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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