

Behavior of Medaka Fish under Distributed Gravity

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Abstract The threshold value of gravity for Medaka fish (*Oryzias latipes*) was determined using parabolic flights of an airplane. Rotating a turntable during a 20 sec of microgravity, a gradient field of centrifugal force was realized in the aquarium. Fish of HO5 strain were used because from the previous studies, in microgravity they were known to exhibit looping behavior more easily than any other strains. Looping fish became stable (i.e., recovered their posture control) when fish swam from a lower-gravity area of the aquarium to an area of a certain gravity value or beyond. On the other hand, stable fish lost their posture control and started looping when fish swam into an area of a gravity lower than a certain value. Using these phenomena, we obtained the gravity value of 0.21 to 0.26G as for the threshold value for Medaka fish to sense the gravity.

Introduction

Vestibular and visual information are two major factors for fish to control their posture on Earth. Under microgravity, various animals exhibit abnormal behavior. When vertebrates are exposed to microgravity, they are confused because their vestibular organs cannot detect microgravity conditions properly. For example, snakes stuck at themselves, and turtles actively extended their limbs and neck (1). Medaka fish (*Oryzias latipes*) exhibit looping and rolling behavior (2).

Animals that cannot control their posture under microgravity can keep their postural stability over a certain gravity value; a value between microgravity and 1 G. In this paper such a critical value is referred to as the threshold gravity. For jellyfish, it is reported that the threshold gravity is 0.3G (3).

It is probable that a threshold gravity also exists for fish. Parabolic flight experiments were carried out to measure the threshold gravity for an inbred strain of Medaka fish, HO5. Medaka fish were used because they are small in size and many inbred strains exist, and the behaviors of various strains have well been documented. Fish of HO5 strain were chosen because in microgravity they are known to exhibit looping behavior more easily than any other strains of Medaka fish (2).

To obtain the threshold gravity, we rotated a turntable to produce a horizontal gradient of centrifugal force. Centrifugal force thus generated by this rotation was regarded as gravitational force (centrifugal gravity) throughout this paper. This paper reports the behavior of HO5 fish in a gradient field of centrifugal force.

Materials and Methods

Fish Strain

Five adult fish of HO5 strain were used in all the parabolas (PBs). The HO5 fish were from the stock of the laboratory of Radioisotope Center, Univ. Tokyo (lab. of K.I.) and kept for a few days at Nagoya for their accommodation.

Experimental Apparatus

A medium-size jet airplane (G-II, operated by Diamond Air Service Co., Nagoya, Japan) was used to realize microgravity that lasted about 20 seconds during each parabola. A total of 12 parabolas were carried out during the flight.

A turntable (d: 300mm) with an observation aquarium (d: 40, h: 70, w: 150mm) and a digital video camera (Canon DM-IXY DV M) were placed inside a box, thus blocking the extraneous light. The box was installed aboard the airplane. To produce a horizontal centrifugal gradient of gravity in the aquarium, the turntable was rotated horizontally. Here it must be noted that vertical acceleration was microgravity because

the entire apparatus was exposed to microgravity. Therefore, centrifugal gravity became directed from the inner side of the aquarium to the outer side. The value of the centrifugal gravity at the outer side of the aquarium (Max) is 3 times higher than that of the inner side (Min).

To observe the behavior of the Medaka fish in various values of centrifugal gravity, the turntable was rotated at various speeds. The rotating speed was switched from one to others during each inter-parabola period. The centrifugal force was set as shown in Table 1. Five Medaka fish were placed in the aquarium filled with oxygenated water and it was sealed tightly with no air volume. Medaka fish could control their posture when centrifugal gravity was large enough to sense it; and otherwise they exhibited postural instability.

Illumination was given from the top of the experimental box by three small frosted tungsten lamps. In order to avoid the visual stimulus for Medaka fish, illumination was kept as low as practical for video recording (around 30 lx on the aquarium surface, measured by an illuminometer). A digital video camera was used to record the behavior of Medaka fish from one side of the aquarium. Figure 1 shows the layout of the experimental apparatus. Because the video-recording was done only from a single direction, behavior of Medaka fish was analyzed as two-dimensional behavior.

Results and Discussion

Estimation of Threshold Gravity

Table 1 shows for each PB the maximum and minimum centrifugal gravity values in the aquarium, and the test results whether Medaka fish exhibited looping behavior or stable state. In respect of fish behavior, the data in Table 1 can be divided into three groups.

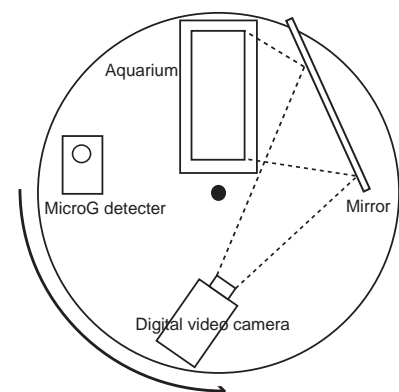


Fig. 1. Experimental apparatus. An adjustable-speed motor rotates the turntable. The rotation generates a gradient of centrifugal force in the aquarium.

- (a) In the first 3 PBs, all the fish exhibited looping behavior.
- (b) Between PB4 and PB10, some fish quitted looping behavior, while the other fish still exhibited looping behavior. To put it more concretely, fish that swam into higher gravity side could stabilize their posture. However, those swam into lower side could not stabilize themselves (shown in Fig. 2).
- (c) In PB 11 and PB12, all the fish swam normally.

In PB4, the first stable Medaka fish appeared. The maximum centrifugal gravity in PB3 was 0.19G and that in PB4 was 0.23G, implying that the threshold gravity value can be between 0.19G and 0.23G. In PB11, all the fish had quitted looping behavior and stabilized their posture. The minimum centrifugal gravity in PB10 was 0.23G and that in PB11 was 0.25G. The threshold gravity may be between 0.23G and 0.25G. Therefore, the threshold gravity is assumed to exist between 0.19G and 0.25G.

For each fish we carried out an analysis of the threshold gravity value beyond which fish exhibited normal swimming. To determine the centrifugal gravity each fish was exposed, its longitudinal position in the aquarium was used.

From the data of two groups (PB1 to PB3, PB11 to PB12), the threshold gravity values could not be obtained, because all the fish exhibited looping behavior in the first three PBs, and all the fish exhibited stable state in the last two PBs. From the data of PB4 to PB10, the threshold gravity was obtained and the data are summarized in Fig. 3. The range of the threshold gravity values are distributed between 0.21G and 0.26G; an average is 0.23G. In such an analysis, the final estimate of the threshold gravity for HO5 fish to sense gravity came to the value between 0.21G and 0.26G.

Evaluation and Characteristic Behavior

The threshold gravity that was concluded through the comparison of PB3 with PB4 is 0.19-0.23G and that of PB10 with PB11 is 0.23-0.25G. A gap between these two estimates resulted from the following reason. In PB4, only one Medaka fish quitted looping behavior; the fish was assumed to be the most sensitive to gravity among the 5 fish. Thus, the former estimate is lower than the average (0.23G). In PB11, all the fish quitted looping behavior; the last fish that quitted looping behavior was assumed the least sensitive to gravity among the 5 fish used in the experiment. Thus, the latter estimate became higher than the average. This argument is supported by the fact that in Fig. 3, all the plots except one point are distributed between 0.19-0.25G.

A characteristic behavior was also observed soon after they were exposed to microgravity. Beyond the threshold gravity, most fish turned their nose to inner side (i.e., the low gravity side) as soon as microgravity was realized. During

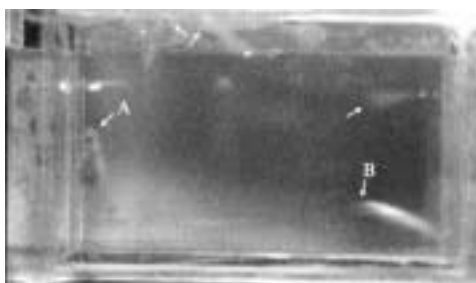
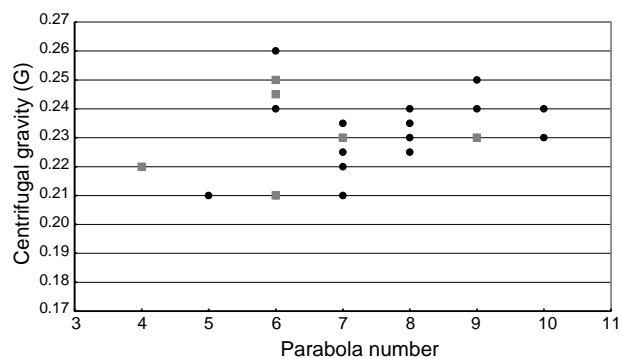


Fig. 2. Stable and instable state of Medaka fish (PB6). Arrows indicate Medaka fish. The centrifugal force was directed from the right side to left side. A Medaka fish (shown with A) in the left side shows stable behavior, on the contrary, one (shown with B) in the right side is unstable.

Table 1 Centrifugal Gravity and Behavioral State.

Parabola	Centrifugal Gravity (G)		State	
	Min*	Max**	Looping	Stable
1	0	0.11	+	-
2	0.05	0.15	+	-
3	0.063	0.19	+	-
4	0.075	0.23	+	+
5	0.1	0.3	+	+
6	0.13	0.38	+	+
7	0.15	0.45	+	+
8	0.18	0.53	+	+
9	0.2	0.6	+	+
10	0.23	0.68	+	+
11	0.25	0.75	-	+
12	0.28	0.83	-	+

* Centrifugal force at the inner side of the aquarium
 ** Centrifugal force at the outer side of the aquarium.
 Looping + denotes presence of looping behavior and Looping - indicates no looping behavior. Stable + denotes presence of stable state and Stable - indicates no stable state.



■ Threshold gravity observed when Medaka fish swam into the lower gravity side and lost their posture control.
 ● Threshold gravity observed when Medaka fish swam into the higher gravity side and recovered their posture control

Fig. 3. Threshold gravity of each Medaka fish.

each parabola, they all gathered to the low centrifugal-gravity side with their belly toward the high centrifugal-gravity side of the aquarium. This may imply that Medaka fish have a tendency to gather in a lower gravity area in a gradient field of centrifugal gravity. This observation may help us in the analysis of the vestibular organs of the fish.

Acknowledgment

This research was supported by the grants from Japan Aerospace Exploration Agency (JAXA) and Japan Space Forum.

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