

## A-5.2 Effects of Enhanced UV-B on Marine Zooplankton

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**Abstract** The influence of enhanced UV-B radiation was investigated with vertical migratory and neustonic copepods. The hatching success of the boreal vertical migrator *Paracalanus* sp. decreased anti-sigmoidally with UV-B dose. The vulnerability of eggs to UV-B was dependent on the developmental stages of eggs. When the UV-B dose was same, the damage was severer for eggs exposed at lower UV-B dose rate for longer periods than those at higher dose rate for shorter period. Hatching success of a temperate vertical migrator *Calanus sinicus* was also decreased by the present-level solar UV-B radiation. On the other hand, no influence of solar UV-B radiation was observed for neustons. Carotenoid and mycosporine-like amino acids contents of the neustons were 4 to 17 times higher than those of the vertical migrator. These indicate that neustons can escape from lethal damages of UV-B using UV-B absorbing compounds. The present study suggests that enhanced UV-B radiation due to the ozone depletion would cause significant damages to vertical migrators, which would further imply that the marine ecosystem would be perturbed through productivity, species composition, species interaction of zooplankton.

**Key Words** UV-B, ozone depletion, zooplankton, copepod, neuston, vertical migration

### Introduction

Zooplankton play an important role in marine food web dynamics. They are the most abundant herbivores and their grazing pressure is one of the controlling factors of the marine primary production. They are also important prey for carnivores, such as fishes, squids, baleen whales, etc. In the present study, we investigated the influences of UV-B on copepods, which are the most dominant in marine zooplankton assemblages. Copepods were divided into two groups, i.e., vertical migrator and neuston, by their vertical distribution. The neustons are distributed on the surface layer throughout a day, on the other hand, the vertical migrators occurred deeper layer during daytime and migrated into the surface layer during night. We examined the effects of UV-B for each group.

### Materials and methods

*Hatching rate of the vertical migrator Paracalanus sp.*

Eggs of *Paracalanus* sp. were put into 100 ml quartz bottles filled with filtered sea water and incubated at 10°C for 3 days. Eggs were exposed to UV-B using Toshiba sunburn lamp FL20SE with white fluorescent lamps under a 12 h light: 12 h dark cycle. Shorter wavelength than 290 nm was cut using Cutting sheet®. After the incubation, hatching success of eggs was examined. Experimental bottles covered with Lumilar® which cut shorter wavelength than 315 nm were treated as control. UV-B irradiance was controlled by the number of UV lamps and the distance between bottles and lamps. In order to investigate the relationship between dose rate and exposure time, eggs were exposed to UV-B (259 mJ cm<sup>-2</sup>) at different dose rates. Influence of UV-B on different stages of eggs were also determined.

Eggs at different stages were exposed to UV-B ( $12 \mu\text{W cm}^{-2}$ ) for 6 h (dose was  $259 \text{ mJ cm}^{-2}$ ) and examined their hatching rate.

#### *Survival rate of the vertical migrator Acartia omorii*

*Acartia omorii* females were incubated in 1.2 l quartz bottles with diatom *Thalassiosira weissflogii* as food. They were incubated at  $10^\circ\text{C}$  under a 12L:12D light cycle for 10 days. Dead animals were removed every day and their survival rates were examined. The UV-B dose ranged between 30 and  $864 \text{ mJ cm}^{-2} \text{ d}^{-1}$ .

#### *Influence of solar UV-B on the vertical migrator Calanus sinicus*

The survival of adult females, egg production rate and egg hatching success were determined under solar UV-B radiation. Experiments were carried out in January, April and June in the Seto Inland Sea. Females of *C. sinicus* were put into quartz bottles (Light+UV) or soda glass bottles (Light-UV). Those bottles were incubated for 5 days in the shallow plastic trays on the deck which were circulated with the surface sea water. Glass bottles covered with black plastic (Dark) were also incubated in the same manner. Dead animals were removed every evening, and also produced eggs were recovered and counted. The eggs were transferred into a plastic bottle with 30 ml of filtered sea water and incubated for 40 h to examine the hatching rate.

#### *Hatching rate of the vertical migrator Calanus sinicus under artificial UV-B*

The hatching rate of *C. sinicus* eggs were determined under different UV-B radiation using Toshiba sunburn lamp FL20SE. Produced eggs were transferred into quartz bottles and exposed to UV-B radiation for 12 h with and without white luminescence lamp. Thereafter, eggs were incubated for 36 h in dark to monitor the hatching success and malformation of nauplii. Experimental temperature was  $14^\circ\text{C}$ .

#### *Influence of UV-B on the neustons Pontella rostraticauda and Pontellopsis tenuicauda*

Collected copepods were incubated in the similar way to the experiments of *Calanus sinicus*. Microzooplankton were concentrated from the surface sea water and added as prey. Average prey concentration was 793 indiv.  $\text{l}^{-1}$ . Experimental bottles were placed on deck as described above.

#### *Carotenoid and mycosporine-like amino acids (MAAs) contents*

Carotenoids and mycosporine-like amino acids (MAAs), which are respectively known as antioxidants and UV absorbing compounds, their contents were determined for the vertical migrators *Calanus sinicus* adult females and the neustons *Pontella restraitecauda* and *Pontellopsis tenuicauda* adult females. Samples were extracted separately in 4 ml of distilled water, acetone, and methanol, and centrifuged. Visible-UV absorption spectra of each supernatant was measured (250-850 nm) using a Hitachi spectrophotometer model 220A.

## Results

#### *Hatching rate of the vertical migrator Paracalanus sp.*

Hatching rate of *Paracalanus* sp. decreased anti-sigmoidally with the increase of UV-B dose (Fig. 1). The relationship between hatching rate (H: %) and UV-B dose between  $17.7$  and  $101 \text{ mJ cm}^{-2} \text{ d}^{-1}$  was expressed as follows,

$$\text{Ln}[(100-H+1)/(H+1)] = 0.103 \times [\text{UV-B}] - 5.50 \quad (\text{F-test, } p < 0.001) \quad (1)$$

Fifty percent inhibition of egg hatching was observed at UV-B dose of  $53 \text{ mJ cm}^{-2} \text{ d}^{-1}$ . The damage was severer for eggs exposed at lower UV-B dose rate for longer periods than those at higher dose rate for shorter periods when the dosage was the same (Fig. 2). It is also obvious that eggs were more vulnerable to UV-B in their early stages of development than in late stages (Fig. 3).

#### *Survival of the vertical migrator Acartia omorii*

No influences were observed on the survival at lower daily UV-B dose than  $56 \text{ mJ cm}^{-2} \text{ d}^{-1}$ . Survival rate of adult female *Acartia omorii* decreased anti-sigmoidally with the increase of UV-B dose (Fig. 4).

#### *Influence of solar UV-B on the vertical migrator Calanus sinicus*

No influence of solar UV-B radiation on the survival, egg production rate and hatching rate was observed in the experiments carried out in January. In April, the survival of adult females decreased in Light+UV bottles (Fig. 5). The egg production rate was highest in Dark bottles and lowest in Light+UV bottles. The hatching rate in the Dark bottles was significantly higher than Light+UV and Light-UV bottles (t-test,  $p < 0.005$ ). The similar results was observed in June. The relationships between egg hatching rate and UV-B dose are shown in Fig. 6. Relative hatching rate in Light+UV bottles to Dark bottles at UV-B dose lower than  $0.2 \text{ mJ cm}^{-2} \text{ d}^{-1}$  was higher than 76 %. On the other hand, the hatching was lower than 23 % at UV-B dose higher than  $0.3 \text{ mJ cm}^{-2} \text{ d}^{-1}$ . It was estimated, therefore, UV-B dose of  $0.2 - 0.3 \text{ mJ cm}^{-2} \text{ d}^{-1}$  is threshold for egg hatching of *C. sinicus*.

#### *Hatching rate of the vertical migrator Calanus sinicus under artificial UV-B*

In the experiment of egg hatching under UV-B exposure without white fluorescent lamps, egg hatching rate (H: %) decreased exponentially with UV-B irradiance ([UV-B]:  $\mu\text{W cm}^{-2}$ ) (Fig. 7). The relationship was described as,

$$H = 72.5 e^{-0.09[\text{UV-B}]} \quad (2)$$

Malformation, e.g., elongated body, shorter appendages, less setae, etc., was observed in hatched nauplii. No normal nauplii were observed at UV-B irradiance stronger than  $15 \mu\text{W cm}^{-2}$ . The relationship between the frequency of the malformation (M: %) and UV-B irradiance was described as,

$$M = -5.6 + 87.9 (1 - e^{-0.4[\text{UV-B}]}) \quad (3)$$

In the experiments of egg hatching with UV-B and white fluorescent lamps, hatching was higher and malformation was less intense than those in the experiments exposed UV-B without white fluorescent lamps (Fig. 8). The hatching rate and malformation percentage were expressed as,

$$H = 76.5 e^{-0.01[\text{UV-B}]} \quad (4)$$

and

$$M = 5.9 + 12.3 (1 - e^{-0.4[UV-B]}), \quad (5)$$

respectively.

#### *Influence of UV-B on the neustons Pontella rostricauda and Pontellopsis tenuicauda*

No obvious influences of the present-level solar UV-B were observed on the survival, egg production, and egg hatching rates of the neustonic copepods.

#### *Carotenoid and mycosporine-like amino acids (MAAs) contents*

Absorbance maxima were observed at 310 nm and 470 nm for methanol extracts, 470 nm for acetone extracts and 310 nm for distilled water extracts. These indicated that the compounds with absorbance maxima at 470 nm and 310 nm are carotenoids and mycosporine-glycine, respectively. Carbon-weight specific absorbance at 470 nm and 310 nm of those neustons were higher than the vertical migrator *Calanus sinicus*.

#### **Discussion**

No influence of solar UV-B radiation was observed on the neustonic copepods. They may have adapted to the sea-surface environment with intense UV-B radiation by containing UV protective compounds, carotenoids and mycosporine-glycine (Mathews and Siström, 1959; Karentz et al., 1991). On the other hand, the present-level solar UV-B radiation can cause serious damage to vertical migrators, especially on their eggs. Inhibition of egg hatching was observed under UV-B radiation stronger than  $0.1 \text{ J cm}^{-2} \text{ d}^{-1}$  for *Paracalanus* sp. and  $0.2\text{-}0.3 \text{ mJ cm}^{-2} \text{ d}^{-1}$  for *Calanus sinicus*. This level of UV-B radiation was observed at mid-latitude seas during the period between spring and autumn. The adult and later developmental stages of the vertical migrators can avoid UV-B by diel vertical migration. On the other hand, the vertical distribution of the eggs is dependent on the vertical physical structure of the water column. When produced eggs are trapped on the surface layer, their hatching may be inhibited by the present-level solar UV-B radiation.

Present study revealed that the sensitivity of zooplankton to the solar UV-B radiation varies depending on developmental stages, species, types of vertical habitats. These results suggest that enhanced solar UV-B radiation due to stratospheric ozone depletion possibly change the zooplankton production and species composition. Such changes would induce the critical influence not only on marine ecosystem but also on fisheries production and/or global geochemical cycle. We should ascertain and monitor the most vulnerable part of the marine ecosystem to UV-B and quantify the effects as soon as possible.

#### **References**

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amino acid compound in Antarctic marine organisms: potential protection from ultraviolet exposure. *Mar. Biol.*, 108: 157-166.

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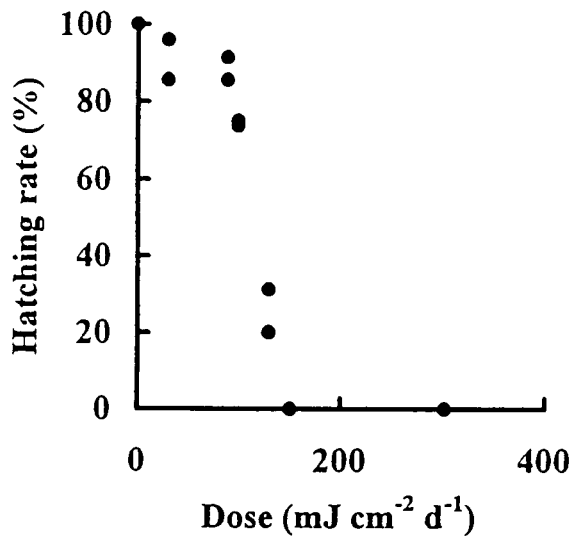


Fig. 1. The influence of UV-B dose on the hatching rate of *Paracalanus* sp.

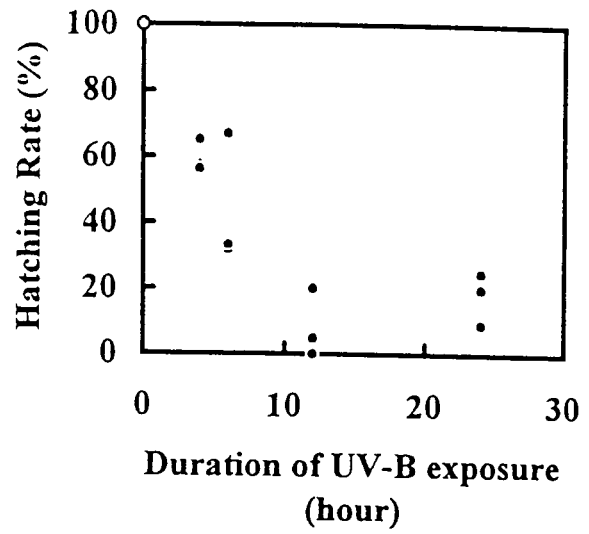


Fig. 2. Hatching rate of *Paracalanus* sp. at different UV-B dose rate.

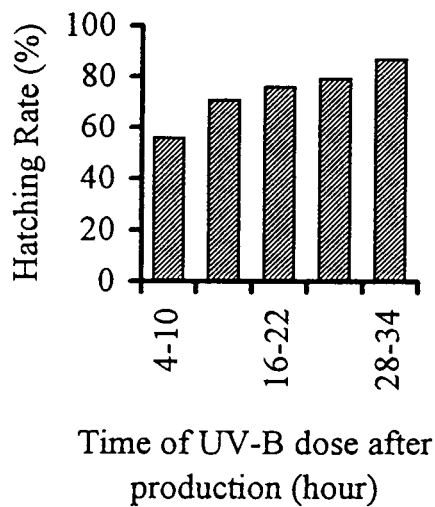


Fig. 3. The relationship between the time of UV-B exposure after production and hatching rate of *Paracalanus* sp.

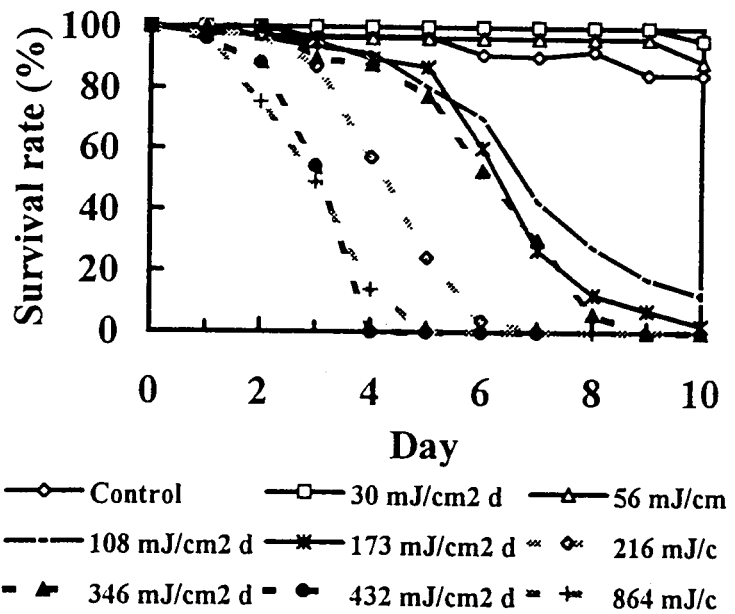


Fig. 4. The influence of UV-B dose on the survival rate of *Acartia omorii* adult females.

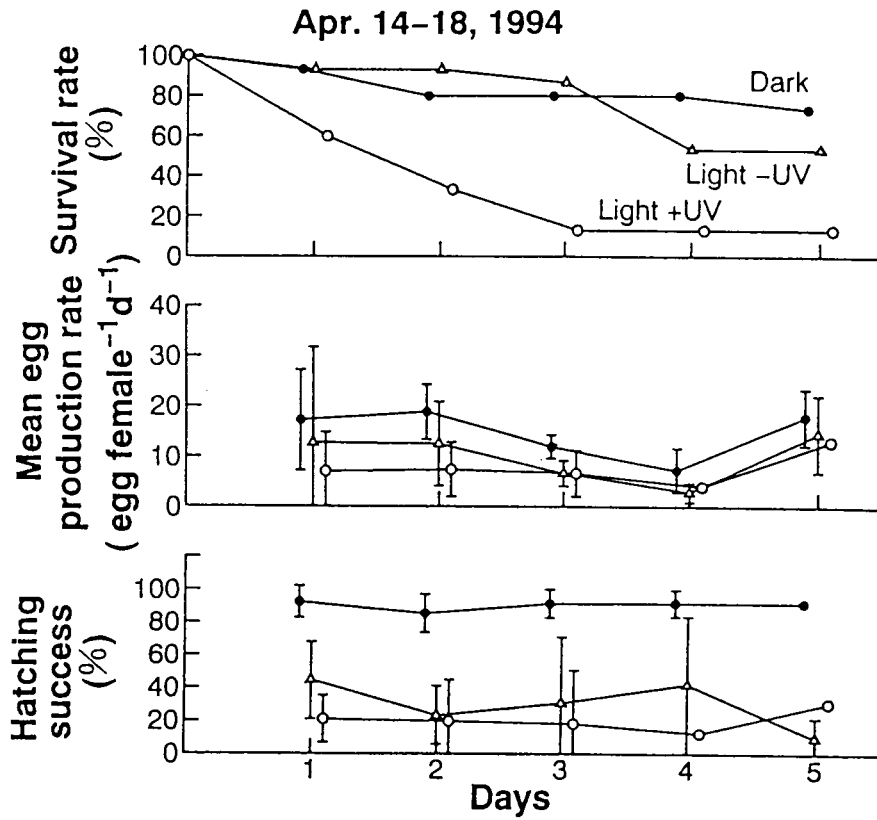


Fig. 5. Influence of solar UV-B radiation on survival rate of adult females (top), egg production rate (middle) and egg hatching rate of *Calanus sinicus* (April, 1994).

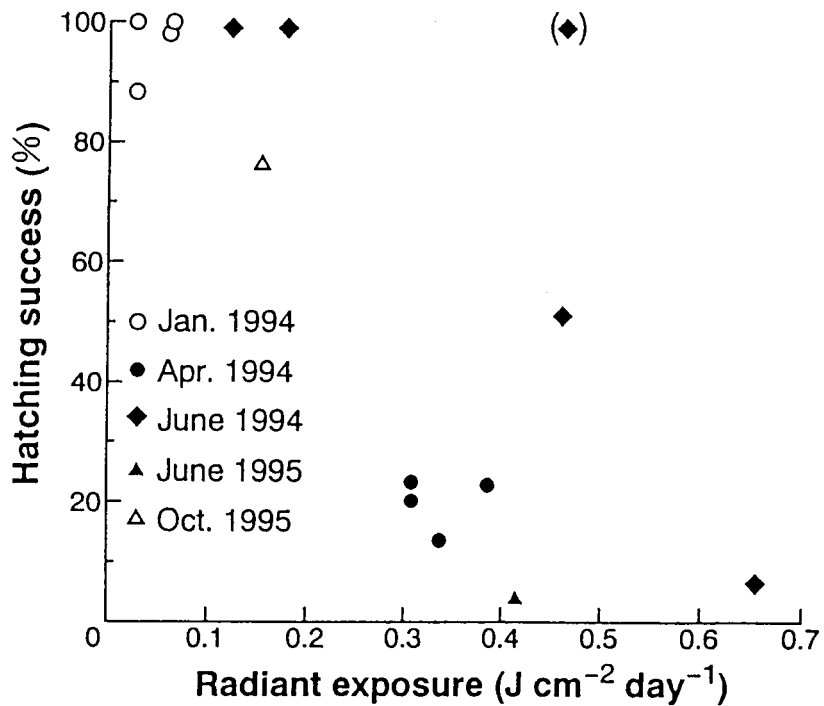


Fig. 6. Relationship between relative hatching success of *Calanus sinicus* to Dark bottle and daily UV-B radiation.

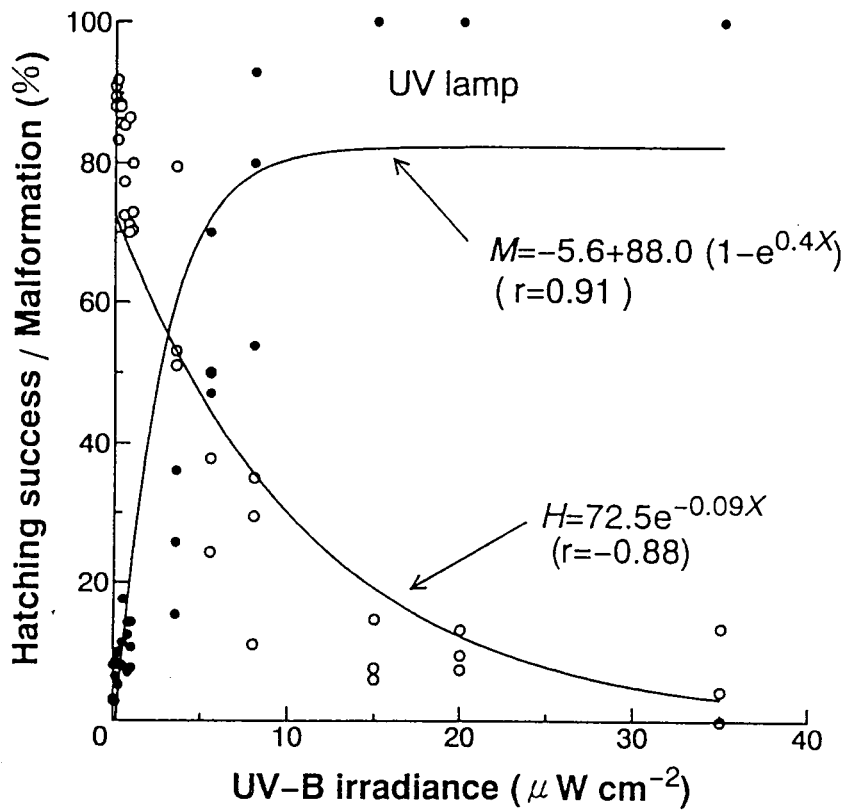


Fig. 7. Egg hatching rate and nauplius malformation rate under artificial UV-B exposure without white fluorescent lamp.

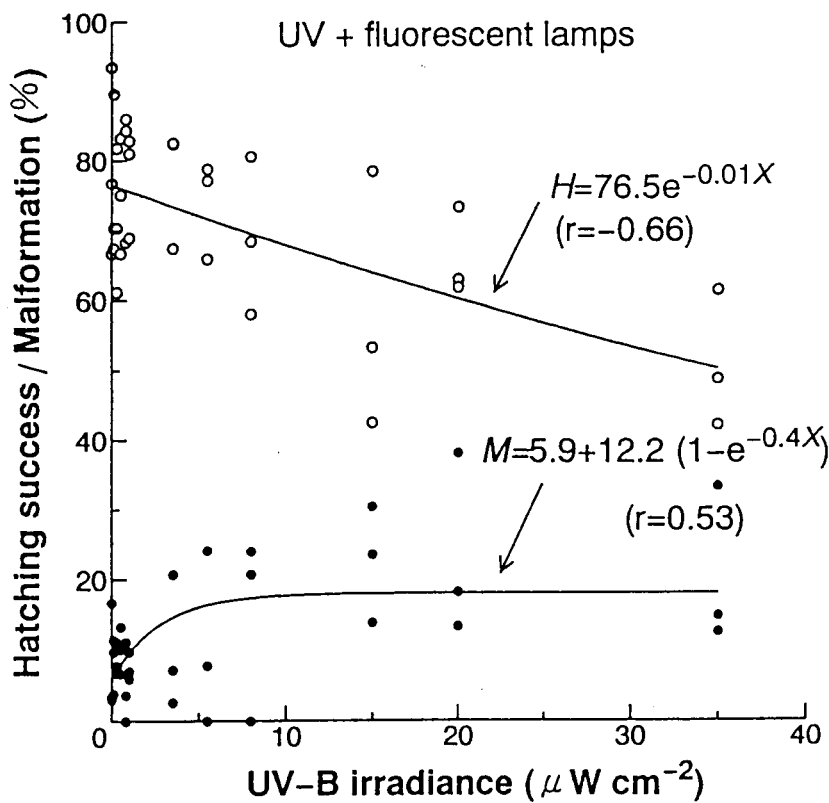


Fig. 8. Egg hatching rate and nauplius malformation rate under artificial UV-B exposure with white fluorescent lamp.