Strong environmental tolerance of *Artemia* under very high pressure

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**Abstract.** It was shown by the present authors group that a tardigrade in its tun-state can survive after exposed to 7.5 GPa for 13 hours. We have extended this experiment to other tiny animals searching for lives under extreme conditions of high hydrostatic pressure. *Artemia*, a kind of planktons, in its dried egg-state have strong environmental tolerance. Dozens of *Artemia* eggs were sealed in a small Teflon capsule together with a liquid pressure medium, and exposed to the high hydrostatic pressure of 7.5 GPa. After the pressure was released, they were soaked in seawater to observe hatching rate. It was proved that 80-90% of the Artemia eggs were alive and hatched into *Nauplii* after exposed to the maximum pressure of 7.5 GPa for up to 48 hours. Comparing with *Tardigrades*, *Artemia* are four-times stronger against high pressure.

1. Introduction

*Artemia*, a kind of planktons and called brine shrimps, are found worldwide in inland saltwater lakes, but not in oceans. They have a biological life cycle of one year. Their eggs in a dried state, called cryptobiosis with a moisture level of below 10 % are metabolically inactive and can remain alive for so many years as to keep the species without evolution since the Triassic period. They hatch into *Nauplii* several hours after soaked in seawater.

A *Tardigrade* also has a cryptobiotic state, and shows very strong resistance to extreme environmental conditions such as in vacuum, at low temperature and under high pressure. It was shown by the present authors group [1] that *Tardigrade* can survive under high hydrostatic pressure of...
7.5 GPa for up to 13 hours. Artemia, therefore, would also be a good candidate for searching lives under very high pressure of several GPa.

2. Experimental method
The present experiments searching for lives under high pressure were performed by using a cubic anvil type high-pressure apparatus [2, 3]. To generate a high hydrostatic pressure in a relatively large volume, this type of high pressure apparatus is extremely useful. In the present case the the inner diameter and the length of the high pressure cell were 1.6 and 1.8 mm, respectively. Dozens of Artemia eggs (INVE Aquaculture, Ogden UT, USA) were sealed in a small Teflon capsule together with a liquid pressure medium of Fluorinate. The capsule was put in the center of a pyrophillite cube (see figure 1). This cube was compressed by six WC-Co second stage anvils with the front edge length of 4.0 mm. These anvils were compressed by a first stage 250-ton press. Figure 2 shows three of the six anvils after generating 7.5 GPa.

The pressure was increased from ambient to the maximum pressure of 7.5 GPa at a rate of 0.3 GPa/min. The pressure was kept constant at the maximum pressure for 5 to 72 hours, and then, decreased down to the ambient pressure with the same rate.

The pressure was determined by using a calibrated curve [2] of the relation between the press load and the actual pressure established before the experiment using the pressure dependence of the superconductivity transition point of Pb. The error of the intensity of pressure was smaller than 0.3 GPa. In this apparatus the press load was controlled to keep the intensity of the pressure constant during the operation at the maximum pressure.

Figure 1. Two halves of the pyrophillite cube and the Teflon capsule.

Figure 2. The second stage cubic anvil press after generating the maximum pressure of 7.5 GPa.

After the pressure was released, the eggs of Artemia were sucked into seawater, and brought under a microscope to investigate hatching for every fifteen minutes up to 36 h after the eggs were sucked into seawater.

3. Results and discussion
Figure 3 shows hatching and survival rate of Artemia exposed to the high hydrostatic pressure, 7.5 GPa for various hours plotted as a function of elapsed time after being soaked in seawater. As seen in this figure almost all the Artemia eggs exposed to the high hydrostatic pressure, 7.5 GPa for up to 48 hours began to hatch around 15-18 hours after soaked into seawater. Then, the ratio reached maximum around 40-70 hours after soaked into seawater. The hatching rate of 80-90 % was as high as for control. Hatching to Nauprius, Artemia Larvae, was observed under a microscope, and no difference
was confirmed between the high-pressure exposed group and control. After hatched into *Nauprius*, the high pressure exposed *Artemia* moved around with no practical differences in speed and frequency with control.

**Figure 3.** Hatching and survival ratio of *Artemia* exposed to the high hydrostatic pressure of 7.5 GPa for various hours plotted as a function of elapsed time after soaked in seawater.

From the present experiment, it was proved that 80-90% of the *Artemia* eggs were alive and hatched into *Nauplii* (see Figure 4 and 5) after exposed to the maximum pressure of 7.5 GPa for up to 48 hours.

![Artemia eggs and Nauplii](image)

**Fig. 4** *Artemia* eggs and *Nauplii* after exposed to 7.5 GPa for 24 h. The photo was taken 30 hours after eggs were soaked in seawater. A *Nauplius* moving around is seen on the left hand side.
After exposed to 7.5 GPa for 72 h, however, the hatching rate was only 3 %. Comparing with Tardigrades, Artemia is four-times stronger in environmental tolerance against very high hydrostatic pressure.

**Figure 5.** Nauplii, hatched from Artemia eggs exposed to 7.5 GPa for 48 hours. The photo was taken 24 hours after soaked in seawater. A Nauplius moving around is seen at the bottom.

The results of the present investigation may give the possibility that a plankton, Artemia [4] and a small animal, Tardigrade as well as the mosses, Ptychomitrium [5] and Venturiella [6] can travel through the space in a large meteorite, and reach the earth alive from other planet or galaxy.

4. Conclusion
From the present experiments we are able to know the effect of high hydrostatic pressure of 7.5 GPa on alive eggs of Artemia and their survival limit. The survival limit of the exposure time to the high pressure of 7.5 GPa for Artemia is determined to be a little longer than 72 hours. It is a surprising result that an animal is still alive after being exposed to such a very high hydrostatic pressure of 7.5 GPa and for such a long duration of 72 hours. This intensity of pressure corresponds to the pressure at the depth of about 180 km below the surface of the Earth and in the middle of the upper mantle.

References
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