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Tolerance of *Artemia* to static and shock pressure loading

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Abstract. Hydrostatic and hydrodynamic pressure loading has been applied to unicellular organisms for a number of years due to interest from food technology and extremophile communities. There is also an emerging interest in the response of multicellular organisms to high pressure conditions. *Artemia salina* is one such organism. Previous experiments have shown a marked difference in the hatching rate of these organisms after exposure to different magnitudes of pressure, with hydrostatic tests showing hatching rates at pressures up to several GPa, compared to dynamic loading that resulted in comparatively low survival rates at lower pressure magnitudes. In order to begin to investigate the origin of this difference, the work presented here has focussed on the response of *Artemia salina* to (quasi) one-dimensional shock loading. Such experiments were carried out using the plate-impact technique in order to create a planar shock front. *Artemia* cysts were investigated in this manner along with freshly hatched larvae (nauplii). The nauplii and cysts were observed post-shock using optical microscopy to detect motility or hatching, respectively. Hatching rates of 18% were recorded at pressures reaching 1.5 GPa, as determined with the aid of numerical models. Subjecting *Artemia* to quasi-one-dimensional shock loading offers a way to more thoroughly explore the shock pressure ranges these organisms can survive.

1. Introduction

Artemia salina, commonly known as brine shrimp, is a parthenogenetic branchiopod crustacean that has long been used in studies of a number of aquatic organisms as a food source [1, 2] as well as for bioassays to test for toxicity in various systems [3, 4]. A hatching rate of 24-48 hr in optimum conditions for *Artemia* cysts make them ideal for testing in a laboratory environment. *Artemia* are typically ovoviviparous; they are born as free-swimming nauplii. In less favorable environments, embryos are developed oviparously; in cysts that wait to hatch until conditions are stable. This dormant state in which encysted embryos reside is known as diapause where they experience reduced metabolic activity [1]. Once they are exposed to water they typically exhibit a hatching rate of approximately 90%, with a minimum of ~75% [5], under normal conditions They are capable of surviving a number of stressors, including salinity and temperature that can vary substantially, with an optimum salt concentration in most *Artemia* species of 60 gL^{-1} and an optimum temperature of 25 °C in laboratory conditions [1]. The temperatures for nauplii viability, however, have a rather considerable range of 5-40 °C [1]. In addition, cysts have been found to remain viable at even more extreme temperatures [6] along with certain evident enzyme activities that are maintained. For

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example, protease activity in the cyst shells of *Artemia franciscana* has been detected, although at a reduced rate, following a 15 min exposure to 100 °C [7].

Artemia exposure to high pressures extending into the multi GPa range has also been investigated more recently. Quasi-hydrostatic tests carried out on *Artemia salina* cysts in fluorinert medium by Ono *et al.* [8] found hatching rates of 80–90% after exposure of several dozen examples to a pressure of 7.5 GPa for up to 48 hr. In contrast, Udagawa and Suzuki [9] showed that shock waves with a pressures in the range of 25-100 MPa produced by underwater detonations resulted in cyst hatching rates of < 2.5% after 48 hr observation [9]. The marked difference between these findings could be related to the nature of the pressure loading, along with the different timescales over which the pressures were applied when compared with the biochemical or physiological changes that determine hatching.

The study presented here focuses on the response of *Artemia salina* nauplii and cysts to quasi-one-dimensional shock pressures. This is in keeping with similar work carried out on *Escherichia coli* [10] in order to analyse the effects of shock pressure on biological systems without the effects of a multi-dimensional wave front. Both hydrostatic and hydrodynamic pressure investigations have been carried out over a number of years to ascertain the survivability of single- to multicellular organisms following pressurisation to several hundred MPa or into the multi-GPa range. The results are of interest to fields ranging from food and agricultural products sterilisation and preservation, deep subsurface biology and exobiology through to panspermia and the origins of life [11, 12].

2. Experimental method

The response of *Artemia salina* was tested at three different shock pressures; 0.78, 0.96 and 1.5 GPa. Dried *Artemia salina* cysts were obtained from Sciento® and used for both cyst and nauplii shock loading experiments. Hatched nauplii were attained by immersing several cysts in a 3% saline solution at 25 °C for 48 hr in a water bath. Sample sizes of 100 cysts were chosen for shock loading at each pressure, while 100 hatched nauplii were examined at the lowest pressure only. These sample sizes were divided into subsets of 20 for the purpose of encapsulating them during the shock. Multiple experiments with reduced sample sizes prevented overcrowding within the sealed capsule used during shock pressure loading. Comparing the results from different runs also provided a measure of statistical significance.

The shock loading experimental set-up, outlined in figure 1, included the plate-impact technique with aluminium flyer plates carried out on a 50 mm bore single stage gas gun for quasi-one-dimensionally loading the *Artemia* samples. The sample capsule assembly was described previously [13]. A capsule containing 20 cysts or nauplii was filled to overflowing with 3% saline solution to avoid any cavitation in the sample during the shock. The cavity in the larger Al capsule was then filled with 20% ballistic gelatin to attenuate the shock and minimise rarefaction waves that may move back through the sample.

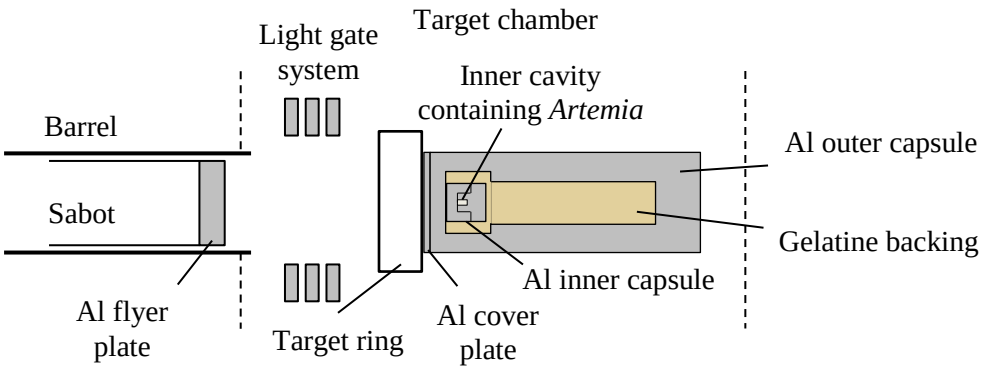


Figure 1. Experimental set-up with the Al capsule and Teflon system in the target chamber of the 50 mm bore gas gun.

In place of pressure gauges, peak shock pressures attained for each sample of cysts and nauplii were evaluated using previously validated numerical models [13]. Our estimates were based on a Lagrangian model of the capsule system implemented within ANSYS® Autodyn. The materials properties used were as listed by Leighs *et al*. [13] and followed the same set-up as used during the shock loading experiments. In order to validate the models the initial shock pressure for one experiment at both the lowest and highest impact velocities were measured using manganin pressure gauges. Subsequent comparison indicated that the pressures derived from the numerical simulations were reliable (figure 2). Fluctuations appearing in the stress-time trace for the experimental data were attributed to wave reflections occurring within the lid of the outer capsule.

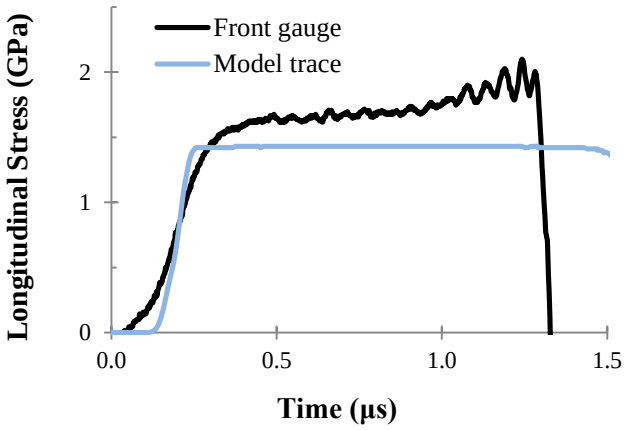


Figure 2. Shock traces from experimental data and numerical model for highest pressure with 230 ms^{-1} impact velocity.

In order to maintain *Artemia* under anaerobic conditions, it was ensured that the nauplii and cysts remained sealed inside their capsules for < 2 hr for each experiment. Control samples were encapsulated for the same period of time. The samples were immediately examined post-shock using light microscopy to search for any visible external damage to the cysts and nauplii. They were then studied after 24 and 48 hr to determine hatching rates and observe motility of the nauplii.

3. Results

Following incubation of the shocked cysts, the hatching rates (i.e., emergence of the embryo from the hatching membrane) were determined after 24 hr and 48 hr in each case (table 1, figure 2). The 'breaking' stage of the cysts was also recorded to observe the emergence of the embryo from the shell (figures 3 and 4). These rates were systematically higher than the hatching rates in each case. At the lowest pressure reached, 0.78 GPa, a breaking rate of 75 % was attained after 48 hr, while applying a pressure of 0.96 GPa resulted in a breaking rate of 70 %. The maximum pressure achieved during shock loading was 1.5 GPa and this led to the lowest breaking rate of 43 %. The hatching rates also showed similar decrease (26, 23 and 18%) with increasing peak pressures. One sample from each different shock pressure was also analysed after 14 days of incubation to search for any further hatched nauplii. However, no additional hatching was observed. Peak temperatures attained during the shock runs were determined through the numerical models (table 1). These ranged from 41^{o}C at 0.78 GPa to 50^{o}C at 1.5 GPa.

Table 1. Breaking and hatching rates of *Artemia salina* cysts observed for each shock pressure after both 24 and 48 hr

Impact velocity (ms^{-1})	Pressure (GPa)	Cyst breaking frequency (%)		Hatching frequency (%)		Shock peak temperature (°C)
		24 hr	48 hr	24 hr	48 hr	
135	0.78	60	75 (± 3)	16	26 (± 3)	41
153	0.96	59	70 (± 4)	12	23 (± 3)	42
230	1.5	30	43 (± 3)	4	18 (± 2)	50

In addition, hatched nauplii were studied following shock loading at 0.78 GPa but only at the lowest pressure due to the temperature increase of the samples during the shock. The motility of their antennae was analysed after shock and observed after 24 and 48 hr for 1 min each. 20% demonstrated motility of their antennae throughout this time, but with a noticeable lack of overall motion compared to nauplii hatched from shock loaded cysts. Many did not appear to have any significant structural damage post shock, as illustrated by figure 5. Nauplii that were found to be unmoving but appeared to be structurally intact were left for up to 14 days in order to check for any subsequent movement of their antennae; however, none was observed in any case.

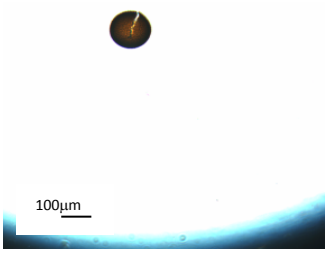


Figure 3. Breaking cyst after shock loading at 0.96 GPa.

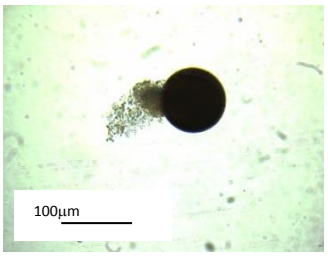


Figure 4. Emerging embryo from cyst after shock loading at 0.96 GPa.



Figure 5. Hatched nauplius after shock loading at 0.78 GPa.

4. Discussion

Exposure of *Artemia* cysts to shock pressures of 0.78 and 0.96 GPa caused a decrease in both cyst breaking and hatching rates, whereas a much larger effect was observed following 1.5 GPa shock. While the nauplii that hatched successfully following 1.5 GPa exposure appeared to be largely undamaged, a small number demonstrated impaired motility. Our results confirm the observation that *Artemia* cysts appear to be significantly more sensitive to quasi-one-dimensional shock compression than static pressurisation into the multi-GPa range. Our experiments resulted in considerably greater hatching rates than had been observed in previous shock experiments, carried out at substantially lower peak shock pressures. However, the previous work used non-planar wave fronts that could have played a role in the reduced hatching success. More studies will be required to elucidate the relative roles played by biochemical changes and the mechanical response of the cyst coating to shock *vs* static pressurisation in determining the rate of successful hatching, and subsequent properties of the hatched nauplii. In order to avoid complications due to heating of the samples during shock compression, we only carried out a single series of experiments subjecting hatched nauplii to 0.78 GPa pressure. Studies of antenna motion indicated that motility was considerably reduced.

In the case of the cyst shells, visible light microscopy showed that in some instances, the breaking stage was initiated, but was not completed (figure 3). Little damage appeared to have occurred to the external structure of the cysts, even for those that did not hatch after 48 hr. This could indicate that delayed hatching or possible death of the embryos contained inside the cysts might be due to some internal biochemical mechanism controlling their shock response, such as particular genes being activated in the encysted embryos but not in the hatched nauplii. Some such genes involved in the production of proteins for embryo protection have already been identified, including p26, but it is not yet known how these genes respond to pressure loading. It is clear that future work lies with studying the internal mechanisms that govern *Artemia salina* response to shock as well as static pressure.

5. Conclusions

By applying quasi-one-dimensional shock wave pressures in the range of 0.78-1.5 GPa, breaking and hatching rates of *Artemia salina* cysts were found to decrease with increasing pressure, unlike static compression results that maintained 80-90% hatching rates after exposure to 7.5 GPa for up to 48 hr. The enhanced effect of shock *vs* static pressurization in reducing *Artemia* cyst hatching is in general agreement with previous studies, although the hatching rates found here were significantly greater than those seen following shock from underwater detonation waves. This implied that the nature of the wave front must play an important role in survival and hatching probability of the cysts, certainly by affecting the mechanical stress fields applied to the cyst envelope. It is apparent that shock and static pressurisation also certainly affect the biochemical and biophysical state of the encysted embryo and these effects could be studied by future genomic and proteomics investigations.

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