

Photobehaviors of the calanoid copepod *Calanus sinicus* from the Yellow Sea to visible and UV-B radiation as a function of wavelength and intensity*

TAO Zhencheng^{1, 2, 5}, WANG Yanqing^{5, 6}, WANG Junjian^{1, 3}, LIU Mengtan^{1, 4, 5, **},
ZHANG Wuchang^{1, 2, 5, **}

¹ Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

² Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, China

³ University of Chinese Academy of Sciences, Beijing 100049, China

⁴ Jiaozhou Bay Marine Ecosystem Research Station, Chinese Academy of Sciences, Qingdao 266071, China

⁵ Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao 266071, China

⁶ Department of Engineering and Technology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

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Abstract The effects of wavelength-specific visible light, white light, and ultraviolet-B (UV-B, 280–315 nm) on selected behaviors, grazing rate, spawning rate, and hatching rate of the marine copepod *Calanus sinicus* collected from the Yellow Sea were studied. *Calanus sinicus* placed in a partitioned experimental system responded positively phototaxis to blue-cyan and yellow light but negatively to orange light and UV-B. No obvious dodge activity was found among *C. sinicus* irradiated with <0.005 mW/cm² UV-B. Under 0.20, 0.30 and 0.50 mW/cm² UV-B radiation, the lethal half times of individuals were 30.47, 2.86, and 1.96 h, respectively. Grazing of *C. sinicus* was restrained at >0.10 mW/cm² UV-B, whereas yellow-red light stimulated grazing. Egg production rate was highest at a white-light intensity of 1.58 mW/cm², with an average rate of 10.04 eggs/(female·d). These results are consistent with the observed phenomenon that *C. sinicus* in the Yellow Sea mostly spawn near dawn. Our results indicate that light intensity and spectrum are important factors affecting the diel vertical migration of *C. sinicus* under natural conditions in the Yellow Sea.

Keyword: *Calanus sinicus*; light; phototaxis; grazing; reproduction; ultraviolet

1 INTRODUCTION

Marine zooplankton live in a continuously changing environment. Light is one of the most influential environmental factors for marine zooplankton. The change of light consists of two aspects: intensity and spectrum. Many planktonic species exhibit significant diel vertical migration in the seawater column, a phenomenon mainly regulated by light (Ewald, 1910; Ringelberg, 1964, 1999; Forward, 1988; Tao et al., 2004). Diurnal solar irradiation is a fundamental ecological factor in the marine ecosystem, playing a key role in controlling thermal stratification and water-column mixing, thereby affecting the entire marine food web. Thus, at

the global scale, many marine processes are influenced by sunlight, such as the marine carbon and biogenic dimethylsulfide cycles (Sunda and Huntsman, 1997; Farquhar and Roderick, 2003; Vallina and Simo, 2007). The spectral composition of sunlight at the earth's surface consists mainly of visible light and ultraviolet A, B, and C (UV-A, UV-B and UV-C).

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** Corresponding authors: wuchangzhang@qdio.ac.cn; liumengtan@qdio.ac.cn

Among the ultraviolet wavelengths, UV-B has the greatest impact on marine zooplankton. Jerlov (1950) confirmed the potential hazard of ultraviolet radiation to marine organisms after measuring UV-B irradiation to depths of 20 m in the eastern Mediterranean Sea. In the past decades, several studies have shown that a decrease in stratospheric ozone allows an increased flux of UV-B radiation to reach the earth's surface (Madronich et al., 1995). Moreover, numerous studies have reported that environmental pollution causes serious damage to the ozone layer, as a considerable amount of UV-B radiation increasingly reaches the Earth's surface, especially at middle latitudes (Smith et al., 1992; Kerr and McElroy, 1993). Increases in UV radiation can directly affect the behavior of zooplankton, thereby indirectly affecting the marine food web (Cullen and Neale, 1994; Karanas et al., 1979; Araseth and Schram, 1999; Mostajir et al., 1999; Speckmann et al., 2000; Piazena et al., 2002).

The planktonic copepod *C. sinicus* Brodsky 1962 is widely distributed in margins of the western North Pacific from Japan to Vietnam (Chen, 1964; Anon, 1977; Kidachi, 1979a, b; Huang et al., 1993). It is the dominant and key zooplankton species in the Yellow Sea and the East China Sea, and it was chosen as the target species in the China-Global Ocean Ecosystem Dynamics research program (Sun et al., 2002). Moreover, *C. sinicus* is generally regarded as one of the most important zooplankton species in shelf waters by virtue of its enormous abundance, large body size, and significant role in the conversion of primary production to higher trophic levels (Lin and Li, 1986; Uye et al., 1986). The mature female *C. sinicus* can be found throughout the year in the Yellow Sea, but its population reproduction and recruitment are mainly in spring. Its distribution pattern, life cycle and population dynamics are in relation to many physical environments (Wang et al., 2003, 2009; Zhang et al., 2005). Significant activities of this species, such as its diel vertical migration (DVM) and tendencies to graze at night and lay eggs near dawn, are presumably related with the diel light cycle.

We conducted laboratory experiments to study the behavioral responses of *C. sinicus* exposed to artificially generated, wavelength-specific visible light and ultraviolet light (UV-B, 280–315 nm). The aim of the study was to characterize the photobehavior of *C. sinicus*, specifically phototaxis, UV-B-induced mortality, and the influence of light on its grazing and reproduction activities.

2 MATERIAL AND METHOD

2.1 Sampling and culture

The zooplankton used in the experiments was collected in the Yellow Sea, using a conical macrozooplankton net (mesh size 500 μm , diameter 80 cm), towed vertically, from 2 m above the bottom and up to the surface, at a rate of about 0.8 m/s. The mature female *C. sinicus* for the egg production experiment were collected during May 2004 in Jiaozhou Bay in the Yellow Sea (120.25°E 36.10°N, depth: 18 m). Other experiments were conducted from December 2003 to April 2004 in the Yellow Sea (123°E 35°N, depth: 70 m). The sampling contents of the cod end were transported to the laboratory in 10-L plastic buckets filled with pretreated seawater (10–15°C, which was the in situ seawater temperature). Actively swimming and visually healthy adult *C. sinicus* were identified and picked out under a stereo zoom microscope (Nikon SMZ-745T, Japan). Before the phototaxis and other incubation experiments, the selected *C. sinicus* were kept in the dark for less than 1 h with an aim to minimize stress. Meanwhile, the preliminary tasks of the experiments will be completed during this period.

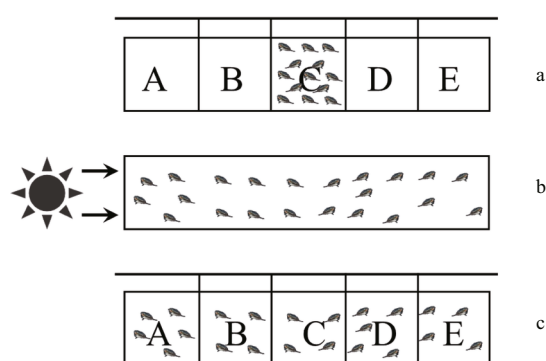
2.2 Photosensitivity of *Calanus sinicus*

Visible light was generated using a metal halide fiber illuminator (Mejiro Precision BMH-250, Japan), which can produce spots of uniform light intensity by transferring light to a compound lens via the optical fiber. The intensity of ultraviolet in the light spectrum produced by this device is very low, and the waveband of visible light in the spectrum is close to that of natural sunlight. The combination of a narrow-band filter and compound lens gives rise to the monochromatic light of different wavebands; hence, with the half-width of the filter being less than 10 nm, seven wave bands were selected (Table 1).

Thirty adult *C. sinicus* were placed into partitioned area C of the experimental device shown in Fig. 1. The individuals were allowed 5 min to adapt to dark conditions, to eliminate external interference on their phototaxis (Fig. 1a). Next, the horizontal partition plate was removed and the *C. sinicus* were irradiated (from the left side of the device) with either visible light or ultraviolet rays of various wavelengths and intensities (Fig. 1b). After irradiation for 3 min, the partition plate was reinstalled and the light source halted. The numbers of *C. sinicus* in each partition

Table 1 The light spectra and intensities of wavelength-specific visible light in phototaxis experiments

Color	Spectrum (nm)	Intensity (mW/cm ²)					
		Lowest	Lower	Low	Normal	High	Higher
Red	649.0	0.009	0.024	0.039	0.053	0.065	0.086
Orange	594.9	0.058	0.136	0.225	0.294	0.364	0.489
Yellow	576.8	0.136	0.331	0.533	0.693	0.848	1.155
Green	545.8	0.108	0.258	0.401	0.527	0.643	0.900
Cyan	509.2	0.009	0.022	0.036	0.048	0.059	0.080
Blue	488.6	0.018	0.044	0.070	0.092	0.114	0.155
Purple	415.3	0.003	0.008	0.012	0.015	0.019	0.026
White	All spectra	1.092	2.908	4.721	6.160	7.633	10.502

**Fig.1** The equipment for phototaxis of *C. sinicus*

a. dark condition for 5 min; b. open illuminant and take off the clapboard; c. turn off the illuminant and put on the clapboard.

area were then counted (Fig.1c). As a control, the entire procedure was repeated using another 30 individuals of *C. sinicus* and all dark conditions in the same device. Each individual *C. sinicus* was used only once in the experiment.

Six different intensities were projected for each waveband (Table 1). It was expected that the phototaxis would cause the *C. sinicus* to move toward the partitioned area nearest the light source or to the partitioned areas separated from the light source by two partitions. Photosensitivity was determined by measuring the phototactic response of the copepods. Phototaxis is defined as directional in relation to a directional light source: a positive response is toward the light source, while movement away is negative (Stearns and Forward, 1984). Therefore, the phototaxis of *C. sinicus* in the partitioned area nearest the light source was deemed positive phototaxis, and that in the partition farthest from the light source was deemed negative phototaxis. Phototaxis ratio (PR) was expressed as the ratio (%) of the number of *C. sinicus* in the partitioned area nearest or farthest from the light source versus the total number.

Phototaxis index (PI) was expressed as the positive phototaxis ratio (PR_p) minus the negative phototaxis ratio (PR_n), namely $PI = PR_p - PR_n$. The phototaxis was divided into five grades based on the determination of the PI, as follows: -1.0 to -0.6, strong negative phototaxis; -0.6 to -0.2, weak negative phototaxis; -0.2 to 0.2, no phototaxis; 0.2 to 0.6, weak positive phototaxis; and 0.6–1.0, strong positive phototaxis. The waveband with the least light intensity was considered to be the one to which the *C. sinicus* were the most sensitive under conditions of the same PI.

We conducted exposure experiments using laboratory-generated ultraviolet light (UV-B, 280–315 nm) to evaluate the phototaxis and tolerance of *C. sinicus* to UV-B. The lethal time of different UV-B intensities on *C. sinicus* was determined using a UV fiber optical transmission system (Mejiro Precision CHG-200, Japan).

2.3 Light-induced grazing behavior

For this experiment, zooplankton in the laboratory was reared on a mixed algal diet consisting of three kinds of algae (*Skeletonema costatum*, *Platymonas subordiformis*, and *Phaeodactylum tricornutum*) at the ratio 1:1:1 in terms of carbon concentrations. The algal mix, which grew well after being cultured for 4–6 days, was fed to the *C. sinicus* at a final diet concentration of 0.42–1.25 µg C/mL, which has been previously determined to satisfy the needs of *C. sinicus* (Frost, 1972; Uye, 1986).

Thirty adult *C. sinicus* were irradiated with different intensities of wavelength-specific visible light and UV-B, for periods of 20 min. After switching off the given light source, the fecal pellets of the copepods were then counted under a Nikon microscope. As controls, the same procedure was repeated using another 30 *C. sinicus* in dark

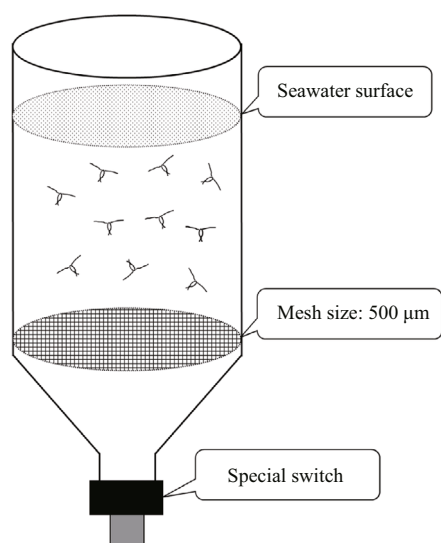


Fig.2 The egg production equipment of *C. sinicus*

conditions. The intensities of the red (649 nm), orange (594.9 nm), yellow (576.8 nm), green (545.8 nm), cyan (509.2 nm), blue (488.6 nm) and purple (415.3 nm) light were 0.121, 0.668, 1.475, 1.375, 0.116, 0.223, and 0.038 mW/cm², respectively. These experiments were done in triplicate for each intensity of light wavelength, and the results of each experiment were expressed as the mean of the three measurements. The number of fecal pellets produced by copepods under the different light intensities reflected the influence of light in different wavebands on the ingestion of *C. sinicus*. Fecal pellet production rate (FPPR) indicated the mean amount of feces discharged of one individual *C. sinicus* within a unit of time.

2.4 Effects of light on spawning and hatching

The zooplankton spawning device used in this experiment is a patented device developed by our laboratory; the device can effectively prevent adult zooplankton from consuming the eggs they spawn (Fig.2). Sexually mature female *C. sinicus* were chosen and then cultured in dark conditions for 48 h so that they could adapt to the environment. Five female *C. sinicus* were placed into each spawning bottle; an experimental group contained five bottles; in advance, 200 mL of filtered seawater was prepared to fill each bottle. The bottles were irradiated by different intensity white light for a period of nine continuous days in thermostat incubators. Every 24 h, eggs of the *C. sinicus* were removed from each bottle along with a portion of the seawater, via a special switch in the bottom of the device, and then the bottles were supplemented with seawater of the same volume,

temperature, and concentration of algal diet. The diet mixture of three kinds of algae aimed to eliminate the influence of the algal type on the copepods' egg production and hatching rates (Li et al., 2006). The dietary algae concentration was $1.02 \pm 0.1 \mu\text{g C/mL}$. The entire duration of the spawning experiment and hatching experiment were performed in thermostat incubators, and the experimental water temperature was the same as that of the seawater from which individual *C. sinicus* was originally collected, and thus maintained at $13.6 \pm 0.5^\circ\text{C}$.

Eggs in each experimental group were counted under the StereoZoom microscope to calculate the daily egg production rate (EPR) of *C. sinicus* exposed to different light intensities. The EPR indicates the number of eggs produced by a female *C. sinicus* in one day, presented as the mean value from five parallel experimental groups. Differences in the EPRs reflect the effects of light of different intensities on the spawning behavior of *C. sinicus* (i.e., the EPR is in direct proportion to the suitability of the corresponding light intensity for the spawning of *C. sinicus*).

In the egg production experiment, eggs produced by all the female *C. sinicus* within 24 h were pooled, and then 30 eggs were taken out at random (all eggs were taken in cases where the total number of eggs was less than 30). The eggs were put in a 100-mL beaker containing 80 mL of filtered seawater, to allow hatching of the eggs in the beaker for 48 h under dark and thermostatic conditions (the temperature being the same with that for spawning), respectively. Nauplii and non-hatched eggs were counted under a StereoZoom microscope to calculate the hatching rate, indicated by the ratio (%) of nauplii to all eggs available for potential hatching. Differences in the hatching rates reflect the effect of different light intensities on the hatching behavior of *C. sinicus*. Zhang et al. (2002a) considered that 43.5 h was needed for *C. sinicus* to achieve a maximal hatching rate at 16°C . The hatching duration for *C. sinicus* was determined to be 12 h at 20°C , and 24 h at 14°C (Zhang et al., 2002b). In the current experiment, the temperature was $13.6 \pm 0.5^\circ\text{C}$, and therefore 48 h was deemed adequate for hatching out spawns of *C. sinicus*.

3 RESULT

3.1 Phototaxis of *Calanus sinicus* under different light spectra

During the phototaxis experiment, the multiple

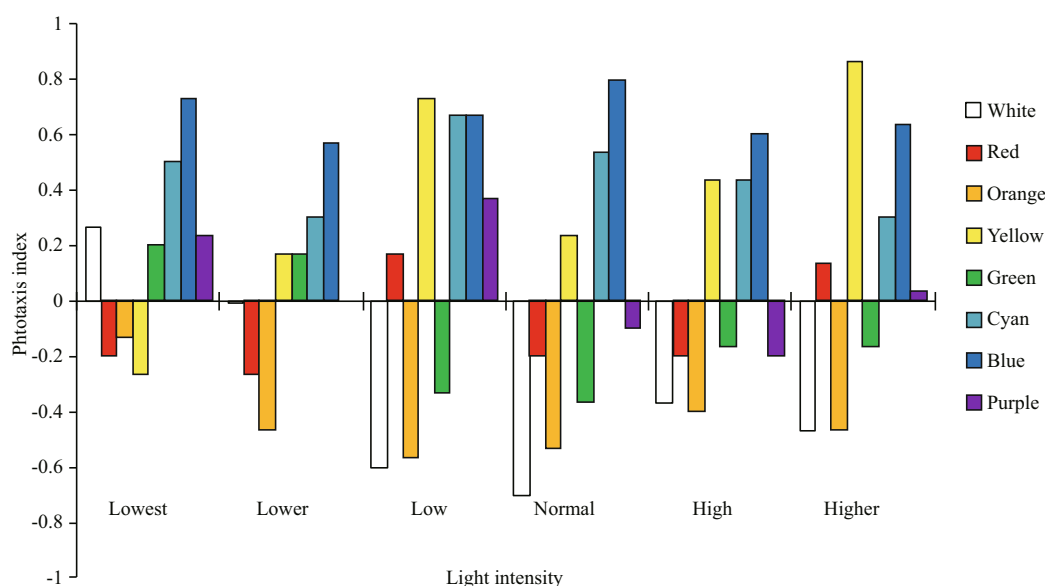


Fig.3 The phototaxis index of *C. sinicus* under the wave-specific visible light of different intensities

The specific light intensity can be checked in Table 1.

control experiments that were performed in dark conditions demonstrated that for *C. sinicus* cultured indoors, the positive and negative phototaxis ratio could be kept at about 40%, hence the positive phototaxis ratio did not differ significantly from the negative phototaxis ratio.

Phototaxis of *C. sinicus* captured in the field and subjected to visible light in the laboratory: The PI of *C. sinicus* was -0.70–-0.37 in the case of white light throughout the spectrum at intensities of 4.72–10.50 mW/cm², manifesting a negative phototaxis. *Calanus sinicus* showed weak positive phototaxis (PI=0.27) to 1.09 mW/cm² white light. The PI was within the range 0.23 to 0.87 in the case of 488.6 nm blue light at intensities of 0.018–0.155 mW/cm², 509.2 nm green light at intensities of 0.009–0.080 mW/cm² and 576.8 nm yellow light at intensities of 0.533–1.155 mW/cm², manifesting a positive phototaxis. In the case of 594.9 nm orange light, the highest PI were -0.13 at the lowest intensity (0.058 mW/cm²) and the other PI was lower than -0.40 at intensities of 0.136–0.489 mW/cm², thus manifesting a negative phototaxis. In the case of 415.3 nm purple light, 545.8 nm green light, and 649.0 nm red light, the PI was mostly between -0.2 and 0.2, and accordingly manifested no phototaxis. According to the average PI of different intensities white light and seven monochromatic light, *C. sinicus* showed positive phototaxis to blue (average PI=0.67), cyan (average PI=0.46) and yellow (average PI=0.36) light, and negative phototaxis to orange (average PI=-0.43) and white (average PI=-0.31) light (Fig.3).

Pre-tests demonstrated that the lowest intensity of UV-B that may be sensed by *C. sinicus* is under 0.016 mW/cm²; therefore, the UV-B intensity was tested at 0.005, 0.010, and 0.016 mW/cm², and three parallel experiments were performed for each waveband and light intensity. *Calanus sinicus* manifested weak negative phototaxis in response to UV-B intensities set at 0.010 mW/cm² and 0.016 mW/cm², with a resultant PI of -0.38 and -0.49, respectively. *Calanus sinicus* showed no apparent phototaxis (PI=-0.18) to the UV-B intensity of 0.005 mW/cm². Together, these results revealed that *C. sinicus* is able to sense the presence of UV-B radiation and avoid its harmful effects through negative phototaxis.

3.2 The lethal effect of UV-B on *Calanus sinicus*

Results of the experiment on the potentially lethal effect of UV-B on *C. sinicus* demonstrated that this species possesses a good defensive response against UV-B intensities of less than 0.10 mW/cm², whereas UV-B intensities higher than those in nature (generally, <0.30 mW/cm²) showed a lethal action on the copepods. The UV-B lethal time (LT₅₀) indicates the time to 50% mortality as a result of the particular UV-B intensity. A lineal regression was adopted to calculate the LT₅₀ of *C. sinicus* exposed to UV-B of different intensities (Fig.4). For the UV-B intensity of 0.020 mW/cm², the equation was $y=0.017x-0.018$, $R^2=0.567$, resulting in LT₅₀=30.47 h. The LT₅₀ of *C. sinicus* was 2.86 and 1.96 h under UV-B intensity of 0.030 and 0.050 mW/cm².

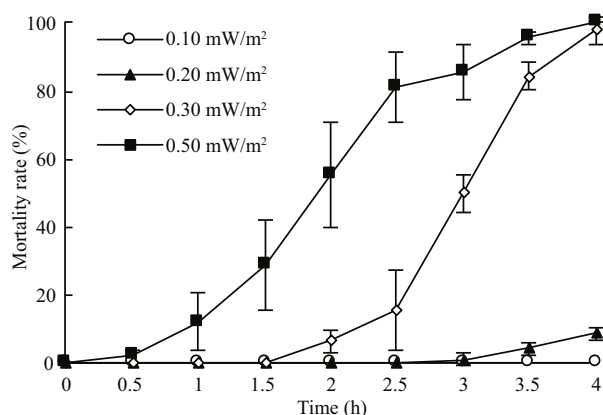


Fig.4 The mortality rates of *C. sinicus* under different intensities UV-B light

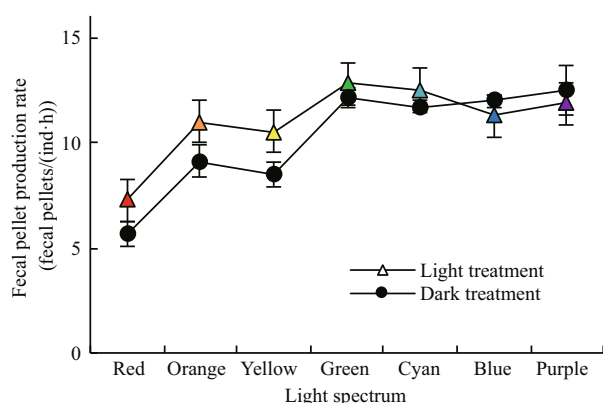


Fig.5 The fecal pellet production rates of *C. sinicus* under wave-specific visible light and dark condition

3.3 Grazing rates of *Calanus sinicus* under visible light and UV-B

For *C. sinicus* irradiated by white light throughout the spectrum with different intensities and for the controls in all dark conditions, the calculated FPPRs were stable, at approximately 6 fecal pellets/(ind.·h), and thus did not significantly differ. Therefore, short durations (20 min) of white-light irradiation throughout the spectrum did not significantly affect the copepods' ingestion.

In the experiments using 415.3, 488.6, 509.2, and 545.8 nm monochromatic light, neither the experimental groups under the different light conditions or the control group under the all dark conditions manifested a high FPPR. The mean FPPR was 12 fecal pellets/(ind.·h) in the case of all monochromatic light types and all intensities, a result attributed to the relatively short culture time. In the experiments using the other five monochromatic light types, the FPPRs of the *C. sinicus* dropped more or less due to the action of the culture time, with mean FPPRs of 5–11 fecal pellets/(ind.·h). In the case of

Table 2 The fecal pellet production rates (FPPR) of *Calanus sinicus* under different intensities UV-B

UV-B intensity (mW/cm ²)	Absolute range of FPPR (fecal pellets/(ind.·h))	Mean FPPR* (fecal pellets/(ind.·h))
0 (control)	3.6–6.6	5.2
0.1	1.5–3.6	2.5
0.2	1.5–3.9	2.3
0.4	1.8–3.0	2.5

*: There was a significant difference between the control treatment and each of the three UV-B treatments ($P < 0.01$).

irradiation with different wavelengths and intensities, the FPPRs of the experimental groups were likewise comparable with that of the controls. The experimental group differed significantly from the control group only in the case of yellow, orange and red light, and the FPPR was relatively stimulated; furthermore, the intensity of yellow light was in direct proportion to the estimated FPPR (Fig.5). These results show that *C. sinicus* ingested slightly but statistically significantly more food under the action the higher intensities of yellow-red light, whereas their ingestion was not significantly affected under the other monochromatic light types.

Irradiation with UV-B at intensities of 0.10, 0.20, and 0.40 mW/cm² significantly inhibited the copepods' ingestion: the highest FPPR in a treatment group was a mere 3.6 fecal pellets/(ind.·h), while the lowest FPPR of a control group was 4.8 fecal pellets/(ind.·h), and the difference was very significant ($P < 0.01$) (Table 2). We suggest that UV-B at the three different intensities did not significantly affect the ingestion of *C. sinicus*.

3.4 Egg production and hatching rates of *Calanus sinicus* under different white-light intensities

In the 9-day spawning experiment, the highest EPRs (the mean value from five parallel experiments) occurred on Day 5, both for the control group in dark conditions and for an experimental group in light conditions. The highest EPR for *C. sinicus* in all dark conditions was 9.89 eggs/(female·d), while the mean EPR in the whole spawning experiment was 4.12 eggs/(female·d). The highest EPR was 20.28 eggs/(female·d) under a white-light intensity of 1.58 mW/cm², while the mean EPR nine days later was 10.04 eggs/(female·d). During the nine days, *C. sinicus* showed the highest single EPR and the highest mean EPR under an intensity of 1.58 mW/cm² (Fig.6). The spawning rate of *C. sinicus* was always higher under light conditions than under dark conditions,

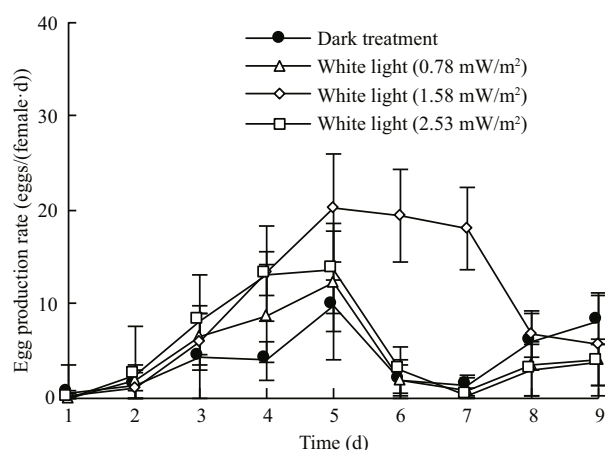


Fig.6 The egg production rates of *C. sinicus* under different intensities of white light and dark condition

demonstrating that irradiation may promote spawning of *C. sinicus*, and indeed that illumination may be essential for *C. sinicus* to spawning.

Spawning of the *C. sinicus* under the different irradiation conditions showed that the hatching rate did not differ significantly among different light conditions within the first three days; furthermore, the hatching rate was relatively high under all conditions, always exceeding 75%. The hatching rates under both light and dark conditions began to change significantly on Day 4; the hatching rate was higher for spawns produced either in dark conditions or under lower light intensities (0.78 and 1.58 mW/cm²) than that produced under high light intensity (2.53 mW/cm²) (Fig.7).

4 DISCUSSION

4.1 Phototaxis and diel vertical migration of *Calanus sinicus*

Ewald (1910) was the first to record that planktonic zooplankton mainly inhabits deeper water during the day and ascend to upper water layers at night. Accordingly, he hypothesized that light affects the diel vertical migration (DVM) of the zooplankton. There are two general hypotheses about how light might regulate the DVM of planktonic zooplankton. The preferendum hypothesis states that this migration of zooplankton occurs as they search for the optimum light intensity (Ewald, 1910; Russell, 1926, 1934; Boden and Kampa, 1967). The relative-stimulus threshold hypothesis proposes that the change of light in the day's changing environment is a signal for zooplankton to determine whether vertical migration should occur (Clarke, 1933; Ringelberg, 1964; Daan

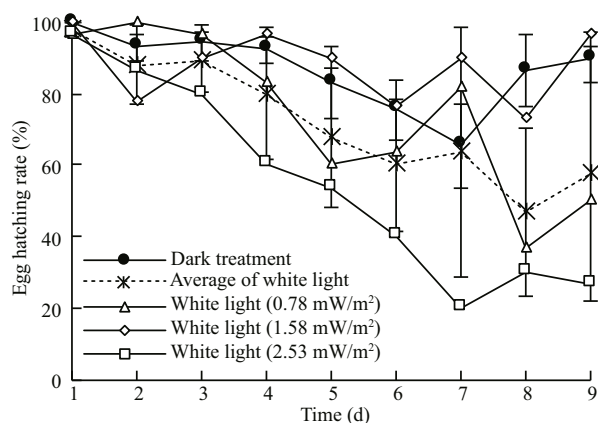


Fig.7 The egg hatching rates of *C. sinicus* under different intensities of white light and dark condition

and Ringelberg, 1969; Buchanan and Haney, 1980; Haney et al., 1990). Ultraviolet radiation is harmful to zooplankton (Klugh, 1929; Dey et al., 1988; Williamson et al., 1994; Chalker-Scott, 1995; Zagarese et al., 1997; Storz and Paul, 1998), thus many researchers have suggested that the tendency of zooplankton to stay in deeper water during the day is a mechanism limiting their exposure to the ultraviolet radiation of sunlight (Williamson et al., 1994; Araseth and Schram, 1999; Speckmann et al., 2000; Leech and Williamson, 2001; Rhode et al., 2001).

As the dominant and key zooplankton species in the Yellow Sea and the East China Sea, *C. sinicus* shows obvious DVM behavior (Uye et al., 1992; Wang et al., 2003; Zuo et al., 2004). Jékely et al. (2008) found that the tiny marine annelid *Platynereis dumerilii* could mediate phototactic swimming using their simple eyespots, with the mechanism of its phototaxis based on molecular tools; this indicated that zooplankton could feel and distinguish light and consequently adjust their behaviors. Stearns and Forward (1984) studied the phototaxis of another marine copepod, *Acartia tonsa*, and found that mature females manifest positive phototaxis in response to light, with a greater degree of phototaxis with increasing light intensities; *A. tonsa* was relatively sensitive to visible light at 453–620 nm, with phototaxis strongest in response to 580 nm visible light. The sensitivity of *A. tonsa* to visible light at wavelengths outside the range of 453–620 nm dropped remarkably; however, *A. tonsa* was still highly sensitive to 380 nm and 700 nm visible light as long as the intensity was strong enough. Sweatt and Forward (1985) found that the planktonic chaetognath *Sagitta hispida* was most sensitive to green-blue light, especially at wavelengths of 500 nm. Smith and

Macagno (1990) found that the relatively large-sized freshwater water flea *Daphnia magna* was fairly sensitive to 434 nm, 525 nm, and 608 nm visible light, and to 348 nm ultraviolet. In our experiments, *C. sinicus* developed noticeable positive phototaxis in response to 488.3 nm and 509.2 nm blue-cyan light, a result which agrees with Forward's (1988) conclusion that zooplankton is most sensitive to blue-cyan light. Wang et al. (2006) found that the absorption coefficients of 488–555 nm light spectra were highest in the Yellow Sea. The *C. sinicus* were relatively sensitive to blue-cyan and yellow light, manifesting relatively strong positive phototaxis, possibly because, as a monochromatic light, blue-cyan light penetrates farthest into the marine waters of its natural region, and thus blue-cyan light may act as a chief signal for the DVM of *C. sinicus*.

4.2 Effects of UV-B on photoresponse of *Calanus sinicus*

In the eastern Mediterranean Sea, at a middle latitude, the intensity of 310 nm ultraviolet decreased by 14% as depth increased by 1 m, and the intensity of 375 nm ultraviolet decreased by 5% (Jerlov, 1950). If the intensity of UV-B was 0.20 mW/cm² at sea level at noon, it would be approximately 0.01 mW/cm² at a depth of 20 m; the latter UV-B intensity may be the degree at which it is sensed by *C. sinicus*. In addition, the UV-B intensity in the morning just as the sun rises is also approximately 10 mW/cm². Therefore, it may be concluded that ultraviolet radiation is an important cause and signal for the DVM of *C. sinicus*. For instance, Rhode et al. (2001) showed that several species of *Daphnia* would descend to deeper water when being irradiated by ultraviolet, again implicating ultraviolet as a primary factor responsible for the DVM of zooplankton. In addition, those authors considered that the DVM was a behavior formed under the synergic action of multiple factors. Schulyer and Sullivan (1997) also suggested that zooplankton can sense ultraviolet. Storz and Paul (1998) found that species of *Daphnia* manifest a negative phototaxis in response to ultraviolet irradiation. Araseth and Schram (1999) demonstrated that the North Atlantic copepod *C. finmarchicus* could sense ultraviolet and also distinguish ultraviolet from visible light, manifesting negative phototaxis in response to ultraviolet. Speckmann et al. (2000) studied the behavior of *Acartiura* spp., *Acanthacartia* spp., and *Tortanus dextrilobatus* under the action of ultraviolet, and reported that early larvae of *T. dextrilobatus* and

C. pallasii were sensitive to ultraviolet and manifested noticeable harm-avoiding behavior by changing their vertical distribution (i.e., they would remain at 50 cm deeper than the normal depth free of ultraviolet); in addition, the mortality of *T. dextrilobatus* increased remarkably when the copepods were fully exposed to ultraviolet. Leech and Williamson (2001) demonstrated that as the surface water was subjected to strong ultraviolet radiation at noon and in the afternoon, most individuals of *Daphnia* would move to lower water layers, but then they would return to the upper layer as soon as the ultraviolet was shielded. Klugh (1929) pointed out that ultraviolet radiation could be lethal to copepods. That conclusion was eventually verified by the indoor experiments of Dey et al. (1988) and in freshwater environments (Williamson et al., 1994). Zagarese et al. (1997) studied the action of ultraviolet on three species of *Boeckella* (*B. brevicaudata*, *B. gibbosa* and *B. gracilipes*), and found that not all of these zooplankton species were necessarily injured or killed by ultraviolet, but only *B. gracilipes* was exceptionally sensitive and fragile to ultraviolet light conditions, while the other two *Boeckella* species possessed a good ultraviolet-defense mechanism.

The current study demonstrated a pronounced lethal action of UV-B on *C. sinicus* when it was artificially generated at higher intensities than found in the natural environment, but that *C. sinicus* appear to possess a good preventive mechanism against UV-B at intensities lower than the natural intensities. However, the intensity of UV-B that might be sensed by *C. sinicus* (~0.010 mW/cm²) was far lower than the intensity that may cause injury to *C. sinicus* (~0.200 mW/cm²), showing that the copepods sensed the presence of UV-B and then escaped it before it could cause injury.

4.3 Grazing behavior of *Calanus sinicus* under different light conditions

According to both field and laboratory experiments, diel grazing is found in many zooplankton species (Zheng and Zheng, 1989; Wang et al., 1998; Strom, 2001). A great number of studies have used feces as an index of the ingestion of zooplankton. Though the FPPR could not be used to calculate the absolute amount of food ingested by the copepods, it might reasonably reflect how ingestion changes along with the condition of light irradiation. Because feces are formed from the ingested feedstuff, the method based on the FPPR should be reliable (Zhang and Wang,

2000).

Zhang and Wang (2000) considered that an individual of *C. sinicus* might discharge 5–10 fecal pellets in an hour when sufficient food is available, an approximation which agrees with the results of the current experiment. In contrast, Karanas et al. (1979) reported that the ingestion rate of common copepods (such as Calanoida) was higher under dark conditions, and dropped with increasing light intensity in indoor light conditions, which disagrees with the results of the current experiment. One explanation may be as follows: first, the *C. sinicus* did not immediately respond to the irradiation conditions while they ingested food, but rather delayed their response, granting that the duration of the current experiment was relatively short; second, the current experiment adopted relatively high intensities of the white light throughout the spectrum. Zheng and Zheng (1989) made continuous observations of the diurnal ingestion behavior of *Calanopia thompsoni* and found a low rate of ingestion during the daytime, as proven by almost empty stomachs, while ingestion tended to increase gradually in the evening and in the small hours of the night. Such ingestion behavior has been similarly observed in other copepods (for example, in *Centropages*). Wang et al. (1998) examined the intestinal contents of copepods and found that as a rule, individual copepods exhibited diurnal ingestion, which was manifested especially in larger-sized zooplankton. The peak of intestinal content occurred from dusk to midnight (18:00–24:00), and the peak intestinal content could be 10 times the minimal intestinal content found in the daytime. This may be because most zooplankton is characterized by DVM and ingest food after dusk and in the early hours of the night.

The experiment on the influence of UV-B on the ingestion of *C. sinicus* showed that high-intensity UV-B manifested a strong inhibiting action on *C. sinicus*. In the present work, we have demonstrated that *C. sinicus* shows negative phototaxis to UV-B even at the low intensity of 0.010 mW/cm²; accordingly, *C. sinicus* appears to possess a good preventive mechanism against UV-B, as demonstrated when continuous irradiation with 0.100 mW/cm² UV-B for 4 h did not cause the death of any individual copepod. Based on this, it may be deduced that within the range of 0.010–0.100 mW/cm² there is likely a critical intensity that ultimately affects the ingestion rate of *C. sinicus*; this possibility offers a good theoretical basis for further studies of the DVM of *C. sinicus*.

4.4 Effects of visible light on the reproduction of *Calanus sinicus*

The spawning and hatching rates of zooplankton are important parameters in marine ecology studies, and numerous investigations of this topic have been made in the past 20 years. Most recent research efforts have emphasized how light indirectly affects the spawning and hatching of zooplankton via direct action on the phytoplankton biomass, as well as the relationship between the light cycle and the formation of diapause spawns and dormant spawns; however, no study to date has investigated the direct effects of light.

Lin and Li (1986) studied the daily egg production of *C. sinicus* and noticed that the species' spawning mode followed a pattern: the *C. sinicus* spawned for six consecutive days, then suspended spawning for four or five days, and then resumed spawning. Zhang et al. (2002a) considered that *C. sinicus* may successively spawn for 35 days under a state of satiation; in addition, *C. sinicus* at peak spawning may spawn for nine consecutive days before an intermission lasting at most two days. The disagreement in the findings of these two studies may be attributable to the different sites where the *C. sinicus* individuals were originally collected. More extensive studies could clarify whether the oögonia of *C. sinicus* grow steadily, and what conditions might affect their growth. The results of the current study are in basic agreement with several previous studies, although differences exist due to differences in regions and temperature. Schulze (1928) conducted the first study of the spawning and hatching of zooplankton under different light conditions and found that the reproductive rate of *Daphnia pulex* dropped under dark conditions, which agrees with our results, but is contrary to the observation that most zooplankton species spawn at night (Uye et al., 1990; Atkinson et al., 1996; Wang et al., 1998). Through a field experiment in the Yellow Sea, Zhang et al. (2002b) found that the spawning of *C. sinicus* showed a noticeable diurnal rhythm: the great majority of *C. sinicus* individuals moved to the upper layers of seawater to spawn from approximately midnight to 03:00–06:00, and most spawns occurred near 06:00, all findings which agree with the results of the current experiments.

Spawns of *C. sinicus* produced under different light conditions did not differ significantly regarding hatching rate in the early stage of the experiment (the

first 3 days), which may constitute a delayed action of the irradiation on the species' spawning. The hatching rate of spawns of *C. sinicus* produced under white light at a high intensity was lower than that of spawns produced under dark conditions or low-light conditions, indicating that high-intensity white light may lower the spawning quality.

5 CONCLUSION

Calanus sinicus distributed in the Yellow Sea showed positive phototaxis to the blue and cyan light but negative phototaxis to orange light and UV-B (≥ 0.01 mW/cm²). High UV-B radiation (≥ 0.20 mW/cm² UV-B) causes a significant lethal effect on the copepod. The yellow-red light stimulates the grazing of *C. sinicus*. The egg production rate of *C. sinicus* was highest under a white-light intensity of 1.58 mW/cm², which are consistent with the observed phenomenon that *C. sinicus* in the Yellow Sea mostly spawn near dawn. Both the light intensity and spectrum are the most important factors influencing the diel vertical migration of *C. sinicus* in the Yellow Sea.

6 DATA AVAILABILITY STATEMENT

The data used in the current study are available from the corresponding author on reasonable request.

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