Effects of Ultraviolet Radiation (UVB) on Marine Zooplankton

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ABSTRACT

The effect of UVB on survival, egg production, and hatching rates was determined for boreal and warm water migratory copepods such as *Acartia omorii, Paracalanus* sp. and *Calanus sinicus,* and warm water neustonic copepods such as *Pontella rostraticuada, Pontellopsis tenuicauda,* and *Labidocera madurae* under solar or artificial ultraviolet radiation (UVR) sources. Significant differences in biological responses to UVB exposure were observed between daily migratory and neustonic copepods. While the metabolic rates of the former species were depressed significantly, the latter species did not show any detremental effect. This can be due to the presence of mycosporine-like amino acid compounds as UV-absorbers in addition to carotinoid pigments. Possible consequence of increased levels of UVB and/or the increased proportion of shorter wavelenths in UVR caused by ozone depletion is discussed particularly regarding the role of daily migratory copepods in the marine ecosystem.

Key words : egg production rate, hatching rate, PAR, survival rate, UVB

1. INTRODUCTION

Marine ecosystems consisted of various trophic levels. The relationship among the trophic levels is usually dependent on "prey-predator systems." The primary producers in the marine ecosystem are mainly phytoplankton in the open sea while the primary consumers are zooplankton. Those two groups are the most fundamental trophic levels in maintaining the structure and biological function of the marine ecosystem. Phytoplankton produce organic matter through photosynthesis using light and carbon dioxide. The effect of light on phytoplankton photosynthesis has been studied for a long time, and photoinhibition has often been recognized (Harris and Piccinin, 1977 ; Belay and Fogg, 1978 ; Smith *et al.,* 1980 ; Belay, 1981) as observed in higher plants (Jones and Kok, 1966). The spectral compostion of solar irradiance has been suspected to affect photoinhibition in phytoplankton (Harris, 1978). Smith and Baker (1980) attributed 50% of photoinhibition to visible light and the other 50% to light at wavelengths less than 430 nm. The effect of UV on phytoplankton photosynthesis was observed at the surface layer (Lorenzen 1979). On the other hand, the effect of UV on zooplankton also has been studied (i. e., Karentz *et al.* 1981). UVB has been shown to decrease the egg production and hatching rates of *Acartia clausii*. The effect of UVB could double the damage obtained by the exposure to the single sex of *A. calusii* adults if both sexes of the adults are exposed. However much more information has to be obtained to understand the effect of UVB on zooplankton to predict future events in a marine ecosystem.

UV penetration into the sea has not been quantitatively determined due to a lack of appropriate instrumentation. However underwater instruments are now available commercially (Smith *et al.,* 1992). In this study, UV penetration is determined in coastal waters in relation to survival, egg production, and hatching rates of zooplankton. Particular attention is given to migratory and neustonic zooplankton since the former species can avoid UV exposure by daily vertical migration while the latter species stays at the surface water for its entire life. Laboratory UV exposure experiments are also conducted to interpretate the results of field experiments. We will report the results obtained during the period of the fiscal years supported from 1993 to 1995 by the Global Environment Research Fund. The specific subjects will be published somewhere else separately.

2. MATERIALS AND METHODS

UV Radiation

An underwater UV radiometer (Biospherical Instrument, model PUV 500) was placed at 15 cm in a lagoon at Coconut Island, Hawaii Institute of Marine Biology, University of Hawaii (22°06′N, 157°47′W) on July 27, 1994, and at 50 cm at St. 3 in Akkeshi Bay, Hokkaido (42°58′N, 144°50′E) on October 9, 1994. The former area was located in subtropical waters while the latter area was located in subarctic waters. UVR with wavelengths of 305, 320, 340, and 380 nm were determined and averaged for every 5 minutes. The depth of the sensor, water temperature, and photosynthetically active radiation (PAR) were also

measured at the same time.

Survival, egg production, and hatching rates of boreal and warm water migratory copepods

Zooplankton samples were collected by a tow net (45 cm in mouth diameter) with 0.33 mm mesh from Kushiro Harbour. Females of *Acartia omorii* as a boreal species were sorted for determination of survival rate under a disecting microscope within 3 hours of collection. The experimental animals were placed in 1.2 liter quartz bottles with diatoms *Thalassiosira weissflogii* as prey. The first group of quartz bottles were covered by lumillar film to shield UVB as a control. The second group of quartz bottles were employed under experimental conditions. Exposure to UVR provided by Toshiba fluorescence tube model FL 20 SE was started the following day. UVB was determined by International Light UVB sensor model SUD 240/UVB/W. The dose of UVB ranged from 30 to 864 mJ cm^{-2}. PAR was provided by a white fluorescence tube at 140 $\mu E\ m^{-2}\ s.^{-1}$ Survival rates were determined every morning and sea water in the quartz bottles was replaced. The exposure experiment lasted for 10 days or terminated at the time when all experimental animals had died.

Females of *Paracalanus* sp. as a boreal species were sorted for the determination of egg production under a disecting microscope within 3 hours of collection. The experimental animals were transferred into 15 ml vials with filtered sea water through glass fiber filter type GF/F. They were incubated in the dark at 10°C for 24 hours. 20 to 25 of the eggs produced were transferred into a 100 ml quartz bottle filled with filtered sea water through a 0.2 μm Nuclepore membrane filter. The incubation at 10°C lasted for 3 days under exposure to UVR produced by Toshiba fluorescence tube model FL20SE in addition to a white fluorescent tube with a 12h light and 12 h dark cycle. The first group of quartz bottles were covered by a cutting sheet to shield UVR shorter than 290 nm, the second group of quartz bottles were covered by lumillar film to shield UVR shorter than 315 nm, and the third group of quartz bottles were employed without any shield (Fig. 1). UVR was determined by International Light UV sensor model SUD 240/UVB/W. Spectral composition of UVR+PAR was determined by Opto Research spectroradiometer model MSR-7000. PAR was set at 140 $\mu E\ m^{-2}\ s^{-1}$.

Females of *Paracalanus* sp. as a boreal species were collected for the determination of hatching rates in Akkeshi Bay in same manner as the survival experiments on October 9-10, 1994. One individual was placed in a 15 ml vial with filtered sea water for 6 hours in the dark to produce eggs. The produced eggs were transferred into 100 ml quartz bottles with 15-20 eggs per bottle. The exposure experiment was conducted at the surface temperature with running surface water for 48 hours. Doses of UVB were controlled by duration of coverage by lumillar films (Fig. 1). UVB in the air was determined by International Light UV sensor model SUD 240/UVB/W and PAR in the air was also determined by LICOR cosin sensor model LI-190 SA.

Zooplankton samples were collected in January, April, and June 1994 in the Seto Inland Sea (34°28'N, 133°25'E). Females of *Calanus sinicus* as a warm water species were sorted and three individuals were placed in 500 ml glasses and 800 ml quartz bottles with sea water filtered through GF/F and *Thalassiosira weisflogii* diatoms ($>10^4$ cells ml^{-1}). The glass bottle shielded all UVB and 90% of UVA. Exposure experiments were conducted at 12.9°C in January, 13.9°C, and 21.8°C in June in the deck incubator. Dark treatment (five replicates) was provided by black sheets. Glass bottles (Light-UV), quartz bottles (Light+UV), and dark bottles (Dark) were incubated for 5 days. The incubated bottles were rotated every 2-3 hours to maintain the cells uniformly in suspension. Dead individuals were counted every day. Produced eggs were removed by a 40 μm mesh concentrater and counted under a disecting microscope. Those

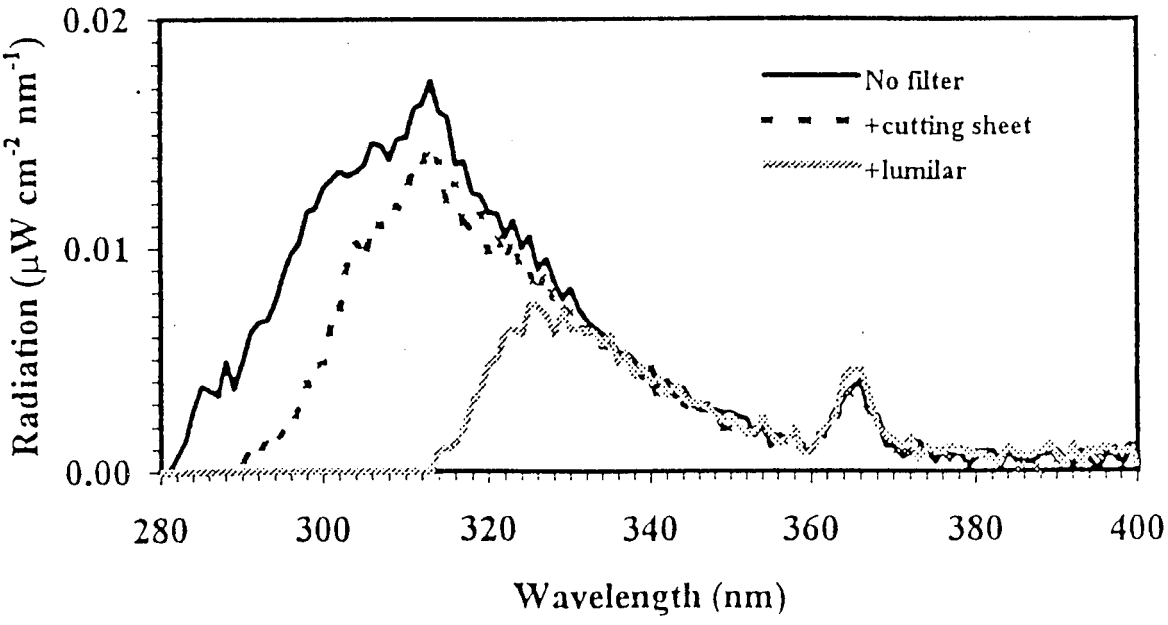

Fig. 1 Spectral distribution of fluorescence tube without and with cutting sheets and lumillar film.

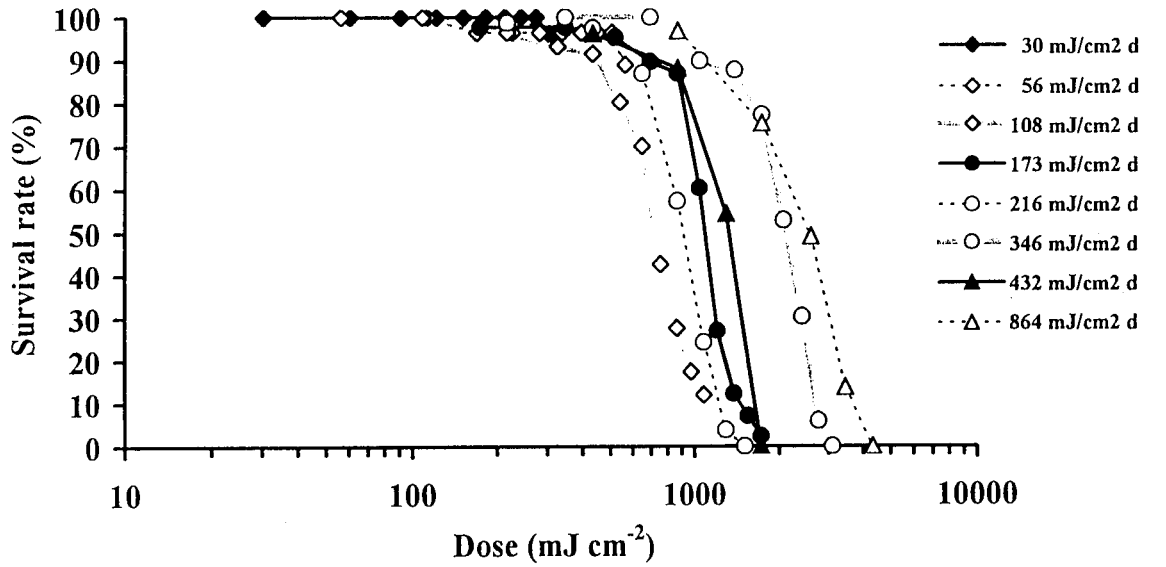

Fig. 2 Relationship between the survival rate of female *Acartia omorii* and UVB dose.

produced eggs were then placed in a 40 ml container. The incubation experiment was conducted at 20°C for 40 hours. The number of hatched individuals was counted under a disecting microscope.

Survival, egg production, and hatching rates of neustonic copepods

Temperate neustonic copepods *Pontella rostraticuda* and *Pontellopsis tenuicauda* were collected in the Seto Inland Sea in September 1994. Three individuals were placed in glass and quartz bottles. Samples concentrated 10 times through nets of 100 μm through 200 μm were added to each bottle for prey since those experimental copepods were omnivores with strong carnivorism. The prey consisted of nauplii and copepodite stages of copepods. The average density of prey was 793 individuals l^{-1} throughout the experiment. They were replaced every day. The exposure experiment was conducted for 5 days at 26.7°C.

Subtropical neustonic copepods *Labidocera madurae,* which were gathered by light at night, were collected by net with 0.5 mm mesh. Females were sorted immediately and placed in 50 ml vials with filtered sea water for 6-8 hours. The produced eggs were transferred into 250 ml quartz bottles with filtered sea water. The dose of UVB was controlled by coverage duration of lumillar film for the first group of quartz bottles (Fig. 1). The second group of quartz bottles was covered by lumillar for the entire experiment. The third group of quartz bottles was covered by black sheets. The exposure experiment was conducted on a water table at 27.0-29.0°C for 48 hours. The UVB dose (305 nm) was determined by Biospherical Instrument underwater UV sensor model PUV 500. Detailed experimental procedures were described in Saito and Taguchi (1995).

Carotenoid pigments and mycosporine-like amino acid compounds

Since neustonic copepods contain some blue or green pigments in their body, female *Calanus sinicus* were collected in March 1984 and female *Pontella rostraticauda* and *Pontellopsis tenuicauda* were collected in September 1994 in the Seto Inland Sea. Experimental individuals were immediately washed with distilled water and thirty individuals were placed in 8 ml vials and frozen at −20°C until further analysis could be performed. After being thawed, the samples were gound and 4 ml of methanol, acetone, or distilled water were added. Extraction was conducted for 2 hours at −20°C for methanol and acetone and at 8°C for distilled water. They were centrifuged at 10,000 g for 10 minutes and absorbance of the supernatant was determined by Hitachi spectrophotometer model 220 A at 310 and 470 nm to determine the relative abundance of carotenoid pigments and mycosporin-like amino acid compounds in relation to the body carbon content of the zooplankton.

3. RESULTS

UV Radiation

Sporadic effects of clouds were observed during the measurements of PAR and UVR at wavelengths of 305, 320, 340, and 380 nm in the lagoon at Coconut Island, Hawaii. Maximum PAR was 2,800 μE m^{-2} $s.^{-1}$ Maximum UVR decreased from 84 at 380 nm to 2.8 μW cm^{-2} nm^{-1} at 305 nm. Relative UVR of longer to shorter wavelength showed diel change with a high during mid day and a low at dawn and dusk, except for 340 nm/380 nm. Similar results were also observed in Akkeshi Bay. Those ratios were significantly dependent on solar zenith angle (β), except for 340 nm/380 nm in Akkeshi Bay.

Migratory copepods

No difference in the survival rate of female *Acartia omorii* between the UVB exposure and shield experiments was observed at daily doses lower than 56 mJ $cm.^{-2}$ Survival rates at UVR higher than 108 mJ cm^{-2} decreased with exposure time. Survival rates were not related to total dose of UVR. Low daily dose gave a sharp decrease of survival rate even at the same total dose (Fig. 2).

Hatching rates of *Paracalanus* sp. without cutting sheets in the incubater (Fig. 2) decreased with dose and were completely suppressed at 101 mJ cm^{-2} (Fig.

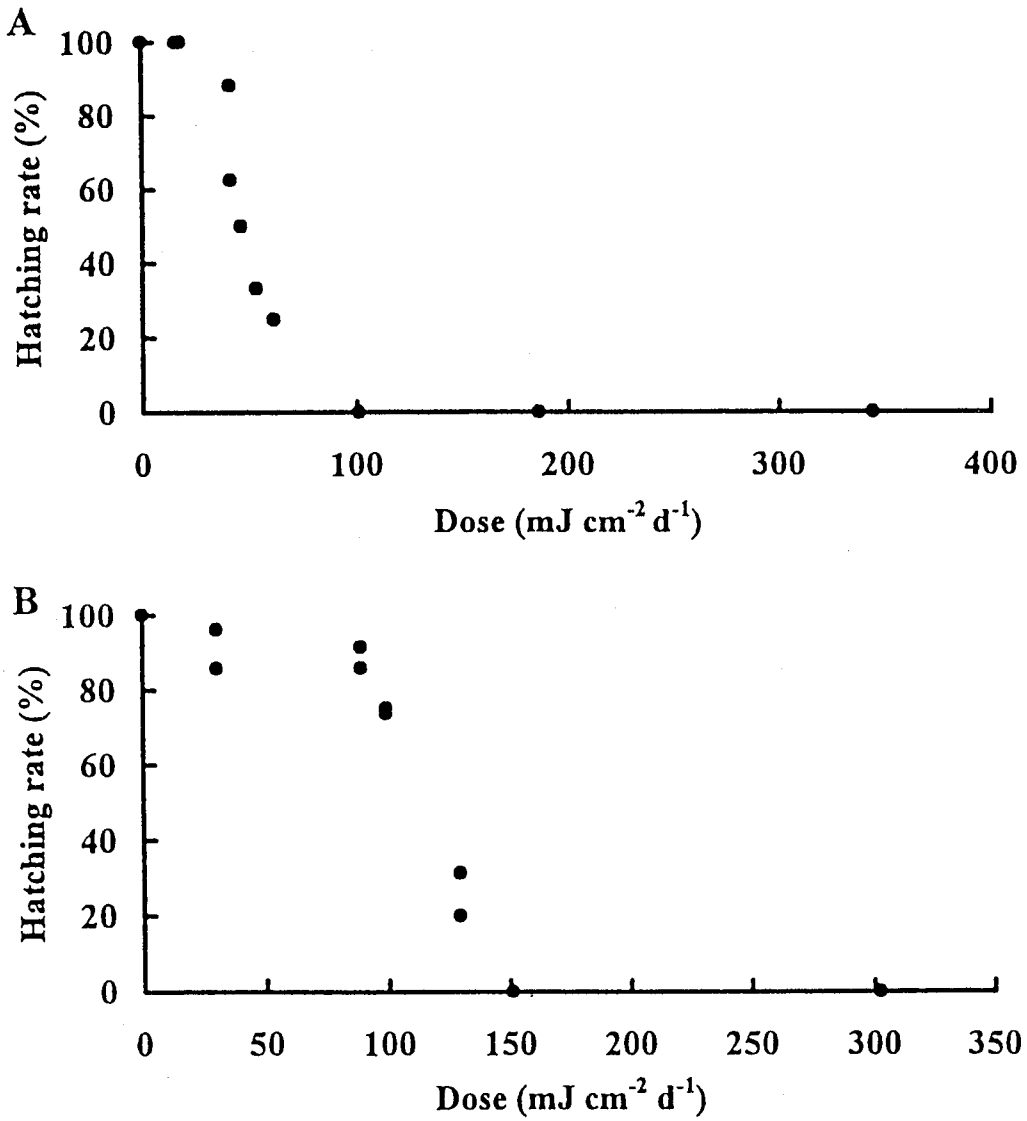

Fig. 3 Relationship between the hatching rate of *Paracalanus* sp. and UVB dose without (A) and with cutting sheet to eliminate UVR of less than 290 nm (B).

3a). The relationship between the hatching rate (H in %) and UVR dose between 17.7 and 101 mJ cm^{-2} was expressed significantly (F-test, $p<0.001$) by the following equation;

$$Ln[(100-H+1)/(H+1)]=0.103\times[UVB]-5.50 \quad (1)$$

The effect of UVB can be predicted from this sigmoid curve for *Paracalanus* sp. in relation to the dose. Equation (1) predicts 50% inhibition of the hatching rate at 53 mJ cm.$^{-2}$ A similar relationship was obtained when the incubation bottles were covered by cutting sheets (Fig. 3 b). The relationship between the hatching rate (H in %) and UVR dose between 30 and 151 mJ cm^{-2} was significantly (F-test, $p<0.005$) expressed by the following equation,

$$Ln[(100-H+1)/(H+1)=0.0502\times[UVB]-4.90 \quad (2)$$

Equation (2) can predict 50% inhibition of the hatching rate at 98 mJ cm^{-2} which is higher than one predicted from the experiment without shielding of UVR.

The hatching rate of *Paracalanus* sp. under a fixed dose of 259 mJ cm^{-2} in the incubater (Table 1) was characterized by a low rate at long exposure with a high rate at short exposure with high doses (Fig. 4). A similar experiment was conducted on board a ship in Akkeshi Bay. The maximum PAR was less than 1,900 $\mu E\ m^{-2}\ s^{-1}$ and maximum UVB was less than 6 μW cm.$^{-2}$ The hatching rate of *Paracalanus* sp. decreased with dose (Fig. 5). The following relationship was obtained between the hatching rate (H in %) and the dose (UVB in mJ cm^{-2}),

$$Ln[(100-H+1)/(H+1)]=0.0379\times[UVB]-4.03 \quad (3)$$

at $p<0.005$ (F-test). Equation (3) can predict 50% inhibition of hatching rate at 106 mJ cm^{-2}.

Maximum UVB were 5.4 in January, 25.3 in April,

Table 1 Dose rate and UVB radiation for the experiment of hatching rate of *Paracalanus* sp.

	Dose (mJ cm^{-2})	UVB radiation ($\mu W\ cm^{-2}$)	Dose rate (h day^{-1})	(day)
Control	0	0	0	0
Experiment 1	259	3	12	2
Experiment 2	259	6	12	1
Experiment 3	259	12	6	1
Experiment 4	259	18	4	1

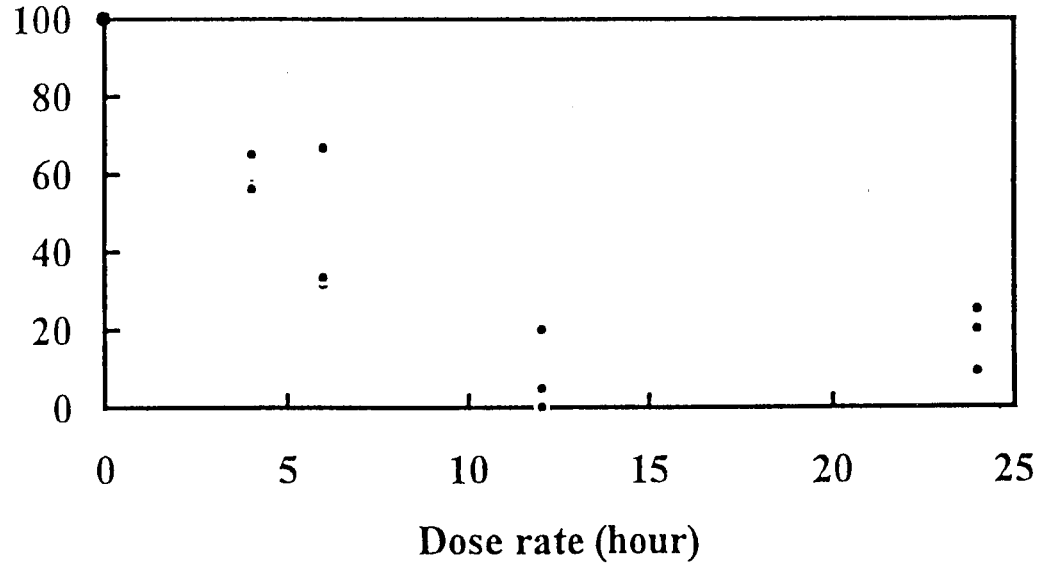

Fig. 4 Relationship between the hatching rate of *Paracalanus* sp. and UVB dose rate.

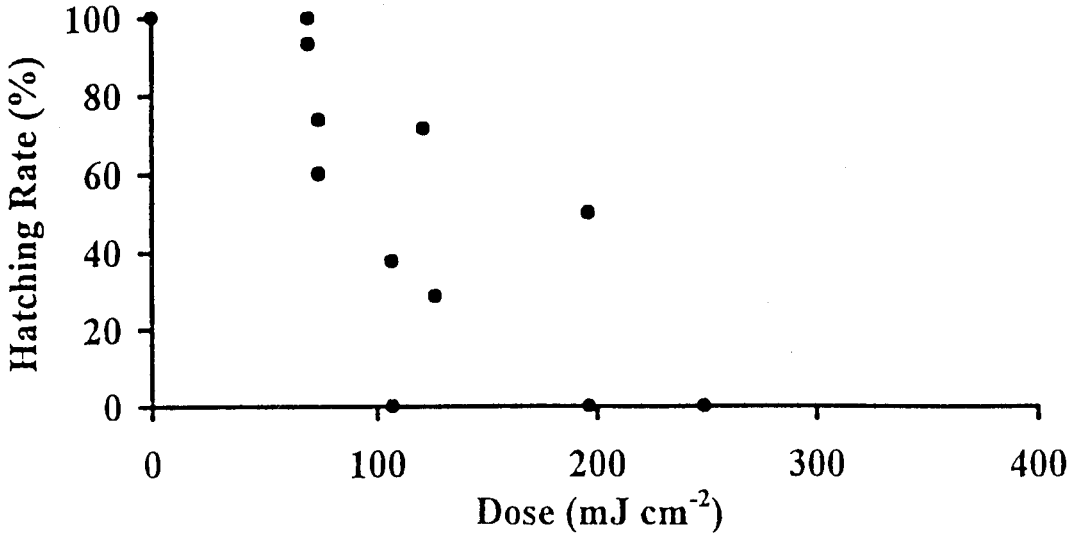

Fig. 5 Relationship between the hatching rate of *Paracalnus* sp. and solar UVB dose in Akkeshi Bay on October 9 to 10, 1994.

and 39.6 μW cm^{-2} in June while maximum daily UVB were 0.06 in January, 0.39 in April, and 0.66 J cm^{-2} in June in the Seto Inland Sea.

The survival rate of *Calanus sinicus* was higher than 87% in January. The egg production rate was high in the dark and all treatments showed a similar value at about 12 eggs female^{-1} d^{-1} on the 5th day. The hatching success decreased with time but little difference was observed between the treatments. Those observations might indicate little effect of UVB on the survival rate, egg production rate, and hatching success in January.

The survival rate of *Calanus sinicus* decreased to less than 20% in the Light+UV treatement in April. The survival rate in Light-UV decreased to about 50 % on the 4th day while that in the dark decreased to 80% on the 3rd day. The egg production rate showed high variability within each treatment. All values were less than 20 eggs female^{-1} d^{-1} with little change with time. The hatching success was the highest in the dark at about 90% and low in Light-UV (31%) and Light+UV (20%). The value in the dark was always significantly higher than in Light-UV or Light+UV at $p<0.005$ (t-test).

The survival rate of *Calanus sinicus* decreased to 40 % in Light+UV on the 1st day and to 50% in Light-UV on the 5th day while it decreased only to 80% in the dark in June. The egg production rate was less than 30%, highly variable within each treatment, and differed little between the three treatments. The hatching success was less than 90% in all treatments but highly variable with time. It differed significantly between dark and Light-UV on the 1st day ($p<0.01$), and dark and Light+UV on the 3rd day ($p<0.005$, t -test).

Survival, egg production, and hatching rate of warm water neustonic copepods

Maximum UVB and daily UVB were 22.5 μW cm^{-2} and 0.36 J cm^{-2} d^{-1}, respectively. The survival rate of *Pontella rostraticuada* decreased to less than 60% with time under all treatments. The egg production rate

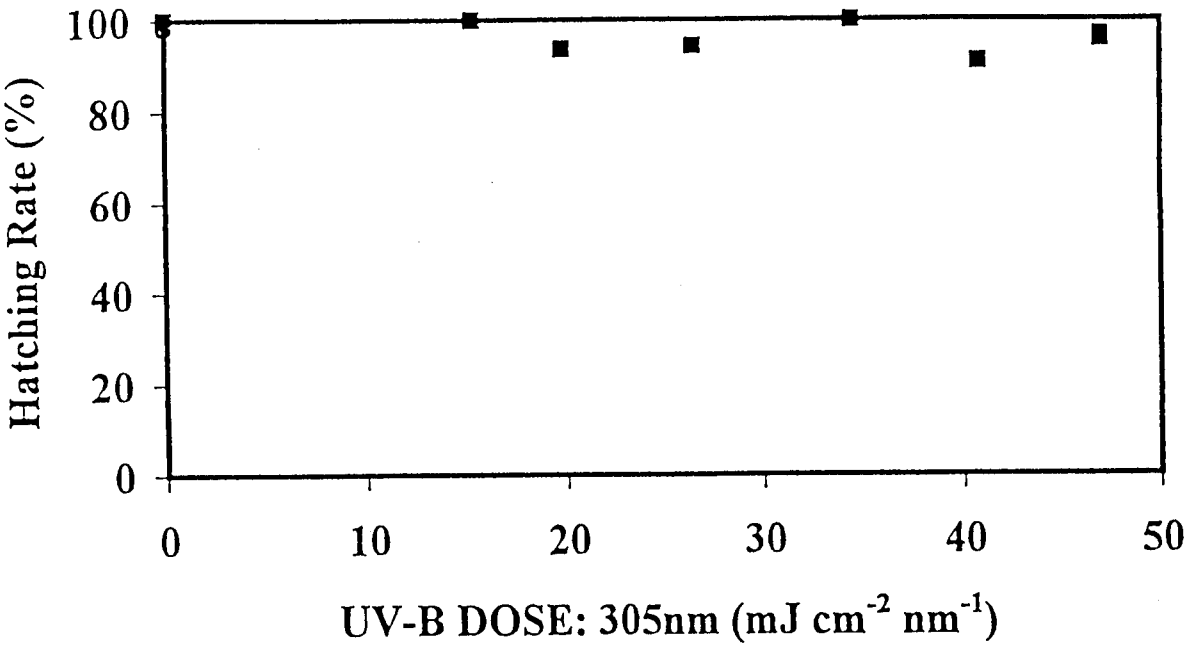

Fig. 6 Relationship between the hatching rate of *Labirocera madurae* and UVB dose on July 29 to 30, 1994.

decreased sharply on the 2nd day and stayed at less than 10% under all treatments. The hatching rate was less than 60% but highly variable. The survival rate of *Pontellopsis tenuicauda* decreased to less than 50% with time under all treatments. The egg production rate decreased sharply on the 2nd day to less than 10 % under all treatments. Hatching success stayed higher than 80% for dark and Light-UV while it decreased to 0% on the 5th day for Light+UV.

The hatching rate of *Labidocera madurae* did not show any significant difference between dark and UVB treatment up to almost 50 mJ cm^{-2} nm^{-1} of 305 nm UVB dose (Fig. 6).

Carotenoid pigments and mycosporine-like amino acid compounds

Absorbance maxima were observed at 310 and 470 nm for ethanol extraction while only at 470 nm for acetone extraction and only at 310 nm for the distilled water extraction of *Calanus sinicus, Pontella rostraticauda,* and *Pontellopsis tenuicauda.*

4. DISCUSSION

The intensity of longer wavelength compared to shorter wavelength of UVB was high during mid day and low at dawn and dusk for both the subtropical and subarctic ocean. Since UVR is related to zenith angle (Kirk 1990), those diel variations were caused by different light path lengths through the ozone layer for the different wavelengths of UVR. A broad band detector was often employed to determine UVR and provided information about diel change of UVR. However the results of the present observation indicate that the biological effect of UVR is much higher during the mid day than those expected from the observation by the broad band detector. Diel variability at each wavelength of UVR has to be considered for further study of biological effects of UVR.

Significant differences in the response to UVB exposure were observed between the daily migratory and neustonic copepods. The latter group did not show negative effects from UVR in terms of survival, egg production, and hatching rates. This observation may suggest little effect of UVR on neustonic copepods. *Pontella rostraticauda* and *Pontellopsis tenuicauda,* which live always at the sea surface, indicate the possesion of mycosporine-like amino acid compounds. Since absorbance was detected in distilled water extract at 310 nm, those materials could be a water soluble mycosporine-like glycine (Karentz *et al.* 1991). Those species also possesed carotenoid pigments, which were detected at 470 nm in methanol or acetone extraction. The presence of carotenoid pigments may suggest that they protect themselves from radiation by absorbing a relatively high energy of blue

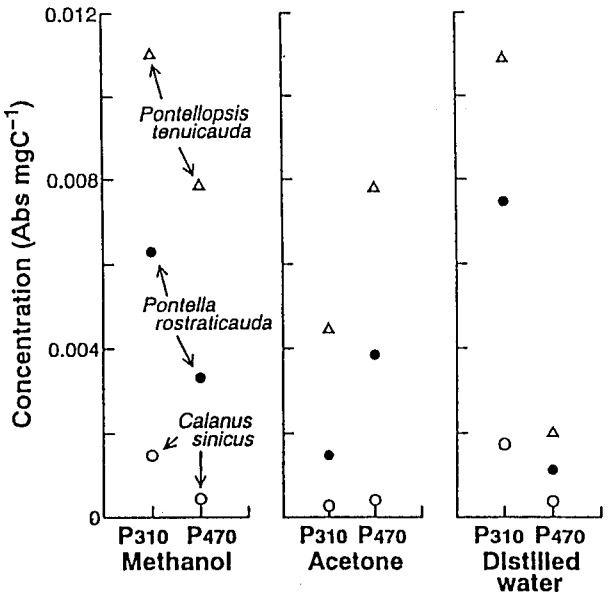

Fig. 7 Body-carbon specific absorption (absorption mg C^{-1}) extracted by methanol, acetone, and distilled water.

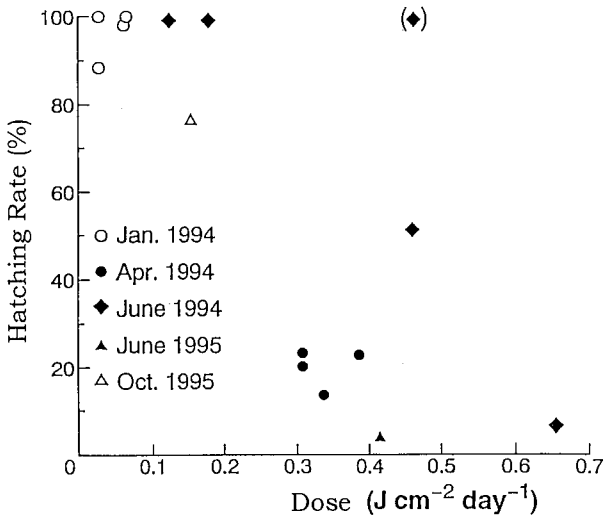

Fig. 8 Relationship between the hatching rate of *Calanus sinicus* relative to darkness and daily UVB radiation.

light (Mathews and Sistrom 1959). This is also supported by higher body carbon specific absorbance at 310 and 470 nm for neustonic copepods than for the migratory copepod *Calanus sinicus* (Fig. 7). *Labidocera madurae,* which lives in shallow coral lagoons in subtropical and tropical water, has never been reported possessing ultraviolet absorbing materials. Since their eggs have a dark green color and sticky surface, those structural characteristics may be responsible for the low UVR effect on the hatching rate observed in the present study.

Migratory copepods exhibited significant UVB effect on survival, egg production, and hatching rates. Field and laboratory experiments showed that the hatching rate of *Palacanus* sp. was depressed by 50% with a dose of 0.1 J cm^{-2} d.$^{-1}$ This species was dominant in boreal waters in fall when daily UVB ranged from 0.2 to 0.3 J cm^{-2} d.$^{-1}$ Once their eggs are trapped at the surface layer by physical mechanisms, they can not contribute to regeneration of the following population. The hatching success of *Calanus sinicus* was depressed by 50% with a dose of 0.2 to 0.3 J cm^{-2} d^{-1} (Fig. 8). Those values are higher than that for the boreal copepod *Palacalanus* sp., but those levels of UVR are usually observed during the period from April to September in the Seto Inland Sea. Those observations may indicate some effects on the regeneration of following *C. sinicus* populations when their eggs were trapped in the surface water due to less body-carbon specific mycosporin-like amino acid compounds. Furthermore survival and egg production rates of *C. sinicus* were depressed by UVR probably due to the transparent body and less body-carbon specific carotenoid pigments.

5. SUMMARY

When the level of UVB and/or the proportion of shorter wavelength in UVR is increased due to the growing ozone hole, the following possiblities may occur ; (1) Decrease of population size of migratory copepods due to the lowered regeneration rate, (2) Shift of habitats of migratory copepods to deeper layers with less phytoplankton due to deeper UVR penetration, (3) Succession of dominant species from migratory to neustonic copepods or other species with UV-absorbing compounds, and (4) Decrease of neustonic copepods due to increase of UVR beyond their tolerant capability. Once those changes are induced, we can predict not only a drastic change in the marine ecosystem but also a decrease in absorption capability of carbon dioxide by phytoplankton. In addition the global environments and economic activities of human beings may deteriorate. A more thorough monitoring program would be necessary to pinpoint the areas in which the biggest UVR effect can be expected so that a conservation program can be applied effectively and immediately.

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