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Effect of UV radiation on the expulsion of *Symbiodinium* from the coral *Pocillopora damicornis*

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ABSTRACT

The variation in density of the symbiotic dinoflagellate *Symbiodinum* in coral is a basic indicator of coral bleaching, i.e. loss of the symbiotic algae or their photosynthetic pigments. However, in the field corals constantly release their symbiotic algae to surrounding water. To explore the underlying mechanism, the rate of expulsion of zooxanthellae from the coral *Pocillopora damicornis* was studied over a three-day period under ultraviolet radiation (UVR, 280–400 nm) stress. The results showed that the algal expulsion rate appeared 10–20% higher under exposure to UV-A (320–395 nm) or UV-B (295–320 nm), though the differences were not statistically significant. When corals were exposed to UV-A and UV-B radiation, the maximum expulsion of zooxanthellae occurred at noon (10:00–13:00), and this timing was 1 h earlier than in the control without UVR. UVR stress led to obvious decreases in the concentrations of chl *a* and carotenoids in the coral nubbins after a three-day exposure. Therefore, our results suggested that although the UVR effect on algal expulsion rate was a chronic stress and was not significant within a time frame of only three days, the reduction in chl *a* and carotenoids may potentially enhance the possibility of coral bleaching over a longer period.

1. Introduction

As one of the most flourishing ecosystems on Earth, coral reefs provide habitat for about 30% of marine fish species, as well as a vast range of goods and services to people, such as food, tourism, and coastal protection [1]. However, coral reefs are also one of the most vulnerable ecosystems in the world [2], and since the late 1980s reef-building corals have been undergoing continuing degradation as a result of more and more frequent mass bleaching events. As one of the main features of coral bleaching, the loss of the unicellular photosynthetic dinoflagellate symbionts (zooxanthellae), which live in the coral tissue, indicates the breakdown of the mutualistic symbiosis between the host coral and the algae. Thus, the algal density is often measured as a bioassay parameter to judge the status of corals. In fact, as early as 1987, a diel cycle of algal expulsion by corals was recognized, and showed that the algal release peaked at noon and that the release rate could reach 0.1% of the standing stock of symbiotic algae [3]. Further studies showed that the released algae were intact and mostly in the process of dividing [4] and that this diel release was related to nutrient levels [5]. Similar changes have also been shown in anemones in response to temperature rises, with higher algal expulsion rates, concomitant with reduced rates of photosynthetic oxygen evolution [6]. Given that the diel pattern is similar to the diurnal change of solar irradiation, this suggests that there is a positive relationship between the expulsion rate and light intensity [7]. Baghdasarian and Muscatine [8] believed the diel pattern was a way for the coral host to regulate symbiont population density, and that the expulsion rate was a function of mitotic index. It can thus be speculated that any change that will induce a higher algal division rate would result in an increase of algal expulsion in order to control the density of algae and avoid hyperoxic stress.

A range of environmental stresses have been identified as promoting coral bleaching, including thermal stress [9,10], eutrophication [11], sedimentation [12], pollution [13], and ultraviolet radiation [14]. Among these, only ultraviolet radiation (UVR, 280–400 nm) has a diel fluctuation pattern similar to the diel algal expulsion rate of corals. Exposure to UVR, especially UV-B irradiances (280–315 nm), has been known to have deleterious effects on organisms, including damaging DNA, inhibiting the synthesis of protein, and inducing the formation of reactive oxygen species (ROS) [15]. Reef-building corals, especially those distributed in tropical shallow reefs, often receive high irradiances because of the high water transparency. According to field observations, the euphotic depth (1% of surface irradiance) for UV-B can reach as deep as 12 m at Heron Reef in the southern Great Barrier Reef, while the corresponding depth for UV-A (315–400 nm) would exceed 20 m [16]. Thus, the reef-building corals may experience significant UVR exposure

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daily, especially at low tide. In this case, the symbiotic algae may suffer damage when exposed to UVR, and have to compensate for this by enhanced repair or stimulated mitosis. At the same time, the host corals have to release the damaged algal cells as well as the dividing symbionts. Therefore, it is possible to hypothesize that UVR stress may lead to a higher algal expulsion rate of corals. In the present study, a shallow water coral, *Pocillopora damicornis*, was chosen and exposed to natural solar irradiation for three days to examine the effects of UVR on the diel expulsion rate of symbiotic algae by corals.

2. Materials and Methods

2.1. Sampling of Coral Colony

Colonies of the reef-building coral *Pocillopora damicornis* (Linnaeus, 1758) were collected from 1 to 10 m depth at Luhuitou Reef, Sanya Bay, Hainan Island (18°13′N, 109° 28′E) in July 2013. They were transported to the Tropical Marine Biological Research Station (Sanya, Hainan), South China Sea Institute of Oceanology, Chinese Academy of Sciences, and placed in a flow-through outdoor aquarium. The aquarium was supplied with flowing, sand-filtered seawater and exposed to natural sunlight, which was partially shaded to allow ~35% photosynthetically active radiation (PAR, 400–700 nm) to pass through, with the mean maximum intensity at midday being 570 μmol m^{-2} s^{-1} (124 W m^{-2}). UV-B radiation was completely filtered by the fiber reinforced plastic ceiling, which transmitted a rather low UV-A radiation of no more than 2 W m^{-2}. After acclimation for one week, coral colonies were fragmented into nine nubbins and affixed to silica caps using epoxy glue to facilitate handling.

2.2. Experimental Setup

To investigate the effects of UVR on the release of symbiotic algae during the daytime, 120 ml quartz tubes were used to collect released algae. Different radiation treatments were carried out using cut-off filters: [1] PAR (P treatment), tubes wrapped with 395 nm cutoff foil (UV Opak, Digefra), so that the coral nubbins received PAR alone; [2] PAR + UV-A (PA treatment), tubes wrapped with Folex 320 filters (Montagefolie, Folex, Dreieich, Germany), so that nubbins received irradiances above 320 nm; [3] PAR + UV-A + UV-B (PAB treatment), tubes wrapped with Ultraphan Film 295 nm cutoff filter (Digefra, Munich, Germany), allowing the individuals to be exposed to irradiances above 295 nm. Triplicate samples were used for each irradiance condition, and the incubations lasted for 3 days.

To avoid oxidative stress and allow gas exchange, one hole was drilled on each silica cap and a PVDF syringe filter (25 mm, 0.22 μm, Membrane Solutions) inserted. Prior to the experiment, each quartz tube, wrapped with cut-off filters, was filled with freshly filtered seawater (FSW, 0.22 μm), and then the nubbins, which had been acclimated for two days, were allocated randomly to the tubes. After mounting the quartz tubes onto the silica caps, the tubes were placed upright in the experimental aquarium ($25 \times 40 \times 28$ cm^3). The temperature was controlled by the flow-through seawater at a rate of 160 ml s^{-1}, and ranged between 28 and 29 °C. Though exposed to natural solar irradiation, in order to narrow the radiation variations between the acclimation and experiment, to recreate the *in situ* irradiance conditions of Luhuitou Reef, and to facilitate a better control of temperature, the experimental aquarium was covered with a neutral density shade filter, which reduced the radiation levels to 28%, 24% and 21% of incident for PAR, UV-A and UV-B, respectively.

The experiments were performed from 08:00 am to 18:00 pm over 3 days, when the quartz tubes were mounted on the nubbins, and the FSW in the tubes was changed once per hour. At night, coral nubbins were uncovered and moved back to the acclimation aquarium. Over the first two days, to allow the coral to acclimate to the UVR exposure environment, we only monitored the photosynthetic efficiency of the symbiont, and did not collect water samples for analysis of algae expulsion until the third day.

Irradiance was monitored every 2 h during experiments by a PMA2100 data logging radiometer (Solar Light Co., Inc., USA) with three radiation detectors for measuring PAR, UV-A and UV-B respectively. In addition, a record of solar radiation was provided by the Tropical Marine Biological Research station. The temperature was recorded at two-hour intervals using a thermometer.

2.3. Photosynthetic Efficiency

Maximum quantum yield of PSII of the symbiont (Fv/Fm) was measured using a Diving Pulse Amplitude Modulated fluorometer (Diving PAM, Walz, Germany). Before measurement, coral nubbins were kept in darkness for 20 min, and Fv/Fm was then obtained. On the first day, the measurements were performed three times, at 06:30 am in the morning, 13:30 pm in the middle of the day and 20:00 pm at night.

2.4. Symbiodinium and Pigment Release Rate

To detect and measure the rate of release of *Symbiodinium*, two methods were employed in our research. One way was by counting the density of algae cells released, and the other was by measuring the chlorophyll *a* content in the water sample. Specifically, on the third day, the incubation medium in the quartz tubes was collected and fixed in 10% formalin. Half of the water sample was centrifuged (10 min at 4000*g*, Anke TDL-40B, China), and we retained the algae pellet, which was resuspended in 1.5 ml FSW. The number of algae was counted manually with a hemocytometer, and the algae population was normalized to living tissue area (cells cm^{-2}), which was determined at the end of experiment with the aluminum foil method [17]. To be brief, the tissue area was determined by weighing the aluminum foil that had been wrapped around the coral nubbins, and the area calculated according to the property that the aluminum had a constant ratio of mass/area. The other half of the water sample was filtered onto a mixed cellulose ester filter (0.22 μm, 47 mm, Xinya, China), extracted in 10 ml absolute methanol and maintained in darkness at 4 °C overnight. After extraction, the sample was centrifuged (10 min at 4000*g*), the absorbance of the supernatant scanned between 250 nm and 750 nm with a spectrophotometer (UV-1800 UV–Vis Spectrophotometer, Shimadzu, Japan), and the concentration of the chl *a* calculated following the equation of Porra [18] and that of carotenoids according to Strickland and Parsons [19]. Pigment concentrations were normalized to nubbin surface area (μg cm^{-2}).

2.5. Symbiodinium Density and Pigment Contents in Coral Nubbins

At the end of the study, coral nubbins were frozen at −20 °C until further analysis for pigment contents and *Symbiodinium* density. Coral tissue was removed from the skeleton using a dental water flosser (Waterpik WP-72, USA) and homogenized. A 3 ml aliquot of total coral homogenate was centrifuged and counted as mentioned above to obtain the final algae density in the nubbins. Another 12 ml aliquot of slurry was centrifuged and extracted in 5 ml absolute methanol to determine pigment contents. *Symbiodinium* density and pigment contents were both standardized to nubbin surface area.

Quantification of UV absorbing compounds (UVAC), with absorbance peaks around 335 nm, in the coral branches was estimated using the peak height ratio between UVACs and chl *a* [20], with the same unit as used for other pigments (μg cm^{-2}). However, the pigment concentration in the cells released per hour in the water sample was sometimes too low to detect, so it was not proper to calculate UVACs by comparison of peak heights. Another calculation method was applied by directly dividing the UVAC peak height (the difference between the absorbance at 335 nm and 353 nm) by nubbin surface area

(OD cm^{-2}). There is a good correlation between the two calculation methods for UVACs (Fig. 3C inset graph).

2.6. Data Analysis

The results are presented as means ± standard deviation. Normality and homoscedasticity were ascertained prior to testing each dependent variable using a Shapiro–Wilk test and Levene's test, respectively. When the data departed from a normally distributed population, they were natural log transformed. Differences in day-time changes of Fv/Fm, the release ratio of symbiotic algae, chl *a* and UVAC content per hour were assessed by two-way repeated measures ANOVA (Two-way RM ANOVA) considering time and UVR effects with a Fisher's least significant difference (LSD) test for pairwise comparisons. For the small sample size, only six time points, i.e. the data of 09:00, 10:00, 11:00, 13:00, 14:00 and 17:00, were chosen for Two-way RM ANOVA. The effects of UV radiation on the cumulative release cell number, total release and algal density within the nubbins, as well as the pigment contents, were compared using a one-way ANOVA ($\alpha = 0.05$).

3. Results

3.1. Diurnal Changes in Photosynthetic Efficiency of Symbiodinium

The results of Fv/Fm measurements showed a significant diurnal change, whether or not the corals were exposed to UVR (Fig. 1B, Two-way RM ANOVA, time $F_{2, 12} = 69.25$, $p < 0.001$). On the first day, the lowest Fv/Fm was detected at noon (Fisher's LSD $p < 0.05$). However, no UVR effect on the diurnal change of Fv/Fm was detected (Two-way RM ANOVA, UVR $p > 0.05$), whereas a significant interaction between time and UVR effects was displayed on the first day (Two-way RM ANOVA, time*UVR interaction $F_{4, 12} = 5.89$, $p = 0.007$).

3.2. Day-time Expulsion Pattern of Symbiodinium

Multivariate tests showed that the variations of both the released symbiotic algae number and the ratio of released cells to cells in the coral were significantly influenced by time ($F_{5, 2} = 22.98$, $p = 0.04$; $F_{5, 2} = 22.23$, $p = 0.04$), while the UVR effect was not obvious ($p > 0.05$). The peak in release ranged from 11:00 am to 13:00 pm for P and PA treatments, and the maximum expulsion rate appeared at 11:00 am, with values of (4.0 ± 5.2) and $(4.2 \pm 2.3) \times 10^4$ cells cm^{-2} h^{-1} 1 respectively, which corresponded to release ratios of (1.9 ± 1.4)% and (2.6 ± 2.3)%. The cumulative release number of algae in PAB treatment was much more than that in the PA treatment at 9:00 am ($F_{2, 6} = 7.90$, $p = 0.02$), and also showed a significantly higher value than both P and PA treatments at 10:00 am ($F_{2, 6} = 7.03$, $p = 0.03$, Fig. 2B). Altogether, the presence of UV-B led to the peak in release time being advanced 1 h, to 10:00 am.

3.3. Day-time Expulsion Pattern of Pigments

As seen from Fig. 3A, the chl *a* content in the water sample was highest around 11:00 am in the control, and the peak extended to 13:00 pm. The addition of UV-A led to a similar peak time, while the concentration of pigment was lower than in the control. Furthermore, with the addition of UV-B the peak release time was advanced, though the highest peak value was lower than in the control. The variation in carotenoid levels was similar to that of chl *a* (Fig. 3B). As shown in Fig. 3C, the peak time of UVACs release was broad and under UV-A plus UV-B was extended from 9:00 am to 14:00 pm, later than the

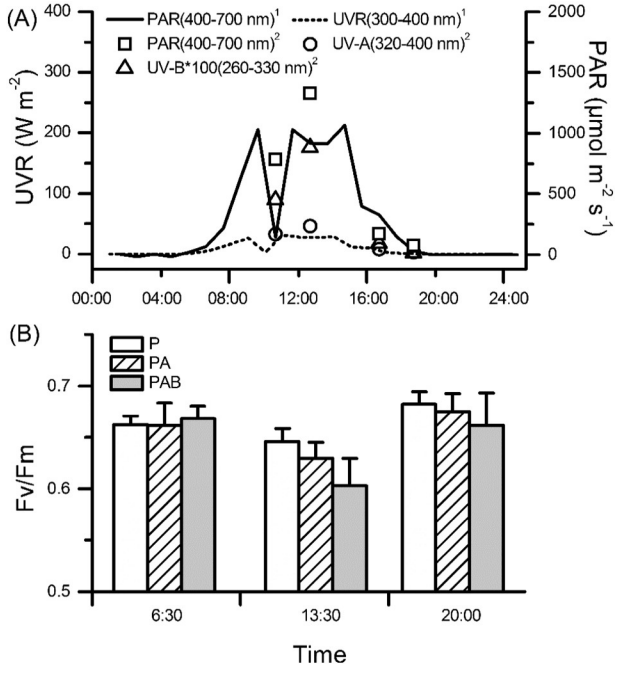

Fig. 1. Daily variability in ambient solar irradiance during the first day (A), and the diurnal change of Fv/Fm (B). Each bar represents the means ± SD, $n = 3$. [1] data from the Tropical Marine Biological Research station; [2] data from the PMA2100 radiometer.

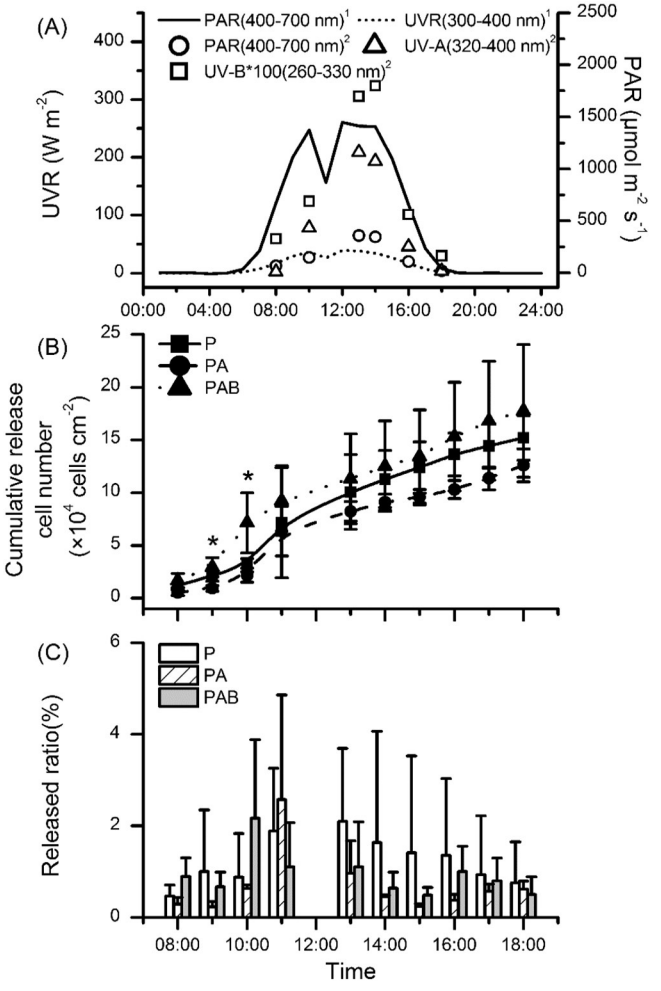

Fig. 2. Daily variability in ambient solar irradiance on the third day (A), day-time cumulative release number of *Symbiodinium* with time (B) and its ratio relative to the algae density in the coral nubbins (C). Each bar represents the means ± SD, $n = 3$. [1] denotes data from the Tropical Marine Biological Research station; [2] data from the PMA2100 radiometer. Treatment means marked with asterisks differ significantly (Fisher's LSD post hoc analysis, $p < 0.05$). In C, no data were collected at 12:00 am.

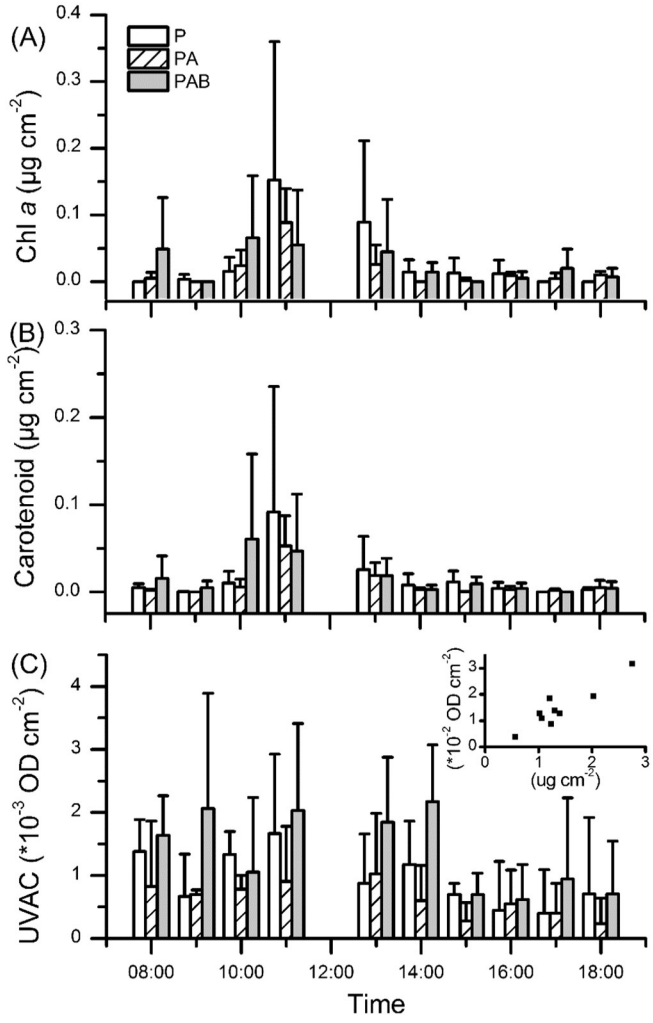

Fig. 3. Day-time release amounts of Chl *a* (A), carotenoid (B) and UVAC (C) with time. The inset graph in (C) shows the correlation between the two calculation methods for UVAC, with the data obtained from coral branches. No data were collected at 12:00 am. Each bar represents the means ± SD, $n = 3$.

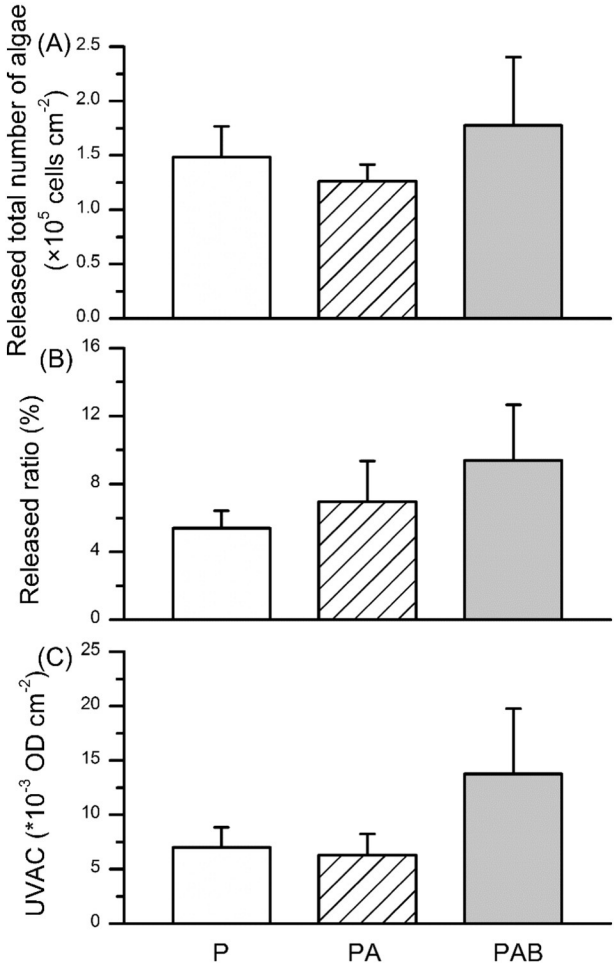

Fig. 4. Total cumulative release of *Symbiodinium* (A), its ratio (B) and UVAC content (C) during the daytime. Each bar represents the means ± SD, $n = 3$.

corresponding releases for chl *a* and carotenoids. For water samples, the pigment content from expelled cells was sometimes too low to be detected, which resulted in the big variation and non-significant difference on the timeline of chl *a*, carotenoid and UVACs ($p > 0.05$). The method for calculating UVACs used here had a good relationship with that used for UVACs in coral branches (Fig. 3C inset graph, $R^2 = 0.83$, Pearson's $r = 0.92$, $p < 0.001$).

3.4. Total Release Amount of Symbiodinium

The maximum cumulative release number of *Symbiodinium* during daytime was ($1.78 \pm 0.63 \times 10^5$) cells cm^{-2} when coral was exposed to the full spectrum radiation, and this accounted for (9.4 ± 3.3)% of the density in the coral branch (Fig. 4). However, the differences between P, PA and PAB treatments were not statistically significant for either the total release number ($F_{2, 6} = 1.09$, $p = 0.40$) or the total ratio ($F_{2, 6} = 1.50$, $p = 0.31$).

Although the mean release ratio appeared higher in nubbins exposed to UV-A and UV-A + UV-B, the effects were not statistically different. Similarly, the cumulative UVACs released from corals were highest when exposed to UV-A plus UV-B, but the differences between the other treatments were not statistically significant either ($F_{2, 6} = 2.93$, $p = 0.14$).

3.5. Photosynthetic Pigments and Symbiodinium Densities in Coral Nubbins

After UVR exposure for three days, the algae density remaining in the nubbins showed no statistically significant difference between treatments ($F_{2, 6} = 0.04$, $p = 0.96$), mainly due to the big variation between coral nubbins under the P treatment, though the mean value was 50% higher than in the other two treatments. However, on multiplying algal density by pigment content per cell, the variation was decreased and the pigment content normalized to surface area showed statistically significant decreases after exposure to UV-A or UV-B ($p < 0.05$, Fig. 5), whereas that normalized to algal cell (pg $cell^{-1}$) had no such change (data not shown). Specifically, the chl *a* fell from (10.6 ± 3.1) μg cm^{-2} to (5.1 ± 0.5) μg cm^{-2} under UV-A and UV-B stress, with that per cell ranging from 3.02–3.07 pg $cell^{-1}$. As for the carotenoids, the concentration per surface area decreased from (4.1 ± 1.2) μg cm^{-2} to (2.0 ± 0.2) μg cm^{-2} under UVR stress, while that per cell stabilized at a level of 0.9–1.5 pg $cell^{-1}$. The ratio of UVAC:chl *a* in coral nubbins showed no obvious difference between treatments, with the value ranging from 0.23 to 0.29 ($F_{2, 6} = 0.67$, $p = 0.55$).

4. Discussion

In the present study, coral nubbins exposed to UVR for three days did not show differences in symbiotic algae density, though their pigment content, including chl *a*, carotenoids and UVAC, decreased under stress. The diel algal expulsion rates between treatments exhibited no significant variation between treatments, with the release patterns being

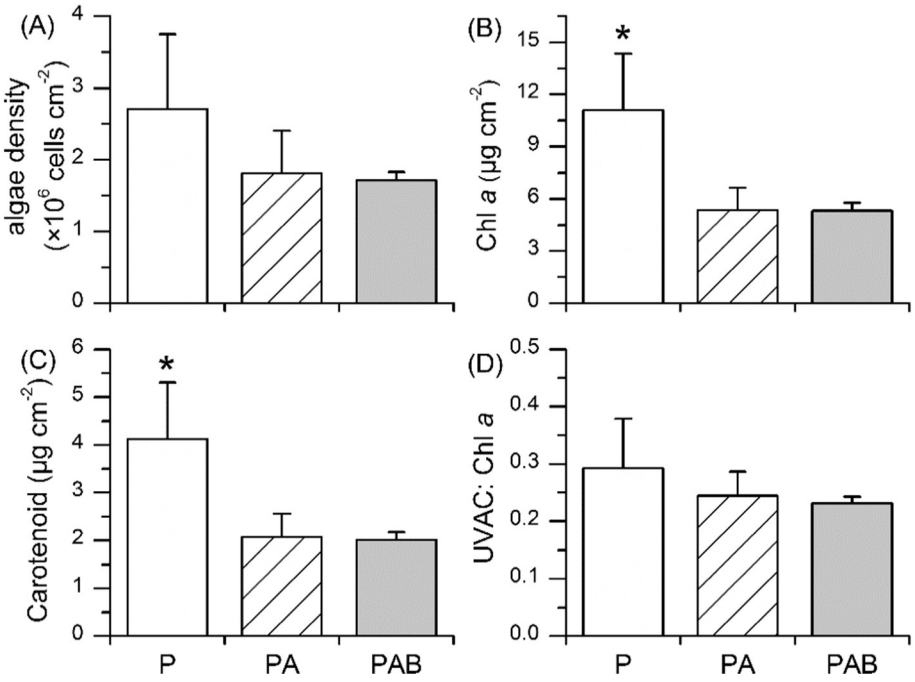

Fig. 5. Symbiotic algae density (A) in the coral nubbins after three-day exposure, chl *a* (B), carotenoid (C) concentration, and the ratio of UVAC:chl *a* in the nubbins (D). Each bar represents the means ± SD, $n = 3$. Treatment means marked with asterisks differ significantly (Fisher's LSD post hoc analysis, $p < 0.05$).

similar, i.e. the peak in release of symbiotic algae was around noon, though UV-B was found to induce an advancement in the peak release time by 1 h.

Here, the peak time algal release rate by *P. damicornis* ranged from $(1.52–9.98) \times 10^4$ cells cm^{-2} h^{-1}, and the release ratio varied from 0.8% to 5.2% of the algal standing stock. These values are rather high when compared with previous studies. There may be two reasons for this. One lies on the method used here to quantity the algal numbers, which was based on conventional hemocytometer counts, which has a low precision. As a result, the values were over seven times higher than the data in a separate study, enumerated with a highly sensitive qPCR approach [21]. We have tried to use the data of chl *a* released per hour combined with the chl *a* content per cell to estimate the algal expulsion rate, and the calculated rate at peak time fell in the range of $(1.07–6.55) \times 10^4$ cells cm^{-2} h^{-1}, which corresponds to values approximately two thirds of the directly enumerated data. This is considerably higher than the values in Stimson and Kinzie [5], also obtained using hemocytometer counts, which showed that the expulsion rate did not exceed 6000 cells cm^{-2} h^{-1} for *P. damicornis* even at the peak release time. In part this may result from the "bottle effect" in our experiments, which conditioned the coral nubbins in a hyperoxic environment. Water flow has been suggested to play a strong role in modulating the physiology of corals, such as photosynthesis [22], calcification [23] and responses to climate change [24]. The reduced flow speed induced by incubations in the quartz tubes would have led to a thickening of the diffusive boundary layer (DBL), and prevented oxygen exchange from the surface of corals into the bulk medium. Thus, even if we exchanged the water in the tubes every hour, it is unlikely the stress would have been relieved.

Though UVR exposure was not found to influence the diel algal expulsion rate or release ratio, the results displayed a potential trend in that the exposure to UV-A or UV-B gradually stimulated the coral to expel its symbionts, which resulted in pigment loss in the coral symbionts after three days. This suggests that the UVR level used in this study may have acted as a chronic stress for the coral *P. damicornis*. Under UVR stress, some *Symbiodinium* cells may become dysfunctional or damaged, but others may remain capable of division to compensate for the loss, thereby minimizing the apparent effects of UVR in the short term, but under chronic stress these factors would start to take effect. Further work exposing corals to UVR for longer than the 3 days used here would clarify this. The synthesis of UVACs, including mycosporine-like amino acids (MAAs) and fluorescent pigments, which act as antioxidant molecules or UV-filters, is one way to mitigate damage from UV stress [25,26]. Interestingly, as the chl *a* content in the coral nubbins exposed to UV-A and UV-B declined, the ratio of UVAC:chl *a* did not change. Accordingly, the UVAC concentration in the coral nubbins exposed to UVR may also decline in the same way as did chlorophyll and carotenoid pigments. The phenomenon is similar to the change in UVAC level observed in the larvae of *P. damicornis* under UVR stress [27]. The difference is that the effect mainly resulted from the potential decrease of algal numbers in nubbins under stress. Though the content of UVACs in corals decreased under UVR, it seemed that most of the compounds were released as cells were expelled to the surrounding waters (Fig. 3C, Fig. 4C), and the treatments exposed to UV-A and UV-B expelled the highest amount of UVACs both on an hourly and a daily basis.

The diel pattern of the algal release rate observed here is consistent with previous studies [5]. Although UV exposure did not affect the diel algal release rate in a statistically significant fashion, the advance in peak release time under UV-A and UV-B exposure is consistent with UVR-induced degradation of symbiotic algae (assuming that the diel expulsion is a circadian change commitment involving algal mitosis) leading to earlier expulsion of degraded algae and a shift in the peak release time. Steele and Goreau [28] have found that the degradation of symbiotic algae happened following the cells were expelled as pellets into gastrovascular cavity, and incorporated into the mesenteries by phagocytosis. Alternatively, because the cell cycle of *Symbiodinium* is dependent on light-dark cycling [29], and assuming that most of the released algae were going to divide, the negative effects of UVR on the algal release may not appear until the following day.

Alternatively, the lack of statistically significant changes in the diel algal expulsion rate between UVR treatments may be a consequence of nutrient limitation. Indeed, field surveys have reported that the N:P ratio of Sanya Bay water has kept increasing since 2004, which has resulted in phosphorus limitation [30]. Moreover, evidence has shown that the turnover time of the PO_4^{3-} pool would increase under UVR

stress [31], thus resulting in the disruption of phosphorus cycling, which is essential to ATP synthesis and membrane transport. Nevertheless, decreasing phosphate availability resulted in higher MAA levels in the dinoflagellate *Glenodinium foliaceum* and the cyanobacterium *Nodularia spumigena* [32,33], though this would not explain the higher amounts of UVACs released to the water when nubbins were exposed to UVR in this study as UVACs per cell were decreased under UVR. On the other hand, UVR has also been proven to inhibit ammonium uptake by phytoplankton [34] through effects on ATP or NADPH availability induced by damage to the photosynthetic apparatus. Furthermore, the synthesis of UVACs such as MAAs also requires the uptake of nitrogen as does the repair of damage to cellular constituents such as the D1 protein and Rubisco [33,35]. Thus, with decreased supply of dissolved inorganic nitrogen (DIN) under UVR stress [34], symbiotic algae cannot complete chromosome duplication and are mostly constrained at the G_1 phase in the cell cycle [36]. In this case, the expelled algae may mostly be dysfunctional cells. Subsequently, in the long term, the interactive effects of UVR and nutrient limitation will result in significant decreases in the concentration of pigments, which was shown here, as well as decreases in the symbiotic algae number. If this is true, additional work has to be done to test whether the expelled algal cells from corals exposed to UVR have a lower mitotic index compared to the control.

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