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Negative effects of full spectrum light exposure along with UV radiation on survival and reproduction of *Cladocera Daphnia magna* (Straus, 1820)

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Abstract

Cladocera *Daphnia magna* (Straus, 1820) is one of the most important cladocerans having immense ecological and commercial significance. It is an important food for many aquatic organisms. Light intensity may have negative effect on its biological activities. That is why the study investigated the direct effects of full spectrum light with different intensities along with UV radiation on life history traits of single clonal cultured *D. magna*. Six-well multi plates were employed for rearing single clonal cultured *D. magna* which were subjected to five different light and UV intensities respectively, with six replications. The results showed that number of neonate production and survival rate were significantly ($p < 0.05$) affected by the intensities of light. Mortality rate was found significantly higher ($P < 0.05$) under the highest light intensity. The offspring production decreased with increasing light intensity. The study reconfirms the negative effect of UV radiation on parthenogenetic reproduction physiology, growth and survivability of single clonal cultured *Daphnia magna*.

Keywords: *Daphnia magna*, full spectrum light, UV radiation, survival, reproduction

Introduction

Light is one of the most important physical factors that greatly influence the life history traits of an organism especially when it's related to planktonic one like *Daphnia magna*. Although a good number of studies have already been conducted dealing with the effects of light on the behavior of aquatic organisms [1-5] but usually, those works had been conducted under artificial light condition (did not identical to full spectrum light of the sun) and there is a scarcity of research works related to the effect of full spectrum light along with UV radiation, excluding photoperiod, on life history traits of *D. magna*. It has already been reported that a positive correlation exists between population density of daphnia and the coefficient of light extinction [6] and the reproduction also influenced by light [7, 8].

Again, due to stratospheric ozone depletion over the past decades, the flux of solar ultraviolet (UV) radiation has increased across the Earth's surface [9-11] and it is speculated that an elevated level of UV radiation is expected to either directly or indirectly affect the photo-induced damage to organisms in aquatic ecosystems [12]. This phenomenon has drawn the attention of considerable researchers to work on the effects of increased UV radiation on terrestrial, marine and freshwater organisms and ecosystems [13-15]. UV radiation especially the UV-B have negative effects on nearly all aquatic organisms including bacteria, phytoplankton [16], zooplankton [17, 18] and fish [19]. However, phytoplankton are more sensitive to photo-induced damage [20] compared with zooplankton and fish. Zooplankton like *D. magna* on the other hand, able to move more actively through the water column and some species even can detect UVB radiation [21-23] which reduce the damaging effects. But still they are susceptible because they are weak swimmers and cannot move deep into water column. The UVB light from the sun even can reach at a depth of 6 m in lakes with low dissolved organic carbons [24]. Therefore, zooplankton have to suffer directly or indirectly through the effects of UV radiation.

The indirect effects of UVB radiation on zooplankton through changes in its food quality and/or quantity has long been studied by several researchers [20, 25]. Feeding on UVB stressed *Selenastrum capricornutum* (Printz 1914) resulted a reduced grazing rate of *D. magna* [26].

Life history experiments on *D. pulex* feeding UVB-irradiated algae showed reduced growth rate and reproduction [27, 28]. Cladoceran including *D. magna* is one of the most common components in the freshwater ecosystem [29] and possesses great ecological importance [30] as a model organism as stress indicator. *D. magna* has been also used as live food for different commercial freshwater fish species at their early stages of life. However, the short life span together with reproduction pattern and relatively faster response against different environmental changes bring the organism suitable as model organism for ecological and ecosystem studies. Feeding behavior along with effects of different environmental parameters has long been studied on this species [30] but very few dealt with direct effects of full spectrum light intensity (mimic to the sunlight) along with environmental level UV radiation. Therefore, the study conducted aiming to evaluate the effects of continuous full spectrum light (nearly identical to sunlight) and UV stress on the life history of *D. magna*.

Materials and Methods

Collection and production of *D. magna*

Experimental Cladocera *D. magna* was obtained from the stock culture of our laboratory; the Laboratory of Shallow Sea Aquaculture of Mie University, Japan. To avoid any bias in the experimental results isolation was applied to the animal and consecutive subculture of the progeny were applied for several generations maintaining set temperature at 20 °C in incubator in order to get similar sized individuals from single mother prior to the onset of the main experiment. For subsample culture 700 mL jars were used maintaining a photoperiod of 12: 12 L: D cycle. Freshly cultured *Chlorella vulgaris* were used as live food for *D. magna* during subsample culture as well as experimental period on a daily basis at a cell density of $\sim 10^6$ cells mL⁻¹.

Preparation of feed for *D. magna*

The stock of *Chlorella* (for feeding the experimental Cladoceran) were collected from the Gene Bank of National Research Institute (NRI), Mie, Japan and cultured in 2L conical flasks with modified f/2 medium [31-33] under permanent light condition (24:0, L:D cycle) maintaining set temperature 25 ± 1 °C. The algae were harvested at their log phase of growth, centrifuged at 3000 rpm for 10 minutes and the precipitate re-suspended in distilled water and then kept at dark, low temperature condition (4 °C) until being used as food. The cell concentration of algae suspension was adjusted by direct counting of cell under light microscope using Hemocytometer (Thoma). The OECD standard procedures [34] (OECD, 2012) were followed to prepare the stock food for the test animals.

Experimental procedures

The effects of natural visual light in combination with UV intensities were examined. Five different light intensities (780-800, 570-610, 390-415, 190-210 and 98-110 μmol) along

with five different UV intensities (6.7, 2.4, 1.8, 1.2 and 0.2 μWcm⁻²) were employed. Fluorescent light was used as control having intensity of 17.33 μmol and 0 mWcm⁻² for light and UV, respectively. For the treatments full spectrum light source (model) was employed which gives closer pattern of sunlight. Light and UV intensities were established by varying the distance between the *D. magna* culture plates and the source. Light and UV intensities were measured using YSI Model 65 radiometer and HD 2302 Delta OHM Light meter and Solar Radiometer UV, Micro Puck Radiometer devices.

D. magna offspring from subsample culture (less than 24 hours old) were placed individually into each of six-well multi plates (Thermo Scientific) where each well contained 4 mL of aerated drinking water and 1 mL of *Chlorella* ($\sim 10^6$ cells mL⁻¹) as food. Every day *D. magna* subjected to new fresh medium along with aforementioned food to avoid other stress regarding water quality and food concentration. All the test cladocerans were grown in incubator maintaining set temperature (20 ± 1 °C) and photoperiod (12:12, L: D).

Parameters observed

For zooplankton like Cladoceran, survival and reproduction of are the two common parameters which can directly interpret the response of organism under unfavorable condition. In this study, daily observation was carried out to examine the mortality and reproduction of *D. magna*. The dead ones were removed immediately and counted and were not replaced. The observation continues until all individuals under experiment died. As soon as the experimental individuals reproduced, the offspring were carefully removed to the new wells and fixed them with 5% formalin for further examination. After completion of the experiment the number of offspring was counted carefully.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) using the software SPSS (Statistical Package for Social Sciences) vers. 16 (IBM®, New York, USA). To meet the ANOVA requirement, Levene test and Shapiro Wilk test were carried out to check the homogeneity and normality of the data, respectively. If there was a significant F-test, subsequent comparisons of treatment means were performed using Duncan's multiple range test (DMRT) at 5% level of significance.

Results

Effects of light intensity on reproduction and survival

Light intensity negatively effects ($p < 0.05$) the offspring production of *D. magna*. Highest average number of neonates (37.50 ± 7.40) were produced under the control light (fluorescent light, intensity 17.33 μmol) and it was gradually decreased with increasing the intensity of fluorescent light (Fig. 1A). Survival of *D. magna* was also influenced by light intensity, where lowest survival was observed in the highest light intensity which was significantly different ($p < 0.05$) from that of control (Fig. 1B).

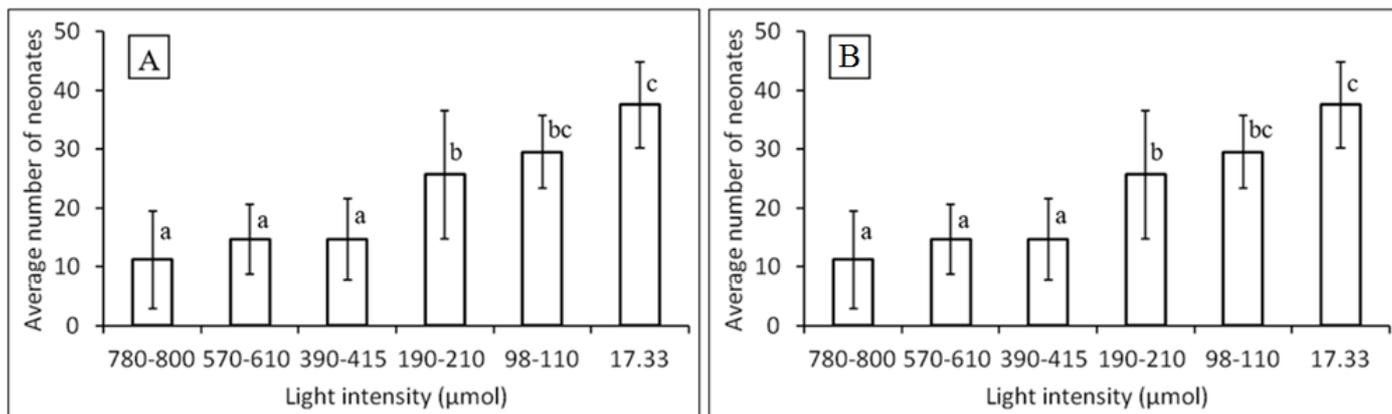


Fig 1: Effects of different intensities of light on *D. magna*. (A) Number of offspring productions. (B) Survival.

Effects of UV intensity on reproduction and mortality

In this present study, full spectrum light and UV-B exposed single clonal cultured *D. magna* was shown gradient reproduction decline under increasing full spectrum light intensity levels however abortion of eggs is not observed in any trials. Neither full spectrum light nor UV-B caused any interruption by gamogenetic (sexual) reproduction and no dormant egg formation observed, since cyclicity is one of the biggest deviation in obtaining monocyclic populations. UV intensity affected the number of neonate production of *D. magna* where the highest number of neonates was found

under the zero illumination (37.50 ± 7.40) which was significantly different from that of highest UV radiation ($p < 0.05$). The number of neonates produced was decreased with increasing the intensity of UV radiation (Fig. 2A). The survivability of *D. magna* and the highest mortality number (19 ± 1.33) was observed under high UV intensity level (6.7 mW cm^{-2}) which was significantly higher from non-UV radiation ($p < 0.05$). As shown in figure B, mortality of *D. magna* was strongly negatively correlated with UV intensity ($r = -0.70$) applied

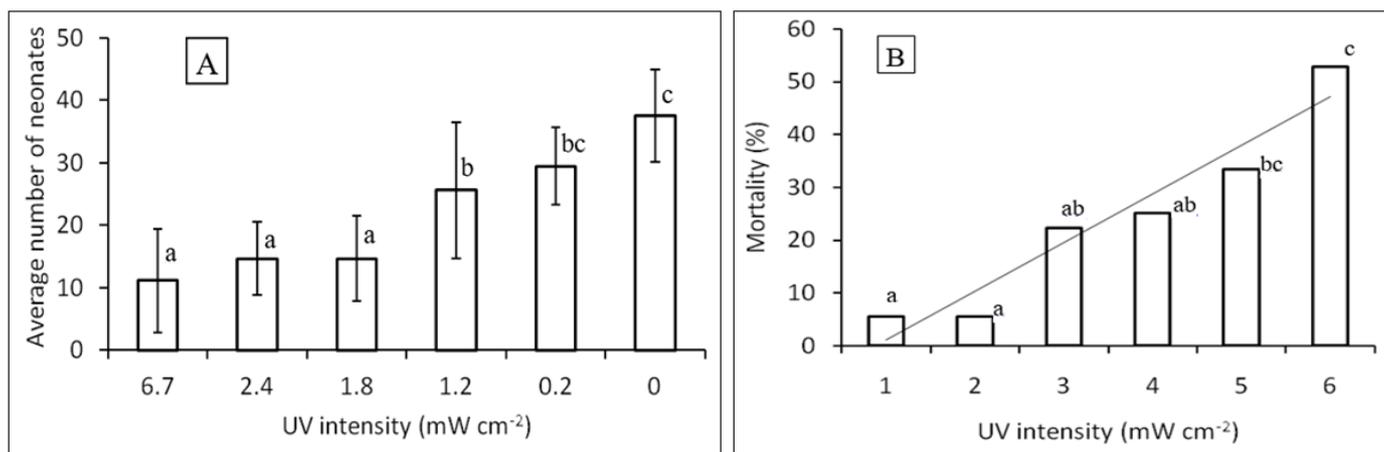


Fig 2: Effects of different UV intensities on *D. magna*. (A) Number of offspring productions. (B) Mortality.

Discussion

Daphnia magna, the crustacean zooplankton plays pivotal roles in the ecosystems of lakes and ponds all over the world. Daphnida are important phytoplankton grazers and valuable food resources for planktivorous fish, and a multitude of other predators. They are also important from commercial view point as they are intensively cultured for used as live food for a number of commercial finfish species. However, the freshwater branchiopod, *Daphnia magna* possesses a multi-chromatic photoreceptor system having capacity to identify the visual lights as well as UV lights [21] photoreceptor. Again, due to climate change and decaying of ozone layer, there is an increasing risk of UV radiation in the aquatic ecosystem which ultimately threatens the aquatic life, specially the planktonic ones. Therefore, sound understanding of daphnid's life history traits in response to different intensities of light along with UV is crucial to better understand how light affects its traits in intensive culture as well in nature.

Daphnia magna, like other *Daphnia* spp. exhibits sensitivity

to light and UV radiation [35] and there is considerable evidence that UV-B exposure is harmful to a variety of aquatic organisms [18, 35-43]. Six different light intensities along with six UV radiations (total UV) were employed in this investigation and the results showed that survival significantly affected with increasing the intensity. UV radiation probably the main cause of mortality of *D. magna* in this experiment. The findings of the present study again confers the negative effects of UV radiation on aquatic planktons and are consistent with other studies demonstrated UV-B radiation reduces survival in *Daphnia* [18, 43]. The *Daphnia* spp., a circumpolar planktonic genus of freshwater zooplankton, is highly vulnerable to UV radiation [41]. The mortality of *Daphnia* increased significantly when was exposed to increased levels of artificial and natural UV radiation [44]. The reasons behind mortality of *Daphnia* in effects of UV radiation might be associated with the efficacy of UV wave lengths, especially UV-B, which can damage deoxyribonucleic acid (DNA) and other biological molecules,

alter carbon distribution, and increase the development of highly reactive oxygen species^[42].

The number of neonate production of *D. magna* also negatively influenced by light intensity and UV radiation where the highest number of neonates (37.50 ± 7.40) were produced under conventional fluorescent light with zero UV^[42] found that UV can interact to affect the abundance and reproduction of zooplankton and for *Daphnia*, UV radiation can reduce the number of broods and number of progeny per brood^[18, 40, 43]. Likewise, both laboratory and field experiments have demonstrated that increased exposure to UV radiation leads to decrease in survival as well as growth and reproduction of both freshwater and marine zooplankton^[13, 19, 44, 45]. However, the magnitude and severity of UV effects showed in this experiment might be quite higher compared with that of natural environment because natural waters contain UV-absorbing substances, such as dissolved organic carbon (DOC) and dissolved organic matter (DOM)^[42]. An increase of terrestrially derived dissolved organic carbon (DOC) offers protection against photodamage by absorbing harmful UV radiation but at the same time natural waters contain some kinds of pollutants like polycyclic aromatic hydrocarbons (PAHs) which accelerate the damaging effects of UV (Phototoxicity 3). Besides these, UV radiation in nature also showed synergistic effects in combination with chemical stressors^[46, 47]. Consequently, UV radiation can create environmental stress resulting in changes in net productivity, trophic dynamics and the taxonomic structure of communities^[48]. On the other hand, the organisms were kept in 5 mL well where there was no opportunity for the organism to escape.

Conclusion

Results from this study suggests that the light spectrum and intensity have drastic effect on cyclicality and reproduction of *D. magna*. Given the importance of *Daphnia* to ecosystems, not only the intensity of light but also its spectral broadband is crucial to obtain realistic results since this experiment demonstrates the utility of survival and reproduction assays in assessing the impact of fluorescence and full spectrum light to better understand at lethal and sublethal levels. This study also reveals that UV radiation certainly affects the life history traits of *D. magna* like other planktonic organisms and for commercial production conventional fluorescent light which produce zero UV gives better result than full spectrum light.

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