Amphibian Amplexus in Microgravity

Authors: Naitoh, Tomio, Yamashita, Masamichi, Izumi-Kurotani, Akemi, Yokota, Shigefumi, and Wassersug, Richard J.

Source: Zoological Science, 12(1) : 113-116

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.12.113

ZOOLOGICAL SCIENCE **12**: 113–116 (1995)

Amphibian Amplexus in Microgravity

Tomio Naitoh[1], Masamichi Yamashita[2], Akemi Izumi-Kurotani[2], Shigefumi Yokota[1] and Richard J. Wassersug[3]

[1]*Department of Biology, Shimane University, Matsue, Shimane 690, Japan,*
[2]*Space Utilization Research Center, Institute of Space and Astronautical Science, Yoshinodai, Sagamihara, Kanagawa 229, Japan, and*
[3]*Department of Anatomy and Neurobiology, Dalhousie University, Halifax, Nova Scotia, B3H 4H7, Canada*

ABSTRACT—We report here on the amplectic behavior of the Japanese treefrog (*Hyla japonica*) in microgravity. Treefrogs were exposed to 35 cycles of altered gravity, including $\approx$1.5 sec of G<0.1 every 3 min and 15 sec, on the FreeFall "G.0" ride at Space World amusement park in Kitakyushu, Japan. During this period a pair of frogs spontaneously entered and maintained amplexus for 1 hr 20 min, before being removed from the ride. In freefall, the pair extended their hindlimbs in the characteristic posture of treefrogs in microgravity.

This is the first report of a vertebrate entering and sustaining a copulatory or amplectic posture under gravitational extremes, including true freefall. These observations bode well for the potential of anurans to breed in microgravity and to be used for biological research in space.

INTRODUCTION

The tenacity of male frogs and toads to hold on during amplexus is legendary. In certain taxa amplexus may last for weeks (e.g., some species of *Atelopus* [3]). Some male anurans, during the height of the mating season, have been found amplexing with inanimate objects (including dead conspecifics; [4]), other males, and even other species. Many frog collectors have personally observed male anurans vigorously clasping females, when the frogs were themselves in the clasp of a predator.

To the list of unusual situations where frogs have been found amplexing, we now add microgravity. We report here on Japanese treefrogs, *Hyla japonica*, that stayed in amplexus during repetitive episodes of freefall. To date, no vertebrates have been successfully raised through a complete life cycle in microgravity. In that regard our observations have positive implications for the potential of amphibians to breed onboard orbiting spacecraft and to be used for biological research in space.

MATERIALS AND METHODS

The treefrogs in our study were collected outside the breeding season (in November of 1993) from wild populations in the vicinity of Matsue, Shimane Prefecture, Japan. They were held in the laboratory for one month prior to experimentation, maintained at room temperature (*circa* 20°C) and exposed to an artificially extended light cycle. The frogs were fed meal worms twice a week. During this period males occasionally called spontaneously.

On December 2, 1993, 20 healthy, active and mature *H. japonica* (8 males and 12 females) were transported 400 km by car to Space World amusement park in Kitakyushu, Fukuoka Prefecture, Japan. The next day they were divided into four groups of five individuals each, with both males and females in each groups. Each group was housed in a clear 1.4 l plastic box (15 cm×8.5 cm×11 cm),with a layer of wet, spongy form rubber on the bottom to help keep the animals moist. Each animal was fed one or two small pieces of beef liver approximately 1 hr before testing. Two of the boxes were then mounted in the passenger chamber of the FreeFall "G.0" ride at Space World and two were retained on the ground as controls.

FreeFall "G.0" cyclically exposes its passengers to $\approx$1.5 sec of microgravity (G<0.1). This reduced G is achieved by a straight freefall drop of 15 m followed by a deceleration phase where the passenger chamber slides down a parabolic slope. The vertical G-forces intermittently rise to as high as 3.6 G because of jolts from shock absorbers during this deceleration phase. At ground level the passenger chamber moves horizontally in a 48 m loop before a vertical rise of 39 meters, which returns it to the top of the drop tower. During this ascent phase, gravity never rises above 1.3 G. The average cycle time for FreeFall "G.0" is 3 min and 15 sec. Our *H. japonica* rode FreeFall "G.0" continuously for 1 hr 54 min, tallying up 35 episodes of freefall.

The frogs on FreeFall "G.0" were continuously videotaped with fix-mounted 8 mm video cameras during their ride. One chamber was maintained in the dark and videotaped in the infrared. The other was illuminated with a 5 watt halogen lamp and filmed in the light. The ground control containers were similarly separated, one in the dark and one in the light.

Gravity meters were attached to the front of the boxes housing the frogs on FreeFall "G.0" and the G-forces in the vertical direction were continually recorded during the filming session. The temperatures in the containers were electronically recorded as well. These temperatures ranged from 25.8° to 28.1°C, a range over which *H. japonica* is naturally active. This range was approximately 10°C above the ambient air temperature ranges on the day of the study. These elevated temperatures were achieved by using warm water at the bottom of the boxes and hot water bottles around the containers, plus insulation.

Accepted November 14, 1994
Received July 7, 1994

RESULTS

One of five male *H. japonica* exposed to FreeFall "G.0" in the illuminated container amplexed with a female during the fifth cycle but at the end of the deceleration phase released the female. The same male again amplexed with a female after the tenth episode of microgravity. That couple then maintained amplexus for the remaining 25 cycles on FreeFall "G.0"; i.e. for more than 1 hr and 20 min.

Shortly before the 14th, 18th and 23rd freefall episodes, the amplectic female extended her neck, while the male kept his body flexed. Together their postures were reminiscent of the typical ovipositioning and fertilization postures of *Hyla* [3]. When performed in tandem, these postures bring the frogs' cloacas into close proximity. No eggs, however, were extruded. These particular postural displays were brief (<5 sec) and intermittent, occurring only three times over a 25 minute period. They were exhibited only during periods of normal or hypergravity (ascent).

Whenever the amplectic couple lost contact with the container's surfaces, they immediately went into an extended hindlimb posture (Fig. 1), which has been described before for *H. japonica* in microgravity on both the MIR Space Station [7] and in parabolic flight [14]. In this posture the hindlimbs are fully or nearly fully extended and abducted, and the torso is simultaneously extended (=lordosis). Individual frogs, not in amplexus, also showed this same posture on FreeFall "G.0" (Fig. 1, left). Those frogs, however, extended their forelimbs forward at the same time as they extended their hindlimbs back. The amplectic male instead held its forelimbs in strong flexion around the pectoral region of the female (Fig. 1, right). During exposure to microgravity, frogs closed their eyes.

The pair were still in amplexus when removed from the ride. Although egg laying did not occur, dissection of the females, which were euthanized and formalin-fixed in the weeks following the experiment, confirmed that the females were gravid. No other animals in the experimental or control groups took up amplexus during the experiment or exhibited any reproductive behaviors.

Neither the amplectic pair nor any of the other *H. japonica* exhibited signs of distress during the experiment. None, for example, vomited during their nearly two hours on FreeFall "G.0".

DISCUSSION

The natural breeding season for *H. japonica* in southern Honshu and Kyushu is between April and September [9]. This hylid frog lays its eggs at night directly in the still water of ponds, pools, and rice fields—not in any exotic or arboreal habitat. Thus the fact that a male amplexed out of season and during the day while: 1) confined in a cage, 2) in the light, and 3) subjected to unusual linear accelerations, is itself somewhat surprising. However, as mentioned above, males of this group had shown signs of breeding activity in the

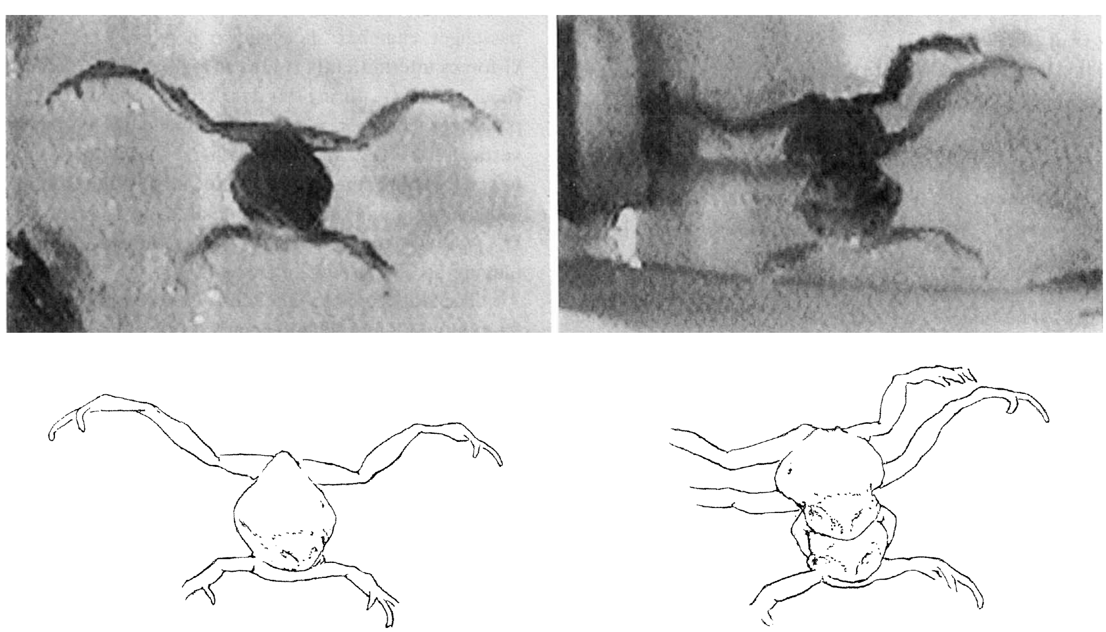

Fig. 1. *Hyla japonica* in freefall. The top images are taken directly from 8 mm videofilm and the bottom images are tracings from the same video frames. The figures on the left show a single individual in the stereotypic "flying" posture taken by treefrogs in microgravity. The hindlimbs are extended and abducted, and the back is arched (see text). The two frogs on the right are in amplexus in microgravity. They maintained amplexus for more than 1 hr and 20 min, over which time they were repetitively exposed to 25 episodes of $G<0.1$. During microgravity the amplectic pair invariably took up the same "sky-diving" posture shown by the single individual on the left whenever they lost contact with the container walls. All frogs close their eyes during exposure to microgravity.

laboratory before the experiment.

We believe the elevated temperatures and prolonged light cycle to which the frogs had been exposed in the laboratory prior to experimentation helped induce the amplexus that we observed. Those environmental conditions closely approximated the temperature and light cycle during the natural breeding period of *H. japonica*. We certainly do not believe that the acceleration profile of the FreeFall "G.0" was itself a stimulus for amplexus in this species. Only one of five males on FreeFall "G.0" amplexed, further supporting the view that exotic accelerations *per se* do not themselves provoke amplexus.

Exposure to altered gravity, and particularly to periods of microgravity, is generally considered stressful for vertebrates [see, for example 2, 6]. Many people experience nausea when exposed for the first time to repetitive cycles of microgravity, such as on parabolic flights. Frogs, including *H. japonica*, can get motion sickness from this type of stimulus [14]. It should be noted, though, that arboreal frogs such as *H. japonica* are relatively resistant to motion sickness from cyclic exposure to microgravity. Wassersug et al. [14] successfully induced emesis in only one out of 17 adult *H. japonica* exposed to 9 to 10 parabolas, where each parabola included more than 15 sec of $G<0.01$ and gravity in excess of 2G during pullout. Furthermore, the one individual in that experiment which regurgitated its stomach contents, did so approximately 24 hr after the provocative stimulus. Adult *H. japonica* on the MIR Space Station have exhibited postures characteristic of motion sickness in anurans, but that was only after those animals were exposed to microgravity for several days [7].

The posture of *H. japonica* in freefall—with abducted and extended hindlimbs and torso arched backward—appears to be identical to the "parachuting" or "flying" frog posture described previously by Stewart [12] and Emerson and Koehl [5] for semi-arboreal and highly arboreal frogs, respectively. It is a high drag posture that would decrease the rate of descent of an airborne anuran. In microgravity, *H. japonica* reflexively takes up this posture when deprived of tactile contact with a substrate (Fig. 1).

No vertebrates are known to enter amplexus while truly falling. Certain swifts though, in the family Apopidae, achieve coitus while airborne [8].

Our observations of amplectic behavior in *Hyla* under an unusual gravitational regime has implications to future microgravity research with vertebrates. Despite many decades of biological research in space and the large variety of vertebrates that have been in orbit so far [reviewed in 1, 11], it is a sad fact that no vertebrate has yet completed a single life cycle in space. Only in the current decade have any vertebrate eggs—those of the African clawed frog, *Xenopus laevis*—been successfully fertilized in microgravity [13]. In 1992, *Xenopus* eggs were not only fertilized on the US Space Shuttle but raised to a free-living tadpole stage while still in microgravity [10]. However, in both the Ubbels et al. [13] and the Souza et al. [10] experiments, fertilization was artificial, using extracted sperm prepared before launch. No vertebrates are known to have spontaneously achieved amplexus or copulated on orbit. It is easy to visualize the mechanical handicaps to copulation for higher vertebrates in space. In that regard our result is a promising sign that at least one species of anuran is capable of sustaining amplexus during unusual linear accelerations, including microgravity. It remains to be seen if amplectic frogs can oviposit and fertilize their eggs in space without experimental intervention. It is noteworthy that a teleost, the medaka (*Oryzias latipes*), has now been observed making mating displays in microgrvity. That occurred on the US Space Shuttle during a July 1994 International Microgravity Laboratory mission (i.e., after this paper was submitted for publication). Those fish laid eggs which proceeded to hatch on orbit (Kenichi Ijiri; personal communication).

ACKNOWLEDGMENTS

This study was supported by the Fund for Basic Experiments Oriented to Space Station Utilization. Masazumi Iwasaki and Mine Fukuda helped with the animal care. Mine Fukuda helped with data collection. We thank Monika Fejtek for assistance in manuscript preparation and Scott Pronych for reviewing drafts of the manuscript. We thank Space World, Inc. for providing us with research access to their facilities.

REFERENCES

1 Ballard RW, Mains RC (1990) Animal experiments in space: a brief overview. In "Fundamentals of Space Biology" Ed by M Asashima and GM Malacinski, Japan Scientific Societies Press, Tokyo, pp 21–41

2 Crampton GH (1990) Motion and Space Sickness. CRC Press, Boca Raton, Florida

3 Duellman WE, Trueb L (1985) Biology of Amphibians. McGraw-Hill Book, New York

4 Eibl-Eibesfeldt I (1950) Ein Beitrag Zur Paarungsbiologie der Erdkröten (*Bufo bufo* L.). Behaviour 2: 217–236

5 Emerson SB, Koehl MAR (1990) The interaction of behavioral and morphological change in the evolution of a novel locomotor type: "Flying" frogs. Evolution 44: 1931–1946

6 Homick JL, Vanderploeg JM (1989) The neurovestibular system. In "Space Physiology and Medicine" Ed by AE Nicogossian, CL Huntoon and SL Pool, Lea & Febiger, London, pp 154–166

7 Izumi-Kurotai A, Yamashita M, Kawasaki Y, Kurotani T, Mogami Y, Okuno M, Oketa A, Shiraishi A, Ueda K, Wassersug RJ, Naitoh T (1994) Behavior of Japanese tree frogs under microgravity on MIR and in parabolic flight. Adv Space Res 14: 419–422

8 Lack D (1956) Swifts in a Tower. Methuen & Co. Ltd, London, pp 44–47

9 Maeda N, Matsui M (1989) Frogs and Toads of Japan. Bun-Ichi Sōgō Shuppan, Tokyo (in Japanese)

10 Souza K, Black S, Wassersug RJ (1995) Amphibian development in the virtual absence of gravity. Proc Natl Acad Sci USA (in press)

11 Stark RE (1993) Ethology in Space, a unique opportunity for behavioural science (ESA STM-246). European Space Agency Publications Division/European Space Research and Technology Centre, Noordwijk

12 Stewart MM (1985) Arboreal habit use and parachuting by a subtropical forest frog. J Herp 19: 391–401

13 Ubbels GA, Berendsen W, Kerkviet S, Narraway J (1990) The first seven minutes of a Zenopus [sic] egg fertilized on a sounding rocket in space. In "Microgravity as a Tool in Developmental Biology (ESA-SP-1123)" Ed by T Duc Guyenne, European Space Agency Publications Division/European Space Research and Technology Centre, Noordwijk, pp 49–58

14 Wassersug RJ, Izumi-Kurotani A, Yamashita M, Naitoh T (1993) Motion sickness in amphibians. Behav Neural Biol 60: 42–51