ANALYSIS OF BEHAVIOUR AND HABITUATION OF FISH EXPOSED TO DIMINISHED GRAVITY IN CORRELATION TO INNER EAR STONE FORMATION A SOUNDING ROCKET EXPERIMENT (TEXUS 45)

Reinhard Hilbig[(1)], Miriam Knie[(1)], Denis Shcherbakov[(1)] and Ralf H. Anken[(2)]

[(1)]*Institute of Zoology, University of Stuttgart-Hohenheim, Garbenstr. 30, D-70599 Stuttgart; email*
[(2)]*Institute of Aerospace Medicine, German Aerospace Center, D-51147 Cologne, Germany; email*

ABSTRACT

The aim of this study was to analyze the time course of the behavioral adaptation to diminished gravity aboard a texus sounding rockrt in correlation with the calcification status of inner ear otoliths. Experimental animals were fish-larvae (*Oreochromis mossambicus*). The adaptation to the two applied levels of low gravity between 10^{-4}g (platform zero-g of the rocket) and 4×10^{-2}g (diminished gravity obtained by centrifugation aboard the rocket = "zero-g" occurring during parabolic air craft flights) was followed and the difference in the time profiles of adaptation were analyzed. The accuracy of symmetry in individual otolith-pairs (left vs. right side) was versed proportional to the time required for regain to normal swimming behavior in the zero-g phase of the flight, e.g. in normal, coordinated swimming fish the symmetry of otholiths between left and right side is high (less than 0.9 % deviation at 10^{-4}g respectively less than 4% at 5×10^{-2}g). In those fish which displayed kinetotic behavior the mean relative difference between left and right ear stone is 12.3% in the case of 10^{-4} g and 6.2% when 4×10^{-2}g were applied. Source of otolith asymmetry, which leads to kinetic behavior are irregular crystallization, differences in growth and unusual adipose of calcium layers. The data give evidence, that nearly all fish were able to sense 4×10^{-2}g and more that 60% at 10^{-4}g.

Keywords

Behavior, adaptation, vestibular system; Otolith; kinetosis, sounding rocket flight, weightlessness.

INTRODUCTION

Previous own, ground based research as well as studies involving parabolic aircraft flights and drop-tower experiments have shown that the susceptibility to the sensation of sensorimotor disorders (kinetoses like motion sickness) at diminished gravity is based – at least in fish – on the genetic predisposition of inner ear stone (otolith) mineralization/calcification (otoliths are mainly composed of calcium carbonate; the most important functional parameters are density of calcium carbonate, form of the otolith – i.e., the distribution of $CaCO_3$ – as well as differences in the calcium [carbonate] incorporation of otoliths from the right vs. the left side of the head [1].

The vertebrate inner ear statolithic organs serve as linear acceleration (e.g., gravity) organ. Basically, a "heavy body" (stato- or otolith; mostly made up of calcium carbonate) is connected to a sensory epithelium by the macula uticuli a collagenous membrane. It is the weight or movement of this heavy body which effects the cilia of sensory hair cells, thus generating action potentials which transmit gravistatic information to the brain. As a matter of fact, the functional capability of otoliths is being heavily impaired by altered gravitational environments. Weightlessness (zero-g) thus induces changes in the central and peripheral interpretation of sensory input leading to alterations in motor behavior (e.g., intersensory-conflict-theory; [2]) including space motion sickness (SMS; [3, 4, 5], a sensory motor kinetosis normally accompanied by malaise and vomiting. SMS comprises a dynamic component, which is likely related to an erroneous interpretation of changing linear acceleration as angular acceleration by the canal organs, whereas the static component of SMS is likely related to the otolithic organs [6, 7]. Some fish of a given batch show a kinetotic behaviour (especially so-called spinning movements and looping responses [6, 8, 9] at the transfer from hypergravity to 1g earth gravity as well as from 1g to µg [6, 8, 9, 10, 11]. Therefore, these animals are highly suitable in the context of addressing the possible role of otolith calcification in regard to the adaptation process towards dimished gravity.

Generally, it is believed that motion sickness is caused by a mismatch between expected and sensed gravity direction (i.e., an intersensory conflict, see above), and in all vertebrates, countermeasures are taken against this mismatch by reweighing the various sensory inputs. In the course of this compensation process, vertebrates "learn" to rely rather on, e.g., visual than on vestibular cues for orientation.

The goal of our experiments was, in brief, to gather clues and insights into the reason of susceptibility and gravity-thresholds of vertebrates to kinetotic movements and inner ear (otolith) related malfunction of swimming behavior caused by microgravity.

MATERIAL AND METHODS

2.1 Maintenance of animals

Larvae (siblings) of Cichlidfish *Oreochromis mossambicus* (Cichlidae) were kept in a hatchery until launch preparation.Experimental animals were housed single tanks of the racks 6 hrs before start of count down (CD) and were maintained in a temperature controlled incubator at 23°C. At –2hrs CD the containers were inserted into the 3rd cargo bay of the rocket.

2.1. Flight implementation plan

Total payload weight was 357kg and the payload capsule carried three different experiments each housed in a separate module. The fish module TEM 06-31 consisted out of several platforms carrying the fishtanks, cameras, video-storing device and housekeeping units for illumination and temperature control. Inside the fish module was 1 fixed platform (Fig.1A) carrying 3 experimental racks with 8 fishtanks (Fig. 1B) each and two centrifuge platforms with 8 fishtanks each.

Fish tanks were milled out of macrolon blocks, as cylindrical chambers (diameter 30mm, high 50mm containing a volume of 35.3cm3). At the respective outer side of each tank a pressure compensation unit was mounted (Fig. 1B). At the bottom of each platform an illumination foil was attached. In each module an observation window was inserted for the respective CCD-cameras (XC ST70) was positioned. The video streams were written on a flash card recorder. Experimental racks were designed for late access and early retrival.

2.2. TEXUS 45 flight parameters

Flight schedule of TEXUS 45: The sounding rocket Texus 45 was successfully launched at February 21 2008 from the Swedish Space Corporation's launch facility Esrange Space Center. The rocket performed a parabolic flight and reached its ceiling at 273km height. After 20 min of flight the payload was recovered and brought back to the lab by helicopter. Launching time was 07:15 and the first thrust acceleration was maximal 7.7g (average 5.0g) at 12.4 sec. The second thrust acceleration was maximal 12.8g (average 7.2g) at 35.7sec followed by YoYo despin and motor separation. Start of zero-g was at 74.5sec and end at 461.0sec total flight time. Reentry deceleration was from 490 until 514 sec with a maximal load of 22.2g.

2.3. Experiments aboard the sounding rocket

The 1st group of fishlarvae (controls) remained in the breeding tank (volume of water: 9L) at 1g. The 2nd group of fish (24 individuals) received 10-4g (HQM) aboard a sounding rocket (TEXUS) on the platform 1.
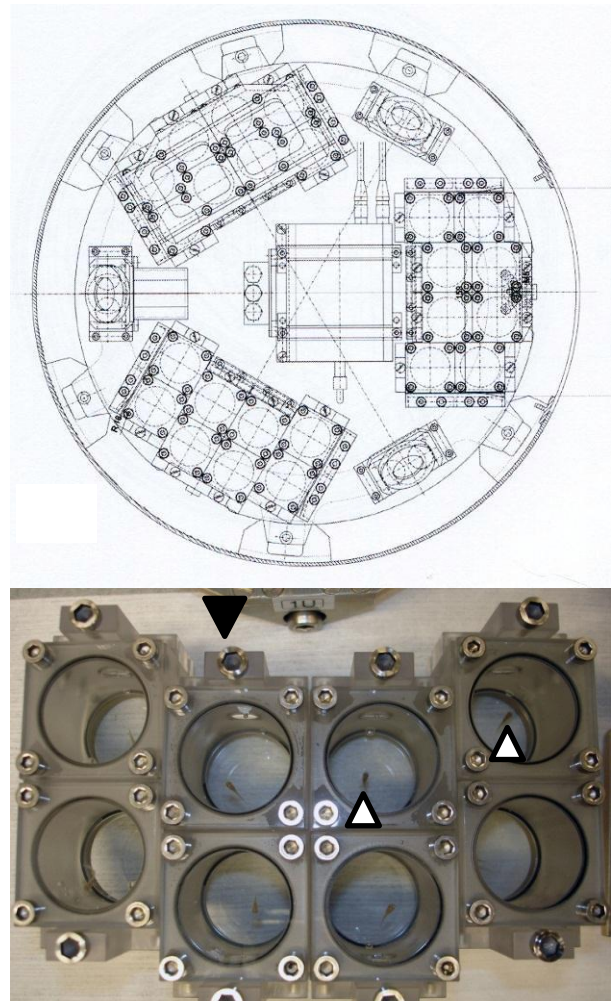

Figure 1. A: Upper platform (fixed) TEM 06-31, B: Rack with 8 chambers with pressure compensation (black arrow head) – ready for flight inserted fish (white arrow head)

The 3rd group of animals (48 individuals) were also kept aboard TEXUS, but received 0.04g (LQM, approximately this G-level is obtained during parabolic aircraft flights) within a centrifuge. After recovery of the payload capsule the experimental rack were removed at Esrange labs and all fish were in a good and healthy condition.

2.4. Analysis of behavior

Objectives of the study were to analyze the swimming behavior of fish by means of a video tracking program "BioMotionTrack D.S." (Shcherba2010), which allowed to determine the threshold for kinetotic behavior in correlation to proportion of asymmetry in otoliths. Categories of swimming descriptive behavior were: normal-, zigzag- and loop-swimming, spinning movements, frozen (without any movements), dorsal light reaction (DLR) and resting. Computerized analysis

of the motions in the zero-g experiments with fish enabled us to quantify the following movement-parameters in addition to the qualitative behavioral descriptions: mean swimming speed, distance covered during swimming, duration of specific types of behavior (e.g. looping, spinning, DLR etc.), number of loops or spinning movements, changes in the swimming direction (left vs. right) or in the angular degree (swimming direction).

This technique of an automated and semi-automated behavioral analyses is a tool for the determination of fitness in different „gravitational" habitats and in consequence a marker for dysfunctions of the vestibular system.

2.5. Otolith sample preparation

All control and experimental animals were anaesthetized with tricain-s (Western Chemical, Inc.) using the procedures approved by the animal care and health officer. After the preparation of the otoliths, all tissue residues where removed under microscopical control, the samples were washed intensively with deionized water, air dried and stored at $-80°C$ until use.

2.6. Morphological analyses of ear stones

The ear stones (lapilli and sagittae) were for each fish separately mounted pairwise on a glass slide. Images were taken with an Axioscope Imager 1 (Zeiss). The images of the otoliths were analyzed with the Axiovision 4.6 software and from Zeiss. For Statistic evaluation average, median, SD and the Student t-test were performed.

RESULTS

1. Behavior of cichlid fish larvae during flight

For a first estimation of the motility of fish during the whole flight, including the phase of launch the mean velocity and the distance covered were calculated. In spite of the high g-load at the beginning of the flight most fish were active during the whole flight (Fig. 2).
There were no significant differences between HQM and LQM in the distance covered, except during launch and YoYo-despin procedures. However, concerning the velocity of swimming LQM-fish swam slower during the whole mission. The higher velocity in HQM-fish was related to the mode of behavior, as these fish displayed rapid and frequently changing kinetic movements (Fig.3). Indicator for kinetic behavior was the relative frequency of spinning and looping movements. Even though a high individual variability was obvious among
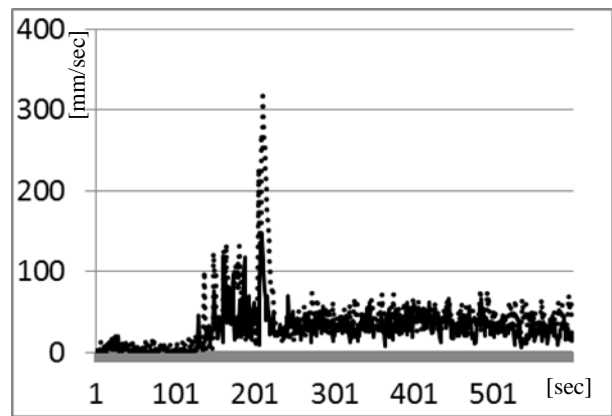

Figure 2. Mean velocity given for 10sec intervals of recording time from -170sec – launch – despin – zeo-g until 590sec
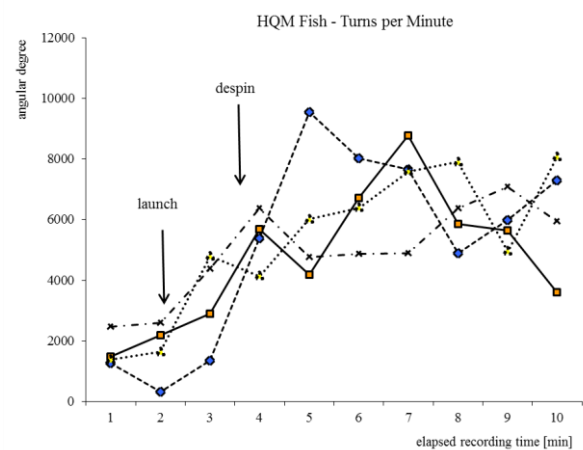

Figure 3. Kinetotic turns in HQM-fish summarized in angular degrees per min.

both groups of fish during the ballistic flight phase ("zero-g") in HQM-fish the kinetotic behavior emained on an constant level. During zero-g the average number of spinning respectively looping movements per minute at HQM was 2.4% / 1.5% whereas at LQM only 0.5% / 0.4% per min were registered. Dorsal light response was performed in the mean 4.3% per min (scattering of data between 0.1 and 11.7%) at HQM and in LQM 6.6% (varying between 0.5 and 8.2%). In contrast normal swimming dominated with 52% per min at HQM and 63% per min at LQM. Typical for the LQM-fish was that at least after 180sec at zero-g kinetotic behaviour had disappeared nearly completely. In general the LQM-fish an adaptation to 4×10^{-2} g was obvious. Especially in the course of adaptation from kinetotic movements (looping, spinning, disorientated
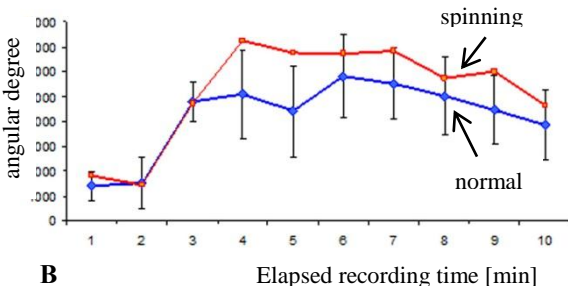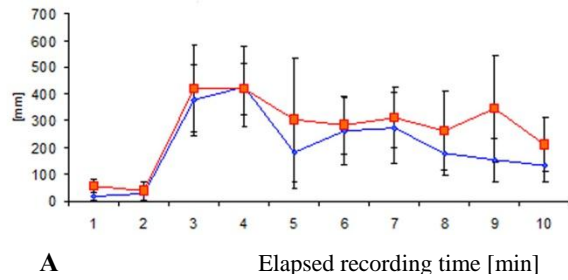
A Elapsed recording time [min]

spinning

normal

B Elapsed recording time [min]

*Figure 4. **A**: Distance covered in LQM [mm/min],*
***B**: Mean kinetotic turns in HQM-fish summarized in*
angular degrees per min.

A Elapsed recording time [min]

normal

spinning

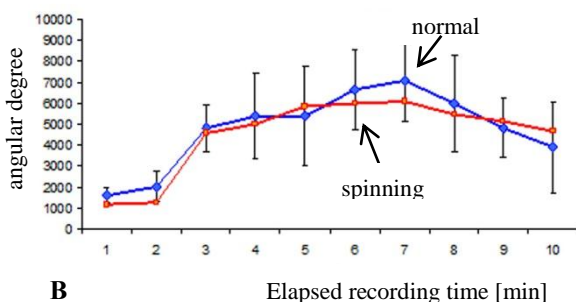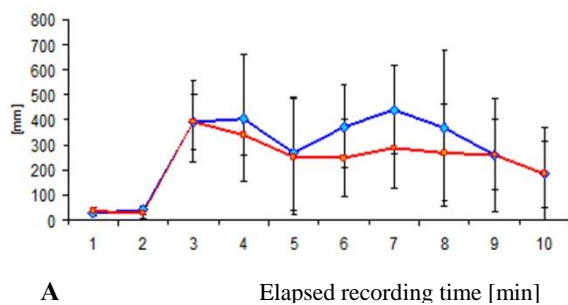**B** Elapsed recording time [min]

*Figure 5. **A**: Distance covered in HQM [mm/min],*
***B**: Mean kinetotic turns in LQM-fish summarized in*
angular degrees per min.

zigzagging) to normal swimming or resting discrepancies in the zero-g phase were evident. Examples of individual adaptation of kinetotic animals

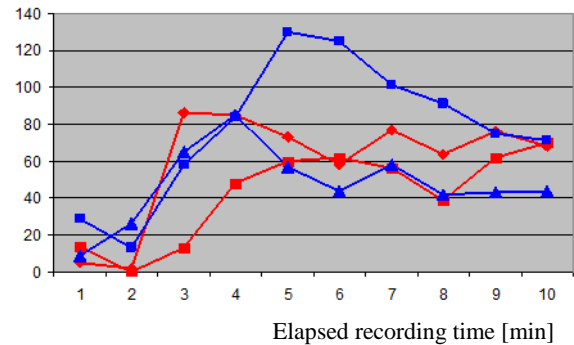concerning the change in swimming direction during the "zero-g" phase are given in Fig. 6.

Elapsed recording time [min]

Figure 6. Adaptation of kinetotic fish in HQM (red) and
LQM (blue), decrease in kinetotic movements
(summarized turns in angular degrees per min).

Characteristic is the onset of adaptation between 160sec and 250sec after launch and heavy g-load. From this time a slowly regain of normal swimming activities at LQM is registered, although the levels are extreme different. The HQM-fish remain on their individual level of movements.

In general we were able to show that fish-larvae (*Oreochromis mossambicus*) are able to adapt to extreme different g-conditions (= Δg habitates; TEXUS-Flight – Launch – Despin-zero-g) within a very short time.

1. Morphological investigations of inner ear stones (lapilli and sagittae)

Otolliths from all flown 72 fish including 18 1g controls were documented and analysed by microscopical morphometric measurements. Most otoliths (lapilli and sagittae) were well shaped and could be correlated with normal swimming fish (Fig.7). Asymmetric morphology in ear stones occurred mainly in fish displaying kintotic behaviour. Origin of asymmetry was unilateral irregular crystallization or addition of material resulting in an excentric or simply bigger ear stone (Fig. 8).
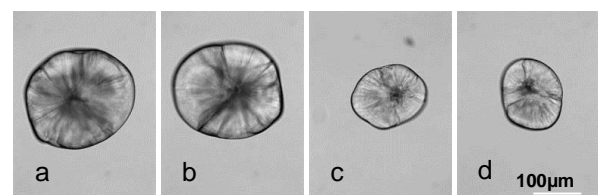

Figure. 7 Light microscopical images (phase contrast)
of sagittae (right, a and left, b) and lapilli (right, c and
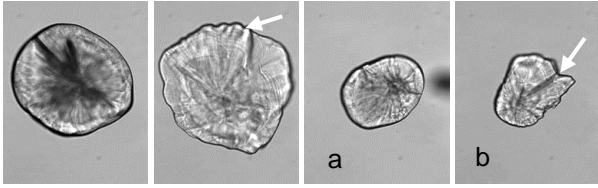*left, b) from normall swimming fish*

Fig. 8 Light microscopical images (phase contrast) of sagittae (right, a and left, b) and lapilli (right, c and left, b) from normal swimming fish.

All ear stones were tested for normal distribution and statistical analyses ensured that 98% of the values were within the standard deviation of the means. Further calculation yielded that mean and media differed only slightly. For spatial orientation the morphometric values of the lapilli are of utmost interest (Table 1).

Table 1: Morphometric data of all fish from the Texus experiment (HQM and LQM)

	Lapilli right	*Laplli left*
Mean	20700.43	20696.75
SD	2206.65	2127.71
Median	20578.68	20517.99
Minimum	14049.68	14169.92
Maximum	26121.38	25834.63

Asymmetry (differences between left and right lapillus) of ear stones are an indicator for possible dysfunction concerning the vestibular sensing. Mean asymmetry in all lapilli was 1127.62µm² (SD 881.16µm², max. 4263.11µm², min. 11.44µm²). For correlation of swimming behavior (normal vs. kinetotic) and the magnitude of left right differences the absolute asymmetry [µm²] at HQM and LQM in the 4 groups was calculated (Tab. 2). In addition the relative asymmetry for each individual lapillus pair was ascertained.

Table 2 Otolith asymmetry in lapilli of kinetotic vs. normal swimming fish at HQM and LQM

	kinetotic		normal	
	HQM	LQM	HQM	LQM
Mean	2285.85	1349,81	568.31	999.95
SD	1350.64	743.27	564.93	484.40
%-diff	12.33	6.20	0.88	3.72

Accuracy of symmetry in individual otolith-pairs (% deviation between left and right ear stones) is determinating the susceptibility for kinetoses in the different gravitational habitats. The lower the gravity level is, the higher has to be the degree in the symmetry in the ear stone pair of the fish (Tab. 2).

DISCUSSION AND CONCLUSIONS

The present study gave evidence that time-course of a habituation to diminished gravity depends on the respective G-level HQM vs. LQM and that an individually different regulation of inner ear otolith calcification plays a role in this process.

The findings concerning the otolith morphometry therefore clearly speak in favour of an asymmetric calcification as the main source for kinetosis susceptibility. The mass of an inner ear otolith and thus its functional capability depends on its size and density concerning $CaCO_3$ incorporation [7] and further components such as strontium salts add few to the total weight [12]. It has been shown in earlier studies that rather an asymmetry in the lapilli (graviperception, [13]) than in the sagittae (hearing process, [13]) causes kinetosis in fish [9, 14, 15]. Regarding otolithic (lapilli) largeness or crystallization, these results clearly show that specimens behaving kinetotically during the zero-g phases of HQM had a significantly higher asymmetry as compared with the fish who swam normally during low gravity. This result is in complete agreement with earlier findings according to which fish, who had developed at hypergravity (hg; 3g for 3 weeks) and exhibited kinetotic movements when suddenly transferred to normal 1g, revealed a higher lapillar asymmetry concerning size [14] and calcium uptake [1]. These findings indicate that an individually different predisposition of otolith mineralisation is of considerable importance regarding an individual susceptibility to kinetosis. Therefore it may be assumed that this calcification status is also correlated with the speed of compensation processes coming into effect in order to re-install a normal postural control.

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