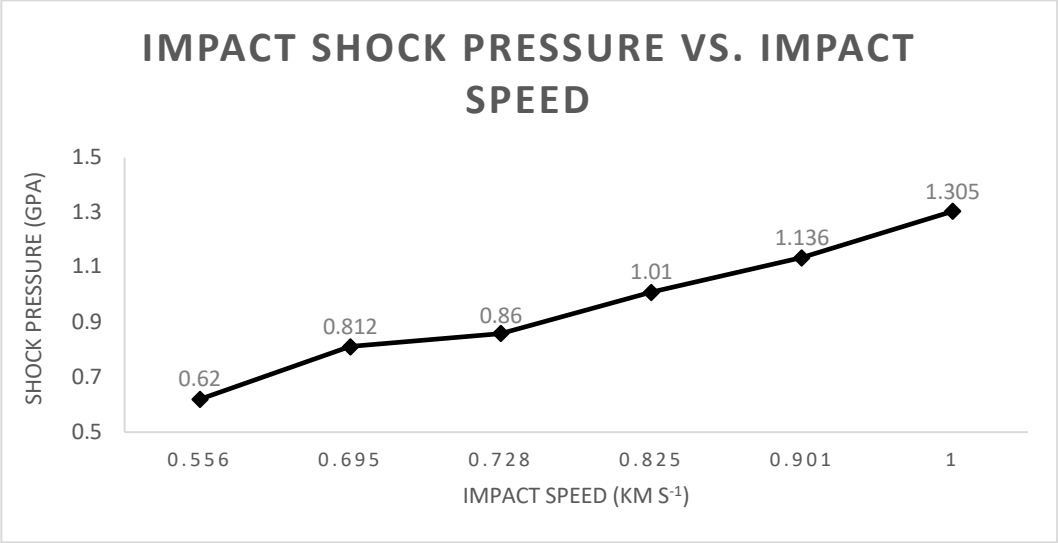

Figure 3-11: Impact Shock pressures calculated from the impact speeds obtained during the experiments

More results and how these pressures affected the tardigrades survivability will be explained in Chapter 4.

3.8 Summary

This chapter explained the two-stage light gas gun set up and how it works. It also described how in the same lab the gun could be used as a single-stage gun or as a 'cold gun', which was the main set-up for this MSc project.

The projectile and target materials and design were described in detail. Due to not being fully satisfied with the method initially used to introduce tardigrades in the nylon sabot, a new, centrifuge, method was devised to improve this, and this was described.

A brief description of the microscope used to image and keep track of the tardigrade's evolution was given. Finally, the Planar Impact Approximation calculations used to determine the impact shock pressures were shown and will be further discussed in Chapter 4. A full detailed analysis of the results obtained in the actual will be given in the next chapter.

4 Chapter 4: Analysis and Discussion

4.1 Introduction

The facilities, apparatus and methods used and carried out during this study were described in the previous chapter. In this chapter, how the samples were prepared for each experiment will be described and a detailed shot programme will be given.

Results and full analysis for both sand and water target will also be given and discussed. The chapter finishes with a discussion of the scientific implications of this study, namely: Why is this project relevant? How can it help when thinking about new astrobiological missions?

4.2 Sample Preparation

During the entire project, tardigrades were kept in 20ml petri dishes (see Figure 4-1). In the petri dishes I would place 1ml of tardigrades (picked from the culture they came in, containing some algae), 1 ml of unicellular algae (obtained from Sciento, the same company the tardigrades were purchased from) and 5 ml of Volvic mineral water (*Hypsibius Dujardini* need this type of water for optimal performance, with Volvic, from a volcanic region of France, specifically named by the supplier). When getting ready to be shot, tardigrades were prepared in a very similar way for the sand and water targets.

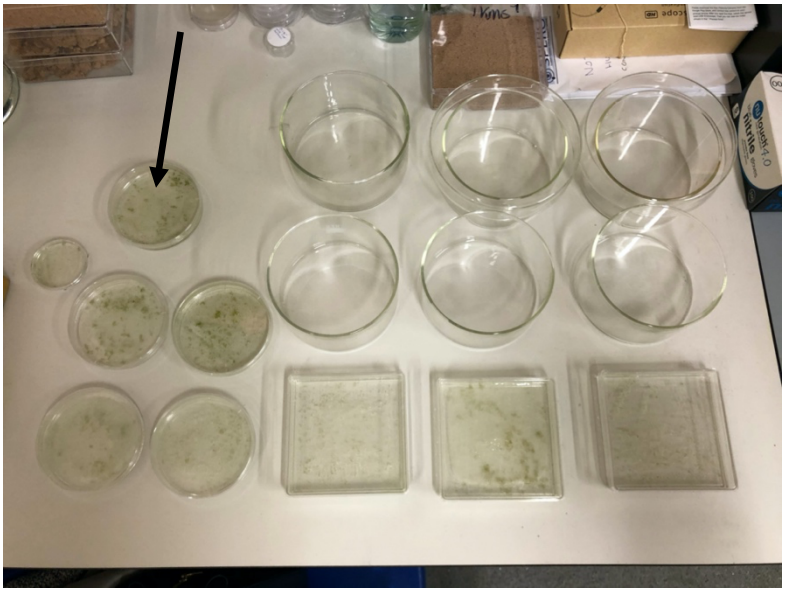

Figure 4-1: Petri dishes containing the tardigrades in their culture. This image was taken after 100 days of being frozen due to COVID 19 pandemic, that is why some ice might be seen in the image. (Black arrow points the 20 ml petri dishes mentioned in the text). To set a scale, the rectangular dishes (bottom centre and right) are 7 cm by 8 cm.

SAND TARGET

The microorganisms were picked from their petri dishes using a 5 ml glass pipet. With the help of the microscope in the Impact Lab, tardigrades (2-3 units at a time) were then positioned inside the nylon sabots. After this, the sabot containing the tardigrades was located in the freezer at ~28 °C for 48 hours, prior to the shot.

WATER TARGET

This set of experiments was planned as check on results obtained with the sand target. For these experiments, tardigrades were placed inside the sabot

after they had been centrifuged (this makes it easier to know the quantity of tardigrades in the sabot). This process was described in Chapter 3. Once the tardigrades were in the sabot, the process was exactly the same as the one followed for the sand target, i.e. the sabot was placed in the freezer at ~ -28 °C for 48 h, prior to the shot.

These are the processes carried out before the shot, how samples and targets are analysed after the impact is described below.

When working with the sand target, finding tardigrades after the impact was complicated and difficult. The way tardigrades were separated from the sand and the sabot residues, which was suggested by Dr. Alesbrook, was by using the density separation method (see Figure 4-2 to see flasks and the sieve used). The steps to do this, are as follows:

1. Fill a glass flask with approx. 200ml of water
2. Take a 75 μm sieve (Note that the typical sand grain size was 0.5 microns pre-shot, but after the shot some finer powdered and was also present – see Fig. 5 in Burchell et al., 2014 [107] for a discussion)
3. Get an empty glass flask of similar dimensions to the one containing water
4. Sprinkle the sand from the target very carefully over the flask with the water (This will allow the sand to settle at the bottom and let the tardigrades float)
5. Using the sieve, pour the mixture on the empty flask - leaving the sand trapped in the sieve and the water with the tardigrades in the new flask
6. Density separation done. Now the water can be transferred into a petri dish and analysed with the microscope. (Usually, a period of 24h

is left between this and the analysis, as the tardigrades need this time to recover from the shock. At higher impact speeds, this time increases).

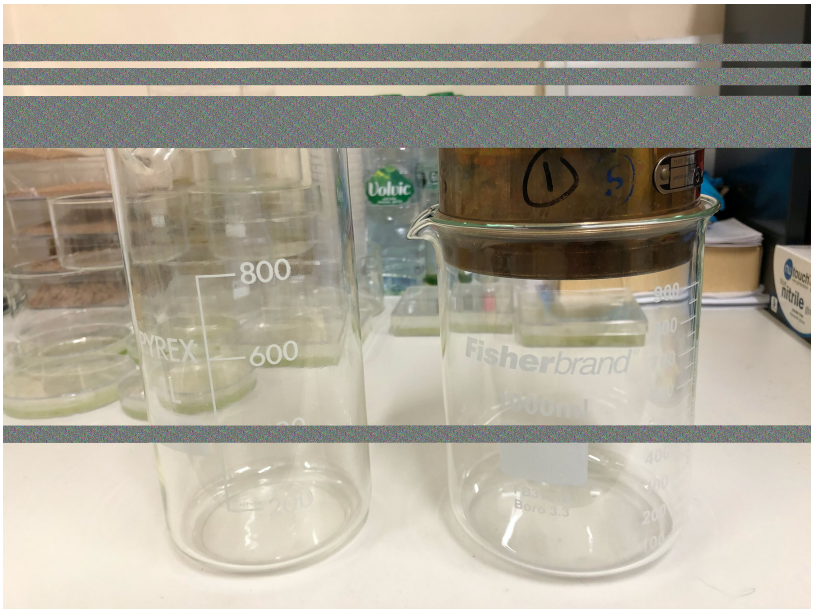

Figure 4-2: Glass flasks used for the density separation method with the sand target. The 75 µm sieve is shown on top of the flask on the right side of the image.

This method was carried out exactly the same way through all the shots done with the sand target. The waiting period between the density separation and the analysis stage varied from 6 h to 48 h, depending on how shocked the tardigrades were.

We questioned whether or not the tardigrades did float during the separation method and whether they stayed trapped in the sand or not. Therefore, this was checked. The sand left on the sieves was stored in different petri dishes with a label showing the corresponding speeds of the shots. They were then located on a baking tray which was introduced into an oven in the Impact Lab. To be safe, tardigrade resistance towards heat was

researched. It was found that *Hypsibius Dujardini* survive up to 275 °C for long periods of time (over 5 days) iffound in their dehydrated state or tun.

A control test (tardigrades were heated in the oven for 10 hours at 250°C) was then done to check that our individuals could actually survive these conditions. And they did, they were visible and moving under the microscope afterwards. Therefore, in the real experiments, the separated sand was placed in the oven at 200 °C for a period of 10 hours. The dry sand was then analysed under the microscope and no organisms were found. The margin of error is not as good as desired however, due to the use of human eye instead of automated apparatus. However, they should be visible after 24 – 48 h when away from the oven, and they were not. This suggests that every tardigrade involved in the shot did float in the water and was filtered through the sieve.

When it comes to the water target, a blank shot (this means that there are no tardigrades in the projectile, it is just an empty sabot) was carried out first to check contamination and the efficiency of the target set up. The method followed after the impact is much simpler than the one done with the sand target. A baking tray was located underneath the target holder, so any water spilled from the target could be collected without any risk of losing tardigrades. Once the shot was done, this water was poured into a petri dish and the content analysed underneath the microscope. In the actual experiments, the no recovery time was found. This is due to time constrains. As it will be explained further in the thesis, future work will involve repeating these experiments and monitor the recovery time of tardigrades after an impact on a water target.

4.3 Shot Programme

To carry out this MSc project as efficiently as possible, a shot programme was developed for both the sand and the water targets. The first half of the year was dedicated to the sand target and the other half to the water target. Due to the COVID 19 pandemic (2020), the water target experiment was carried out in a shorter period of time and after the tardigrades were frozen for more than 100 days. This could have altered the results obtained, by introducing the extra factor of 100 days frozen. Also, a reduced number of shots was done; 3 shots instead of the 6 originally planned. Crucial speeds/pressures were prioritised in these shots, so we could have a better understanding of the sand target results. As stated, whilst not desirable, the closure of the laboratory for 4 months forced these changes on the planned work.

First of all, I explain the number of shots and characteristics of them for the sand target, see Table 4.1:

Table 4.1: Table showing speeds, peak shock pressures (from the PIA), number of tardigrades in the projectile when fired, and survival percentages for the sand target experiment. This shows briefly the results obtained which are discussed in more detail in the main text in this chapter. TGS stands for "tardigrades in sand" and gives a unique code for each shot.

Shot no.	Speed km/s	Shock Pressure GPa	No. tardigrades in projectile	Survival rate %
Blank shot	0.560	0.625	0	N/A
1TGS	0.556	0.62	3	100
4TGS	0.695	0.812	2	100
3TGS	0.728	0.86	2	100
5TGS	0.825	1.01	3	65 (Found 2/3)
2TGS	0.901	1.136	3	0
6TGS	1.000	1.305	2	0

To plan the water target experiment, we did not want to just shoot at random velocities or at the same speeds done with the sand target. The aim of this experiment was to find what speed would be needed to recreate the same shock pressures found in the sand target experiment. This would allow us to check that the results obtained were reliable. Therefore, by selecting key shock pressures and using the Planar Impact approximation calculations in reverse as it were, the speeds for the water target were found.

The speeds and pressures needed so that the water shots covered the same shock pressure range as with the sand targets, were calculated and are given in table 4.2. The PIA is an approximation, although past studies comparing it to more detailed hydrocode modelling have shown it to be accurate to within typically 5 to 10%. Given that here we start with impact speeds, calculate the related shock pressure with the PIA, and then reverse the process with a new target material to obtain an equivalent impact speed, we should accept that the resulting predicted required impact speeds are estimates, rather than exact quantities.

Table 4.2: Table showing speeds and shock pressures for the originally planned water target experiments. The speeds were calculated by requiring similar shock pressures to those obtained for the sand target experiment (TGS). TGW stands for tardigrades in water. Due to time pressure only 3 shots were carried out and the selected speeds are highlighted.

Shot no.	**Shock Pressure GPa**	**Planned speed km/s (Range)**
Blank Shot	0.73	0.673
1TGW	0.62 (1TGS)	0.590 – 0.600
2TGW	0.812 (4TGS)	0.750 – 0.800
3TGW	0.86 (3TGS)	0.790 – 0.850
4TGW	1.01 (5TGS)	0.900 - 1
5TGW	1.136 (2TGS)	1 -2
6TGW	1.305 (6TGS)	2 - 3

As mentioned before, only three of the planned 6 shots were carried out. The highlighted speed ranges were the ones selected for these 3 shots. They were chosen to try and prove the results obtained with the sand target. With the one at 0.86 GPa being the critical point. This means, that if we also obtain a sudden decay in survival within this range, the results obtained in the previous experiment are confirmed.

The speeds obtained from a specific shock pressure were specific values. But because reaching a very precise speed value with the light gas gun is not easy (we can measure speed to within a fraction of 1%, but when judging in advance what speed any particular set up will achieve, there is an uncertainly of about > 15%). Therefore, we give an indicative speed range for each shock pressure. This means that as long as the impact speed stays within those ranges, the results are a reasonable match to what was desired. And the actual shock pressure will then be calculated once we know the actual speed in a given shot.

4.4 Results and Analysis

During this project, not just the survivability of tardigrades against high speed impacts was observed, but also, what happened to them after they survived. Are they able to lay eggs again? And how often? Is this survival a long term one, or does it damage the organism to a point they end up dying prematurely? And how can this be applied when studying panspermia or the origin of life? We start by looking at how the impacts on sand affected these hardy creatures.

4.4.1 Sand Target

Following the results shown in Table 4.1, a radical change in survivability can

be observed at higher speeds/pressures. It becomes clearer when looking at Figure 4-3:

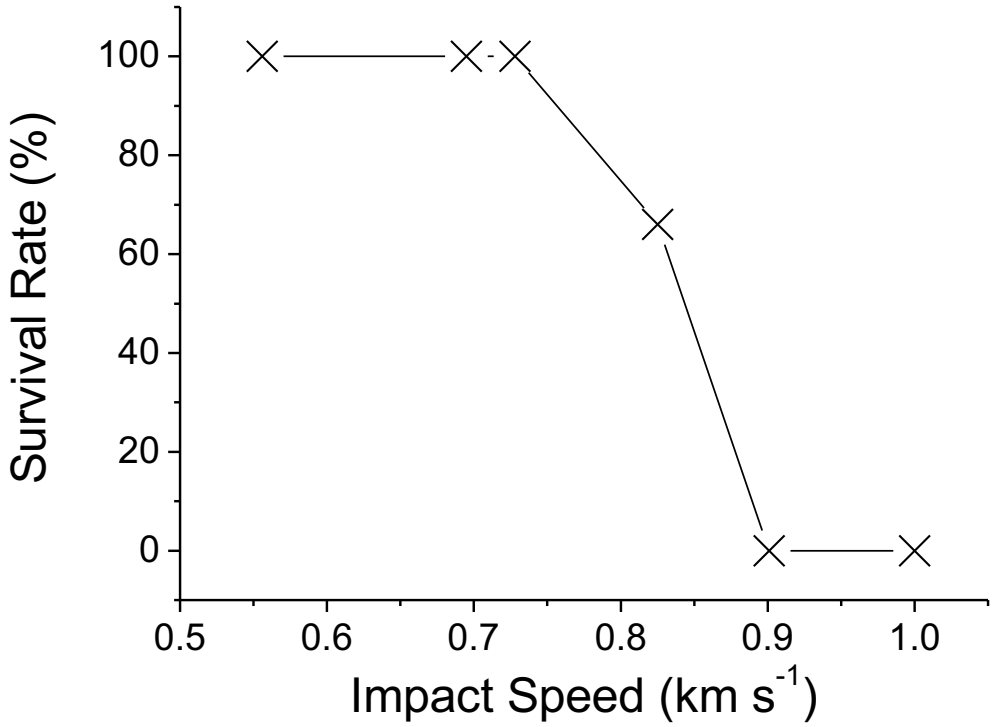

Figure 4-3: Diagram showing survival rate vs impact speed of tardigrades in the sand target experiment (plotted vs. speed as it is the parameter varied during the experiment).

This clearly shows that at low speeds, the pressure exerted on the projectile when impacted in the sand is low enough for the tardigrades to survive (see Figure 4-4 for a pressure vs survival rate diagram). Above 0.73 km s^{-1}, a decay in survival can be seen, which falls to 0% by 0.9 km s^{-1}. More shots over the same speed regime would add to the statistics behind this work and might define the range better. This could be done if this project is continued at any time by another MSc or PhD student.

However, an observation can be made namely that after ~ 0.86 GPa, tardigrades survival starts to decrease and falls to zero by 1.2 GPa.

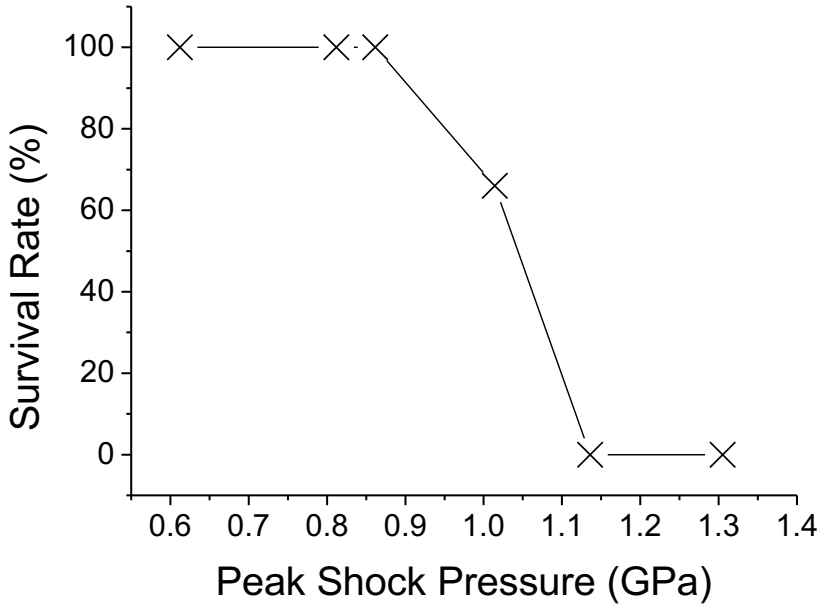

Figure 4-4: Diagram showing survival rate of tardigrades vs. impact shock pressure in the sand target experiment (plotted vs. pressure to generalize the results).

From both Figure 4-3 and 4-4, it can be seen that the transition from survival to death occurs in the region between 0.86 – 1.2 GPa (0.73 – 0.9 km s^{-1}). Here is where the survivability is affected. This discovery is the main result of this work. However, it would now be interesting if this experiment was repeated multiple times, with a larger number of organisms, so that the transition regime can be mapped out more fully. We are also assuming for example that, the peak impact pressure is uniform throughout the entire projectile and is given by the PIA, but this is not true. The variation in peak pressure across the interior of a similar sized but water-filled sabot has been studied by [107]. They found that the peak pressure inside the sabot varied about a mean value by ±12%. This could explain why the result here includes a shot (5TGS) where some survived and one did not (it was in a part of the sabot

where the peak pressure was greatest). Then at higher speeds, the whole of the sabot interior is subject to peak pressures above the "lethal" value.

The best way to understand what happened, is to carry out more repetitions of shots within the speed/peak pressure range mentioned above. And also, another key point, would be, if possible: Increase the number of tardigrades

in the projectile. This would help creating survivability statistics. Longer sabots could be used for example. Now that a better technique has been developed to introduce these organisms into the sabot, this could be done efficiently, and better results and possible answers to our questions will be found.

It is interesting that up 0.73 km s^{-1} we have full survival of the tardigrades after a shot but, how does this affect them internally? Even though at low speeds the survivability is of 100%, the lifespan of these creatures after the shock may not be as promising. As a check, the recovery time of the tardigrades to normal levels of mobility was monitored during the entire project. Not just after the impacts, but also after different stress tests like being frozen and defrosted (see Table 4.3).

Table 4.3: Table showing the number of tardigrades frozen in a sample, the time in hours they spend in that state, and how many hours it took the majority of the sample to recover their normal behaviour once defrosted. In each case all the tardigrades in the sample recovered, and none were observed to remain immobile indefinitely.

Hours Frozen (h)	No. of tardigrades	Hours to recover (h)
24	1	2
24	10	2
48	10	8
48	10	9
72	9	16
72	10	16

The results in Table 4.3 are plotted in Fig 4-5. The result of each experiment is plotted on the x axis in order of increasing time frozen. The results increase systematically, suggesting a possible linear relation. Given that there were two experiments for each time frozen, and that the results in each case seemed similar, the data for each time period frozen are combined. Thus, there are three data points (24, 48 and 72 hours frozen). The y value at each point is the mean value between the observation times, and the error bar shows the full range of time between observations.

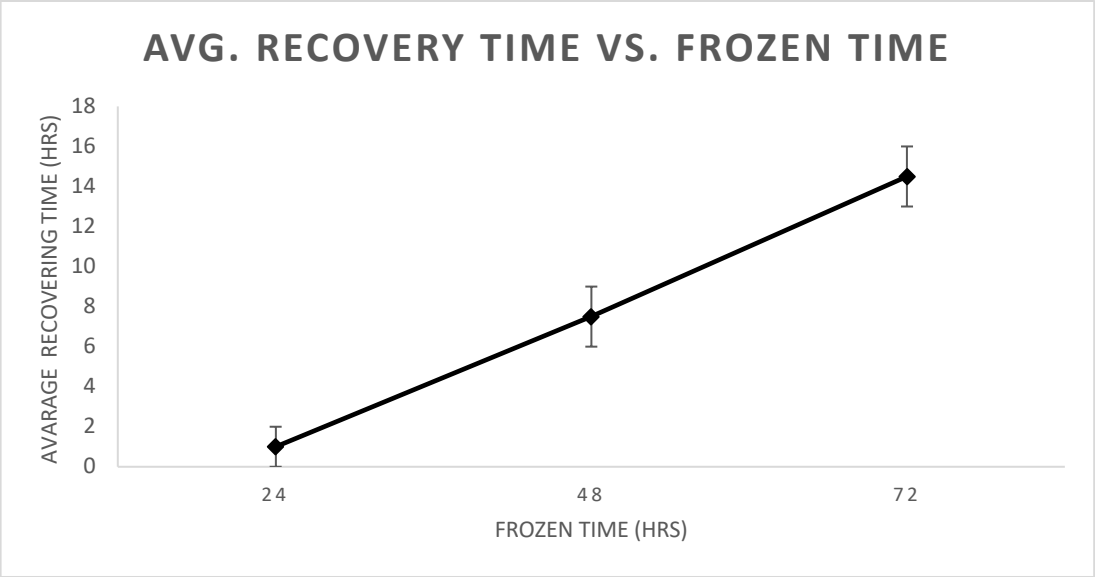

Figure 4-5b: Diagram showing the average number of hours it took the control tardigrades to recover after being frozen and defrosted. *For the 24 hour data point we use 1±1 hr recovery time, for the 48 hr data point, use 7.5±1.5 hrs and for the 72 hour data point, the y value would be 14.5±1.5 hrs. This clearly shows a linear relationship.*

The recovery of tardigrades after these high-speed impacts, did not show a linear increase with increasing shock pressure (see Table 4.4). Instead, what was observed was a nonlinear trend, with tardigrades at some speeds recovering faster (this not being the fastest recovery time) than others that were shot at lower speeds. They were check every 2-3 hours. This is not the most accurate method that should be used but was the only resource

available during the experiments. However, the limited accuracy cannot explain changes of 12 or 20 hours in the recovery time from one sample to the next. This could be the result of Gaussian Statistics. If more experiments were carried out and more data was collected, this could be studied.

Table 4.4: Table showing the hours it took the tardigrades to recover from the high speed impacts. The two highlighted times in green show a discrepancy. What might be expected is that the tardigrades that were shot at 0.86 GPa took less time to recover than the ones at 1.01 GPa, but this was not the case. See main text for a discussion.

Shot no.	**Speed km s^{-1}**	**Shock Pressure GPa**	**Hours to recover from Impact (h)**
Blank shot	0.560	0.625	n/a
1TGS	0.556	0.62	24
4TGS	0.695	0.812	24
3TGS	0.728	0.86	36
5TGS	0.825	1.01	16
2TGS	0.901	1.136	None alive
6TGS	1.000	1.305	None alive

This recovery time phenomena could be seen as a very interesting result. While expecting that at higher speeds the recovery was slower and even find premature death, it didn't always happen like this. Some organisms recovered within a day , after being shot at 0.825 km s^{-1} (see as it can be seen in Table 4.4). Different possibilities as to why this happened come to mind:

a. The location of a particular tardigrade in sabot was closer to the rear of the sabot than usual, therefore the pressure exerted on it was slightly

lower than if they were located at the front. It could reduce the shock slightly from the mean value given by the PIA, so this could explain why it took them less time to recover.

b. That some tardigrades had a higher recovery efficiency, even though the impact pressure was higher, could be due to them being from a healthier part of the population. Maybe they were younger than the tardigrades used in the other shots, and this helped them to recover more easily.
c. Or it may simply be that we are seeing the effect of small statistics, combined with irregular sampling in time, along with the difficulty of seeing if they have recovered by observing mobility (maybe they were just resting normally at the moment of observation). Given the small numbers used in each shot, this is always a risk, again indicating why more shots with higher statistics would be a good follow on experiment, combined with more regular observations, and a variety of methods testing for metabolic activity and not just mobility).

Clearly more shots are needed to probe this further.

Even if they survived and they recovered after a set number of hours, a common fact in every individual that was shot is that they did not subsequently lay eggs whilst being observed. This very interesting change in behaviour could mean three things. One is, that the organisms are so damaged internally that the reproduction function has been affected their ability to reproduce. The second though is that, because they have experienced such a shock after the impact, as an evolutionary reaction, they will not reproduce until feeling fully safe to do so. And unfortunately, this does not happen as they end up dying prematurely due to damage. And finally, the possibility exists of all the tardigrades being males in the sabot that was fired, which would naturally reduce the laying eggs factor down to 0. Again, more statistics would help clarify this.

4.4.2 Water Target

When performing the experiment for the water targets, only 3 shots were carried out. The speeds and pressures are shown on Table 4-5. The survival rate was not detected - this will be discussed in the following paragraph.

Table 4.5: Table showing speeds and shock pressures for the water target experiment. Selected from the speed ranges obtained on table 4.2. TGW stands for tardigrades in water. Tardigrades were frozen 48h prior the shot. But before this, as described earlier, they had been stored in the fridge at around 4 °C for ~100 days.

Shot no.	Impact speed (km s^{-1})	Shock Pressure (GPa)	Survival rate %
1TGW	0.662	0.63	n/a
3TGW	0.819	0.82	n/a
5TGW	1.393	1.2	n/a

In March 2020, the lab had to be shut down due to the COVID 19 pandemic. This was after the sand target shots had been complete, but before the water target shots could be done, although preparations were underway. The closure meant that, to keep all the tardigrades alive during an extended period of time the laboratory had to be closed (which was initially unknown), the tardigrades had to be kept in the freezer at ~ -28 °C. In parallel, some tardigrades had been centrifuged as described in Chapter 4. These centrifuged tardigrades were planned to be used for the water target experiment, so they were kept in the fridge at around 4 °C. This kept their vital metabolic activity on standby, not quite suspended as when frozen, but not active either.

Once the lab reopened, 130 days after closure, the tardigrades were both defrosted and taken out of the fridge. Both samples were looked at under the microscope, and something interesting was observed. In both cases the adult

tardigrades looked like they had their metabolic activity close to 0 but were not in the tun shape (see Figure 2-7 for reference). However, their eggs looked in perfect state. If time had permitted, a test could have been done with a green nucleic acid stain to detect metabolic activity in organisms,

usually used in tardigrades to check whether they are dead or alive. This dye/stain is known as SYTOX. Unfortunately, it was not available in the lab, and ordering it, with the time constrains of the MSc, was not possible given the time lost during the closure.

Therefore, it was unclear if the samples in the lab were alive or not. Given the time constraints it was decided to proceed with the tardigrades in their inert state. The centrifuged sample was used to provide specimens for the TGW shots, and part of the same sample was set aside as a control. This control was then used to compare the recovery time for both shot and rested tardigrades ('rested' meaning not being shot).
After 7 days observing the control, the transparent look had started to fade, and it looks like their bodies had gained some more definition. The process these tardigrades are followings seems reasonable. If we look at table 4-3, it took the tardigrades observed during the first half of the project (before lockdown) 16 hours to recover after being 72 h frozen. Therefore, it will take at least a month for these creatures to recover full functionality if the linear relationship observed earlier holds over this longer period.

Taking into account that the 'rest' tardigrades were not exposed to any other stress apart from being frozen and defrosted, and with this it will take them roughly 30 days to recover; the tardigrades that have been shot, have an extra stress situation to recover from: the impact. This means that, to see any survival rate in these tardigrades, we would have to wait more than a month for them to show any physical activity. Due to time constraints, this is currently not feasible, but it would be interesting if it could be followed up.

We can report the following observations however:

- In the shot at 662 m s^{-1} we observed intact bodies of tardigrades after the shot.
- In the shot at 819 m s^{-1} we again observed intact tardigrade bodies after the shot.
- In the shot at 1.393 km s^{-1} we did not observe anything, not even tardigrade fragments.
- The specimens from each shot and the control (rest) sample have been stored and it is hoped they will be inspected one month after shooting, but it is currently (at time of submission) not possible to say what they will reveal if anything.

Overall, this water experiment was inconclusive when it comes to comparing results with the sand target experiment. However, structurally, the results are consistent with the ones from the sand experiments, but more work would be required to check if the survivability is similar. Also, interesting information about tardigrades behaviour has been revealed.

4.5 Scientific Implications and Discussion

Now that we have observed the results of the various experiments, how does this affect the concept of panspermia? And what scientific applications could these results have?

Thinking about the experiments that were performed in the lab and the types of targets that were used, how could we extrapolate this to a future mission? If a mission to Enceladus is used as an example, we would need a collector to be able to capture small fragments ejected from the plumes in the surface of this icy moon. Possible shock pressures and impact speeds should be

calculated beforehand using the PIA method, the same way it was done during this project. We used sand and water as targets, but for this specific case we are discussing regarding Enceladus, a spacecraft would use a collector, which if it were a metal would typically be aluminium, which can

retain impact residue. The ejecta would be made of ice, and probably it will contain some organic material or even microorganism: a very similar set up to the one used during the experiments with the tardigrades.

Why aluminium as a preferred option to any other material for the collector in the spacecraft? In our first scenario let us consider a spacecraft orbiting Enceladus at low altitude so it can intercept a plume.

If we use the constants provided by Melosh 1989 [8] mentioned in Chapter 4 to calculate the shock pressure using the PIA, then from equations 3.9, 3.10, 3.11, 3.12 and 3.13, we can see that an impact speed of ~100 m s^{-1} will generate a shock pressure of 0.85 GPa in a detector in orbit aroundEnceladus. We obtain this by finding the orbital speed as a function of altitude. This assumes a circular orbit, with orbital speed given by $v = \sqrt{[GM/r]}$, where r is the radius of Enceladus plus the chosen altitude. We assume the impact speed is dominated by the spacecraft orbital speed. In Table 4.6 we show v for various choices of altitude and with this we use the PIA to calculate the associated peak shock pressure. It is clear that to achieve survival of tardigrades in such a collector, a high altitude is required.

As it can be seen in Figure 4-6 the higher the detector is found, the lowerthe orbital and thus the impact speed. This implies, the higher the detector is placed, the lower the impact shock pressure (see Figure 4-7). Although this is good for the survival rate, being at a very high altitude reduces the amount of ejecta the detector could collect as the plume becomes more diffuse.

Table 4.6: Table showing the impact speed dominated by the spacecraft orbital speed calculated using the formula $v = \sqrt{[GM/r}$, where G stands for Gravitational constant (6.67×10^{-11} m^3 kg^{-1} s^{-2}), M is Enceladus Mass (1.08×10^{20} kg) and r is the radius of Enceladus (252.10 km) plus the chosen altitudes for the detector. And the impact shock pressure is calculated using PIA with the constants needed for aluminium (c = 5.3 km s^{-1} and S = 1.37).

Detector Height (km)	r (Enceladus r. plus detector hight, km)	Impact speed (ms^{-1})	Impact Shock Pressure (GPa)
25	277	161	0.207
50	302	154	0.197
75	327	148	0.188
100	352	143	0.181
150	402	133	0.167
200	452	126	0.157
250	502	119	0.148
300	552	114	0.141
350	602	109	0.134
400	652	105	0.129
450	702	101	0.123
500	752	98	0.120

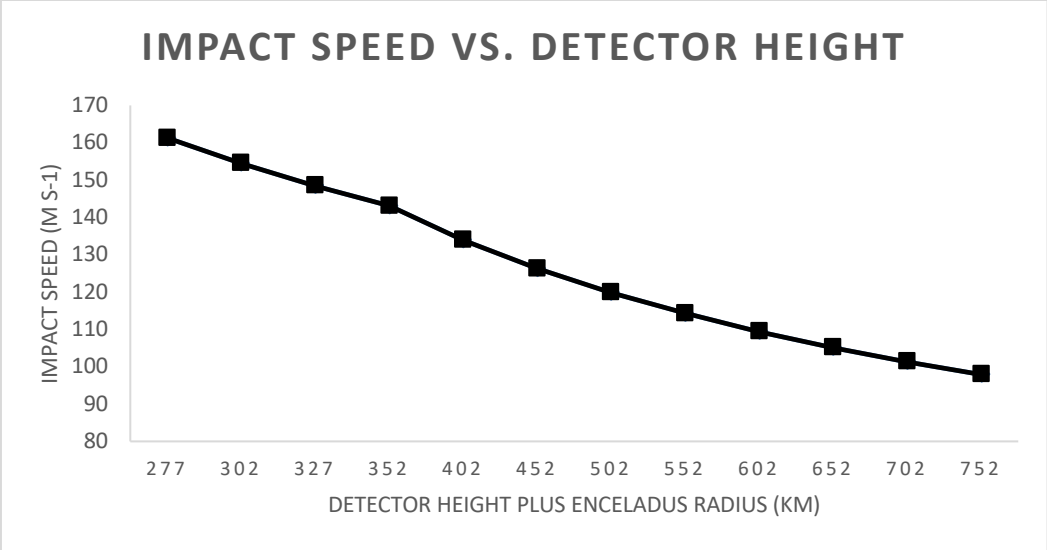

Figure 4-6: *Impact speed vs Height of detector in spacecraft on Enceladus. Blue line corresponds to experimental data and orange dotted line corresponds to the linear fit.*

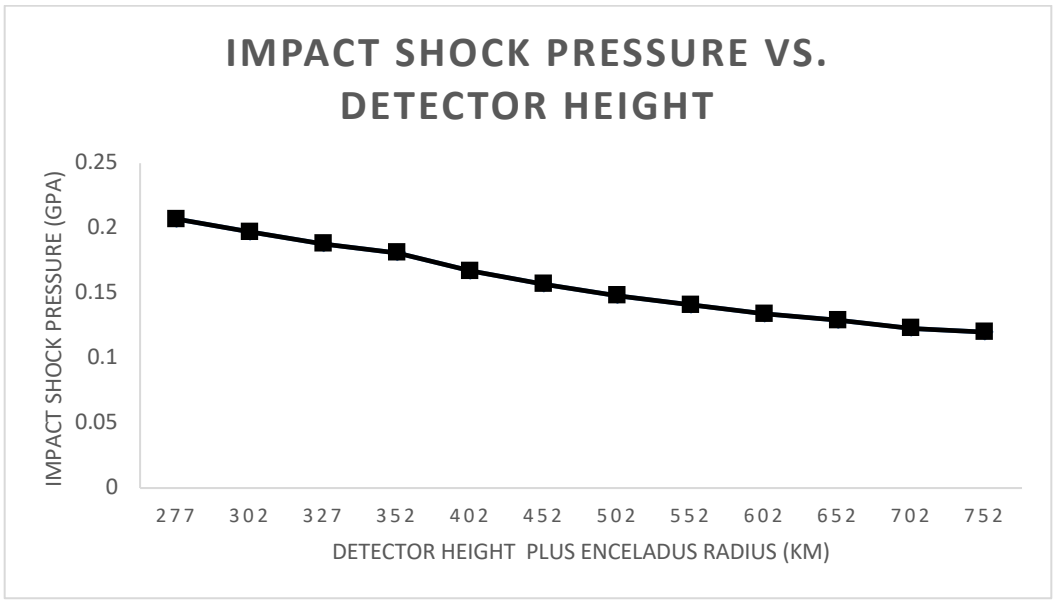

Figure 4-7: *Graph showing the decrease in shock pressure as the detector in the spacecraft moves further away from the surface of Enceladus. Blue line corresponds to experimental data and orange dotted line corresponds to the linear fit.*

Having plotted the experimental data as it was obtained vs. speed, we need to make this more general, and this is done by calculating shock pressures.

This will help as shock pressure is universal, i.e., pressure will give a generalized result. This will allow other scientists to compare their particular experiments to the generalized results presented in this work.

For a tardigrade the outcome is a steep decrease in survival once reaching the pressures around 1 GPa. These conditions will not benefit the survival of any microorganisms with a similar complexity to tardigrades but will probably allow the collection of small prebiotic molecules, organics or even some types of bacteria – if these were to be found on Enceladus plumes.

A second scenario is where the spacecraft flies past Enceladus without going into orbit, In this case, we take the encounter speeds as those reported by the Cassini mission, where speeds ranged from 5 - 10 km s^{-1} . [108] The associated shock pressures are in the range from 19 GPa to 30 GPa, and for a tardigrade the outcome is they fragment. So there is no chance of survival in such a scenario.

It is true that there are other materials that will generate smaller shock pressures, but aluminium has a high resistance to extreme space conditions: vacuum, radiation, etc. Gold would resist even better, and being soft may retain more of what impacts it. However, its greater density means the peak shock pressure will be higher. This is why aluminium is chosen, is also cheap and it is abundant; all this makes it the perfect candidate for this supposed mission.

However, if a different metal were chosen, whilst the exact peak shock pressure can be found from the PIA, it will not be greatly different from that for aluminium. This is shown by [109], where impacts on 5 metals (aluminium, copper, silver, indium and gold) were considered at 2 km s^{-1}. The lowest peak shock pressure was in aluminium, and the highest in gold (some 30% greater in value).

There is a class of materials, however, that would significantly lower the peak shock pressure, this is porous materials. A typical example is aerogel. The NASA Stardust mission used this to collect relatively unshocked samples of dust from comet 81P/Wild-2 [34]. Aerogel can be a very low density material, and this reduces the peak shock pressure. For example, on the Stardust mission, at 6.1 km s^{-1} impact speed, a typical mineral grain was estimated to experience a peak shock pressure of 60 – 80 GPa when hitting aluminium [110]. But, when it struck low density aerogel, this fell to just below 1 GPa [111]. The exact pressure in aerogel depends on the density of the aerogel as well as the impact speed, but clearly this suggests that even for a fly-by it might be possible to capture tardigrades in aerogel and have them survive. However, a great depth of aerogel would be needed, at least 10 cm or maybe more.

Other issues arise when considering a mission. Firstly, we could think about the fly-by. This will capture some ejecta on the collector, but how much? And what is the density of interesting objects in the plume? This means, if the collector measured say 1m by 1m (which is large), and we collected 1mg of material during the fly-by, is this enough to fully characterise the contents of the plume? Not just the type of ice and possible organics that are found on this icy moon, but also any more complex living material like a tardigrade. To improve this, either multiple fly-bys are needed, or a mission to *orbit* Enceladus could be sent. This will collect plume material on every orbit and the chance to collect material of interest increases. Thus, a higher time spent orbiting the moon, means more data can be collected, and a good representation of the material in the plume obtained.

Is there another way to minimise shock pressure during collection? It is known that the shock pressures and impact speeds are the highest when the

collector is facing 90° to the impact direction. To reduce the shock, we could plan an oblique impact. This could be done by placing a mechanism that allowed the detector to be moved to a chosen inclined angle as desired by the research team when reached the icy moon. This will probably increase the survival of any organic material or microorganisms, if present, in the ejecta. For example, it is the vertical component of the speed that is held to be important in the PIA, and since this is reduced in an inclined impact so is the shock pressure. With an appropriate choice of impact angle, we could envisage halving the peak shock pressure.

If this was possible, with test results were obtained and the science better understood, a spacecraft with a collector could be send to the rest of the icy moons in our Solar System, like Enceladus or Europa.

4.6 Summary

This chapter showed the results for both the sand and water experiments. It also discussed what was obtained, and what scientific implications do the results suggest.

An overview of the sample preparations was given, explaining the processes followed for both before and after each shot. Slight differences were described for the after shot sample analysis between the sand and the water experiments.

Also, attention was paid to the effect of COVID-19 pandemic on the results obtained. Finally, a brief conclusion regarding both experiments and what was accomplished during the MSc project.

The main conclusions will be summarised in Chapter 5, as well as an in depth review on 'Future work' that could be done after this project finishes.

5 Chapter 5: Conclusion

5.1 Main Conclusions

The aim of the research presented in this MSc thesis was to show whether or not microorganisms like tardigrades can survive hypervelocity impacts. We wanted to prove that not only organic compounds, sugars and spores could survive the phenomenon known as panspermia, but also, complex organisms like tardigrades.

It is known that there are many objects in space. It may be unlikely that life evolved there, but if material was removed from a planetary surface as impact ejecta it could contain biological materials and then distribute it across space just like rocks. Lithopanspermia suggests that life could survive the shock pressures associated with this impact. So, we wanted to see if tardigrades could survive this.

After introducing the research topic, on Chapter 2 the concept of how life originated on our planet was explained. It was understood that we roughly know when it did start but how is still a very well-kept secret. The uncertainty of not knowing how, has driven scientists to develop different experiments that could explore this mystery. As an example, Miller and Urey were mentioned with their experiment trying to recreate the primitive Earth's atmosphere and show how it was conducive to forming amino acids, key building blocks for life.

The idea of panspermia as a vehicle for live to arrive to Earth was also discussed, and it is seen that is still a hypothesis that can neither be proved or disproved. This helped drive the development of experiments on space missions to low Earth orbit, like Foton-M3, trying to check survivability of different samples in space, including tardigrades.

On Chapter 3 the two-stage light gas gun set up and how it works was explained in depth. How in the same lab the gun could be used as a single-stage gun or as a 'cold gun' was also explained, being this the main set up of the project.

The projectile and target materials and design were described in detail. Due to not being fully satisfied with the method initially used to introduce tardigrades in the nylon sabot, a new, centrifuge, method was devised to improve this, and this was described.

A brief description of the microscope used to image and keep track of the tardigrade's evolution was given. Finally, the Planar Impact Approximation calculations used to determine the impact shock pressures were shown.

Chapter 4 showed the results for both the sand and water experiments. It also discussed what was obtained, and what scientific implications do the results suggest.

An overview of the sample preparations was given, explaining the processes followed for both before and after shot. Slight differences were described for the after shot sample analysis between the sand and the water experiments. Finally, a brief conclusion regarding both experiments and what was accomplished during the MSc project. The key result that emerged was that tardigrades can survive impacts with an associated shock pressure up to about 1 GPa. This places them in the same category as seeds etc., which also suffer critical damage at such impact pressures. This does not prevent a mission to a body such as Enceladus from successfully capturing such complex materials from the Enceladus plume, but it does impose restrictions on how such a collection should be carried out.

And finally, in Chapter 5, a global overview of the project is given. Showing different options of future work that could be done and how this potentially could help to improve and prove the consistency of the results obtained.

This is the first successful study of this type and the statistics are small, although sufficient to show a result. If it gets reproduced, this will increase statistics and might improve the accuracy on pinning down the transition regions from viable to dead.

Another area for research arises from this, which focuses on controlled samples of tardigrades after impact (making sure the number of tardigrades is >100 for statistics reasons) and studies how long they live after that, and how long it takes them to reproduce again. This can also be done by exposing the tardigrades in the controlled samples to extreme conditions and see how their behaviour changes.

5.2 Future Work

The experimental procedures could be further enhanced and complemented following the next steps:

Add at least two more shots to the sand experiment programme. This will give us enough data to see the trends better. Having more experiments done with the same target allows us to see if the results obtained during this project are consistent. Also, a major focus should be put in the speed/pressure region were the tardigrade's survival starts to decrease (see Fig. 4-3 or 4-4), this will help us observe in detail which specific section of the region between the speed 800-900 m s^{-1} (with associated shock pressures of 0.82 GPa – 1 GPa) is were tardigrades survival starts to be affected.

1. Use in collaboration with the Optics department the OCT (Optical Coherence Tomography). This will allow us to get very detailed images of the tardigrades before and after the impacts. Giving the opportunity to appreciate the already though rearrangement of the tardigrade's organs after the shot. Also, it would be interesting to do the same for the eggs. As they have also been shot as projectiles with the adult tardigrades. Do they modify their shape? What damage do they suffer? All these could be answered using OCT.
2. And finally, something I considered interesting was to look at the microbiome of the tardigrades after the impact. It would be useful if we could see if there is or not any damage in it. It would be of medical interest, as microbiomes are of great use to make blood donation bags last longer, improve our gut health… Imagine what could be done with a resistant microbiome like the one of the tardigrades.

All these ideas could be performed or at least considered by another student carrying out their MSc or PhD project following this one.

5.3 Final Summary

In conclusion, this MSc project has shown that complex organisms like tardigrades have a survival threshold at around 1 GPa, corresponding to impact speeds of around 825 m s^{-1} in rocky planets and around 1 km s^{-1} in water planets.

This implies that only in, what by Solar System standards, are relatively low impact speeds and shock pressures can they survive. This limits their role in possible examples of Panspermia. It suggests for example, that the Israeli mission to the Moon in 2019, and which crashed at several km s^{-1}, did not leave viable creatures on the lunar surface. However, this process need not

only be active in our Solar System. Exoplanet systems are also now known to have moons, e.g. the TRAPPIST – 1 system would be a good candidate, as possible habitable planets with their moons have been detected there.

This result is similar to that for seeds, complex structures that are damaged as shock waves pass through them. Previous work on seeds also found a critical threshold for survival around 800 MPa to 1 GPa [18, 105]. This suggests that complex structures of mm size are susceptible to damage at these shock pressures.

When comparing to results for survival of spores and microorganisms, although some survival is reported at impact speeds up to 5 km s^{-1} (shock pressures of order 40 to 50 GPa – [112] this is at a low level of around 1 per 10,000 or less. Such levels of survival cannot be checked for tardigrades in the experiments described here.

As a next step, a new method of experiment is needed, which can handle samples of say 100 to 1000 tardigrades per experiment, perhaps large flying plate experiments, like the examples mentioned on Chapter 4 with the scientific implications.

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