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UV-B Damages Eyes of Barnacle Larvae and Impairs Their Photoresponses and Settlement Success

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The impact of enhanced UV-B radiation on marine ecosystems due to ozone depletion has caused growing global concern. Barnacle larvae have evolved complex photoreceptors and elaborate phototactic behaviors, which enable them to identify suitable habitats for feeding and settlement. For the first time, we demonstrate that environmentally realistic levels of UV-B radiation can induce ocular damage in barnacle larvae, thereby impairing the phototactic behavior of naupliar larvae and reducing settlement success of cypris larvae. Significant disruptions of rhabdomeres (the photosensitive structures in which phototransduction takes place) occurred in the retinular cells of naupliar eyes when naupliar larvae were exposed to a UV-B dose of 7.2 kJ m^{-2}, and impairment was dependent upon dose rather than irradiance. Our experimental data also showed that phototaxis of nauplii was ca. 4 times more sensitive to UV-B than settlement of cyprids. Since barnacles play an important role in the function and structure of coastal systems worldwide, any impairment of phototactic and settlement behavior of the larvae would pose a significant threat to the sustainability of this ecologically important species. The fact that enhanced UV-B radiation can induce ocular damage and subsequent phototactic impairment in barnacle larvae suggests that UV-B may also cause similar damage to other zooplankton species.

Introduction

Although the use of chlorofluorocarbons (CFCs) and halons has been significantly reduced in recent years, large scale and widespread ozone depletion is still expected to continue (*1, 2*). The impact of the consequential increase in UV-B radiation on marine ecosystems has caused growing concern, and dose response data on ecologically important marine species are urgently needed in order to assess the ecological risks involved (*3*−*5*). Solar UV-B radiation can penetrate to significant depths (at least 20−30 m) in marine water (*6, 7*) and may affect zooplankton in the euphotic zone. Although some zooplankton might actively avoid UV-B radiation (*8, 9*), meroplankton such as barnacle larvae cannot completely stay away from solar light and must forage and settle in the euphotic zone. Thus, enhanced UV-B radiation is likely to impair their phototactic behaviors, thereby reducing their fitness or survival.

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Animal photoreceptors are particularly susceptible to an increase in solar UV-B radiation (*10*), and exposure to excessive UV-B is known to induce ocular damage in vertebrates (*1, 11*−*13*). Barnacles undergo six planktonic naupliar stages and one cypris stage before settling as sessile adults. Barnacle nauplii have a prominent naupliar eye, while the cyprid possesses a pair of compound eyes and a single naupliar eye (*14*−*17*). Whether UV-B impairs photoreceptor function of barnacle larvae and thus affects the phototactic behavior of the larvae (which is essential for their foraging and settlement (*18*−*20*)) is virtually unknown.

We exposed nauplii and cyprids of *Balanus amphitrite* to a range of environmentally realistic UV-B doses under laboratory conditions, studied impairments of their phototactic behavior and settlement success, and then related the behavioral changes to the observed ocular damage. Results of these studies are expected to help understand UV-B impacts on the health and fitness of barnacle larvae and allow us to evaluate the ecological risk of UV enhancement on barnacle populations, and other zooplankton in general.

Experimental Section

Adult barnacles (*Balanus amphitrite*) were collected from the intertidal zone of a clean site 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 phototaxis experiments. To culture cyprids, stage II nauplii were allowed to grow in filtered seawater under continuous aeration and a 15 h:9 h light:dark cycle at 22 °C. The larvae were fed daily with the diatom Skeletonema costatum. Nauplii developed into cyprids within 10 days, and active cyprids were harvested from the cultures for settlement experiments.

UV-B Exposure Experiments. All UV-B treatments were carried out in an UV chamber maintained at 22 °C. Artificial UV-B irradiation (280−315 nm) was generated using an overnight-stabilized UV-emitting fluorescent lamp (6 W, Cole-Palmer, VL-6.M, France) with peak intensity at 312 nm (range 280−390 nm). In addition to the UV-A region (315−390 nm) already included in 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. Maximum UV-B irradiances of 1.4 W m^{-2} and 3.85 W m^{-2} were measured at the South Pole, Antarctica (90°00′ S) in 1998 and Ushuaia, Argentina (68°19′ W, 59°49′ S) in 1990, respectively (*21*). In Hong Kong, UV-B irradiance typically peaks at ca. 4 W m^{-2} during midday in summer (Au, D. W. T., unpublished data). If the attenuation of UV-B is 50% at 1 m (*7*), the UV-B irradiance at 1 m depth should be ca. 0.64−2 W m^{-2}. Consequently, a low UV-B irradiance of 0.72 ± 0.03 W m^{-2} and a high UV-B irradiance of 1.99 ± 0.1 W m^{-2} were used in the present study. A Macam SR9910−V7 spectroradiometer (Macam Photometrics Ltd., Scotland) was employed to quantify the actual level of UV irradiance in this experiment. Three hundred barnacle naupliar/cypris larvae were placed in a 50 mL glass beaker with 10 mL of filtered seawater. The UV-B treatment groups were covered with a UV transparent acrylic sheet (Rainbow, Hong Kong) and the control group with a UV opaque acrylic sheet (Mitsubishi Rayon Co. Ltd., Japan) to allow passage of visible light only. Mild aeration was provided throughout the experimental period. At the end of the UV-B exposure, larvae were divided for phototaxis and settlement studies and tissue processing for transmission electron microscopy.

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Phototaxis Assay. The phototactic behavior of stage II nauplii after exposure to low and high UV-B irradiances for 1, 3, 5, and 7 h, was examined. The setup of phototaxis assay and statistical analysis followed the procedures described in our earlier study (*22*). In essence, the numbers of UV-B treated nauplii found in each section of the phototactic chamber (with light intensity decreasing from 2600 to 25 lx along the five nonpartitioned sections) were counted, and percentage distribution was calculated and compared with that in the respective time control using a Chi-square (χ^2) goodness-of fit test ($\alpha = 0.05$) (*23*).

Settlement Assay. The settlement success of cyprids after exposure to both UV-B irradiances for 2, 4, 8, and 12 h was examined. In each treatment, three replicates were set-up together with their corresponding controls. Each replicate consisted of 100 cyprids in a glass beaker filled with 90 mL of filtered seawater. The external surface of the beaker (except the open-top) was covered with a black plastic film. Cyprids were incubated for 6 days at 22 °C on a 12 h light/12 h dark cycle. Numbers of cyprids settled were counted and settlement success expressed as percentage. Data on percentage settlement success were arcsine-transformed to achieve homogeneity of variances prior to two-way ANOVA. Where significant effects were found, post-hoc multiple comparisons between treatment groups were carried out using a Tukey Honest Significant Difference (HSD) test ($\alpha = 0.05$) (*23*). ED_{50} values (median effective dose of UV-B, at which 50% of the test population was affected) for each parameter were estimated, using probit-analysis (*24*).

Ultrastructural Study. Morphometric analyses were carried out on thin sections at the median region of the naupliar eye, where a large area of retinular cytoplasm/rhabdom could be examined.

Rhabdoms can be morphologically classified into three types: Type I, intact rhabdom with well-organized and closely packed microvilli; Type II, rhabdom with loosened microvillar arrangement, and Type III, disorganized rhabdoms with electron-dense, disrupted microvilli. For each treatment group, a total of 15 rhabdoms (5 larvae × 3 rhabdoms) were examined and categorized, and the percentage occurrence of each morphological type was calculated. The proportions of different morphological types of rhabdom in the treatment groups and their corresponding controls were compared using a Chi-square (χ^2) test ($\alpha = 0.05$) (*23*).

Stereological volume density (V_v) was studied for mitochondria. Measurements were made on images using a square test point lattice with 36 points spaced at $d = 30$ mm. A total of 12 records of retinular cells were sampled systematically for each eye (at 11 000×). Five larvae were examined for each treatment. A two-way ANOVA was performed to test the effects of UV-B exposure, UV-B dose, and their interactions on V_v (mitochondria, cytoplasm of retinular cell). Tukey HSD test ($\alpha = 0.05$) was used to identify differences between individual treatment groups (*23*).

Results and Discussion

Good dose−response relationships between cumulative UV-B doses and phototaxis of nauplii as well as settlement success of cyprids were demonstrated, regardless of irradiance levels (Figure 1). Phototaxis of nauplii was impaired, and cypris settlement success was reduced, at unweighted UV-B doses of 7.2 kJ m^{-2} and 28.6 kJ m^{-2}, respectively (equivalent to CIE erythemally weighted doses of 2.3 kJ m^{-2}_{CIE} and 9.2 kJ m^{-2}_{CIE} correspondingly). Thus, our results show that both phototaxis and settlement were impaired under environmentally realistic levels of UV-B and that phototaxis of nauplii was ca. 4 times more sensitive to UV-B than settlement of cyprids.

Results of our parallel ultrastructural study revealed the extent of cytological damage in the eyes of UV-B treated barnacle larvae. It was found that the naupliar eyes of *B. amphitrite* nauplii were the most sensitive to UV-B, followed by the naupliar eye of cyprids, while the compound eyes of cyprids represented the least sensitive photoreceptive structure. Such differences may be explained by the respective position and/or structure of the larval eyes: the naupliar eye of the nauplii is situated just beneath a very thin cuticle and epithelium (Figure 2A), which UV-B can impinge upon. The naupliar eye of cyprids is situated deeper inside and is surrounded by layers of body tissue and oil cells (Figure 2B). A significant amount of UV-B radiation would be absorbed before reaching the naupliar eye, and the residual UV-B may not be sufficient to induce damage to photoreceptor cells. Surprisingly, although the compound eyes of cyprids are located immediately underneath the carapace (Figure 2B), the least cytological damage was found. Similar to the lens in the mammalian eye (*10*), the crystalline cones in the compound eyes (Figure 2B′) of barnacle cyprids probably serve as UV-blockers or filters and offer protection to the light-perceiving elements in the eye, e.g., the rhabdom underneath (Figure 2B′). This can be verified by measuring the UV absorption ability of the crystalline cones.

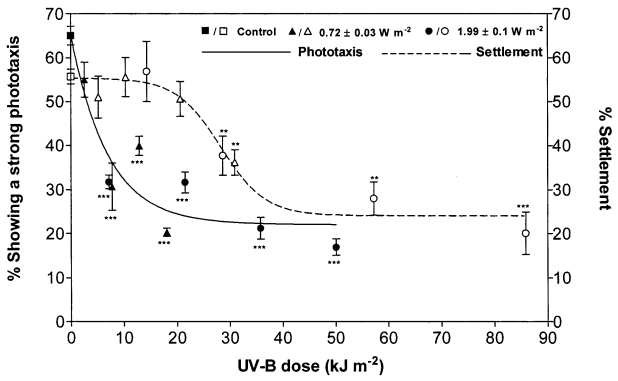

FIGURE 1. Dose−response relationship between UV-B dose (UV-B irradiance × exposure time) and percentage of nauplii showing the strongest phototaxis, i.e., moving toward a light intensity of 1500−2600 lx (—), and percentage of cyprids settled (− − −). The curves were fitted by exponential regression ($y = 22.07 + 43.14 \exp(-0.1442x)$; $r^2 = 0.8816$) and Boltzmann sigmoidal regression ($y = 24.10 + 31.26/\{1 + \exp[(28.21 - x)/-4.231]\}$; $r^2 = 0.7664$), respectively. The filled (■, ▲, ●) and open (□, △, ○) symbols represented the data of phototaxis and settlement assays, respectively. Data are expressed as mean ± S.E.M. ($n = 3$). Values significantly different from their respective control, are indicated by asterisks ($p < 0.01$; *** $p < 0.001$).**

Significant disruption of rhabdomere integrity (Figure 2C−E) and mitochondrial swelling were clearly evident in retinular cells of naupliar eyes (Figure 3A,B). Significant damage to rhabdomeres occurred at a UV-B dose of 7.2 kJ m^{-2} (Figure 3A), a level at which phototactic impairment of nauplii also occurred (Figure 1). Rhabdomeres are the photosensitive structures in invertebrate photoreceptors (*25*). The disruption of organization and integrity observed in rhabdomeres in naupliar eyes suggests impairment of photoreception and reduction of phototransduction efficacy. Swelling and disruption of mitochondria suggests that the ATP supply needed for phototransduction in the retinular cells might be affected. These cytological changes could have accounted for the observed phototactic impairment. The cypris compound eyes were not afflicted by UV-B, and mild rhabdomere disorganization only occurred in the naupliar eyes of cyprids at a dose higher than that required for retarding cypris settlement. Our results suggest that rhabdomeric alteration alone could not fully explain the reduction of settlement success caused by UV-B.

The most pertinent question is whether the present experimental data implies any deleterious effects of UV-B

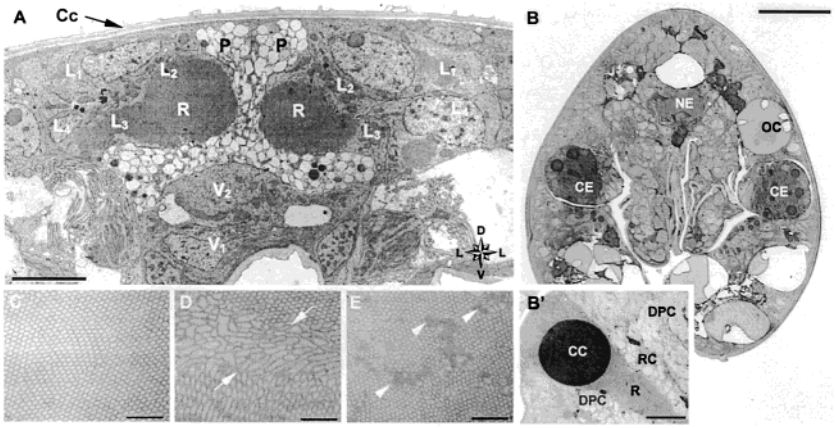

FIGURE 2. Larval eyes of *Balanus amphitrite*. A, Section of eye of nauplius in frontal view. The naupliar eye is situated beneath the dorsal cephalothoracic cuticle (Cc). It contains two bilaterally symmetrical pigment cells (P) in the center and three groups of retinular cells, two on the lateral sides and one on the ventral side. Four retinular cells (L_1−L_4) are present in each lateral component and two retinular cells (V_1−V_2) are found in the ventral component. The microvillar borders (rhabdomeres) of each retinular cell meet to form a well-developed rhabdom (R). The navigator indicates the orientation of the micrograph: D, dorsal; V, ventral; L, lateral. B, Cyprid (t.s) with a naupliar eye (NE) and a pair of compound eyes (CE). B′, Each ommatidium (t.s) in the compound eye is made up of 3 crystalline cone cells (CC), 5−6 retinular cells (RC), and 2 distal pigment cells (DPC). R = rhabdom. OC = Oil cell. C−E, Rhabdom (t.s.) C, Type I rhabdom. Rhabdomeres in cross-section display a regular array of closely packed microvilli. D, Type II rhabdom. Rhabdomeres near the basal region show a less regular membrane network (*arrows*), in which the more loosely organized rhabdom regions contain enlarged microvilli of irregular sizes and shapes. E, Type III rhabdom. Severe disruption of rhabdomere organization, in which the most disrupted areas appear to be electron-dense (*arrowheads*). Scale bars: A and B′ = 4 μm; B = 50 μm; C−E = 100 nm.

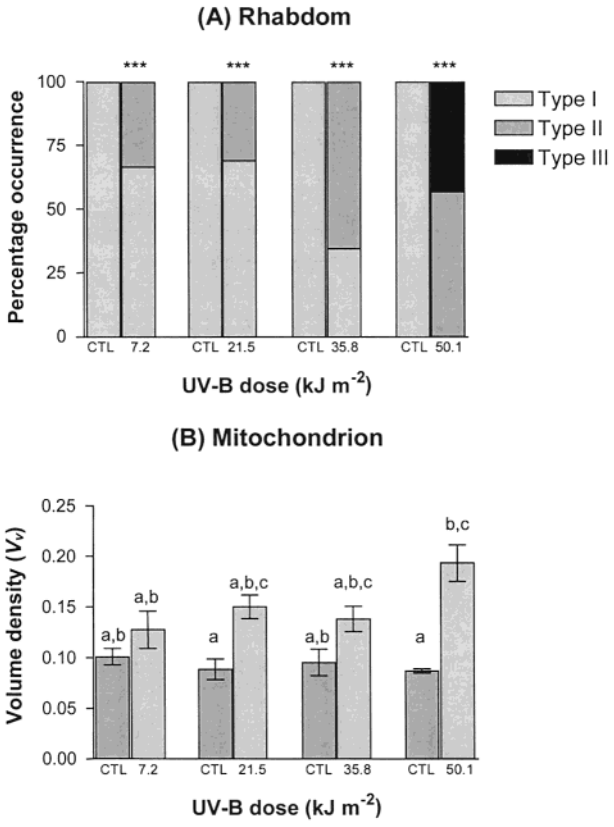

FIGURE 3. Naupliar eyes of stage II nauplii after exposure to UV-B dose for up to 50.1 kJ m^{-2}. A, Percentage occurrence of Types I−III rhabdom, found in the retinular cells. Percentage occurrences in the UV-B treatment groups significantly different from the corresponding controls are indicated by asterisks ($p < 0.01$; *** $p < 0.001$). B, Volume density of mitochondria. Data are expressed as mean ± S.E.M. ($n = 5$). Treatments marked with the same letter are not significantly different from one another in the Tukey Honest Significant Difference (HSD) test ($p \geq 0.05$).**

on *B. amphitrite* in their natural environment. In Hong Kong, for example, the highest UV radiation in summer is ca. 0.325 W m^{-2}_{CIE} (*26*). In Antarctica, the solar UV-B at the water surface ranged from ca. 9.76 to 21.14 W m^{-2} (*21, 27*−*29*), but much higher levels (>30 W m^{-2}) have been reported in the Palos Verdes peninsula, Southern California (*9*). For similar solar spectra, the estimated CIE erythemally weighted UV irradiance from these data could range from ca. 0.75 to 2.33 W m^{-2}_{CIE}. According to Smith et al. (*7*), the spectral irradiance at 300 nm was attenuated by about 80% at 10 m in Antarctic waters, which would translate into an attenuation of about 15% at 1 m for an exponential decay. Therefore, even using a conservative assumption of 50% attenuation of UV-B radiation at 1 m water depth, the level of UV irradiance at 1 m in the environment would still be higher than 0.634 W m^{-2}_{CIE} (the high irradiance level used in this study). Accordingly, exposure to the above levels of solar radiation in the natural environment for <1 h would be sufficient to acquire the equivalent dose of CIE erythemally weighted ED_{50}s which have been shown to impair phototaxis and settlement of barnacle larvae (i.e. ca. 0.8 kJ m^{-2}_{CIE} and 3.4 kJ m^{-2}_{CIE}, respectively). In fact, in the natural environment, periods with the strongest solar radiation (during spring and summer) may last considerably longer than 1 h, although factors such as cloud cover and solar zenith angle may reduce incident UV radiation.

In the natural environment, barnacle nauplii tend to concentrate in the euphotic zones for feeding, while cyprids of many barnacle species are commonly found 1−2 m below the sea surface prior to their settlement (*30*). For example, adult *B. amphitrite amphitrite* and *B. amphitrite hawaiiensis* mainly colonize at 1 m above chart datum (the lowest mean water level), while adult *B. amphitrite cumunis* are most abundant at 1.5−3 m below the sea surface (*31*). As such, the majority of barnacle nauplii and cyprids dwell near the water surface and would be exposed to a UV-B dose higher than that used in the present study. The above experimental evidence and analysis indicate that the prevailing UV-B radiation in the natural environment is likely to impair phototaxis and settlement of barnacle larvae in the field. Barnacles play an important role in the function and structure of coastal water systems worldwide (*32*). Impairment of their phototactic response may reduce their foraging efficiency, and reducing their settlement success will directly threaten

their survival. Assuming the susceptibility to UV-B of other barnacles and zooplankton is similar to that of our *B. amphitrite* larvae, many other species of meroplankton may be expected to be affected by increased levels of UV-B radiation, which would then pose a significant ecological risk to marine ecosystems, generally.

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