

Are barnacle larvae able to escape from the threat of UV?

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Abstract Earlier laboratory experiments suggested that environmental levels of UV-B radiation can damage the eyes of barnacle naupliar larvae and impair their phototactic behaviors. However, since barnacle larvae may avoid UV by migrating to deeper waters, it is not known whether such impairment would actually occur under field conditions. For the first time, this study provides both field and laboratory evidences to show that prevailing UV-B in the natural habitat of barnacle larvae could be an important environmental factor affecting natural barnacle populations. We here showed that although barnacle nauplii may avoid UV-B irradiation by downward migration, the amount of UV energy (9.8×10^{-6} J) received by a naupliar eye during downward migration in the natural water column is within the same order of magnitude as the total energy (7.5×10^{-6} J) sufficient to cause damages to naupliar eye and impair their phototactic responses. It is possible that solar UV-B prevailing at shallow waters would pose a similar threat to other zooplankton species over large geographic scale.

Introduction

Despite recent satellite data of NASA showed a slowdown of ozone depletion in the upper stratosphere

(22–28 miles altitude), geophysicists warn that there is still little sign of recovery in ozone levels in the stratosphere and the Antarctic (Newchurch et al. 2003). The international effort in phasing out ozone-destroying substances by 2000–2005 have significantly reduced rising levels of chlorofluorocarbons (CFCs) and halons, which have been used extensively as coolants, Styrofoam insulation, propellants and fire suppressants. However, since CFCs and halons are very stable chemicals with an expected life of over 100 years in the atmosphere (UNEP 1992), recovery of the ozone layer will take many decades (Rex et al. 2004) and is not expected before 2050 (WMO 1999, 2003). Furthermore, an increase in emission of green house gases and nitrogen dioxide has been shown to be the major cause of ozone loss in the Arctic (Bouwman 1998; Waibel et al. 1999). Indeed, many studies confirmed that there is an increasing trend of solar UV-B irradiance globally as a result of loss in stratospheric ozone (UNEP 1992, WMO 2003). Taken together, enhanced global solar UV-B irradiance is expected to continue and pose a significant threat to life on Earth in the current century.

Most zooplanktons dwell in the euphotic zone (Bothwell et al. 1994), and therefore are highly vulnerable to increasing UV-B irradiation. Although some zooplankton may avoid UV by migrating to deeper waters (negative phototaxis), or by increasing pigmentation, there is little doubt that significant biological damage will occur if UV-B irradiation exceed their tolerance limit in their natural habitat (Roy 2000). Thus far, there are very limited dose-response data and field data to enable us to assess the risk of UV-B radiation on marine zooplankton species (WHO 1994).

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Barnacles are found in great abundance in intertidal and subtidal zones ranging from the tropical to the temperate regions (up to the Aleutian Islands, AK, USA). They play an important role in controlling energy flow and production in the coastal ecosystems (Wu 1979). Their free-swimming larvae have evolved complex photoreceptors and elaborate phototactic behaviors, which enable them to identify suitable habitats for feeding and settlement (Kauri 1962; Walley 1969; Hallberg and Elofsson 1983). Barnacle larvae are found in great abundance in shallow coastal waters (<1 m) during day time (Pyefinch 1949; Macho et al. 2005), and may therefore be exposed to high level of UV in their natural habitats. Our previous experimental data demonstrated that environmental realistic dose of UV-B radiation (7.2 kJ m^{-2}) can induce ocular damage in barnacle *Balanus amphitrite* stage II nauplii, thereby impairing their phototactic behaviors (Chiang et al. 2003). However, it remains uncertain as to whether the prevailing UV-B irradiation in coastal waters is sufficient to cause such damage. Thus, we measured the solar UV-B irradiance in coastal marine water (where barnacle larvae were commonly found) in Hong Kong. Based on the data of the field measurements, we carried out further laboratory experiments to investigate how much solar UV-B energy may be received by a naupliar eye in their natural habitat. We also determined the observable effective UV-B dose that impairs phototactic behavior of nauplii, delaying larval development and increasing larval mortality. The objective is to provide concrete field and laboratory data to test whether prevailing UV-B in coastal waters is sufficient to damage barnacle larvae and pose a real threat to the sustainability of natural adult populations.

Materials and methods

Irradiance quantifications

A Macam SR9910-V7 spectroradiometer (Macam Photometrics Ltd., Scotland) was employed to quantify UV-B irradiances and to measure spectral distribution of the solar UV radiation and ultraviolet and visible lamps used in the present study. The spectroradiometer consists of a double grating monochromator for stray light rejection, and a thermoelectrically cooled photomultiplier tube detector connected to a quartz fiber light guide fitted with a cosine diffuser assembly. The spectral response was calibrated against a deuterium and quartz halogen lamp traceable to National

Physical Laboratory standards. The spectrum from 280 to 400 nm was measured at 0.5 nm intervals.

Test organism

Adult barnacles (*Balanus amphitrite*) were collected from the intertidal zone at a clean site in Sai Kung, where according to Environmental Protection Department of Hong Kong (HKEPD 2002), the water quality is amongst the best in Hong Kong. Brood sacs containing mature stage I nauplii were dissected and released in filtered seawater. Stage I larvae molted to stage II within 3–4 h. Stage II nauplii swimming actively toward light were collected for experiments.

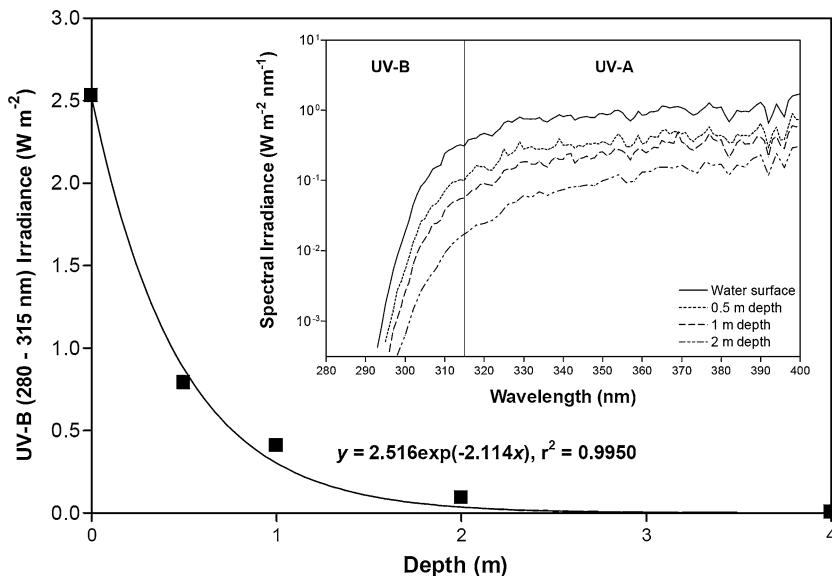
UV-B exposures

All UV-B treatments were carried out in an UV chamber maintained at 22°C. Artificial UV-B irradiation was generated using an overnight-stabilized UV-emitting fluorescent lamp (6 W, Cole-Parmer, VL-6.M, France) with peak intensity at 312 nm (range 280–390 nm) and free of UV-C. In addition to UV-A (315–390 nm) which was also generated by the UV light source, visible light emitted by a fluorescent lamp (11 W/21-840, 900 Lumen LUMILUX Hellweiss Cool White, Osram, Italy) was also provided.

Vertical migration experiment

The effect of UV-B radiation on the vertical distribution of stage II nauplii was conducted using an open-top upright rectangular Perspex chamber with internal dimensions of 3 cm L × 3 cm W × 40 cm H. The frontal and rear sides of the chamber were made with transparent acrylic for easy observation of nauplii distribution at different water depths, while the two lateral sides of the chamber were black, opaque and non-reflective. The chamber was divided into seven equal-sized, non-partitioned vertical sections (by engraving with lines at 5 cm intervals) and filled with natural filtered seawater up to 35 cm. We exposed the nauplii to a profile of UV-B irradiance at $2.7\text{--}0.2 \text{ W m}^{-2}$, assuming that the surface solar UV-B irradiance is at 2.65 W m^{-2} (Fig. 1) and the UV-B attenuation rate in water is ca. 10–90% of the surface irradiance at 1 m depth (the active region of barnacle nauplii). About 100 larvae were transferred to the vertical migration chamber and allowed to adapt for 15 min. The UV opaque acrylic sheet (Mitsubishi Rayon Co. Ltd., Japan) covering the UV treatment chamber was then replaced by an UV transparent acrylic sheet (Rainbow, Hong Kong), while the control chamber (no UV

Fig. 1 Measurements of UV-B flux at different water depth at a typical site with clear water (Sai Kung). The curve is fitted with exponential regression. Insert: Solar UV-B spectral irradiation measured at a typical mid-summer day in Hong Kong



radiation) remained covered with the UV opaque cover. Three replicates were set up for each treatment together with their corresponding control. To measure the vertical migration pattern of these larvae under the influence of UV-B radiation, the numbers of nauplii found in each of the seven vertical sections were counted every 5 min for up to 30 min. We then calculated the weighted vertical mean depth of nauplii in the test column at particular examination time following Bollen and Frost (1990):

$$\text{WMD} = \frac{\sum n_i d_i}{\sum n_i}, \quad (1)$$

where WMD is weighted mean depth (cm); n_i is number of organism at each depth d_i ; d_i is mean depth (cm) that the organism counted. Effects of UV-B radiation and duration of exposure, as well as the interaction of these two factors on weighted vertical mean depth of nauplii was tested by two-way ANOVA. Where significant effects were found, post-hoc multiple comparisons were carried out to identify differences between different time intervals at each UV-B level, and also between different UV-B levels at each time interval, using a Tukey honest significant difference (HSD) test. Significance level (α) was set at 0.05 level (Zar 1996).

Phototactic experiment

Result of our earlier study (Chiang et al. 2003) showed that phototaxis of stage II nauplii was significantly impaired after exposure to UV-B dose of 7.2 kJ m^{-2} , and the impairment was dependent upon dose rather

than irradiance. Consequently, a lower range of UV-B doses ($0\text{--}7.2 \text{ kJ m}^{-2}$) were used in the present study to investigate the observable effective UV-B level of phototactic impairment in nauplii. One hundred barnacle nauplii were placed in a 50 ml glass beaker with 10 ml filtered seawater and exposed to UV-B doses of 0 (control), 2.4, 4.8 and 7.2 kJ m^{-2} , respectively ($n = 3$ for each dose). The UV-B treatment groups were covered with an UV transparent acrylic sheet (Rainbow, Hong Kong), and the control group with an UV opaque acrylic sheet (Mitsubishi Rayon Co. Ltd., Japan) which only allow passage of visible light. Mild aeration was provided throughout the exposure period.

The phototactic behavior of stage II nauplii was examined immediately after exposure to UV-B irradiances. The set up of phototaxis assay and statistical analysis followed the procedures described in our earlier study (Wu et al. 1997). In essence, the phototactic chamber was an open-top rectangular Perspex chamber (internal dimensions: 150 mm L \times 30 mm H \times 40 mm W) with a single transparent window at one longitudinal end. The internal surfaces of the chamber, except the window, were black, opaque and non-reflective. The chamber was divided into five equal-sized, non-partitioned sections by engraved lines on the bottom: namely S1, S2, S3, S4 and S5. S1 occupied the window end of the chamber; S2 was located at 30–60 mm from the window, S3 at 60–90 mm, and so on. An artificial light source (Kyowa dissecting microscope illuminator with a 6 V tungsten bulb, Japan) was projected 10 cm in front of the transparent window so that light intensity gradually decreased from S1 to S5. Light intensity at S1, S2, S3, S4 and S5 was

found to be: 50.7–29.2, 29.2–5.3, 5.3–1.2, 1.2–0.6 and 0.6–0.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. About 100 larvae from each treatment group were transferred to a phototactic chamber, and three replicates were set up for each treatment together with their corresponding control. The numbers of nauplii found in each section were counted 10 min afterward and larval distribution in each of the five sections calculated. Nauplii moving toward a light intensity of 29.2–50.7 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (i.e., S1) were considered to show the strongest positive phototaxis. The observed distributional pattern of nauplii in each treatment was compared with that in the respective control, using a Chi-square (χ^2) Goodness-of fit tests ($\alpha = 0.05$) (Zar 1996).

Larval development, mortality and settlement

The observable effective UV-B levels for significant impairment of larval development, mortality and settlement success were investigated. Three hundred stage II nauplii (100 larvae/replicate \times 3 replicates) after UV-B exposures (2.4 and 4.8 kJ m^{-2}) and their corresponding controls were cultured in a Petri dish (90 mm diameter) with filtered seawater (salinity 32‰, pH 8.2 with 36.5 mg/l streptomycin sulfate and 21.9 mg/l penicillin G sodium salt, which do not have photosensitizer activity), and maintained under a 15-h light and 9-h dark cycle at 28°C. Seawater was changed once a day and the larvae were fed with the diatom *Skeletonema costatum* (in late log growth phase) at ca. 1×10^6 cells/ml daily. Planktotrophic stage II nauplii developed through planktotrophic stage III nauplii to stage VI nauplii and then molted to a non-feeding cypris stage within 4 days. The larval stage and mortality were checked daily for 9 days. A multivariate analysis of variance (MANOVA) was preformed to determine if the dependent variables as a group (nauplii stages II, III, IV, V and VI; cyprid; dead larvae) were affected by UV-B dose, cultivation day and their interaction on larval stage distribution. The significance level was set at $\alpha = 0.05$, and Wilks' lambda was used as the multivariate test statistic (Johnson and Wichern 1992). Compositional data were centered logratio transformed prior to MANOVA test to eliminate the problem of a constrained sample space (Aitchison 1986). Where significant effects were detected, post-hoc multiple comparisons between treatment groups were carried out using a Tukey test ($\alpha = 0.05$) (Zar 1996).

Larvae developed into cypris stage were then transferred to a 24-well microtitre plate (10 cyprids/plate) and the percentage of successfully settled cyprid (metamorphosed to juvenile) was checked after 6 days.

Data on percentages of cyprid developed and settlement success were arcsine-transformed to achieve homogeneity of variances prior to one-way ANOVA. Where significant effects were detected, post-hoc multiple comparisons between treatment groups were carried out using a Tukey HSD test ($\alpha = 0.05$) (Zar 1996).

Results

Field measurements

We measured the solar UV-B irradiance in coastal marine water (where barnacle larvae were commonly found) in Hong Kong during full sun between 12:00 and 13:00 in the mid-summer period of July–August, 2003. UV-B irradiance at different water depth was measured at 0.5 m intervals, from water surface to 4 m depth. Figure 1 shows measurements of UV-B flux at different water depth at a typical site with clear water in Hong Kong (Sai Kung). The surface solar UV-B irradiance level would reach to ca. 2.65 W m^{-2} during a typical summer mid-day. We then derived the following exponential equation and calculated the attenuation rate of UV-B_{280–315 nm} in water column:

$$I_B = 2.516 \exp(-2.114x), \quad (2)$$

where I_B is the irradiance of UV-B_{280–315 nm} (in W m^{-2}) and x is water depth (in m).

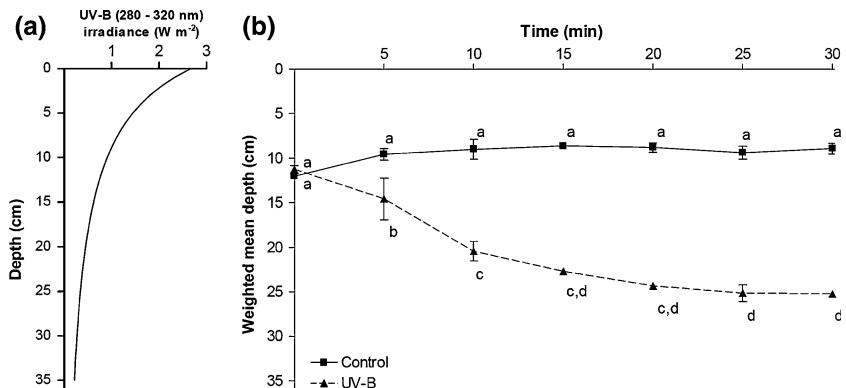
Vertical position of nauplii under the influence of UV-B irradiation

Figure 2 illustrates the comparison of vertical position (in terms of weighted mean depth) of barnacle nauplii under the influences of visible light only (control) and UV-B irradiation. The control nauplii showed positive phototaxis and maintained at a relative high position closed to the light source. In contrast, for the UV-B treatment group, the nauplii migrated further downward, within 5 min UV-B irradiation, to position where UV-B irradiance was significantly lower (Fig. 2a).

Effects of UV-B on phototaxis of nauplii

Over 80% of nauplii from the control group showing a positive phototaxis and a significant decline in percentage of nauplii showing a positive phototaxis was observed after they were exposed to UV-B doses $\geq 4.8 \text{ kJ m}^{-2}$. In general, a good dose response relationship was demonstrated between UV-B doses

Fig. 2 **a** UV-B irradiance profile ($0.2\text{--}2.7 \text{ W m}^{-2}$) along the migration chamber. **b** Weighted mean depth of *B. amphitrite* stage II nauplii at time interval upon exposure to UV-B irradiance. Data are expressed as mean \pm SEM ($n = 3$). Data marked with the same letter are not significantly different from one and other in the Tukey HSD test ($p \geq 0.05$)



and percentage of nauplii showing a positive phototaxis (Fig. 3).

Effects of UV-B on larval development, mortality and settlement

MANOVA test showed that exposure to UV-B dose of 4.8 kJ m^{-2} significantly delayed the larval development (Fig. 4, Tables 1, 2). In addition, the total larval mortality was also significantly increased. Although the percentage of larvae reaching to cypris stage was significantly reduced after exposed to UV-B dose of 4.8 kJ m^{-2} , there was no significant effect on the settlement success of survived cyprids (Fig. 5).

Discussion

Barnacle nauplii tend to concentrate in the euphotic zones for feeding. After metamorphosis into cypris

larvae, most of them will settle in shallow waters (less than a few meters below surface). For example, *B. amphitrite amphitrite* and *B. amphitrite hawaiiensis* settle mainly at 1 m above chart datum (the lowest astronomical tide), while *B. amphitrite cumunis* mostly settle at 1.5–3 m below the sea surface (Wu 1999). Under field conditions, the abundance and active region of barnacle nauplii are predominantly at 0.5–1 m water depth (Macho et al. 2005). Based on our field UV measurement at Sai Kung (Fig. 1), penetrated UV-B radiation at such water depth is equivalent to 12–33% of the surface UV-B irradiance (Eq. 2). In highly transparent waters, the UV-B could only be attenuated by 10 and 40% at 0.5 and 1 m, respectively (Au et al., unpublished data). Thus, our field measurements indicated that barnacle nauplii would be exposed to ca. $0.8\text{--}0.3 \text{ W m}^{-2}$ (or even higher in highly transparent waters) in their natural habitat. We then exposed *B. amphitrite* nauplii to a similar profile of UV-B irradiance under laboratory conditions (see Materials and methods and Fig. 2a). Barnacle nauplii migrated downward within minutes (Fig. 2b) to where UV-B irradiance was significantly lower (Fig. 2a), with an attempt to avoid the deleterious effects of environmental UV-B.

However, it is not known whether UV-B irradiation in the water column is sufficient to damage eyes of the naupliar larvae during their downward migration. We exposed naupliar larvae of *B. amphitrite* to a range of environmental realistic UV-B doses based on our previous study (Chiang et al. 2003), with an attempt to investigate the observable effective UV-B doses for phototactic impairment, larval development, mortality and subsequent settlement success of cypris larvae. A good dose-response relationship was generally demonstrated between cumulative UV-B doses (kJ m^{-2}) and all the parameters investigated (Figs. 3, 4, 5). At a UV-B dose of 4.8 kJ m^{-2} , significant phototactic impairment of naupliar II (Fig. 3), delayed larval development (Fig. 4) and increased larval mortality

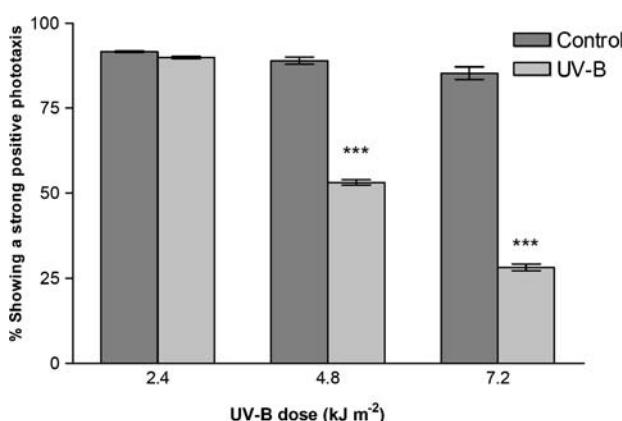


Fig. 3 Percentage of *B. amphitrite* stage II nauplii showing the strongest positive phototaxis, i.e., moving toward a light intensity of $29.2\text{--}50.7 \mu\text{mol m}^{-2} \text{ s}^{-1}$, after exposure to UV-B radiation. Data are expressed as mean \pm SEM ($n = 3$). Values significantly different from their respective control are indicated by asterisks (***) $p < 0.001$

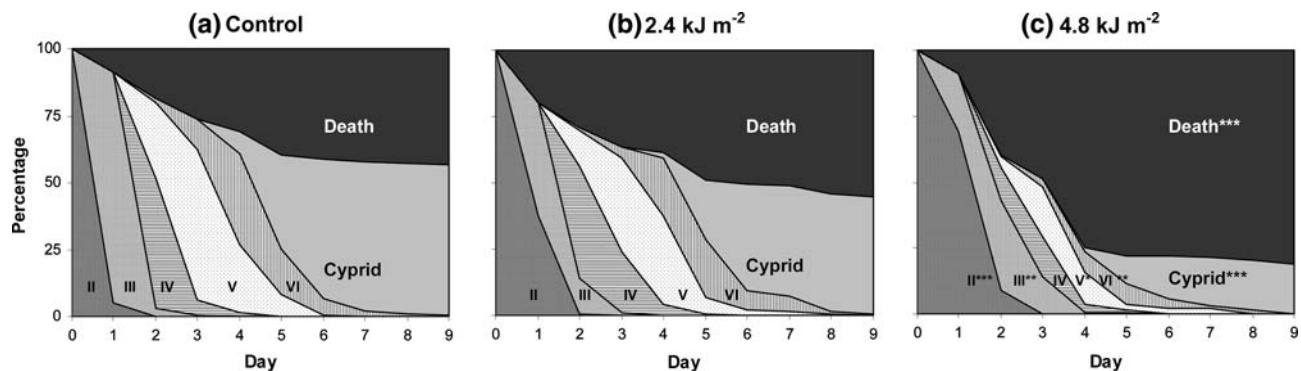


Fig. 4 Daily percent mortality and larval stage distributions of *B. amphitrite* larvae after exposure to UV-B doses of 2.4 and 4.8 kJ m^{-2} . Data expressed are pooled from three replicates.

Values significantly different from the control are indicated by asterisks (** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$)

(Tables 1, 2) were clearly evident. Although there was no significant effect on the settlement success of the survived cyprids (Fig. 5b), the percentage of successful cyprid development was significantly reduced at 4.8 kJ m^{-2} UV-B dose (Fig. 5a). However, no significant changes could be observed for any of the above parameters at a UV-B dose of 2.4 kJ m^{-2} . Our findings suggest that the observable effective UV-B dose for significant phototactic impairment and subsequent development of barnacle larvae therefore, is in the range of >2.4 to $\leq 4.8 \text{ kJ m}^{-2}$.

Table 1 Results of MANOVA for the effects of UV-B doses and cultivation days on the overall larval stage distribution (stage II, III, IV, V, VI nauplii, cyprid and death larvae)

Factor	Wilks' lambda	F value	p value
UV-B dose	0.289	7.877	<0.001
Day	0.000	46.683	<0.001
UV-B dose \times Day	0.027	2.606	<0.001

Table 2 Pairwise comparison for the overall larval distribution of UV-B treatments with control

Larval stage	p values	
	Control vs. 2.4 kJ m^{-2}	Control vs. 4.8 kJ m^{-2}
Naupliar II	0.262	<0.001***
Naupliar III	0.790	0.004**
Naupliar IV	0.626	0.997
Naupliar V	0.999	0.032*
Naupliar VI	0.866	0.002**
Cyprid	0.057	<0.001***
Dead	0.134	<0.001***

Data significantly different from the control are indicated by asterisks (** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$)

Our previous study has demonstrated that naupliar eyes of barnacles are particularly vulnerable to UV-B damage and sightless larvae were unable to swim toward light (Chiang et al. 2003). At a deleterious UV-B dose of 4.8 kJ m^{-2} , the energy received by a naupliar eye with a surface area of ca. $1.57 \times 10^{-9} \text{ m}^2$ (Au et al. unpublished data) is equivalent to ca. $7.5 \times 10^{-6} \text{ J}$. In other words, if such amount of energy is inflicted on naupliar larvae while traveling down the water column, adverse impacts will likely result. In addition to predator avoidance, downward migration is particularly important for barnacle larvae to escape from enhanced solar UV-B in water, which occurs during the day when clouds blocking the solar radiation are displaced.

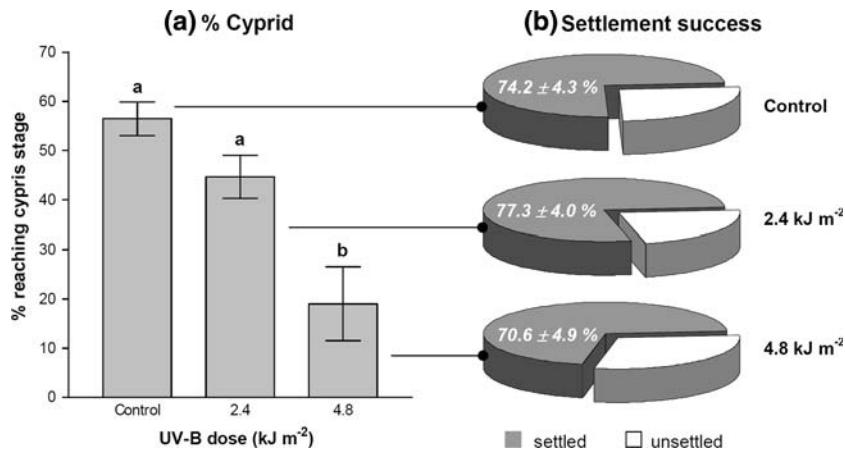
A calculation, based on our field UV data and the vertical migration data, is deduced to estimate the energy received by naupliar eyes during downward migration. Based on the attenuation UV in natural waters (Eq. 2 and Fig. 2), the total energy E (in J) incident on an object (with a cross-sectional area A in m^2) within a given time T (in s) is given by

$$E = \int_0^T I A dt = A I_o \int_0^T \exp(-kx) dt, \quad (3)$$

where A and I_o are constants, and assuming the object travels at a constant velocity “ v ” to a depth “ D ” during this period, we have

$$\begin{aligned} E &= A I_o \int_0^D \exp(-kx) \frac{dt}{dx} dx = \frac{A I_o}{v} \int_0^D \exp(-kx) dx \\ &= \frac{A I_o}{kv} [1 - \exp(-kD)]. \end{aligned} \quad (4)$$

Fig. 5 **a** Percentage of larvae reaching cypris stage after UV-B exposure and **b** their respective settlement success rate. Data are expressed as mean \pm SEM ($n = 3$). Data marked with the same letter are not significantly different from one and other in the Tukey HSD test ($p \geq 0.05$)



Based on the vertical migration data (Fig. 2b), the nauplii traveled downward about 5.01 cm after UV-B exposure for 5 min. Thus, the downward migration velocity v of barnacle larvae is ca. $1.67 \times 10^{-4} \text{ ms}^{-1}$. The area A of the larval eye is $1.57 \times 10^{-9} \text{ m}^2$. For a traveled distance D of 1 m, the total energies E_B (in the wavelength ranges 280–315 nm) inflict on the larval eye is calculated as below

$$E_B = \frac{(1.57 \times 10^{-9})(2.516)}{(2.114)(1.67 \times 10^{-4})}[1 - \exp(-2.114 \times 1)] \\ = 9.8 \times 10^{-6} \text{ J.}$$

Thus, the theoretical amount of energy received by naupliar eyes during downward migration (up to 1 m deep) in the natural water column was found to be $9.8 \times 10^{-6} \text{ J}$. This is within the same order of magnitude as the total energy $7.5 \times 10^{-6} \text{ J}$ received by a naupliar eye ($1.57 \times 10^{-9} \text{ m}^2$) at the observable effective UV-B dose of 4.8 kJ m^{-2} (Figs. 3, 4, 5), at which impairments of phototaxis and larval development would occur.

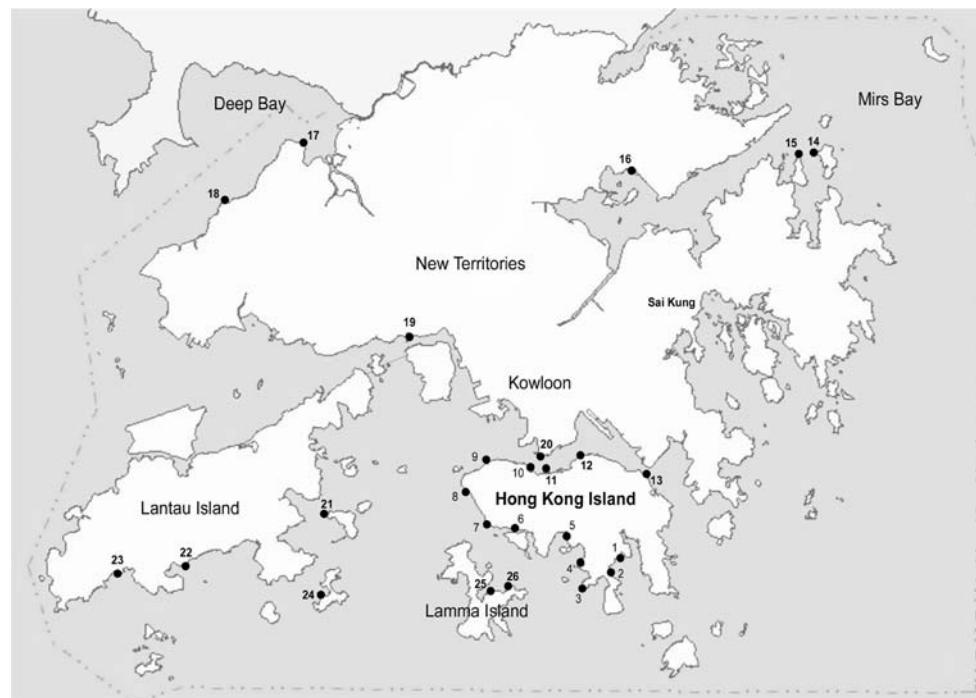
In fact, despite barnacle larvae attempt to migrate to deeper waters in their natural environments, they are generally unable to maintain their vertical positions against water movements due to strong tidal or wind mixing. The fact that barnacle larvae (nauplii and cyprids) are found in great abundance in shallow water (<1 m) (Pyefinch 1949; Macho et al. 2005) showed that barnacle larvae are living at the edge of UV-B irradiation sufficient to pose a real threat to their normal phototactic behavior and larval development. Such behavioral impairment will subsequently affect the settlement success and therefore barnacle populations in their natural habitats.

We have analyzed the results of two comprehensive surveys on the distribution and occurrence of

B. amphitrite in Hong Kong waters: the first one conducted in 1973 (Wu 1975) and the second one in 2003 (CCPC 2003) (Fig. 6). A clear reduction in the occurrence of *B. amphitrite* populations was found in 10 of the 26 study sites between the two surveys. Amongst these ten sites, at least two sites are free of anthropogenic pollution and activities between the two surveys. Although long term UV data in Hong Kong are not available, the total atmospheric ozone in Hong Kong has been decreasing between 1979 and 1993, and then stabilized at a lower level thereafter (Lam et al. 2002), implicating that UV-B radiation has been increasing between the two study periods. Indeed, a similar decrease in total atmospheric ozone has been also reported widely in Asia, including Taiwan (Hsu and Yung 1999). Taken together, these field data tend to substantiate our findings that enhanced UV-B radiation might have threatened the sustainability of barnacle populations. In summary, our laboratory and field data show that the level of UV-B irradiances prevailing in marine coastal waters is sufficient to impair the normal phototactic behavioral and settlement success of barnacles in their natural habitats. Such impairments inevitably reduce the fitness and survival and hence threat the sustainability of barnacle populations in nature. Being a key species in coastal system, its population decline may in turn lead to major changes in marine coastal ecosystems over a large geographic scale. Assuming other zooplankton have a similar tolerance to UV, similar damage may also occur in other zooplankton species.

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Fig. 6 Comparison of the occurrences of *B. amphitrite* in 26 locations in Hong Kong in 1973 and 2003. The table underneath shows the location name of the site number and barnacle occurrence, with “plus” and “minus” signs representing presence and absence, respectively



Site No.	Site Name	Substratum	GPS		1973	2003
			N	E		
1	Turtle Back Cove	Boulder	22°13.950'	114°13.443'	+	+
2	Stanley	Rocky	22°13.351'	114°12.881'	+	-
3	Chung Hum Kok	Rocky	22°12.616'	114°11.883'	-	-
4	Middle Bay	Rocky	22°13.738'	114°11.788'	-	-
5	Deep Water Bay	Rocky	22°14.520'	114°11.226'	+	-
6	Aberdeen	Artificial seawall	22°14.885'	114°08.629'	+	-
7	Wah Fu	Rocky	22°15.102'	114°08.032'	-	-
8	Mount Davis	Rocky, boulders	22°16.767'	114°07.052'	-	-
9	Kennedy Town	Artificial seawall	22°17.023'	114°07.540'	+	-
10	Central Pier	Artificial seawall	22°17.206'	114°09.656'	+	-
11	Wan Chai Pier	Artificial seawall	22°17.015'	114°10.330'	+	-
12	North Point Pier	Wharf pile	22°17.491'	114°12.697'	+	+
13	Heng Fa Chuen	Artificial seawall	22°16.748'	114°14.427'	+	-
14	North Tap Mun	Boulder	22°28.591'	114°21.396'	-	-
15	North Hoi Ha	Boulder	22°28.411'	114°20.722'	-	-
16	Tai Mei Tuk	Boulder	22°28.108'	114°13.977'	+	+
17	Tsim Bei Tsui Pier	Boulder	22°29.173'	114°00.688'	-	-
18	Pak Nai	Boulder	22°26.504'	113°57.019'	-	-
19	Ting Kau Wan	Boulder	22°22.156'	114°04.816'	+	-
20	Tsim Sha Tsui	Artificial seawall	22°17.587'	114°10.258'	+	-
21	Peng Chau	Rocky	22°17.410'	114°02.035'	-	-
22	Tong Fuk	Boulder	22°13.591'	113°56.060'	-	-
23	Tai Long Wan	Rocky, boulder	22°13.223'	113°53.362'	-	-
24	Cheung Po Chai	Rocky	22°23.464'	113°55.065'	+	-
25	Mo Dat Wan	Rocky, boulder	22°12.472'	114°08.626'	+	+
26	Lo So Shing	Rocky	22°12.314'	114°07.297'	-	-
					14+	4+

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