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Impact of UV Radiation and Elevated Temperature on Growth of Phytoplanktons, *P. micans*, and *S. costatum*

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The growth of two phytoplanktons was studied in a natural environment and in the laboratory under artificial radiation conditions in the presence or absence of UV radiation. The effect of an elevated temperature on the two phytoplanktons was also examined. UV radiation resulted in a decrease in the growth of the two phytoplanktons ; *P. micans* was more affected by UV than *S. costatum*. Four hours of UV radiation decreased the motility of *S. costatum and P. micans* by 20 % and 40 %, respectively. Accordingly, an elevated temperature and UV radiation decreased the growth rate of the two phytoplanktons investigated.

Key words : growth of phytoplankton, UV radiation, elevated temperature

1. Introduction

Many oceanographers and limnologists have made significant progress in the quantitative modeling of balanced phytoplankton growth. These models have a common basis in depicting phytoplankton growth in terms of mass and energy fluxes[1] that describe light absorption and carbon assimilation for a cell with a specified light-harvesting composition. Photoadaptation kinetics are typically modeled with empirically determined first-order rate constants, although expressions other than a first-order rate equation may be more appropriate[2]. Atmospheric ozone is a strong selective absorber of UV radiation. The shortest and most damaging UV wavelengths, UVC(200~280 nm), are absorbed strongly by the atmosphere so that only negligible amounts reach the earth's surface. UVB(280~320 nm) is also extremely injurious to organisms and this radiation increases most significantly when the stratospheric ozone is reduced. The longest wavelengths of UVA(320~400 nm) radiation are known to induce both photodamage and photoreactivation processes in living cells[3], and this radiation is relatively unaffected by variations in stratospheric ozone concentrations. Photosynthetically available radiation(PAR, 400~700 nm), like UVA, is nearly independent of the ozone concentration. Consequently, stratospheric ozone depletion leads to a dramatic increase in damaging UVB irradiance, whereas the corresponding irradiance in the UVA remains relatively constant.

Recent field studies have shown that an increase in UVB radiation is associated with a decrease in phytoplankton productivity[4]. UVB radiation affects different biological processes including photosynthesis, nitrogen metabolism, growth rate, motility, and phytoplankton orientation[5-7]. Milot-Roy and Vincent demonstrated that natural phytoplankton assemblages in a subarctic lake are moderately inhibited by UV radiation, with UVB radiation accounting for approximately 30 % of the total inhibition by UV radiation[8].

Phytoplankton includes a number of photoreactivation and photoprotective strategies to partially compensate for the photoinhibitory effects of different wavelengths of light[9]. The above strategies can be examined by determining (i) the impact

of UV radiation on the growth potential of phytoplanktons ; (ii) the potential lethality of UVB radiation on phytoplanktons ; (iii) the pres-ence of UV photoprotectants in a water column ; and (iv) the distribution of UVA and PAR-photoprotective carotenoids in phytoplankton communities. In this study, the impact of UV radiation and elevated temperatures on the life of phytoplanktons was investigated and reported.

2. Materials and Methods

2.1. Organisms and culture conditions

Samples of seawater were taken from near the middle of the sound with a 5 l bottle equipped with a teflon-covered spring. The samples for the experimental incubations in the temperature-controlled water baths were taken at a 5 m depth. The marine phytoplanktons were then isolated and the cells were grown in a f/2 medium in 100 ml Erlenmeyer flasks[10]. Glass flasks were used for the controls, whereas quartz glass was used for the treatments with UV radiation. The cells were grown in growth rooms at 19°C with a 16h-8h light-dark cycle. The control cultures were grown in white light with an irradiance of 19 Wm^{-2} (400-700 nm). The Erlenmeyer flasks were placed at a 45° angle to the light.

2.2. Determination of growth

The organisms used for the determination of the specific growth rate were cultured for 7 days. Cell counts were taken at the beginning and after 7 days using a haematocytometer. Based on the initial number of cells(N_o) and the number of cells at the end of the period(N), the growth rate(v) was calculated by the formula $v=k \times \log(N/N_o)/t$, where t is the time in days and k=3.3222. For the growth curves, the cells were counted every day and the exponential growth was observed from the beginning of the control experiments until the tenth day after inoculation. The experiments were replicated at least three times.

2.3. Measurement of motility

The determinations of motility($\mu m\ s^{-1}$) were carried out with organisms grown under different light conditions(control and UV radiation). The motility was determined using an automatic computer-controlled video analysis system. The program was written by D.P. Hader et al. using various methods for cell detection and tracking[11]. An IR-sensitive video camera was mounted over a microscope and the built-in lamp was used in combination with an IR transmitting cut-off filter to produce radiation. The organisms were introduced into a flat glass cuvette and the cuvette was then placed under a microscope. The cell motility was calculated based on tests of 1000 organisms and replicated at least three times, as a result, the motility patterns of two phytoplanktons were established.

2.4. Artificial light conditions

Control PAR(Visible, 400-700nm, 19 Wm^{-2}) light was produced by four 400W halogen lamps. The treatment conditions consisted of a control, plus 1, 2, and 4 h of UV radiation per day from one or two 40W sunlamps. Cellulose acetate film was preburnt for 48 h at a distance of 1 m from the UV lamps to minimize any change in its filter properties.

2.5. Natural sunlight experiments

The planktons were placed in cuvettes directly from the culture room thereby avoiding any transmission of UV radiation. The cuvettes were then cooled with water ; the temperature in the cuvettes was maintained at 17~20 °C. Next, the glass or quartz bottles were placed on a holder(maximum, 8 bottles) at 90° to the water surface for 4 h. The holders were kept at 35 and 120 cm below the water surface.

2.6. Effect of elevated temperature and UV radiation

The effect of an elevated temperature and UV radiation on the cultures of two phytoplanktons was tested. The cultures not exposed to UV radiation were subsequently split into two new cultures, one that remained unexposed to UV and the other that was exposed to UV as a semicontinuous culture. The temperatures of these cultures were then increased from 19 to 35 °C, and the effect of the elevated temperature on the two phytoplanktons

was examined.

3. Results and Discussion

The organisms used for the determination of the specific growth rate were cultured for 7 days. For the growth curves, the cells were counted every day and exponential growth was observed from the beginning of the control experiments through until the tenth day after inoculation. The specific growth rate represents the number of population doublings per day. The experiments were replicated at least three times.

The natural and artificial PAR and UV radiation in the air were measured. The spectral response curve for the UV sensor is shown in Fig. 1. The UV values from different depths were not adjusted for the transmission differences in the water with various wavelengths or the angle at which the light penetrated the water. Accordingly, the UV values from the underwater measurements should be considered as semiquantitative.

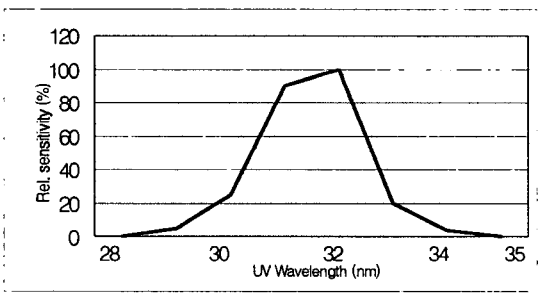

Fig. 1. Spectral response curve for broad-band UV sensor when scanned from 280 to 350 nm.

The effect of UV radiation on the growth rate was studied over 7 days in the laboratory under PAR and 2h of UV radiation per day. This resulted in a decrease in the growth rate for both species(Fig. 2). The 2 h UV radiation was used to simulate natural light conditions when UV irradiance is highest during the 4 h around noon. With 4 h of artificial UV radiation over 7 days, there was no phytoplankton growth. Four hours of UV radiation from UV lamps decreased the motility of S. costatum and P. micans by 20% and 40%, respectively(Table 1, Fig. 3).

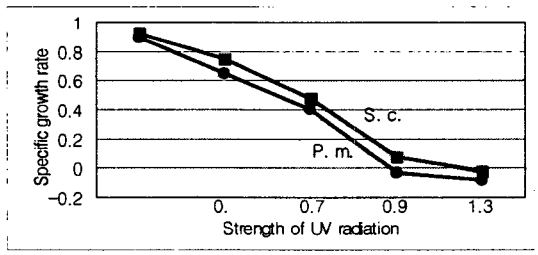

Fig. 2. Growth rate(doublings per day) with daily UV radiation for 1 week corresponding to UV radiation of 0.5, 0.7, 0.9, and 1.3 $kJm^{-2}day^{-1}$.

Table 1. Effect of 4h of artificial UV radiation(2.6 $kJm^{-2}day^{-1}$ UV) on the motility($\mu m\ s^{-1}$) of two phytoplanktons.

laboratory (organism)	control	UV (time)	not inhibited (%)
S. costatum	220±14	198±7 (1h)	90
		194±5 (2h)	88
		180±6 (3h)	82
		176±4 (4h)	80
P. micans	140±9	118±8 (1h)	84
		105±7 (2h)	75
		101±5 (3h)	72
		84±4 (4h)	60

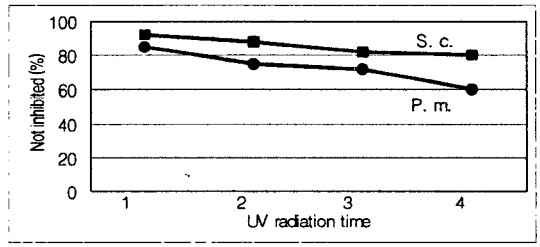

Fig. 3. Effect of UV radiation on the motility($\mu m\ s^{-1}$) of two phytoplanktons based on Table 1 data.

The effect of elevated temperature and UV radiation on the cultures of two phytoplanktons was tested and the results are shown in Fig. 4. The higher temperature(35 °C) and UV radiation treatments resulted in a decrease in the growth rate of *S. costatum* and *P. micans by* 0.3 and 0.25, respectively. When compared with the results from shorter term experiments, the combined findings

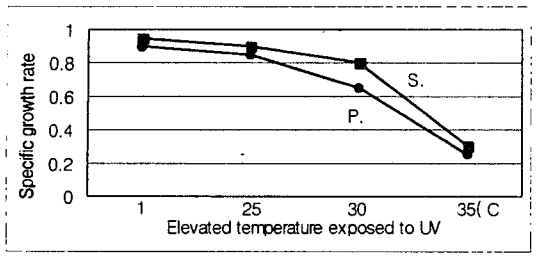

Fig. 4. Effect of elevated temperature and UV radiation on the growth of two phytoplanktons. The culture was exposed to UV radiation and the temperature was increased from 19 to 35 °C.

strongly suggest that (i) the UVB inhibition of surface samples seems to be greatest during the morning hours and is mitigated by photoinduced photoprotective mechanisms during the day ; and (ii) the UVA photoregulation of UVB photoprotective mechanisms may significantly control the UVB inhibition pattern of primary production. The difference in response between the field and laboratory conditions indicate that UV radiation and PAR are both important when studying the effects of UV radiation. Accordingly, the environmental effects that can affect the growth rate of an organism include the temperature difference between the field and laboratory experiments. In the present work, the laboratory and field temperatures were 19～20 °C and 16～17 °C, respectively. Short exposure to high levels of UV resulted in more damage to phytoplankton growth and a stronger inhibition of photosynthesis than longer exposure times at a lower irradiance[12]. The difficulties in simulating natural light regimes require the use of a proper weighting factor that can accommodate the wavelength dependence of the biological action[13]. Generally, the shorter the wavelength, the more effective the radiation.

The findings that tolerance and exposure to solar UV are approximately equal confirm that solar UV is a significant ecological factor, thereby suggesting that the capacity for resistance that can cope with altered solar UV exposure is seriously limited without physiological or behavioral modification. Doubtless most organisms could adapt if the solar UV were to increase, either through increased pigmentation, a repair capacity, or avoidance. However, such physiological and behavioral adaptation to solar UV would reduce the resources available for other purposes. Future studies concerning the overall impact of increased UV radiation in the ecosystem should consider the inhibition of primary production in short- and long-term experiments, plus potential mechanisms for protection and adaptation in phytoplankton species.

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