A-5.3.1 Evaluation of the Enhanced UVB Radiation on the Interrelationship between Phytoplankton and Zooplankton

Contact Person Hiroaki Saito
Senior Researcher, Biological Oceanography Section
Hokkaido National Fisheries Research Institute, Fisheries Agency
116 Katurakoi, Kushiro, Hokkaido 085-0802, Japan
Phone +81-154-92-1723 Fax +81-154-91-9355
E-mail hsaito@hnf.affrc.go.jp

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Abstract

Effects of enhanced UVB radiation on survival, grazing and growth rates were investigated for copepods and smaller zooplankters such as ciliates. Also, the effects on the interaction between phytoplankton production and microzooplankton grazing were investigated using dilution culture. Most vulnerable developmental stage of copepod was egg, which lacks swimming ability and contains less pigment. Tow distinct groups were observed in the copepods for their vulnerability for UVB radiation. Neustonic copepods were less vulnerable, in contrast, water-column dwelling copepods were vulnerable than formers. The most vulnerable zooplankter was tintinnid ciliate *Favella taraikaensis*, they could not survive under the radiation of 1.1kJm^{-2}d^{-1}. Results of dilution culture suggested that negative effects on phytoplankton production and zooplankton grazing were same level, or slightly higher effects on zooplankton in average. The magnitude of the effect were different between on phytoplankton and zooplankton depending on season. Microscopic examination suggested that naked ciliates, the most dominant taxa in the ocean, were always the most vulnerable in the microzooplankton community.

These results suggests that both phytoplankton and zooplankton are likely suffered by enhanced UVB radiation, and zooplankton, especially microzooplankton were relatively more vulnerable than phytoplankton. Then the balance between phytoplankton production and zooplankton grazing will be most likely disrupted by enhanced UVB radiation.

Key Words UVB, ozone-depletion, phytoplankton, zooplankton, interrelationship, copepods, protozoans, dilution method

1. Introduction

Due to stratospheric ozone depletion, the mid-ultraviolet ray (UVB, 280-320 nm) reaching the surface of the earth is increasing. This harmful ray may cause some serious

damage to aquatic organisms, since UVB can penetrate to ecologically significant depths of the water. The impact of increased UVB penetration in the aquatic ecosystem will likely be focused on the survival and physiology of plankton which have no or limited escaping ability from the harmful ray and the subsequent disruption of the food chain dynamics.

2. Research Objectives

Phytoplankton which form the base of the aquatic food chain have been found to be affected by the present level of solar UVB, as was clearly demonstrated by several investigations [1)2)3)4)] that exposure to increased UVB reduces primary productivity. In the second trophic level, being constituted by primary herbivores (zooplankton), increased UVB may also have significant effects as evidenced by induction of reproduction impairment [5)6)], reduced survival and development rate [5)6)7)8)9)10)], sex shift [11)] and deformation [12)]. One of the characteristic features in the marine food chain dynamics is the tight coupling between phytoplankton and herbivores, and hence it is necessary to investigate the effects of UVB on trophic coupling between phytoplankton and zooplankton in order to assess the disruption of the marine ecosystem by enhanced UVB.

3. Effects of solar radiation on the phytoplankton-microzooplankton coupling in the Oyashio region

3-(1) Introduction

The balance between phytoplankton production and zooplankton grazing is mainly maintained by microzooplankton in the ocean. In this section, we investigated the effect of UVB on the interaction between them using the natural solar radiation by dilution culture. We also investigated the effect of UVB radiation on the growth rates of major taxa of microzooplankton by microscopic examination.

3-1(2) Research methods

Sampling and experiments were done during cruises of the R/V *Tankai MaruH*, Fisheries Agency, during the 1996-98. Experiments were conducted 4times from spring to fall in the center of Oyashio current and Akkeshi Bay.

Microzooplankton grazing rates (including nano-heterotrophs grazing on phytoplankton) were estimated by the dilution culture [13)]. Surface water was obtained with a plastic bucket and filtered through a gauze (100 μm mesh opening) to remove macrozooplankton. The prefiltered surface water was sequentially diluted with filtered seawater (0.22 μm Millipore or GF/F filters) from the same source. Then, a series of HCl prewashed polycarbonate bottles (0.5-1L) and quartz bottles (0.25L) were filled with the diluted seawater. Each experiment consisted of series of 4 dilution treatments each in

duplicate. The bottles were incubated in a deck-mounted tank with flow-through surface seawater, under natural light conditions. Incubations lasted one day. The waters (200 ml) were filtered through a glass-fiber filter (GF/F) at the beginning and the end of the experiments. Filters were then placed into disposable tubes containing either n,n dimethylformamid and kept in a freezer for several days for pigment extraction. The chlorophyll-*a* concentration of the extract solution were determined with a fluorometer (Turner Designs model 10) before and after acidification. Phytoplankton growth rate (μ) and microzooplankton grazing (g) rates were calculated from linear regressions of apparent growth rate in chlorophyll-*a* against dilution factor. Phytoplankton growth and microzooplankton grazing rates were determined as the y-intercept and the slope of the regression lines, respectively.

3-(3) Results and discussion

Phytoplankton growth rates and microzooplankton grazing rates from the dilution cultures were summarized in table 1.

Table 1. Effects of UVB radiation on phytoplankton growth and microzooplankton grazing (d^{-1}) by the dilution cultures. AK: Akkeshi Bay, OY: Oyashio current

Date	May 98	Jul 98	Aug 98			Oct 97		Avg
Location	OY	OY	OY	OY	AK	OY	AK	
Phytoplankton growth								
UV+	0.07	0.57	0.54	0.77	0.44	0.48	0.40	
UV-	0.09	0.52	0.65	0.74	0.72	0.70	0.63	
Percent increase by UV cut	29	-9	20	-4	64	46	58	29
Microzooplankton grazing								
UV+	0.09	0.54	0.14	0.84	0.45	0.28	0.27	
UV-	0.06	0.68	0.22	0.96	0.74	0.35	0.43	
Percent increase by UV cut	-33	26	57	14	64	25	59	30

Phytoplankton growth rates were generally higher in summer, and positive correlation between growth rate and PAR (photosynthetic available radiation) were observed. In average, phytoplankton growth rate increased 29% by UVB cut, but the increase during summer was 18% and that was 43% for other seasons. The same trends were observed in the measurements of primary production by ^{13}C

uptake experiments. Microzooplankton grazing changed seasonally with phytoplankton production. In average, microzooplankton grazing rate increased 30% by UVB cut, and summer average (40%) was larger than the average of other seasons (13%). However, the seasonality was not clear compared with phytoplankton production. If we neglect May's value, which was erroneous because of low grazing rate, the total average was 42%. Then, this should suggest that microzooplankton is more vulnerable than phytoplankton for UVB radiation.

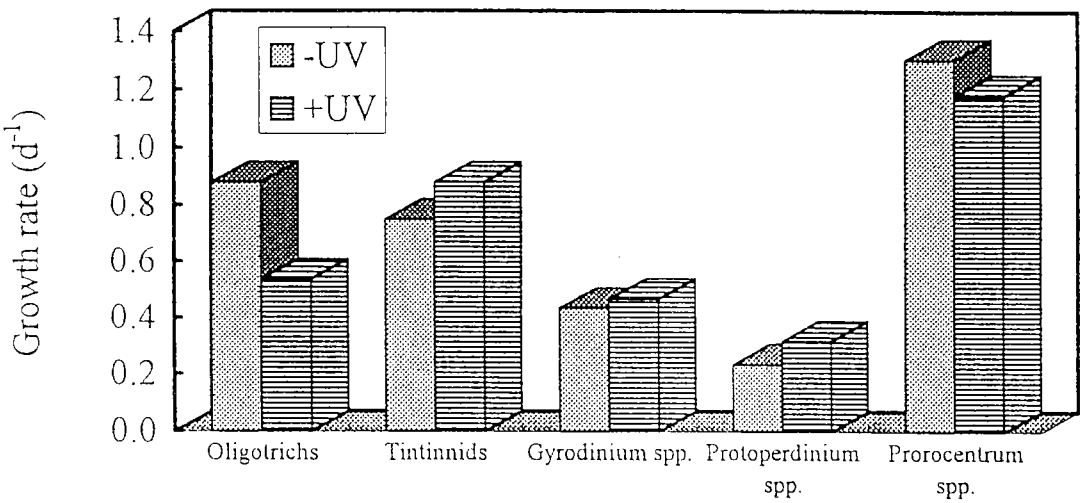

Fig.1. Growth rates of each microzooplankton taxa during the dilution culture at October 1997.

Seasonality and geographical variation of the effects of UVB radiation should partly be caused by difference of sensitivity of each organism. Figure 1 shows growth rate of each taxa of microzooplankton during the dilution cultures. The growth rate of oligotrichs (naked ciliate) decreased 40% by UVB radiation. In contrast, heterotrophic dinoflagellates (*Gyrodinium* and *Protoperidinium*) were less sensitive to UVB radiation. These phenomena were observed for all dilution cultures. These results suggest that enhanced radiation of UVB should effect not only on the balance between phytoplankton production and microzooplankton grazing, but also on community structure and species diversity of grazers.

4. Effects of UVB on phytoplankton-zooplankton interrelationship in warm-water region

4-(1) Introduction

Copepods are the major constituents of marine zooplankton, and their life history consists usually of 13 developmental stages, i.e. egg, nauplius stage 1 to 6, and copepodite stage 1 to 6 (or adult). The occurrence of copepods are often seasonal and their spatial

distribution varies according to spatio-temporal variations in the environment. It is of primary importance to elucidate which developmental stages and which species are most vulnerable to UVB in order to assess the effects of UVB on phytoplankton-zooplankton coupling. First, we examined the egg hatching success and mortality of various developmental stages using a brackish-water copepod *Sinocalanus tenellus*. Second, we examined the difference in egg hatching success among 8 copepod species from the Inland Sea of Japan and Lake Nakaumi. Third, we examined the effects of UVB on the physiology of adult copepods by measuring their feeding and egg production rate under various UVB conditions.

It is now well-known fact that microzooplankton, the monomial body size ranging from 20 to 200 μm, play important roles as grazers of phytoplankton as well as secondary producers [14]. Tintinnid ciliates are the major group in the microzooplankton in the Inland Sea of Japan [15]. Since effects of UVB on this protozoan microzooplankton have never been studied before, we examined the growth of a tintinnid under various UVB conditions in the laboratory.

4-(2). Research methods

4-(2)-① Effects of UVB on egg hatching success and survival of various developmental stages of a copepod

Twenty 0-6 h old eggs were transferred to each quartz test tube (volume: 30 ml), and duplicate tubes at each radiation level were exposed for 12 h. The eggs were then transferred into glass crystallizing dishes containing *ca.* 40 ml of filtered water and incubated in the dark for 12 h, and the number of hatched nauplii and unhatched eggs were counted. Hatching was defined by the complete decapsulation from the egg inner membrane. Ten individuals of each developmental stage (i.e. N1/N2, N3, N4/N5, N6, C1, C2/C3, C4, C5 female and C6 female) were pipetted into each quartz test tube and were exposed to different radiation condition for 12 h. After exposure, the animals were kept in the dark for 12 h, and the number of live and dead individuals were counted.

4-(2)-② Effects of UVB on egg hatching success among 8 copepod species

Newly spawned eggs of 8 copepods (i.e. *Acartia omorii, A. sinjiensis, Calanus sinicus, Centropages tenuiremis, Paracalanus* sp., *Pontellopsis tenuicauda, P. yamadae* and *Sinocalanus tenellus*) were introduced into quartz test tubes and exposed at different UVB doses, as described above. Experimental temperatures (18-25°C) varied depending on copepod species.

4-(2)-③ Effects of UVB on feeding and egg production of copepod adult females

Fifteen adult females of *S. tenellus* were introduced into each quartz bottle (volume:

780 ml) containing glassfiber filtered water without phytoplankton food, and were exposed to different radiation conditions for 12 h. Then, the culture of *T. weissflogii* was added and animals were fed for 1 h in the dark. A sample consisted of 4 copepods was extracted in a small plastic tube containing 6 ml DMF, and its gut pigment contents (*i.e* chlorophyll *a* and pheopigments) were determined using a Tuner Design fluorometer.

Five adult females of *S. tenellus* with dark oocytes in the oviducts were transferred into each quartz bottle containing the food medium. After the exposure to different radiation condition for 12 h, they were transferred individually into glass crystallizing dishes containing the same food suspension under a 12L (without UVB):12D light periodicity for 3 days. Every day the number of eggs produced was counted and adult females were transferred into fresh food suspension.

4-(2)-④ Effects of UVB on growth of a tintinnid ciliate

Stock culture of a tintinnid *Favella taraikaensis*, which had been isolated from Hiroshima Bay, was maintained in the laboratory by fed with cultured dinoflagellates *Prorocentrum minimum* and *Heterocapsa triquetra* at 20°C and 12L:12D photoperiodicity. Ten or 100 individuals from the stock culture were introduced into quartz bottles containing the same food medium, and 3 bottles were placed under each radiation condition. The number of individuals were counted periodically by taking aliquots from the culturing bottles.

4-(3) Results and discussion

4-(3)-① Effects of UVB on egg hatching success and survival of various developmental stages of a copepod

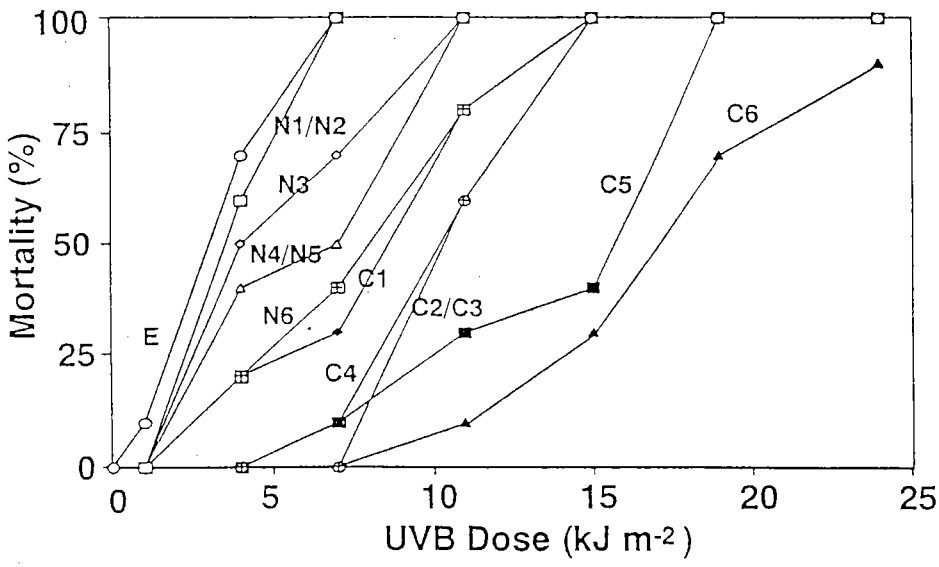

Fig. 2. Mortality of different developmental stages of a brackish-water copepod *Sinocalanus tenellus* exposed at different UVB doses.

Eggs incubated in the dark and those exposed to combined UVA+PAR and PAR alone exhibited 100% hatching success, whereas eggs exposed to radiation containing UVB showed decreased hatching success with increasing UVB. The hatching success abruptly decreased from 90% at UVB dose of 1.0 kJ m^{-2} to 30% at 4.0 kJ m^{-2} and no hatching occurred at 7.0 kJ m^{-2} (Fig. 2). The mortality of the each developmental stage also increased with increasing UVB, and younger stages were more susceptible to UVB than older stages (Fig. 2). These results show that the effects of UVB is most critical for eggs and young nauplii among life stages of copepods.

4-(3)-② Effects of UVB on egg hatching success among 8 copepod species

In all species, the hatching success decreased with increasing UVB, whereas neustonic copepods such as *P. tenuiremis* and *P. yamadae* were much stronger than the rest which are largely water-column dwelling species (Fig. 3). Previous studies[15] showed that some neustonic species contained much higher concentrations of micospolin-like amino acids and carotenoids, which act as sun-screen and quencher of activated oxygen, respectively. These compounds may also be contained at higher amount in the egg of *P. tenuiremis* and *P. yamadae*.

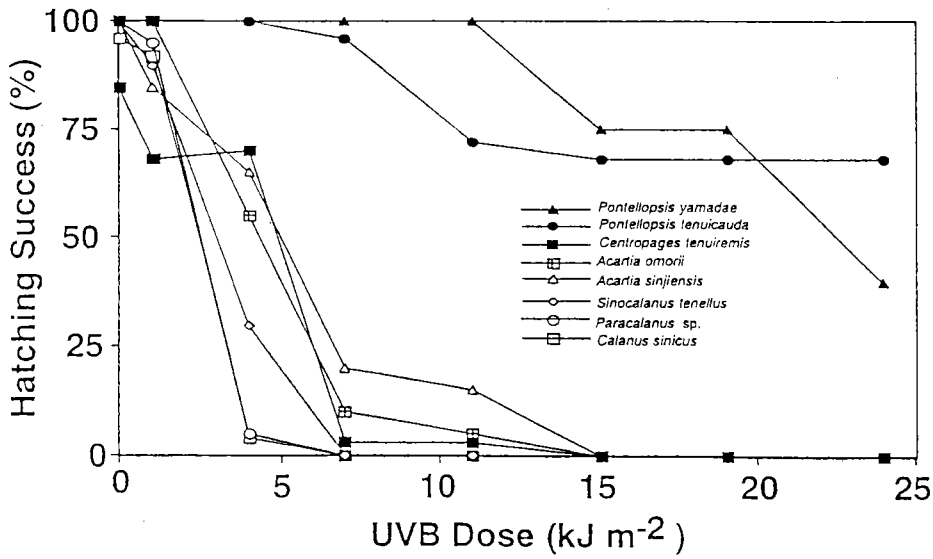

Fig. 3. Species-specific difference in susceptibility to UVB based on hatching success among 8 copepod species.

4-(3)-③ Effects of UVB on feeding and egg production of copepod adult females

Females incubated in the dark, exposed only to PAR and exposed to combined UVA+PAR contained mean gut pigments of 11.8, 10.7 and 10.2 ng $indiv.^{-1}$, respectively (Fig. 4). The mean gut pigments when irradiated at UVB doses of 1.0, 4.0 and 7.0 kJ m^{-2} were 9.7, 5.4 and 7.0 ng $indiv.^{-1}$, respectively, showing no significant difference from those without UVB. At 11.0 kJ m^{-2}, the gut content was reduced to 1.6 ng $indiv.^{-1}$, which was significantly

lower than those without UVB.

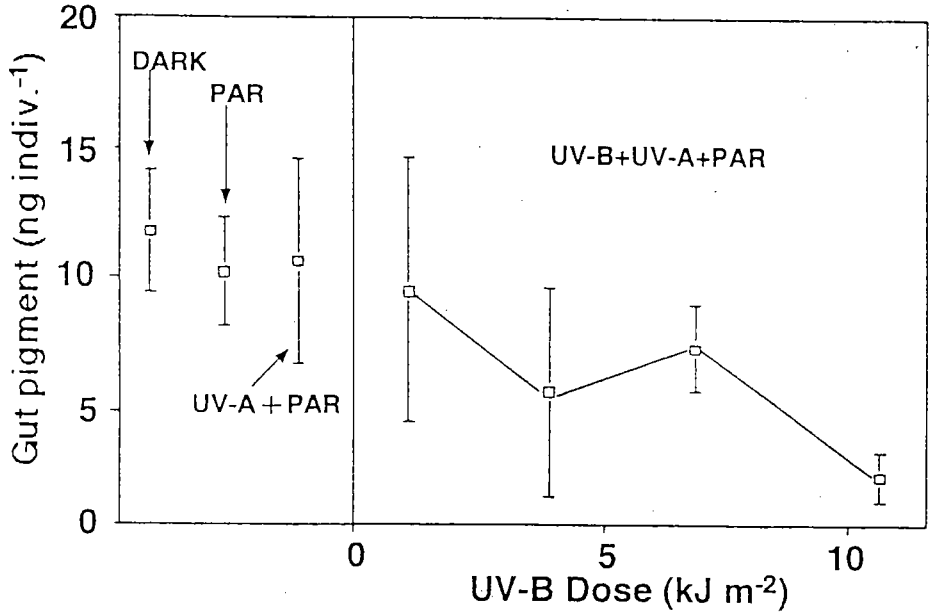

Fig. 4. Gut pigment contents of adult females of a brackish-water copepod *Sinocalanus tenellus* exposed at different radiation condition.

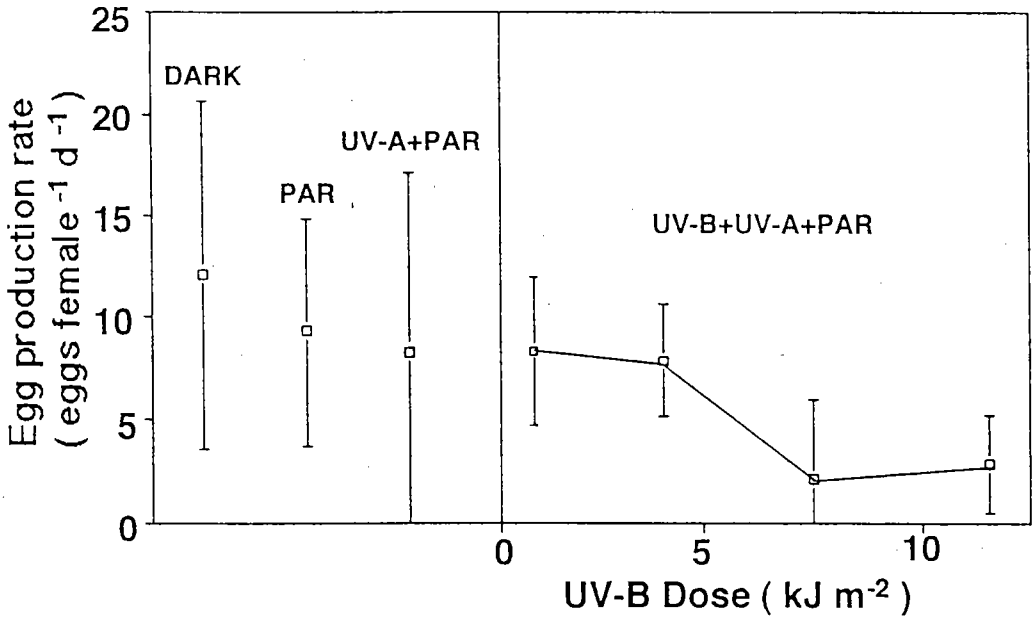

Fig. 5. Egg production rate of adult females of a brackish-water copepod *Sinocalanus tenellus* exposed at different radiation condition.

Egg production rate was not significantly reduced at UVB doses of 1.0 and 4.0 kJ m^{-2} (mean: 7.9 and 7.3 eggs female^{-1} d^{-1}, respectively) compared to those without UVB (mean: 7.2, 6.4 and 8.3 eggs female^{-1} d^{-1}, in dark, PAR and UVA+PAR, respectively) (Fig. 5). However, it was significantly reduced when exposed to 7.0 and 11.0 kJ m^{-2} (mean: 2.2 and 2.9 eggs female^{-1} d^{-1}, respectively).

From these results, UVB irradiance may cause feeding and reproduction disorders in adult copepods. Such case is not likely in the ocean since adult copepod can escape from the surface irradiated layer, but conceivable in shallow water body, such as fresh-water ponds and tide pools.

4-(3)-④ Effects of UVB on growth of a tintinnid ciliate

The abundance of *F. taraikaensis* increased exponentially throughout the experimental period of 9-11 days when incubated at daily UVB doses <0.43 kJ m^{-2} (Fig. 6). At 0.61 and 0.86 kJ m^{-2}, however, such an exponential growth was obtained only during the former few days, and thereafter the density declined. This tintinnid species could not reproduce at 1.1 and 1.5 kJ m^{-2}. The specific growth rate is plotted against daily UVB dose in Fig. 7.

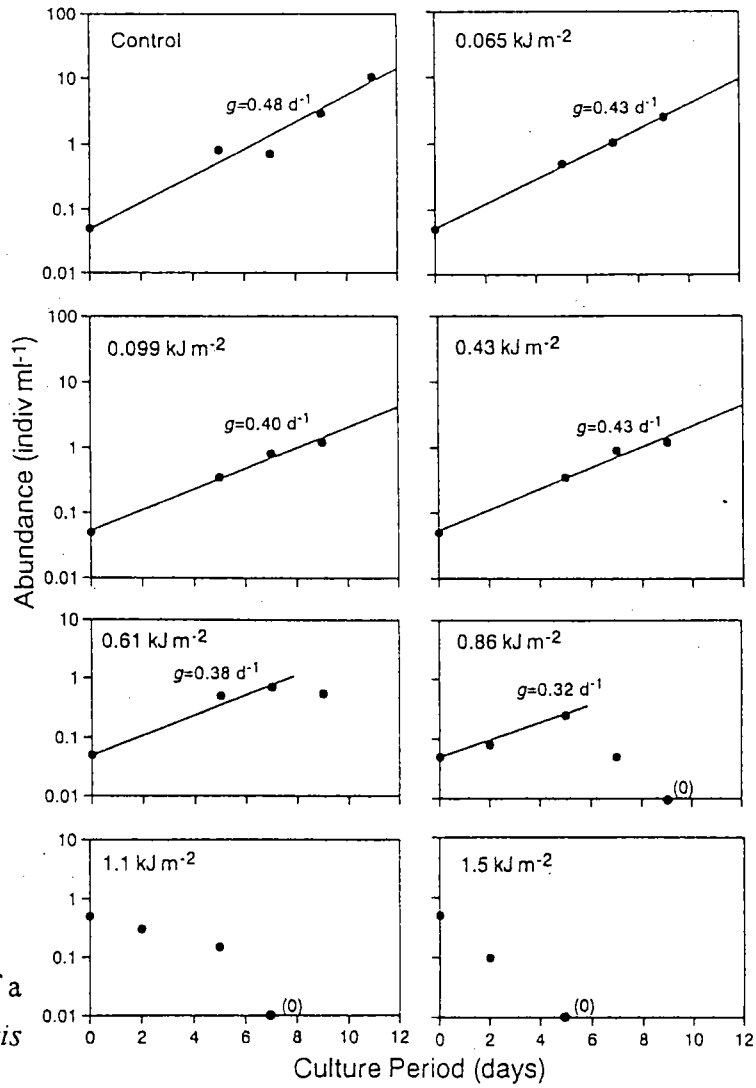

Fig. 6. The population growth of a tintinnid ciliate *Favella taraikaensis* exposed at different UVB doses.

Diet phytoplankton (i.e. *P. minimum* and *H. triquetra*) did not show any deleterious effect by UVB in this experiment, indicating that this protozoan microzooplankton is much

more sensitive than phytoplankton. This further suggests that under certain UVB irradiated conditions the coupling between phytoplankton and microzooplankton is lessened, leading to inefficient energy transfer from primary production to zooplankton secondary production.

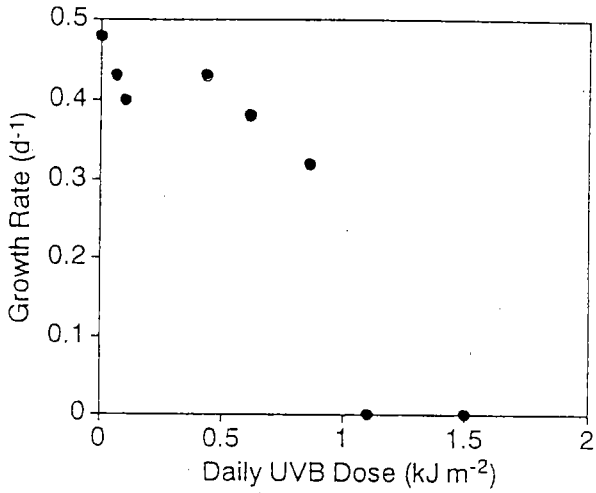

Fig. 7. Relationship between specific growth rate of a tintinnid ciliate *Favella taraikaensis* and daily UVB dose.

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