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Chapter 11

Approaches to Assess the Suitability of Zooplankton for Bioregenerative Life Support Systems

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Abstract

Future manned space exploration will send humans farther away from Earth than ever before (e.g., to Mars), leading to extended mission durations and thus to a higher demand for essentials such as food, water and oxygen. As resupplying these items from Earth is nearly impossible, aquatic bioregenerative life support systems (BLSS) appear to be a promising solution. Due to its central role in aquatic ecosystems, zooplankton could act as a key player in aquatic BLSS, linking oxygen liberating, autotrophic producers and higher trophic levels. However, prior to the utilization of BLSS in space, organisms proposed to inhabit these systems have to be studied thoroughly to evaluate any space-borne adverse traits, which may impede a proper function of the system. To investigate the impact of microgravity (μ*g*), in particular, several platforms are available, providing μ*g* periods ranging from seconds (Bremen drop tower and parabolic flights), to minutes (sounding rockets), up to even days and months (space flights and the International Space Station (ISS)). Furthermore, ground-based facilities, such as clinostats, enable the of candidate organisms to variable periods of simulated/functional μg. In this book chapter, research on zooplankton utilizing these methods is summarized.

Keywords: zooplankton, bioregenerative life support systems, microgravity, drop tower, parabolic flight, sounding rocket, clinostat, space

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1. Introduction

At the beginning of the spaceflight era in the late 1940s and early 1950s, it was uncertain whether humans will be able to survive a journey into space as it poses a hostile environment to life. Therefore, animals have been used to test the survivability under space conditions before the first manned space missions were launched. Initially, fruit flies acted as test organisms aboard a German V-2 rocket [1]. Up to now, a variety of vertebrate (e.g., monkeys [2], fish [3] and gerbils [4]) and invertebrate animals (e.g., spiders [5], snails [6] and ants [7]) has been used to investigate the impact of microgravity (μ*g*) and cosmic radiation on various biological processes ranging from behaviour [8] to embryology [9]. Fourteen years after the first animal experiment, a manned mission followed with the launch of the spacecraft Wostock I. Since then, the sojourn times of humans in space have significantly increased from nearly 2 hours up to several months aboard the International Space Station (ISS) (e.g., [10]). The present objective of space exploration (e.g., to Mars [11]) will send humans farther away from Earth than ever before, leading to even longer mission durations. One of the key issues to be solved in advance of those missions is the supply of essentials, such as food, water and oxygen, since regular supply is only feasible to low-orbit platforms. A solution to create independency from regular delivery is to rely on autochthonous production using bioregenerative life support systems (BLSS). Besides the production of food, future BLSS could fulfil functions such as the regeneration of atmosphere, purification of water and waste, as well as food processing [12]. As the reaction of aquatic organisms to μ*g* [3, 13–15] given the increased viscosity of their habitat compared to terrestrial systems [16, 17], most BLSS are based on aquatic systems.

The first systems for housing aquatic animals in space that led to the current BLSS were designed quite simple and did not aim to fulfil the functions of a BLSS, but provided initial insights into what needs to be taken into account for future systems. An example of such a primary system is special plastic bags filled with water and oxygen to keep killifish aboard Skylab 3 [13]. A further advanced system, the so-called *STATEX* container (derived from *STAT*olith *EX*periment), was used to test tadpoles of the South African clawed toad in the German-D1 mission in 1985 [18] and in addition with cichlid fish larvae in the Spacelab-D2 mission in 1993 [19]. The *STATEX* container was equipped with a centrifuge that allowed to perform inflight reference experiments under the same conditions, but exposed the animals to the same acceleration as the gravitational force on Earth (1 *g*). The animals were housed in small, petri-dish like, mini-aquaria into which oxygen transfer was provided by a gas-permeable biofoil. In 1980, also the National Space Development Agency of Japan (NASDA) started to develop experimental hardware to support aquatic animals in space. All these facilities included an artificial lung for the supply with oxygen and a feeding system. One of the major aims from the technical point of view was to achieve a completely closed water circuit and an effective water purification system, requiring a limited amount of water to be used in the space shuttle [20]. The first of these systems was the *Vestibular Function Experiment Unit* (VFEU) that was used to study the behaviour of Japanese carp in 1992 [21]. The subsequent *Aquatic Animal Experiment Unit* (AAEU) was flown in 1994 and included four different experiments with fish and newts (for an overview of the experiments, see [20]). The original VFEU was later on improved to accommodate marine fish under low temperature

for two shuttle missions in 1998 [22]. In parallel, two more facilities to conduct space research with aquatic animals have been introduced: the *Aquatic Research Facility* (ARF) by the Canadian Space Agency and the *Autonomous Biological System* (ABS) by the National Aeronautics and Space Administration (NASA). Both systems were employed in the space shuttle mission STS-77 in 1996 [23, 24]. In the ARF, a highly sophisticated facility to study the development of sea urchins in μ*g* [23], oxygen was transferred into the experimental units via a gas-permeable biofoil. In the ABS, however, the oxygen needed for the animals (water fleas, small snails and small shrimps) was produced within the facility itself by an aquatic plant [24]. With this, the ABS represents the first facility brought into space, which combined organisms from different trophic levels and mimicked natural ecosystems. The ABS was used in the space shuttle and aboard the Mir Space Station [15]. In 1992, another German setup, the *Closed Equilibrated Biological Aquatic System* (C.E.B.A.S.), was introduced as a possible precursor for long-term multi-generation experiments with aquatic organisms on Earth and in space missions [25]. For the application in space, C.E.B.A.S. was miniaturized (C.E.B.A.S. minimodule) to fit into a Spacelab middeck locker [26]. It consisted of four subcomponents (zoological, botanical, microbiological and electronic component) and was successfully flown onto two space shuttle missions in 1998 [27]. However, due to the limited space preconditioned by the Spacelab locker, it was impossible to establish a self-sustaining artificial ecosystem. Hence, food for animals inhabiting this system still had to be provided by an automated feeder, causing limited mission duration. The impossibility to harvest the oxygen-producing plant *Ceratophyllum demersum* inside the running setup was another obstacle to reach the goal of a self-sustaining system, as a rapid increase in biomass led to mutual shading and thereby to a reduced photosynthetic activity. This issue might be solved by using unicellular algae for oxygen production, as their automated harvesting is less complex, rendering phytoplankton a foundation of aquatic BLSS.

Several systems based on phytoplankton have already been successfully tested (AQUARACK [28], BIORAT [29], CAES [6], AQUACELLS [30] and SIMBOX mini-ecosystem [31]), and it was shown that the amount of oxygen produced by *Euglena gracilis* is sufficient to sustain fish (OMEGAHAB [32]). However, fish food still has to be provided by an automated feeder. A solution to this problem is to produce it within the BLSS itself by the introduction of herbivorous zooplankton. In aquatic food webs on Earth, these organisms link oxygen-producing phytoplankton (microalgae) and higher trophic levels, such as fish, and may thus play this very role also in BLSS [33]. However, zooplankton is well studied with regard to ecological and evolutionary aspects (e.g., [34, 35]), but so far little is known about its performance in μ*g* (e.g., [15, 36, 37]). Gaining knowledge on aspects such as behaviour, survival and reproduction of zooplankton under space conditions is thus of great importance with regard to the establishment of stable biomass production in BLSS. Therefore, potential effects of μ*g* on candidate organisms have to be evaluated. To this assignment, different methods are available to researchers in order to achieve μ*g* conditions, ranging from short-term μ*g* in drop towers and parabolic flights to prolonged μ*g* phases aboard sounding rockets and the ISS. Furthermore, ground-based facilities, providing simulated/functional long-term μ*g*, such as clinostats, are a valuable tool for the assessment of zooplankton used for biomass production in BLSS for space application. In the following, examples of gravitational research on zooplankton using these methods are explained in detail.

2. The Bremen Drop Tower

Since its inauguration in 1990, the Bremen Drop Tower has been widely used for a variety of experiments, predominantly in fluid mechanics, physics and space research. The drop tower facility in Bremen consists of three basic elements: the tower itself with a height of 146 m (110 m free fall in 120 m vacuum drop tube) (**Figure 1A**), the catapult and drop apparatus, each allowing a different method to obtain microgravity (μ*g*), and the experiment integration area (**Figure 1B**) including the control room, laboratories and a mechanical as well as an electronic workshop. With a g-value of about 10^{-6} *g*, the quality of the μ*g* in the Bremen Drop Tower is exceptional compared to other facilities (cf. other chapters). In addition to the excellent μ*g* quality, further favourable features are the daily accessibility and unproblematic safety regulations in comparison with parabolic flights and ISS experiments. Also, air traffic is not affected and risk of fire or contact with harmful substances can be mitigated. Yet another key benefit in using the drop tower represents the possibility to test experimental hardware as well as obtaining biological data and optimizing the procedures in preparation for subsequent campaigns under μ*g* conditions. Easy accessibility of the tower and hardware, constant consultation and evaluation between researcher and the *Zentrum für angewandte Raumfahrtechnologie und Mikrogravitation* (ZARM) technicians allow a productive environment for experimental design as well as theoretical and practical implementation of hardware and test objects. This is especially useful when adjustments on the experiment on short notice are necessary. Furthermore, multiple launches and drops per week are possible, so configuration and hardware errors become evident almost immediately after experiment recovery.

There are two ways of using the drop tower for microgravity research. First, there is the drop apparatus, through which the drop capsule is lifted to a height of 120 m at the top of the drop

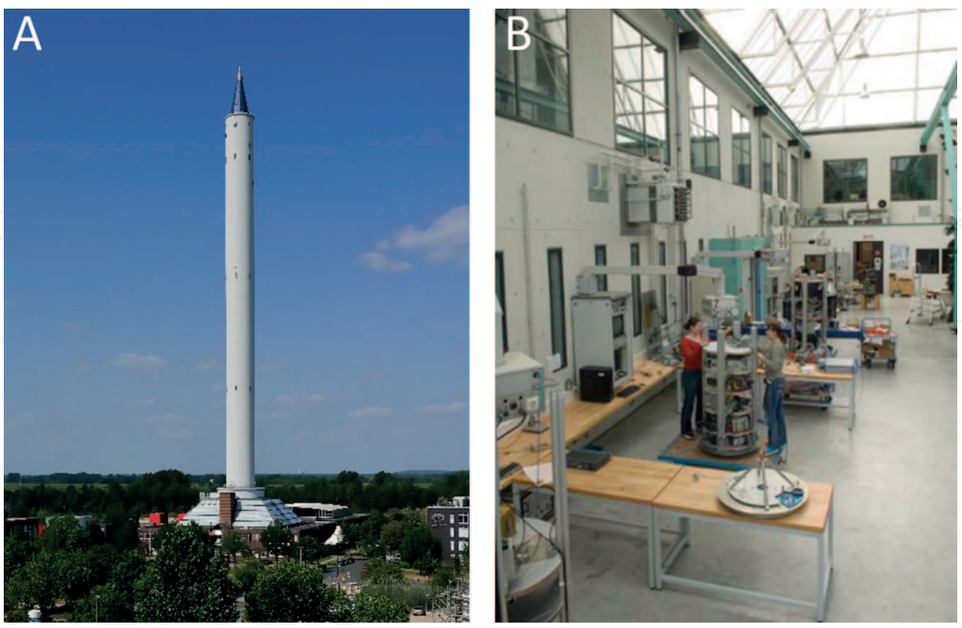

Figure 1. A: The Bremen Drop Tower (photo: ZARM). B: Experiment integration area (photo: ZARM Drop Tower User Manual 2012).

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tube by a hoisting winch and finally released into free fall, resulting in about 4.7 seconds of μg. This method is the original one and was the only way to operate until the installation of the catapult system. The catapult system enables a second way of using the drop tower in shooting the drop capsule in a vertical parabola to the tip of the tower and back, resulting in about 9.3 seconds of real μg.

In order to withstand deceleration forces and to maintain an environment at constant atmospheric pressure, the experiments are integrated into one of two drop capsule types of different sizes. The drop capsule is a modular cylindrical container with a diameter of 800 mm and a length of 1.6 m in the first type or 2.4 m in the second type, while the space for experiments extends to a length of either 953 or 1718 mm according to the needs of the experimental hardware (**Figure 2**). The payload area is subdivided in experiment platforms on which the hardware can be placed. A maximum mass of up to 500 kg for the integrated capsule is possible. Subtracting the capsule net weight, a maximum payload mass between 161.5 and 264.4 kg is feasible, depending on the capsule type in use. The drop capsule is assembled with the experimental setup in the integration hall of the drop tower facility. The whole experiment integration process is monitored and assisted by specially assigned technicians of the drop tower staff (**Figure 3**). The integration process usually starts 10 days to 1 week before the first drop or

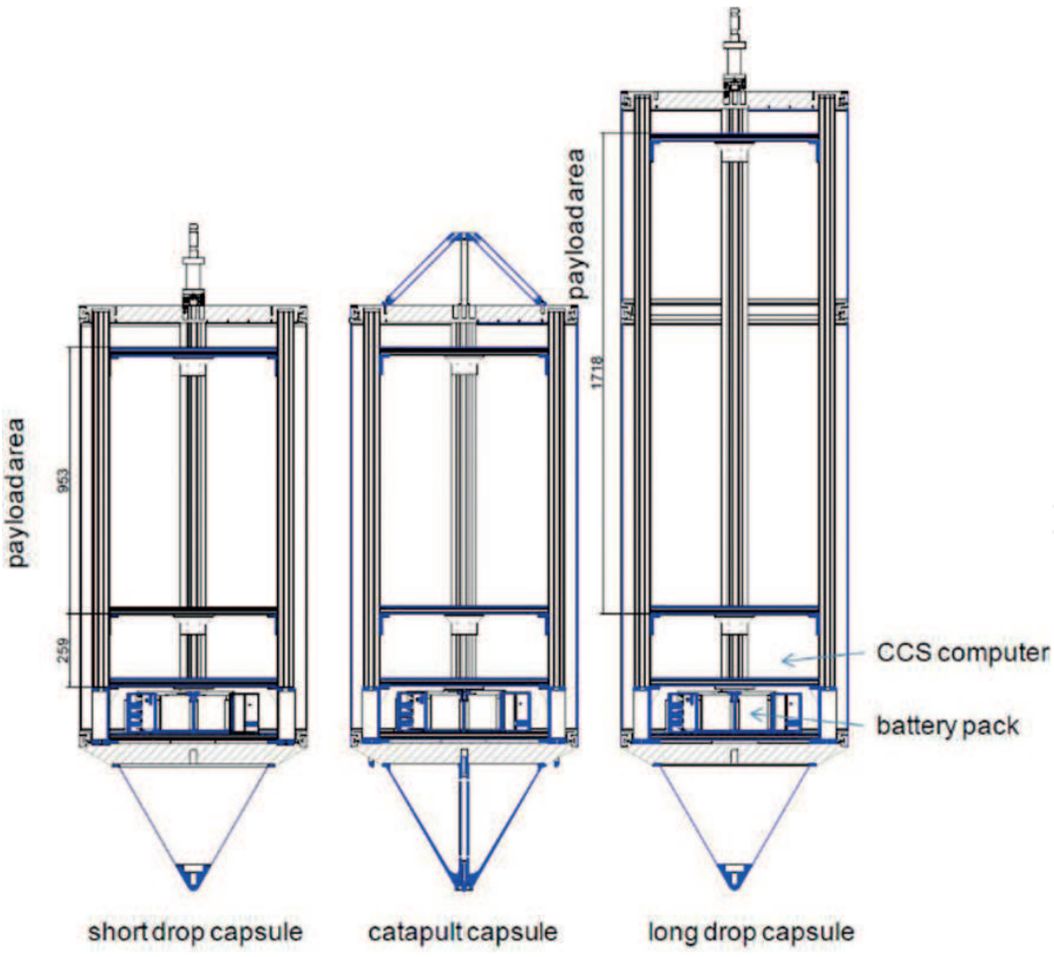

Figure 2. Different types and sizes of the drop capsule (photo: ZARM Drop Tower User Manual 2012).

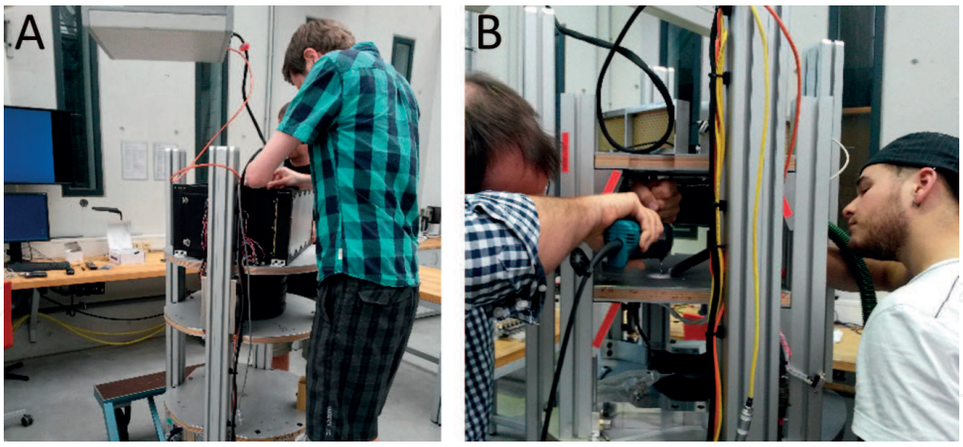

Figure 3. Different stages of the assisted integration process. A: Early stage of capsule assembly. B: Preparation of experiment platform by technicians of the drop tower staff.

catapult launch. It is usually possible to get extended preparation time as needed, e.g., in some biological experiment setups, when organisms require to reach a certain developmental stage and size. Acclimatized laboratories and storing areas for all chemical compounds are provided by the ZARM facility and installed in close range to the drop tower integration area. All elements essential to the experiment setup in the capsule, such as electronic devices, experiment containers and controllers, are mounted on the experiment platform and wired accordingly to controllers and power supply units. Sensitive samples are transferred to the corresponding containers shortly before finalization of the integration. Also, during this time, all preparations and integration details are organized in close correspondence with the drop tower staff. This procedure is important as it ensures a flawless and a highly adjusted operation of the experiment.

The basic setup of the capsule contains the base structure, the four-stringer-rack for experiment accommodation and a lid plate with interfaces and a release bolt (**Figure 4A, B**). The base structure always consists of a switchable power supply unit, a radio telemetry and telecommand system, a WLAN unit on top of the capsule and also the capsule control system (CCS) for experiment control [38]. The CCS is programmable and controls the units of the base structure and experiment sequences. It also controls any electronic device attached to the experiment platforms, such as high-speed cameras, servomotors and illuminating devices like LED arrays. It is therefore of highest importance to adjust the CCS programme individually to the present experiment hardware. This is done by the staff of the drop tower, i.e., the ZARM FAB mbH, in correspondence with the experimenters. For example, in experiments on the water flea *Daphnia* using RNAlater as preservative to determine the effect of μg on gene expression at specific moments in time, the exact time of RNAlater release from hydraulic-driven syringes for each fixation unit could be set and programmed in the CCS (**Figure 5**). The programming enabled a fixation at four different time points on each shot [39].

When operating on catapult mode, once the integration is completed, the drop capsule has to be balanced in order to ensure a safe launch procedure, as an unbalanced setting could lead to deviations from the flight vertical vector and in turn cause fatal damage. After balancing, the drop capsule is covered by a pressure-sealing aluminium sheath (**Figure 6**), before it is

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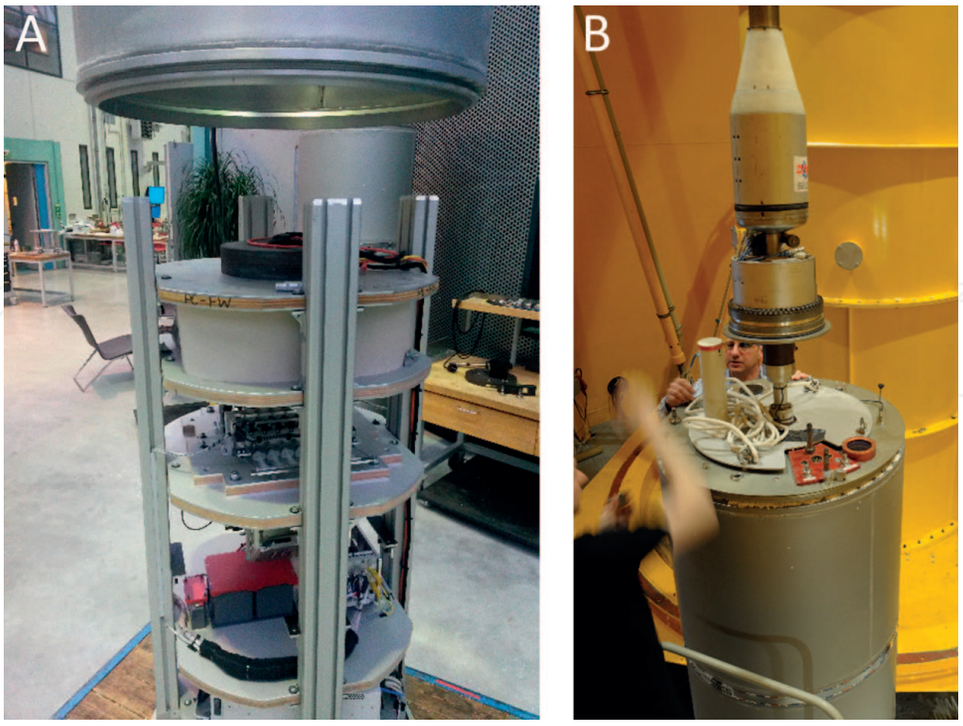

Figure 4. A: Four-stringer-rack for experiment accommodation. B: Drop capsule lid plate with interfaces and release bolt (photo: ZARM).

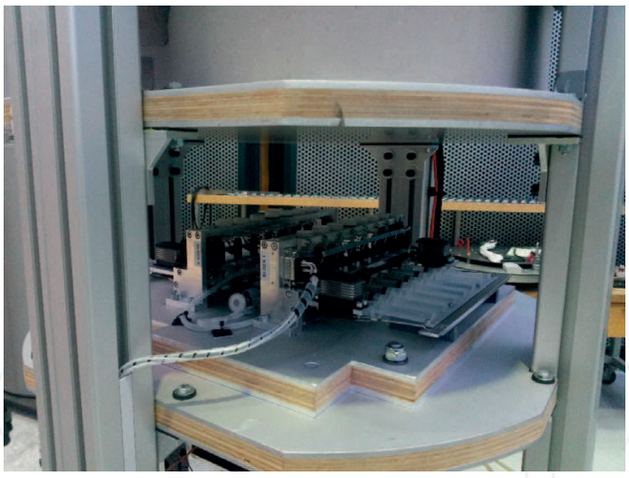

Figure 5. Hydraulic-driven syringes controlled by the capsule control system for fixation experiments with zooplankton.

transferred to the catapult or drop system of the tower. Before launching the experiment, the tower has to be evacuated for approximately 1.5 hours to create a vacuum in the drop tube in order to reduce the air resistance to a minimum.

The catapult has been installed 11 m below the base of the tower. It is based on a combined hydraulic-pneumatic system and consists of 12 pressure tanks located around the prolonged drop tube in the chamber. The catapult utilizes a pneumatic piston that accelerates the drop

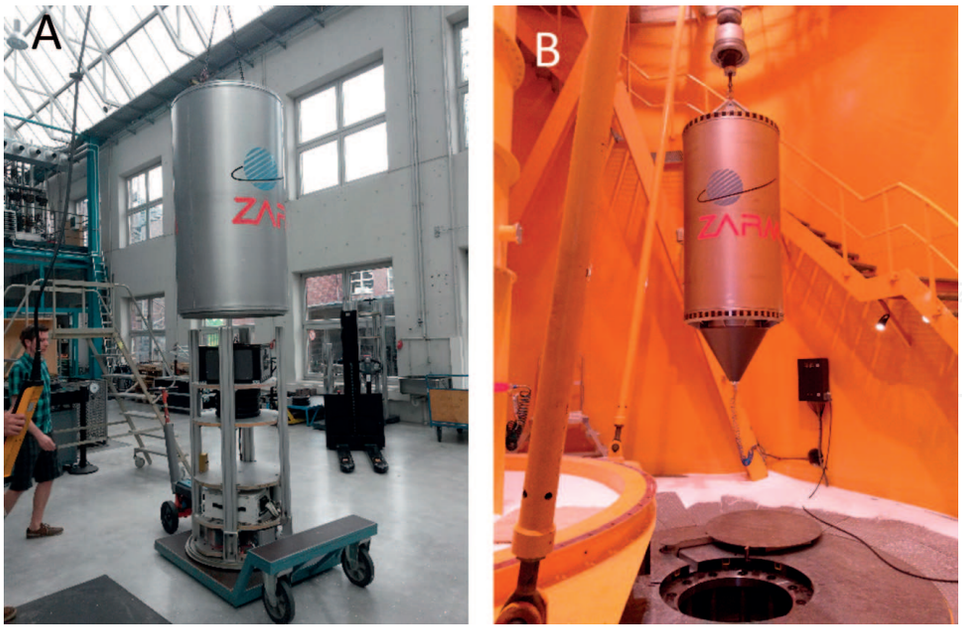

Figure 6. A: Sealing process of the capsule with the aluminium sheath for pressurisation. B: Sealed capsule in the drop tower.

capsule to speeds up to 48 m/s within 0.28 s leading to a capsule velocity of around 175 km/h during the parabola [40]. The launch is started at the control room by the technical staff. While both methods achieve the same high quality of μ*g*, the catapult operation involves a very short time frame (around 280 ms) of hypergravity at start acceleration. This may need to be taken into account depending on experiment design and requirements. In any case, the drop capsule is received at ground by a deceleration container filled with small polystyrene pellets, which ensure a safe landing of the capsule. The container is positioned below the drop tube during the catapult launch or the drop. After the shot or drop, the drop tube is ventilated again and the experiment can be recovered from the drop capsule for analysis. All experiment and monitoring data can be retrieved from the CCS. Following this procedure, up to three launches or drops per day are possible.

In the past, a variety of biological experiments were performed at the drop tower in Bremen. Research in plant biology focused on auxin transport, stress reactions in roots already present in the first few seconds in μ*g*, and could show fluxes of nitric oxide, reactive oxygen species and oxygen in the apex zone of seedlings of *Zea mays* under μ*g* conditions [41]. Already in the 1990s, the gravitactic orientation and its respective thresholds in the unicellular green alga *Euglena gracilis* were analysed using real-time image analysis [42, 43]. Present drop tower experiments are now being focused on gravity-related signalling pathways and adaptation mechanisms in *Euglena* as well as their helical swimming patterns via a three-dimensional motion analysis system. Experiments in the drop tower facility have been used not only to study swimming patterns, but also to investigate the effects of gravity on animal behaviour and postural control mechanisms under μ*g* conditions. For instance, the catfish *Synodontis nigriventris* shows a ventral substrate response (VSR) behaviour. This behaviour is characterised by a turning of the fish's ventral side towards a respective surface, when a suitable substrate is close by. Without a

substrate present, it swims upside down. In order to elucidate if the VSR is affected by a lack of gravity or coupled to a gravitational stimulus, some specimens of *S. nigriventris* were exposed to μ*g* in the drop tower. The experiments showed that the VSR can override the vestibular input in this particular species [44]. In order to analyse kinetotic ("motion sickness") behaviour in fish over a short period of time under μ*g*, Anken and Hilbig [45] exposed *Oreochromis mossambicus* to various gravity environments, ranging from 0.1 to 0.9 *g*. Along with establishing suitable thresholds for kinetosis levels, the drop experiments revealed that the short time frame of 4.7 s in specific gravity conditions is sufficient to induce kinetosis and also confirmed the feasibility of the procedures with the used hardware.

In zooplankton research, the search for suitable organisms for bioregenerative life support systems (BLSS) has risen in importance. Knowledge on graviperception and the involved organs in zooplankton organisms give an insight into how these animals cope with gravity. Future experiments in the drop tower with different planktonic species can help to find answers to these questions. By using drops under different μ*g* levels, thresholds for gravity perception of the selected species can be determined, as it has already been shown for some protists in clinostat experiments [46]. In order to establish such BLSS, the comprehension on how food webs function and how zooplankton organisms are affected by altered gravity conditions is of utmost importance. To test whether predator-prey interactions are affected by μ*g* is a first step to investigate the functioning of a BLSS based on multi-trophic levels. If the food chain is interrupted because the predators do not feed on their prey, energy transfers to higher trophic levels and hence biomass production is not possible, thus preventing food production for humans in space missions. Experiments focusing on the first trophic level at the Bremen Drop Tower showed that foraging and feeding of *Daphnia magna* are not significantly altered in μ*g* [37]. So far, little is known on how μ*g* acts on a molecular level in zooplankton. In human cells, studies in parabolic flights have revealed that already short-time exposure to μ*g* affects gene expression patterns [47], and also various studies in simulated microgravity report disturbances and changes of the cytoskeleton (e.g., [48, 49]). Also in *Daphnia magna*, a structural disruption of the cytoskeleton and an upregulation of energy metabolism-related proteins during clinorotation could be observed [50]. In further drop tower experiments, preservation of *Daphnia* with RNAlater in different gravity conditions can help to elucidate the first response as well as adaptation processes in altered gravity on the cellular basis [39].

In conclusion, the Bremen Drop Tower represents an extraordinary facility for various applications in space and microgravity research. A milestone in the drop tower history was undoubtedly the construction and inauguration of the catapult system in December 2004, which enabled μ*g* conditions up to almost twice the time achieved at free fall mode [40]. The engaging working environment coupled with an outstanding quality of μ*g* ensures a high quality of data and inspiration for future experiments. Easy handling and relatively low costs by using commercial hardware allow changes on short notice and step by step refining of experimental hardware and procedures during the integration phase and to some extent even during the actual experiment campaign. With up to three possible catapult launches or drops per day, not only good data quality, but also a sufficient amount of replicates are provided. The opportunities at the drop tower facility will continue to foster further research on biological systems related to varying gravity conditions and therefore secure its key position for short-

term tests and as preparation environment for campaigns in more complex gravity-related research environments, like parabolic flights with aircrafts, sounding rocket launches and suborbital flights with new commercial vehicles. Thus, drop tower experiments are an indispensable tool for research on plankton as a key element for bioregenerative life support systems for future crewed long-term space missions.

3. Parabolic flights

The term parabolic flight describes a special flight manoeuvre where an aircraft follows a free-fall ballistic Keplerian trajectory [51]. Thereby, the resultant of all forces acting on the occupants of the aircraft other than gravity is nulled. This manoeuvre is started by accelerating the aircraft to gain velocity before pulling up to convert horizontal velocity into vertical velocity. During this climb, the gravity level increases. Upon reaching a sufficient upward velocity, the pilots reduce the thrust, compensating the effect of air drag and the aircraft starts to "fall" uphill (parabolic free fall) and the microgravity phase starts. The aircraft then passes the apogee of the parabola and starts to dive downwards. At the end of the parabola, the pilots pull up to stop the dive and the g-level increases again [52, 53] (**Figure 7**).

The first-ever parabolic flights were performed by National Aeronautics and Space Administration (NASA) pilot Scott Crossfield and Air Force pilot Charles E. Yeager at Edwards Air Force Base in California and at Wright Field in Ohio in 1951 [54]. From then on, parabolic flights became a valuable and frequently used tool for training astronauts (e.g., [55]), medical and physiological experiments on human subjects (e.g., [56]), space technologies (e.g., [57]), physics and material sciences (e.g., [58]), medical engineering and biotechnology (e.g., [59]) and life sciences (e.g., [60]).

The United States Space Agency NASA operated its own parabolic aircrafts until 2014. Since 2015, the *Zero Gravity Cooperation* (ZERO-G) and their aircraft, the G-FORCE ONE, a modified Boeing 727-200 are used for research [61, 62]. Further, the Canadian National Research Council (NRC) in association with the Canadian Space Agency (CSA) irregularly utilise parabolic flights for research aboard their Falcon-20, which has been modified for parabolic flights [63].

The European Space Agency (ESA) launched its own parabolic flight programme in 1984 [53], and since then, on average six scientific parabolic flight campaigns are carried out each year, reflecting the great interest in this platform. Since 2015, parabolic flights for research are performed using the Airbus A310 ZERO-G. It is the largest airplane for parabolic flights worldwide [64], owned and operated by Novespace, a subsidiary of the French National Space Center (CNES). A parabolic flight campaign usually consists of 3 consecutive flights conducting 31 parabolas each. The μg phase of each parabola has a duration of approximately 22 s and the residual acceleration acting on experimental set-ups is in the order of 10^{-2} g. Furthermore, modified parabolas can be flown during which partial g-levels (including lunar (0.16 g) and Martian (0.38 g) gravity) can be achieved.

In comparison to experiments aboard research satellites or the International Space Station, parabolic flights have several advantages. They have a short turnaround time of approximately

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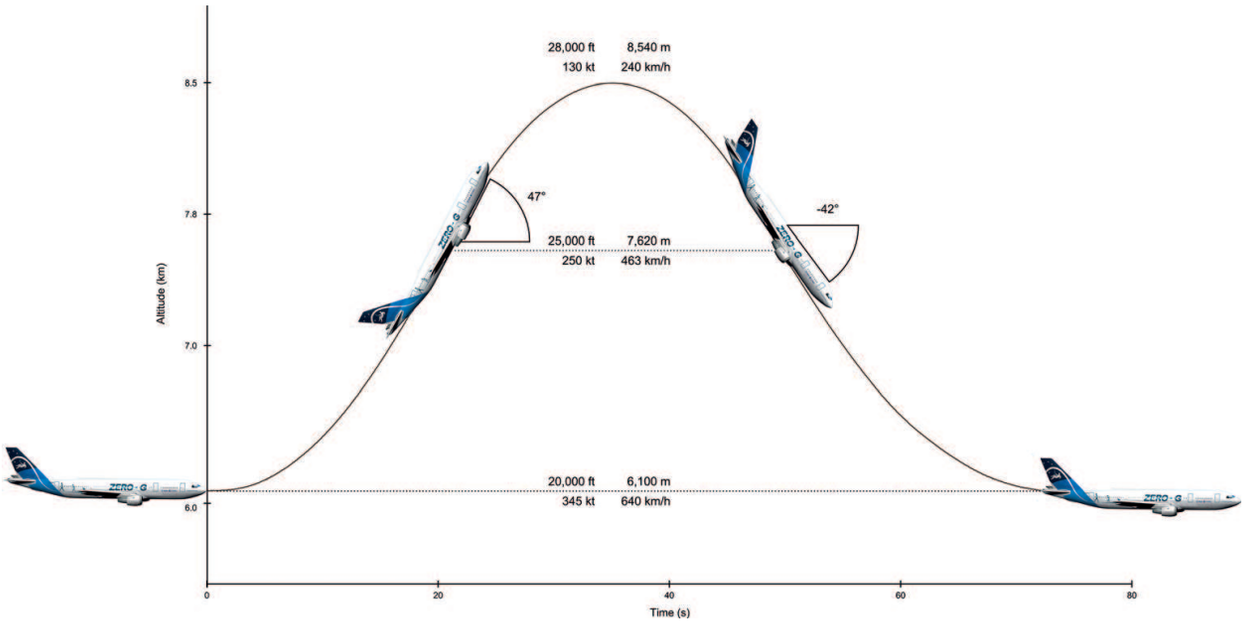

Figure 7. Schematic illustration of the parabolic flight manoeuvre. Courtesy of Novespace, France.

8 months between the experiment proposal and its performance. The scheduled campaign dates are reliable and the campaigns take place regularly. Furthermore, it is possible to use laboratory-type instrumentation, which also provides a high flexibility in the experimental approach. The major advantage is that investigators can directly interact with their experimental set-up or change experimental parameters during and in between parabolas. However, major disadvantages are the hypergravity phases interspaced between the phases of reduced gravity (microgravity or partial *g*), which can present a substantial prejudice to some research questions. The short duration of the reduced gravity phase is a further disadvantage since some issues may require a longer time period. Nevertheless, these periods are still sufficient to address a lot of questions in the area of life sciences. They range from the impact of μ*g* on the cellular level to shifts in physiological parameters and to the behaviour of whole organisms in reduced gravity conditions. Some of these variations/shifts are also likely to occur in zooplankton and are thus of interest with regard to the suitability of zooplankton for bioregenerative life support systems. A selection of those studies will be presented in the following paragraphs.

Parabolic flights are a well-suited tool to analyse the effects of μ*g* on the cellular and molecular level, such as the change of the electrophysiological properties of various cell types and the propagation velocity of action potentials [65]. Furthermore, bone cells are studied on a regular basis, as mechanical loading plays a critical role in their function and differentiation [66, 67]. Likewise, cytoskeleton experiments are frequently carried out, as it is redistributed and reorganized under reduced gravity [68, 69]. In order to find possible explanations for these phenomena, the impact of altered gravity on gene expression is increasingly being investigated in different organisms [47, 70]. Another research area in which parabolic flights are frequently utilized is physiology. A number of physiological changes are caused by μ*g*, such as an initial shift in the distribution of blood [71], which happens within a very short time frame. This might have an impact on the blood flow and could thus influence the pulmonary diffusing capacity [72]. Also isometric force production was examined during parabolic flights [73],

since it is known that deviations from 1 *g* affect sensorimotor performance and thereby the ability to grasp objects or to operate the pedals, buttons and levers of machines. The behaviour of animals from different habitats under reduced gravity conditions was examined in a multitude of studies, showing that behavioural changes occur quite fast and that their responses are fairly diverse. In pigeons, flight movements are provoked at the transition from hypergravity to μ*g* at the beginning of the parabola and they display random head movements while free floating [74]. Likewise, the reaction of different species of amphibians and reptiles to diminished gravity was analysed in parabolic flights [75]. In terrestrial and semi-arboreal lizards, long-axis thrusting body motions and high-amplitude, high-frequency tail thrashing movements have been observed [75], whereas terrestrial and arboreal frogs take up a "sky diving" posture [76], to name just a few examples. Fish also show altered behaviour when subjected to parabolic flights, the so-called loop-swimming, which is exclusively exhibited in μ*g* [77].

In planktonic research, parabolic flights are mainly used to study spatial orientation, locomotion behaviour, physiological and cellular responses to reduced gravity conditions and to elucidate the gravireceptive organs. As an example, the removal of the graviceptor (rhopalia) of an asexually produced life stage of the jellyfish *Aurelia aurita* led to an inability to swim in 1 *g* conditions and a missing response to the *g*-force changes occurring during the parabolic flight, whereas unharmed control individuals swam loops or became immobilized [78]. This experiment showed the importance of intact rhopalia for orientation in *Aurelia aurita* at 1 *g* and during the *g*-force changes in parabolic flights. In the protist *Paramecium biaurelia* [79], the changes of graviorientation and gravikinesis were investigated at different accelerations. At first, *P. biaurelia* showed a significant preference for upward swimming (negative gravitaxis), but after 7 s of μ*g*, no significant swimming direction could be recorded. Another aim of this experiment was to test whether parabolic flights are suitable to examine the threshold of gravikinesis in these organisms. It was shown that parabolic flights are in principle suited for preliminary threshold studies on fast-reacting biological systems. The unicellular freshwater flagellate *Euglena gracilis* was used in a number of parabolic flights. These organisms are of special interest with regard to aquatic bioregenerative life support systems, as they could act as a foundation of such systems. *E. gracilis* shows a pronounced negative gravitaxis, which is most likely mediated by an active physiological mechanism involving changes of internal calcium concentrations and the membrane potential [80]. It was shown that the μ*g* phase leads to a pronounced loss in the precision of orientation in *E. gracilis* and to a decrease of the intracellular calcium concentration, which indicates that calcium signalling is involved in the graviperception and orientation. Similar experiments have been performed with *Astasia longa*, a close relative of *E. gracilis* [81]. They show a negative gravitaxis in the absence of other external stimuli apart from gravity. During μ*g*, however, a clear deterioration of gravitactic orientation was detected, which improved during the subsequent hypergravity phase. Also, the cytosolic calcium levels showed acceleration-dependent changes, with a transient increase upon increasing acceleration. Like in *E. gracilis*, these findings confirm the model of gravitaxis, which assumes the presence of mechanosensitive channels, activated upon deviation from the vertical swimming direction.

In addition, parabolic flights can be used to address responses of microcrustaceans to altered gravity. It was previously shown that the locomotion behaviour of the water flea *Daphnia*

magna and of ostracods is modified in μ*g*, due to a disturbance of spatial orientation [15, 36]. As this could impair food uptake of the animals, a parabolic flight experiment was performed to analyse if the ostracod species *Heterocypris incongruens* is still able to forage [37]. Since the direction of incident light is used as orientational cue by many crustaceans, the possible influence of illumination on the behavioural response was included in the experimental design (for technical details, see [37]; **Figures 8** and **9**). As feeding could not be directly observed in ostracods, due to the non-transparent carapace valves covering the mouthparts, the sojourn time on food was used as indirect proxy for the feeding duration. The fact that feeding behaviour was not significantly affected in μ*g* renders *H. incongruens* as suitable candidate for future BLSS.

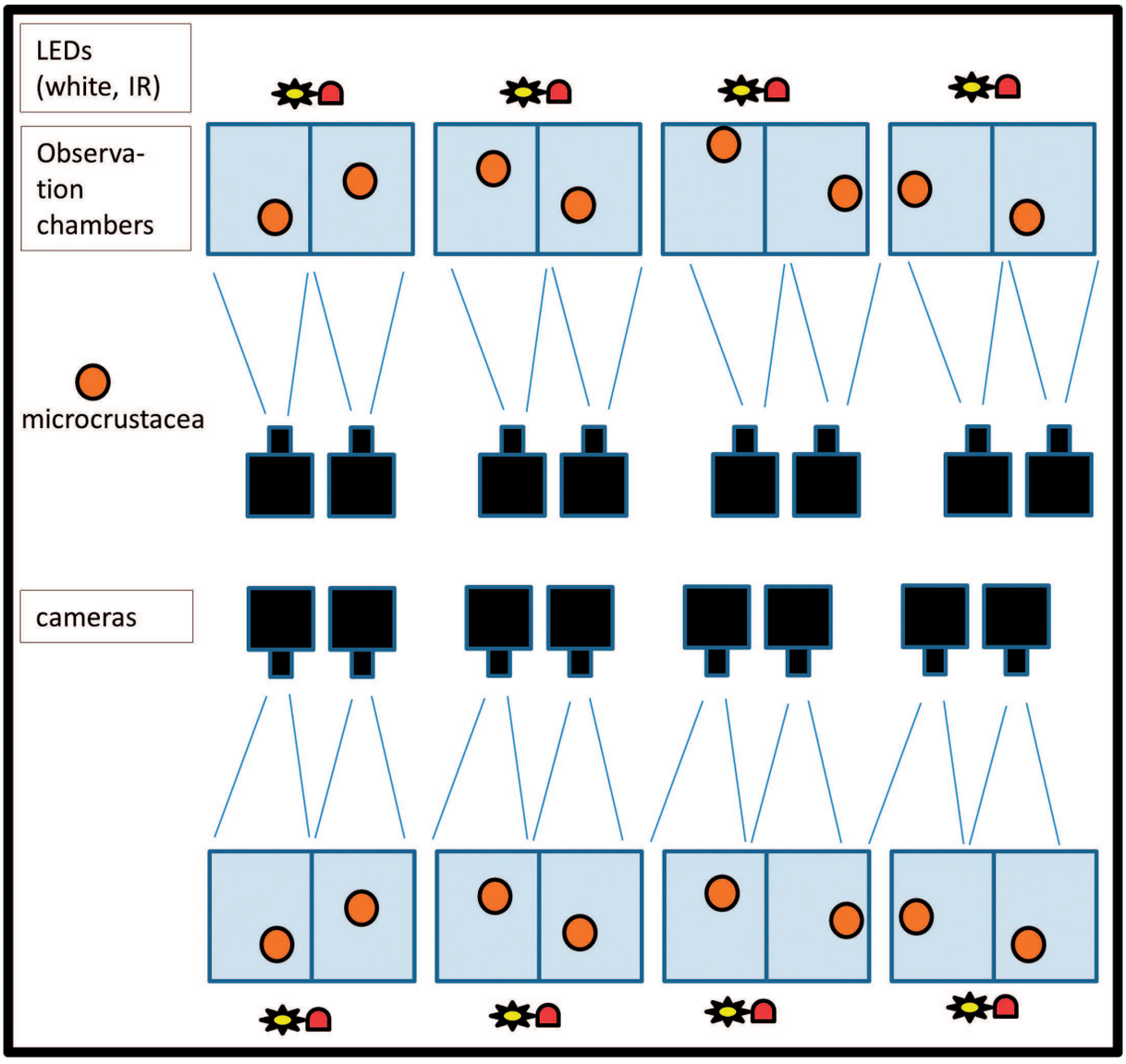

Figure 8. Schematic drawing of experimental set-up for the analysis of microcrustaceans in altered gravity conditions during parabolic flights.

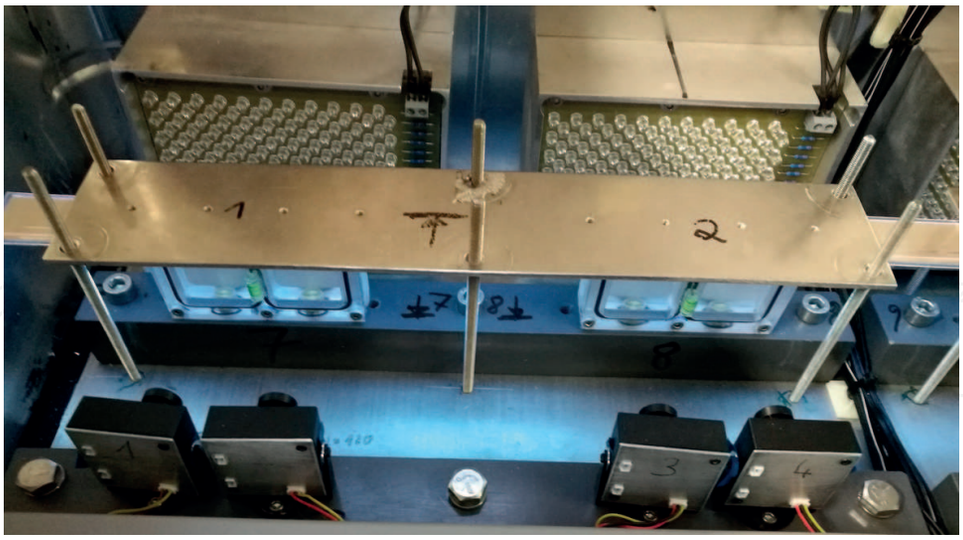

Figure 9. Picture of the experimental set-up used with microcrustaceans in parabolic flights.

Taken together, all the results gained with different planktonic organisms show that parabolic flights are an excellent method to analyse their responses to altered gravity conditions, especially μ*g*, and the underlying mechanisms. In order to obtain a comprehensive assessment of the suitability of planktonic organisms for bioregenerative life support systems, it is thus necessary to include data from other platforms such as clinostats, the drop tower and sounding rockets.

4. Sounding rocket experiments

The use of rockets for research was already proposed by Robert H. Goddard at the beginning of the twentieth century [82]. In 1933, the first instrumented, liquid-fuelled sounding rocket was launched by the Russian Tikhonravov [83]. The history of sounding rockets was profoundly affected by the beginning of the German military rocket programme in 1930 and the beginning of rocket research at the Guggenheim Aeronautical Laboratory, California Institute of Technology (GALCIT), in 1936 [84]. The German work culminated in the development of the V-2 rocket. After World War II, the American army was the first to inherit an underground V-2 factory. The seized rocket parts were transferred to White Sands Missile Range in New Mexico and assembled into complete rockets to provide an immediate source of high-altitude vehicles and a test bed for further developments. The first American V-2 was launched in 1946 [85], and in total, 67 V-2s were launched from White Sands as part of the Hermes programme [86]. Even though almost half of the V-2 s launched were classified as failures, the experience gained with this rocket provided the knowledge to build sounding rockets tailored specifically to space research. A comprehensive overview on the first sounding rockets developed in the United States is given in [84]. A breakthrough in the use of rocket technology for civilian purposes was initiated by the International Geophysical Year (IGY) from July 1957 to the end of 1958, which

was devoted to global atmospheric research and prompted the launch of some 200 sounding rockets worldwide [87]. The impetus given by IGY to sounding rocket activities led to the establishment of national sounding rocket programmes in many countries. An elaborate overview on the European developments is given in [87]. Up to now, sounding rockets are frequently used in microgravity and space research all over the world embedded in well-structured programmes (e.g., NASA Sounding Rocket Program [88], Australian Space Research Institute Small Sounding Rocket Program [89] and European Sounding Rocket Programs [90]).

The TEXUS Sounding Rocket Programme (Technologische EXperimente Unter Schwerelosigkeit) was first funded in 1976 by the German Ministry for Research and Technology, as a preparatory programme for the first Spacelab mission in 1983, and from the end of 2005 by the Federal Ministry of Economics and Technology, both acting through the DLR Space Agency in Bonn. The European Space Agency (ESA) joined in that project from 1981 and the first experiment flew on the German TEXUS 6 mission in 1982.

Skylark VII two-stage solid fuel launchers (first stage: Goldfinch IID; second stage: Raven XI) manufactured by British Aerospace were usually employed in the TEXUS programme. The mission-related tasks, such as the provision of the rocket motor, the service systems and the launch service, are covered by an industrial consortium led by Airbus Defence and Space (Bremen, Germany). Since TEXUS 42, launched in December 2005, the Brazilian two-stage solid propellant VSB30 rocket motor has been used for TEXUS missions.

The European long-duration sounding rocket programme MAXUS started in 1990 and finally the MiniTEXUS was added in 1993 to the family of sounding rockets. The TEXUS/MiniTEXUS and MAXUS rockets consist of two major sections: the motor and the payload, which is mounted on top of it. The modular payload comprises the Recovery System with the parachute, the Service Module and the Experiment Modules. On a MAXUS rocket, additionally a Guide Control System (GCS) and a Telemetry and Tracking Unit (TTU) complement the payload (see **Figure 10**). The MiniTEXUS flight offers 3 1/2 minutes of microgravity flight, the TEXUS more than 6 minutes and the MAXUS flight about 13 minutes of microgravity [91, 92].

All missions are launched from the European rocket launch site ESRANGE near Kiruna in the north of Sweden. On its ballistic flight, microgravity (μg) conditions (10^{-4} g) prevail for 3 1/2 minutes on a MiniTEXUS flight, for more than 6 minutes on a TEXUS flight and for about 13 minutes on a MAXUS flight [91, 92]. The payload of the rocket, meaning the tip that contains the Experiment Modules as well as the Recovery and Service System, comes down on a parachute and is transported back to the launch site by a helicopter. Scientific experiments are housed in modules stacked one atop the other within the rocket. Each experiment is directly monitored and controlled by researchers on the ground through telecommanding and TV systems. Scientific data are either directly transmitted during the flight by telemetry or saved after the payload has been recovered. The TEXUS/MAXUS missions offer a unique environment especially for biological research due to the availability of well-equipped laboratories close to the launcher for the preparation and post-flight analysis of biological specimen and due to the possibility of late access to the rocket and early retrieval of samples after the flight. Furthermore, safety requirements for this unmanned programme are less stringent than those of manned missions and experiments are less expensive.

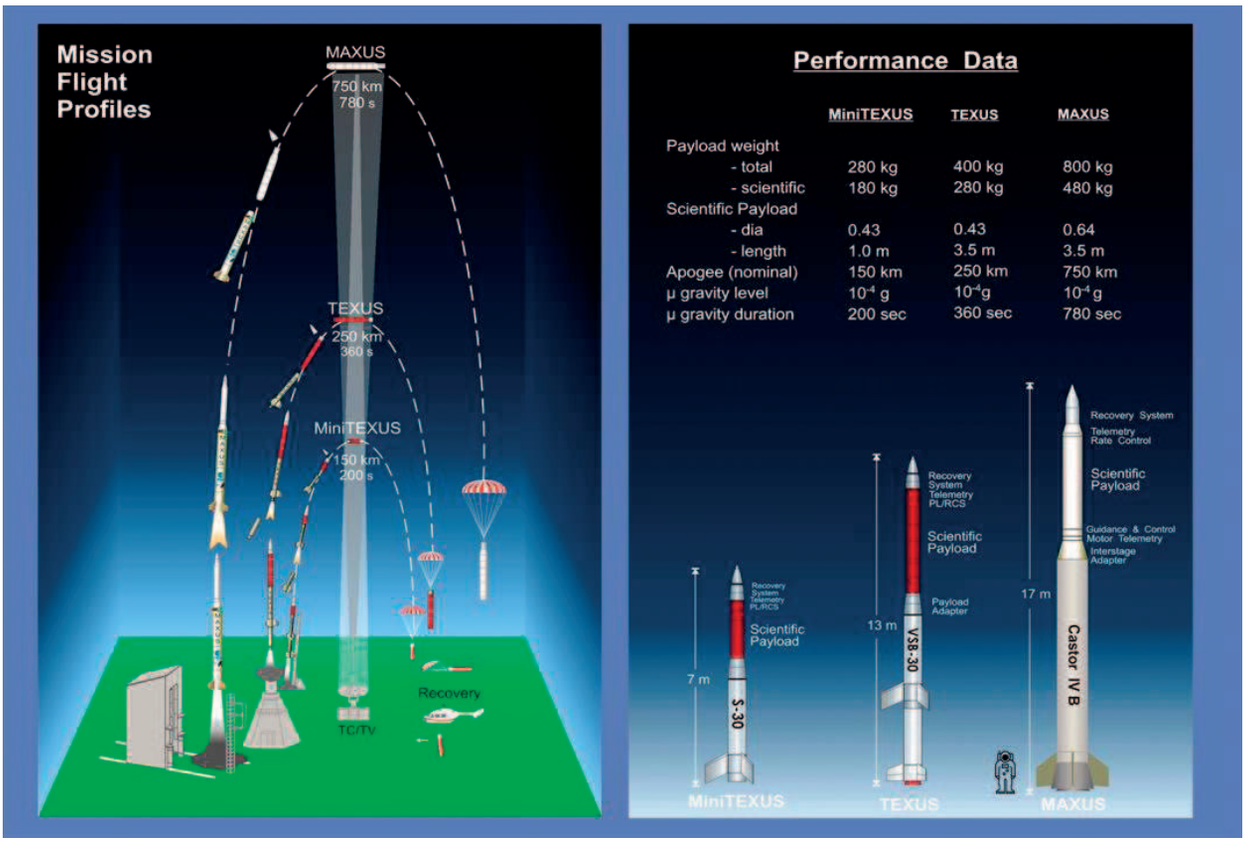

Figure 10. TEXUS/MAXUS launcher and flight profile. Courtesy of Airbus Defense & Space, Germany.

The major difference to other microgravity platforms such as parabolic flights and drop tower tests is the extended duration of the μ*g* phase and the high microgravity quality compared to parabolic flights.

Hence, a plethora of basic research was performed with different organisms during the various TEXUS and MAXUS missions. The gravitaxis and phototaxis in the flagellate *Euglena gracilis* were studied during the TEXUS 23 and TEXUS 28-30 missions [93], and the scientists were able to show that in the absence of light, the Earth's gravitational field is responsible for orientation in *E. gracilis*, as the directed upwards swimming was replaced by swimming in random distributions in μ*g*. Through the application of light during the flight, it was demonstrated that the cells showed both positive and negative phototaxis in μ*g* and the precision of orientation was higher than under terrestrial conditions. In the TEXUS 35 mission, the threshold for gravitaxis in *E. gracilis* was determined and it was shown that reorientation of the cells started at 0.12 *g* and the precision of gravitaxis increased with the increase of applied acceleration [94]. A further experiment in the TEXUS 36 mission dealt with the physiological mechanism of gravitaxis in *E. gracilis* and it was demonstrated that cAMP is involved in gravitaxis and the increase in this second messenger is triggered by mechanical stimuli [95]. A close relative of *E. gracilis*, the flagellate *Astasia longa*, was studied in the MAXUS 3 mission with respect to intracellular calcium levels at different accelerations, and a clear change due to changes from μ*g* to accelerated conditions could be observed [80]. During the TEXUS 27 and 28 missions, the

swimming behaviour of the ciliate *Paramecium biaurelia* was analysed under μ*g* conditions. A random distribution of swimming directions was observed after 80 s of μ*g*, showing that gravity is the stimulus for the directed upwards swimming at 1 *g* [96]. Another organism whose locomotion behaviour was studied with a TEXUS mission (TX 48) is the cichlid fish *Oreochromis mossambicus*. This experiment gave evidence that fish are able to adapt to extreme gravitational habitat, as about 40% of the animals immediately started to swim normal after the launch and about 14% were able to regain normal swimming during the μ*g* phase [97].

Just recently, also the locomotion behaviour of zooplankton (*Daphnia magna*, *Daphnia cucullata* and *Heterocypris incongruens*) and predator-prey interactions between different trophic levels (predators: *Triops cancriformis*, larvae of the phantom midge; prey: *D. cucullata*) were studied in the TEXUS 52 mission. The long phase of μ*g* achieved with TEXUS facilitated to analyse which adaptation strategies towards weightlessness may occur in zooplankton and, in addition, a molecular study was carried out to investigate the influence of μ*g* on the expression of different genes, for example, stress markers.

For the observation of the organisms during the flight and preparation for post-flight analysis, specific experiment modules were developed that provide specific features like cameras with different magnifications, manipulators or centrifuges to apply a well-defined gravitation stimulus and fixation systems for the in-flight preservation of the specimen.

A typical experiment module design configuration is the TEXUS experiment module 06-31 (TEM 06-31). This TEM was accommodated on several TEXUS flights, namely TEXUS 45, 48 and 52, in the years 2008–2015 [98]. The design of the experiment module was always adapted to the scientific objectives of each dedicated flight campaign. For the last flight on TEXUS 52, the experiment module was equipped with a combination of any of the previous design features for observation and fixation. Thus, a description of this configuration covers all of the previous flight configurations.

The experiment module TEM 06-31 is presented in **Figure 11**. It consists of three different experiment platforms dedicated to a specific research objective and the Experiment Service System, which houses the electronic system for the automatic operation and control of the experiment module during the flight preparation phase and the flight phase.

Each of the experiment platforms is equipped with so-called late access units (LAU). These units house the aquatic systems in water-filled containers. The containers are sealed and connected via a short capillary tube to a small gas volume to compensate volume variations caused by temperature variations. The pressure inside the sealed containers is maintained at ambient pressure during the flight of the sounding rocket when the LAUs are exposed to a vacuum environment.

For the launch preparation, the LAUs are equipped with the organisms a few hours prior to the nominal rocket lift-off, checked for leak tightness and finally integrated into the rocket via late access hatches about 1 1/2 hours prior lift-off.

The three experiment platforms provide the following technical features for the observation or fixation of the specimen.

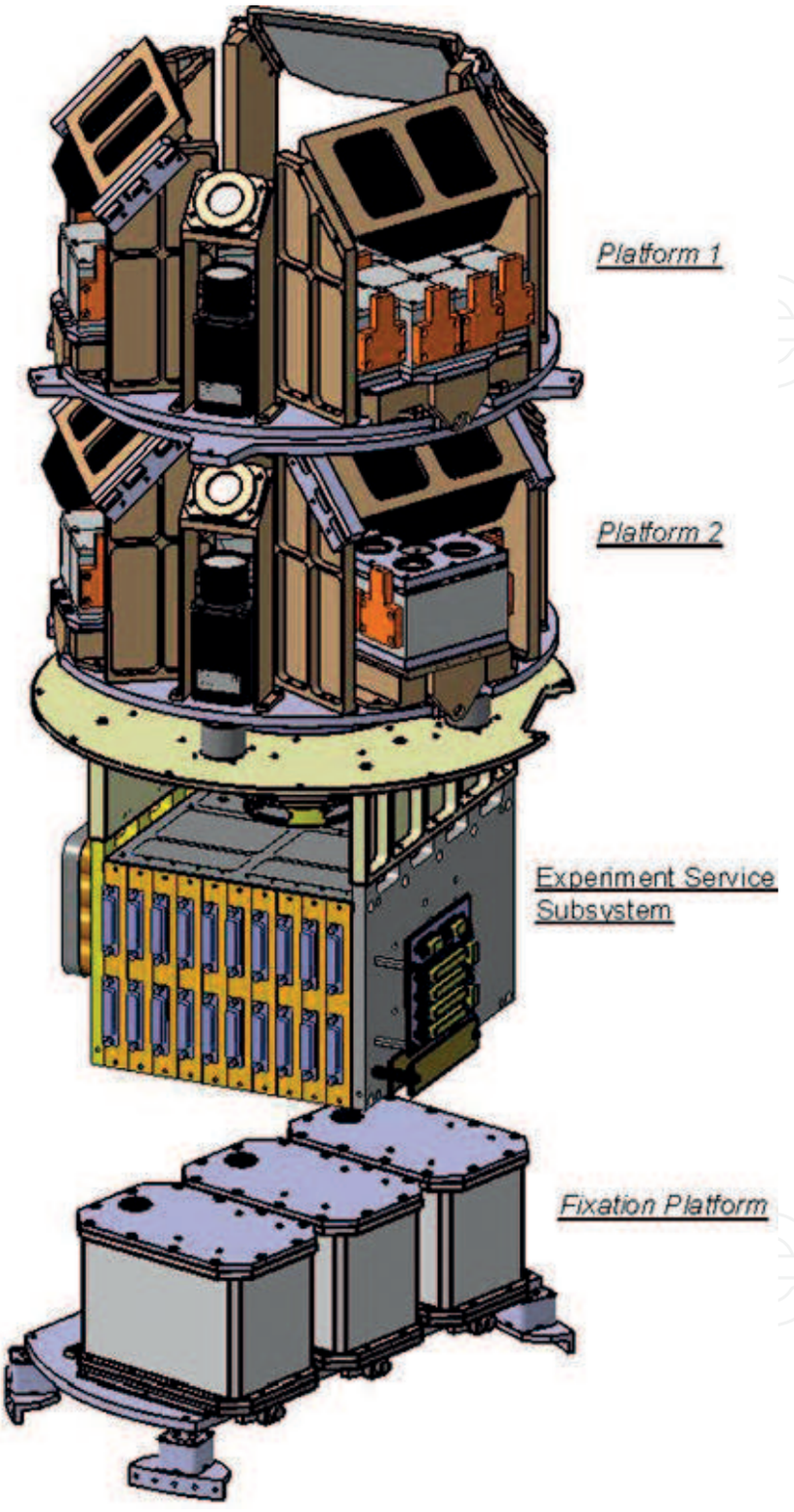

Figure 11. TEXUS experiment module 06-31. Courtesy of Airbus Defense & Space, Germany.

Platform 1: This platform is dedicated to the observation of the aquatic organisms' swimming behaviour under microgravity. Three LAUs with each accommodating eight sealed and water-filled containers are installed on this platform. The observation is performed from the top side of the containers via a set of mirrors and three CCD cameras equipped with an on-board video recording system for post-flight analysis of the video data. The illumination of the containers is performed via a backlight infrared illumination system, which is located beneath the late access units. The wavelength of the infrared light is not visible by the organisms.

Platform 2: The platform 2 design is based on the concept of platform 1 with the following main modification to study predator-prey interactions under microgravity conditions during microgravity: The sealed containers are equipped with additional mechanism for removing a barrier to a second small volume (see **Figure 12**). In the small volume, the prey is housed during the launch and released by operating the mechanism in the μ*g* phase. In the main volume of the container, a predator is housed. The observation system is identical to platform 1.

Fixation Platform: This platform accommodates three LAUs. Each of these LAUs contains two sealed containers housing the aquatic specimen in a water volume of approximately 15 ccm. These LAUs are equipped with a mechanism containing a fixative volume of up to 20 ccm. This mechanism can be activated at any time during the mission. When activated, the water in the container will be completely substituted by the fixative solution within seconds.

Sounding rockets represent a highly suitable tool not only to analyse the behaviour and behavioural adaptability of zooplankton but also to study interactions between organisms from different trophic levels, which are a basic prerequisite for the establishment of food chains in bioregenerative life support systems (BLSS).

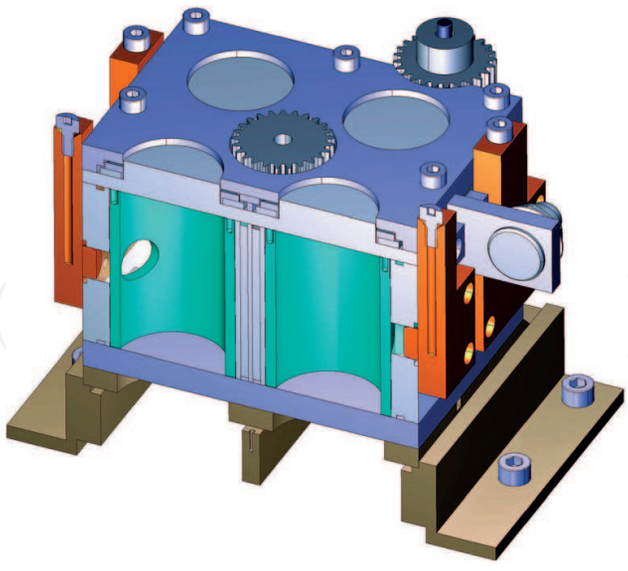

Figure 12. Observation unit with separation mechanism. Courtesy of Airbus Defense & Space, Germany.

5. Zooplankton experiments on space flights

Experiments that have directly assessed the survival and behaviour of zooplankton under microgravity conditions in actual space flights are few and basic. The first experiments more specifically observing the behaviour of microcrustaceans (cladocerans and ostracods) on board a spacecraft were carried out in basic ABS units (containing hornwort, amphipods and gastropods; [99]) on an Endeavour and Atlantis/Mir mission in 1996–1997, the latter for 4 months [15, 36]. Besides rudimentary observations on survival, which was more successful for ostracods than for cladocerans, and on aberrant swimming behaviour, no additional data were collected on zooplankton [36]. The most recent experiment was launched on the SpaceX Mission-8 in April, 2016. The experiment, developed by NanoRacks LCC and King's College London (UK) and sponsored by NASA, was carried out on the ISS as part of the educational International Space School Educational Trust (ISSET) Mission Discovery [100]. Behaviour of *Daphnia* was observed weekly by the astronauts (for the duration of a month) in small closed cell culture flasks fed with phytoplankton (*Chlorella* sp.) through a syringe connected to the flask, fitting into the dimensions of the ISS NanoRacks Platform, where light and temperature can be controlled (see [101]). The short trial illustrated the challenges of designing and conducting strongly size- and time-constrained experiments with zooplankton in the ISS and showed the limitations of what can be deduced from short observations during allocated crew time, indicating the importance of (and the need for) aimed ground-based work to improve space experiments on microgravity in zooplankton and expected outcomes.

From the few space experiments, we know that survival of live zooplankton up to several months is possible. Experiments are also possible after the animals are revived from dormancy. Besides being crucial components as intermediate consumers in the miniature artificial freshwater ecosystems, an additional important feature of zooplankton, in particular cladocerans, is their ability to produce drought- and cold-resistant dormant eggs. Exposure of *Daphnia* and *Eucypris ornate* (Ostracoda) resting stages (dormant eggs, which are encased in a chitinous casing, called *ephippium*, in *Daphnia*) to outer space and subsequent hatching on earth have shown that dormant eggs remain viable after having been transferred to outer walls of space platforms during missions (Biorisk experiment; [102]). Although undefined, effects of cosmic radiation and of microgravity on the viability of the dormant eggs are present. In the EXPOSE-R project, researchers showed that after 18 months in space, 11–35% of *Daphnia* ephippia and 7% of ostracod resting eggs hatched in comparison to Earth controls [103], still comparatively higher than dormant eggs of killifish under the same conditions. In general, animals with an anhydrobiotic stage or state show a higher tolerance to gamma radiation when desiccated than when hydrated due to the presence of high levels of protective molecules (e.g., tardigrades; [104]) and efficient DNA repair systems. As a by-product of adaptations against desiccation and freezing, such organisms show a high tolerance to a wide range of extreme conditions, and the study of dormancy in zooplankton is useful for space exploration [105].

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6. Simulation of weightlessness

Space flight experiments offer long periods of microgravity, but research in the near-Earth orbit is expensive and limited by the small amount of flight opportunities [106]. Other facilities such as drop towers, parabolic flights and sounding rockets provide only limited periods of microgravity ranging from few seconds to several minutes. In addition, application of the latter methods includes phases of hypergravity (reaching up to 30 *g* catapult acceleration at the Bremen Drop Tower [40]) before the onset of microgravity, which can present a considerable prejudice to some research questions.

The history of clinostats started at the end of the eighteenth century when Sachs and Pfeffer exposed plants on a device, which enables rotation of an object around an axis perpendicular to the direction of the gravity vector. By means of this so-called clinostat, the role of gravity for plant gravitropism could be visualized.

To some extent, weightlessness (microgravity) can be simulated on ground. Though gravity is a unique natural force - permanently present and acting - experimenters try to create a condition in which the organism "looses" its orientation with respect to gravity [107]. Gravity cannot be switched off on Earth, but its direction can be randomized. This situation is achieved when a test system is rotated on a horizontal axis, perpendicular to the direction of the gravity vector. The aim of this 2D clinostat principle is that the exposed organism can no longer detect the gravity vector. Consequently, several parameters have to be considered. From physical principles, it is obvious that speed of rotation and diameter determine the quality of the simulation. Furthermore, physiological parameters such as reaction time and thresholds for stimulus perception of the respective organism are of relevance, which are, however, in most cases not known. Many organisms have been exposed to simulated microgravity on clinostats, which have been adapted to several experimental demands (for review, see [108]) (**Figure 13**): clinostats for adherent or suspended organisms, aquatic systems and in combination with online analysis such as photomultipliers or microscopy. In some cases, a direct comparison with results obtained in real microgravity was possible and validated 2D clinorotation as appropriate method to simulate microgravity. An example is the random swimming displayed by previously gravitactic organisms [109].

Clinostats are essential parts to provide a comprehensive view on the impact of altered gravity on biological systems. Otolith growth in cichlid fish is slowed down by hypergravity, while microgravity during space flight increases their growth. Long-term clinorotation on a 2D fast-rotating clinostat confirmed these results in late-stage zebrafish [110]. Callus cells of *Arabidopsis thaliana* were exposed for 8 hours on a horizontal or a vertical clinostat [111]. The amount of glucose and fructose decreased while the starch content increased. In order to understand the physiological mechanism, the proteome was analysed after clinorotation. Eighty proteins were found to show qualitative and quantitative differences after horizontal rotation. Eighteen proteins showed a significant expression alteration under horizontal rotation in contrast to a vertical rotation. Rat alveolar macrophages (NR8383) were exposed on a

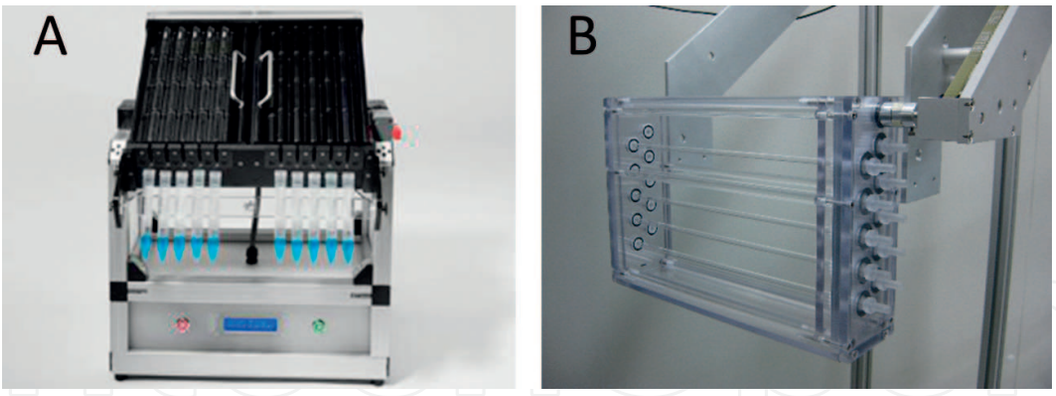

Figure 13. Clinostat with one rotation axis (2D clinostat), constantly running to simulate microgravity conditions for planktonic organisms and in general for cells in suspension. A: Clinostat with up to 10 pipettes, which can be emptied during clinorotation in prepared tubes. B: Clinostat constructed for aquatic systems to be placed into an aquarium.

2D clinostat to study the effect of gravity on the oxidative burst reaction, which is a key element in the immune response and cellular signalling [112]. The results indicate that reactive oxygen species (ROS) release is reduced in microgravity and enhanced in hypergravity and the adaptation to altered gravity occurs within seconds. Flagellates and ciliates were also observed under 2D clinorotation as well as on the random positioning machine (RPM) [49]. 2D clinorotation (60 rpm) was found to mimic the results obtained under real microgravity: the swimming direction was random, swimming paths kept their high linearity and the velocity was not impaired. In contrast, the protists showed erratic movements and frequent spontaneous changes in swimming direction on the RPM.

Exposure of the microcrustacean *Daphnia cucullata* in a 2D clinostat demonstrated that this method does not generate small scale turbulences, which resemble those generated by predators during their movement in the water column [113]. Otherwise, these organisms would express predator-induced defences such as peaked and elongated helmets as well as longer tail spines [114, 115]. However, the narrow tubes in a clinostat - a prerequisite to limit residual accelerations - limit long-term (embryonal) developmental studies as oxygen and food supply are severely restricted. Using a proteomic approach, key proteins and pathways involved in the response of *Daphnia* to simulated microgravity on a 2D clinostat (60 rpm with a residual gravity of ~0.008 *g*) were identified. Assuming that clinorotation is an appropriate simulation for *Daphnia*, the data indicate that microgravity will have an impact on the actin cytoskeleton disruption and breakdown of protein structures in general as well as an increase of energy demands. Interestingly, most of the proteins found to be affected are well-conserved throughout taxa and suggest that a lack of gravity affects similar molecular processes in a variety of organisms [50].

The studies with microcrustaceans and other organisms support the assumption that experiments in space can partly be replaced by studies using clinostats but also underline the need for experiments in real microgravity with respect to long-term exposure and adaptation processes [37].

A further technical approach to simulate weightlessness is the principle of 3D clinorotation. In contrast to a 2D clinostat, a second rotation axis perpendicular to the other axis characterizes a 3D clinostat. Furthermore, the operation is performed in a constant or randomly changing

mode with respect to speed and rotation direction; the latter configuration has been named "random positioning machine (RPM)". So far, no evidence for an advantage of a 3D clinostat and a RPM over a 2D clinostat has been presented. Even more, induced side effects have to be critically considered. By using dinoflagellates (*Pyrocystis noctiluca*) as fast and sensitive reporter system, shear forces were made visible as they provoked bioluminescence [116]. The results show that the mechanical stress is higher on a RPM than during constant clinorotation, thereby proofing fast and constant 2D clinorotation as simulation method with negligible small side effects in contrast to random operation modes tested so far.

Magnetic levitation has been shown to be unsuitable to simulate microgravity in unicellular organisms, as magnet forces have a severe influence on the behaviour of the exposed organisms, as demonstrated in the cases of *Euglena* and *Paramecium* [117].

To sum up, ground-based studies in combination with long-term space flights are valuable tools to provide a comprehensive view on the role of gravity on the behaviour, physiology and genetics of motile microorganisms, which promise further insights into the complex molecular machinery of graviperception, signal transduction and responses. 2D clinostat running fast and with a restricted effective radius has been approved as the optimal simulation approach. However, due to the size limitations on clinostats, only small objects can be exposed under optimal simulation conditions on these instruments in order to avoid effects of radial accelerations. All results obtained under simulation conditions should be verified in selected space experiments in real microgravity.

7. Synopsis

Gravity plays a dominant role for spatial orientation of planktonic organisms and probably all eukaryotic organisms are capable of perceiving gravity [118]. Typical representatives of planktonic unicellular protists are *Euglena* and *Paramecium*. Both show a precise gravitactic orientation. The flagellate *Euglena*, which can grow photoautotrophically or heterotrophically, changes its swimming direction age-dependently ranging from preferentially downward swimming in the water column (positive gravitaxis) in young cells to pronounced upward swimming (negative gravitaxis) in older cells [119]. The underlying reason remains unknown so far, but a pure physical reason, i.e., changes in shape, could be excluded. The ciliate *Paramecium* shows negative gravitaxis, the precision of which is modulated by the oxygen concentration in the water [120, 121]. *Euglena* and *Paramecium* respond to a variety of other environmental stimuli such as oxygen gradients and light, assuming a complex interaction of underlying signalling pathways. Here, microgravity and hypergravity have become important analytical tools.

By using a plethora of experimental devices and designs on ground and in space, such as 2D and 3D clinostats, random positioning machines, sounding rocket flights, drop towers, satellites and shuttles, the mechanism of gravitactic orientation in unicellular organisms, which is summarized here as an example, was elucidated [30, 109, 122–124]. The first proof that gravitactic orientation in *Euglena* and *Paramecium* is due to the detection of the Earth's gravity field (1 *g*) and not due to the magnetic field lines or a chemical gradient was obtained during a

TEXUS (technical experiments under microgravity) sounding rocket flight [125, 126]. In microgravity, the cells swam randomly, while the 1 *g* controls displayed precise negative gravitaxis. In order to determine the threshold of gravitaxis, the slow rotating centrifuge microscope NiZeMi was installed in the Space Shuttle Columbia during the second international microgravity mission (IML-2). In orbit, *Euglena* and *Paramecium* cells were subjected to increasing accelerations via a centrifuge. The threshold for gravitactic orientation was found at ≤0.16 *g* and saturation at 0.64 *g* for *Euglena* [127] and in the range of 0.16–0.32 *g* for *Paramecium* [128]. This was confirmed in a subsequent sounding rocket flight [43]. Using the same instrument on ground showed that the *Euglena* and *Paramecium* cells even increase their gravitactic orientation >1 *g* (hypergravity) and can orient with respect to a centrifugal acceleration up to 9 *g* [129, 130].

One early hypothesis that *Euglena* cells orient themselves using a passive buoy effect [131] could be clearly falsified [132, 133]. Rather they use mechanosensitive ion channels as gravisensors of the large transient receptor potential protein (TRP) family [134], which is found in many organisms serving diverse functions such as photoperception, nociperception, thermosensation, taste, osmolar sensation and mechanosensing [135, 136]. Using RNA interference (RNAi) [137], the specific gravireceptor in *Euglena* could be identified as TRPC7 [134]. This Ca^{2+} gating channel can be efficiently blocked by gadolinium [138, 139]. Also, in *Paramecium*, mechano(gravi-)sensitive ion channels have been identified, which modulate the membrane potential and the ciliary activity [140]. Several calmodulins are found in *Euglena*. During gravistimulation, Ca^{2+} ions are gated into the cell where they bind specifically to one of the calmodulins (CaM.2), which is involved in the gravitaxis signal transduction chain. This was confirmed by RNAi [141]: after blocking the synthesis of CAM.2, gravitaxis was impaired. The changing Ca^{2+} concentrations during gravistimulation could be recorded using the fluorescence of Calcium Crimson induced by microgravity and hypergravity phases during parabolic flight manoeuvres [80] as well as on a centrifuge during a sounding rocket flight (MAXUS 3) [142]. After gravitactic stimulation, calmodulin activates an adenylyl cyclase, which converts ATP to cAMP, which was confirmed on a sounding rocket flight (TEXUS 36) [95]. The gravitactic sensory transduction chain in *Paramecium* also involves cAMP [143, 144]. A phosphodiesterase quenches the cAMP signal and decreases gravitactic orientation in *Euglena*, while inhibition of the enzyme or the application of the analogue 8-Bromo-cAMP enhances the precision of gravitaxis [145, 146]. In the final step, cAMP activates one (PK.4) of the five protein kinases A found in *Euglena* [147], which is thought to finally control the flagellar reorientation [118].

Euglena is an excellent candidate for bioregenerative life support systems. Being photosynthetic, it can absorb carbon dioxide exhaled by astronauts during long space flights, e.g., to Mars, and emit oxygen, which can be used by the astronauts. In addition, it is able to utilize ammonia, which is toxic to many other organisms. As a proof of principle, a closed system was constructed and run for more than 600 days. The system was composed of an 11-L tank populated by *Euglena* and a zoological compartment, which contained 15 snails (*Biomphalaria glabrata*) and 4 adult swordtail fish (*Xiphophorus hellerii*) [148]. Subsequently, several closed environmental life support systems (Aquacells, Omegahab) have been developed for space flights on Russian Foton satellites. The *Euglena* suspension was located in a 1450-ml cylindrical aquarium (in the Aquacells experiment on the Foton M2 mission) connected to a fish tank (35

larval cichlids (*Oreochromis mossambicus*)), via membrane tubes, which allowed exchange of oxygen, carbon dioxide and ammonium excreted by the fish [30]. This assembly was successful in sustaining the fish during the space flight. In addition, the swimming behaviour and cell shape of the flagellates were video recorded. Another closed aquatic ecosystem, containing the chlorophyte *Chlorella*, *Euglena* and the snail *Bulinus*, was flown 17.5 days in orbit in cooperation with Chinese scientists on board the Shenzhou 8 spacecraft [31, 149]. After return, transcription of genes involved in signal transduction, oxidative stress defence, cell cycle regulation and heat shock responses were analysed using quantitative PCR. The analysis showed that *Euglena* suffered stress upon short-term exposure to microgravity since of the 32 tested genes, 18 stress-induced genes were upregulated. These results confirm the suitability of *Euglena* within a biological life support system.

Each of the approaches presented in this chapter is suitable to gain knowledge on how organisms respond to altered gravity conditions, especially microgravity, and can be regarded as stand alone. However, as the example of *Euglena* impressively shows, it is advisable to combine the different approaches in order to achieve a comprehensive understanding of how organisms deal with the absence of gravity and what the underlying physiological and genetic mechanisms are. These studies play a crucial role in the selection of suitable zooplankton organisms for bioregenerative life support systems.

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