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Effects of solar PAR and UV radiation on tropical biofouling communities

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ABSTRACT: We investigated the effect of solar ultraviolet radiation (UVR) and photosynthetically active radiation (PAR) on the development of tropical micro- and macrofouling communities for 30 d. The experimental design involved 3 treatments: full spectrum (PAR+UVR), PAR only, and minimal light (reduced PAR and UVR). Terminal restriction fragment length polymorphism analysis demonstrated that different light conditions resulted in the formation of highly different microbial communities. The lowest densities of bacteria were found under the full spectrum treatment, while the lowest densities of diatoms were found in the minimal light treatment. Macrofouling communities consisted of 13 species and differed among light treatments. In the presence of UVR, communities had low species diversity, evenness, and richness, while in minimal light and PAR treatments, communities had high species diversity, evenness, and richness. Similarity percentage (SIMPER) analysis revealed that the tubeworm *Hydroides elegans,* the alga *Ulva* (*Enteromorpha*) sp., and the bivalve *Perna viridis* were the species responsible for most of the dissimilarities in macrofouling communities among treatments. While densities of *H. elegans* were similar in the PAR and minimal light treatments, this polychaete had higher growth rates under minimal light conditions. We conclude that UVR and PAR directly control the development of shallow micro- and macrofouling communities by inhibiting the recruitment and growth of sensitive species and promoting the growth of resistant species, but also that these forms of solar radiation influence the surface cues available to competent larvae by altering the development of the microbial community.

KEY WORDS: Ultraviolet radiation · Visible light · Microbial communities · Biofilm · Biofouling · Community structure · Larval recruitment · Juvenile growth · South China Sea · Photosynthetically active radiation

INTRODUCTION

Solar ultraviolet radiation (UVR) has harmful effects on a variety of marine organisms (Bornman & Teramura 1993, Williamson 1996), affecting adult organisms as well as propagules (Ban et al. 2007). UVR can affect the larvae and adults of marine invertebrates by damaging the DNA of single organs, receptors, or cells (Chiang et al. 2003, Hoag 2003, Bonaventura et al. 2005) or affect whole physiological processes (Franklin & Forster 1997, Wiencke et al. 2000), such as the development, settlement, and survival of the organism (Chalker-Scott et al. 1992, Bingham & Reitzel 2000, Kuffner 2001, Lesser et al. 2003). However, only a few studies have investigated the effects of UVR on marine benthic and biofouling communities under natural conditions, and the findings of these studies are not always consistent (Santas et al. 1998a,b, Lotze et al. 2002, Molis et al. 2003, Molis & Wahl 2004, Wahl et al. 2004, Dobretsov et al. 2005). For example, field studies

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in Nova Scotia, Canada (Lotze et al. 2002), and Hong Kong (Dobretsov et al. 2005), and mesocosm studies in the Caribbean (Santas et al. 1998a) have found that UVR influences macrofouling and benthic community structure and development. Similarly, the growth of UV sensitive diatom species was reduced in a field experiment in the Caribbean (Santas et al. 1998b). However, another study in Namibia (Molis & Wahl 2004) did not detect any clear trend of UVR effects on macrofouling communities because some UV-tolerant species provided protective shading for UVR-sensitive species (Wahl et al. 2004).

Propagule settlement and the formation of marine biofouling communities (Glasby 2000, Glasby & Connell 2001, Dobretsov et al. 2005) can also be affected by photosynthetically active radiation (PAR), especially for photosynthetic species such as algae (Han et al. 2004, Jiang et al. 2007, Steinhoff et al. 2008, Wulff et al. 2008). Glasby (1999) reported that communities developing on substrates exposed to minimal light (shaded) were characterized by low algal and spirorbid density and high density of bryozoans, serpulid polychaetes, ascidians, and sponges.

Microbial communities can also regulate the recruitment of macrofouling species by induction or inhibition of larval settlement and metamorphosis of invertebrate larvae and algal spores (see review by Qian et al. 2007). UVR and PAR might therefore affect the structure of macrofouling communities indirectly by modifying the growth and composition of microbial communities.

The present study expands our understanding of the effect of UVR on biofouling communities. In comparison to previous studies, which separated microbial and macrofouling components or studied them under laboratory controlled conditions, we investigated the effect of both UVR and PAR on the overall development of micro- and macrofouling communities in field experiments. The specific goals of this study were to examine the effect of UVR and PAR on (1) the densities of bacteria and diatoms; (2) the composition of bacterial communities; (3) the density and diversity of macrofouling communities; and (4) the growth of a dominant macrofouling species, the polychaete *Hydroides elegans.*

MATERIALS AND METHODS

Experimental site. This field experiment lasted 30 d in July and August 2004, and was conducted at the perimeter of a fish farm in semi-enclosed Yung Shue O Bay, Hong Kong (22° 24′ N, 114° 21′ E). The study area is characterized by a tidal range of 0.5 to 2.5 m. The water depth at the site of the fish farm was 5 m at low tide, and currents were relatively modest with an average flow rate of 0.1 m s^{-1}. Sediments in the bay were a combination of fine sand and silt. The water temperature and salinity during the study period varied from 29 to 30°C and 28 to 30‰, respectively. The mean UV-A (320 to 400 nm) and UV-B (280 to 320 nm) radiation at noon in Hong Kong during the experimental period was 29.4 ± 2.3 W m^{-2} (minimal = 22.7, maximal = 36.5 W m^{-2}) and 1.5 ± 0.4 W m^{-2} (minimal = 0.38, maximal = 2.6 W m^{-2}), respectively, as measured by the Hong Kong observatory. Although the actual radiation levels received by Petri dishes were not measured in this study, our previous experiments (S. Dobretsov unpubl.) in the same area showed that about 39 ± 8% of UV-A and UV-B reach a water depth of 20 cm.

Experimental design. The effects of UVR and PAR on the biofilm and macrofouling communities were examined using 18 custom-designed experimental units (Fig. 1). These units were suspended, about 50 cm apart from each other, from a floating pontoon. Each unit consisted of 1 large (37 cm diameter) acrylic disk and 1 small (24 cm diameter) disk; the 2 disks were held 15 cm apart by a section of PVC tubing. The large upper disk acted as a light filter that shielded the lower disk; the lower disk was opaque and served as a base to which we attached a set of sterile Petri dishes (50 mm diameter) to monitor the biofilm and macrofouling communities. All Petri dishes were attached to the upwards-facing side of the lower disk. These experimental units were attached to ropes and suspended at a depth of 15 to 20 cm below the surface, with a weight attached to the lower end of each rope to stabilize the unit and to keep the disks aligned vertically (Fig. 1). The experimental design involved 3 treatments (full spectrum, PAR only, and minimal light) with 6 replicate units $treatment^{-1}$ and 6 Petri dishes

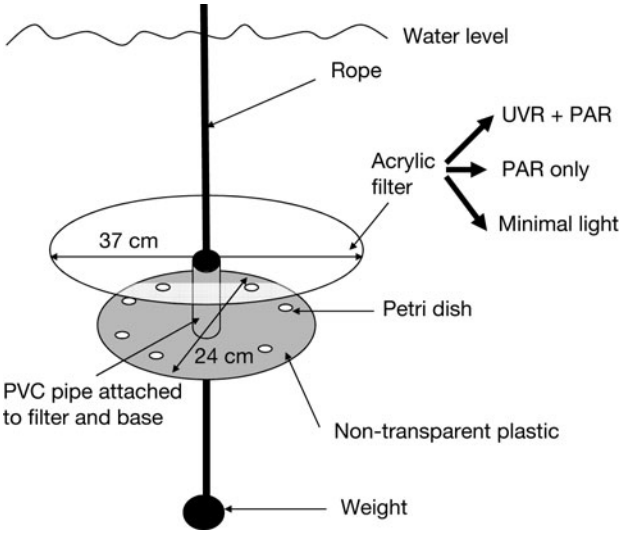

Fig. 1. Experimental set-up

unit^{-1}. For the full spectrum (control) treatment, the top disk of each unit consisted of 3 mm thick acrylic that was 90% transparent to all UVR and PAR wavelengths. The PAR only treatment was obtained using 4 mm thick acrylic that was opaque to UVR (290 to 400 nm) but allowed 90% transmission of PAR. The third treatment, minimal light, was obtained using 3 mm thick grey acrylic that was opaque to all light wavelengths and thus shielded the Petri dishes from all direct solar radiation; only modest levels of oblique diffuse light could reach the Petri dish surfaces. To minimize the effects of sedimentation and fouling on the transmission properties of the filter disks, all upper disks were cleaned with a soft sponge at least twice a week.

Two Petri dishes were taken from each replicate unit on Days 7, 14, and 30 after the start of the experiment. On each of those collection dates, for each of the 3 treatments, 6 Petri dishes (1 dish unit^{-1} × 6 replicate units treatment^{-1}) were used for the analysis of macrofouling communities and measurements of *Hydroides elegans* growth. The other 6 Petri dishes (1 dish unit^{-1} × 6 replicate units treatment^{-1}) were divided into 2 sets of 3 dishes. One set of 3 dishes treatment^{-1} was used for bacterial community composition analysis and the other set was used for counts of bacterial and diatom densities. We report data for microbial analysis only for Days 7 and 14; by Day 30, the bacteria and diatoms were layered on top of each other and could no longer be counted, and the presence of PCR inhibitors and DNA-degrading enzymes in the dishes prevented the amplification of bacterial DNA.

Microbial community analysis. ***Bacteria and diatom densities:*** Prior to counting bacteria and diatoms on the inside surfaces of the Petri dishes, biofilms were fixed with a 4% formalin solution prepared with autoclaved 0.22 µm filtered seawater. Bacteria were then stained with the DNA-binding fluorochrome 4,6-diamidino-2-phenylindole (DAPI; Fluka). DAPI was first dissolved in distilled water at 50 µg ml^{-1} (stock solution) and then diluted in filtered (0.22 µm) sterile seawater to achieve a working concentration of 0.5 µg ml^{-1}. For each dish, bacteria were counted in each of 10 haphazardly selected fields of view under an epifluorescence microscope (Axiophot, Zeiss; magnification 1000×; λ_{Ex} = 359 nm, λ_{Em} = 441 nm). Densities of bacteria and diatoms were log-transformed (Zar 1996) to improve normality of the data. The normality assumption was verified with the Shapiro-Wilk test (Shapiro & Wilk 1965). Densities of microorganisms developed under different light regime treatments were compared using repeated measures analysis of variance (ANOVA; Zar 1996). Post hoc multiple comparisons were performed using the Tukey HSD test at a significance level of $\alpha = 0.05$.

Bacterial community composition: The composition of the bacterial community that developed in 3 replicated Petri dishes treatment^{-1} was examined using terminal restriction fragment length polymorphism (T-RFLP) analysis (Dunbar et al. 2001). The entire surface area of each Petri dish was swabbed with a sterile cotton ball. One swab from each Petri dish was extracted with 1 ml of buffer (100 mM Tris-HCl, 100 mM EDTA, 100 mM sodium phosphate, 1.5 M sodium chloride, 1% CTAB; at pH = 8). For lysing, the samples were subjected to 3 cycles of freezing and thawing followed by 30 min incubation in Proteinase K solution (10 mg ml^{-1} in TE buffer) at 37°C, and then a 2 h incubation in 20% sodium dodecylsulfate (SDS) at 65°C. The cotton swabs were then removed and the solution was centrifuged (7599 × *g* for 5 min). DNA from the supernatant was extracted and purified twice in a volume of 24:1 chloroform:isoamylalcohol, followed by precipitation in isopropanol at room temperature for 15 min. The precipitated DNA was washed with cold 70% ethanol, resuspended in 50 µl of autoclaved double-distilled water, and then frozen at –80°C until use.

Polymerase chain reaction (PCR) of the 16S rRNA bacterial gene was performed in a total volume of 25 µl containing 1 µl of DNA template, 250 µM of each deoxyribonucleotide triphosphate (dATP, dCTP, dGTP, dTTP; Pharmacia Biotechnology), 1 U of DNA *Taq* polymerase (Amersham Biosciences), and 0.8 µM of each universal primer: 341F forward (5′-CCT ACG GGA GGC AGC AG-3′) and 926R reverse (5′-CCG TCA ATT CCT TTR AGT TT-3′). The 926R primer was labeled at the 5′ end with 6-carboxy fluorescine (FAM) dye. The thermocycling conditions were as follows: 95°C for 2 min (1 cycle); 95°C for 30 s (15 cycles), 60°C for 3 min (1 cycle), and 72°C for 3 min (1 cycle). The annealing temperature started at 60°C and was reduced to 45°C in increments of 1°C cycle^{-1}. Amplified DNA (4 µl of PCR mixtures) was visualized by gel electrophoresis on a 1.5% agarose gel in Tris-acetate-ethylenediaminetetraacetic acid (TAE) buffer.

Fluorescently labeled PCR products from triplicate PCR amplifications were purified with the Wizard® PCR preps DNA purification system (Promega) according to the manufacturer's protocol. Purified amplicons were digested with 20 U *Msp*I (Boehringer Mannheim Biochemicals) at 37°C for 6 h. Aliquots of digested products (10 µl) were mixed with 0.5 µl of internal size standard (ET550-R, Amersham Biosciences) and applied to capillary electrophoresis on a MegaBACE™ genetic analyzer (Amersham Biosciences) operated in the genotyping mode. After electrophoresis, the lengths of the fluorescently labeled terminal restriction fragments (T-RFs) were determined by comparison to internal size standards by using the 'Fragment Profiler' software (Amersham Biosciences). The lengths of T-

RFs were rounded up to the nearest integral values. Peaks that were less than 1.0 bp apart from a larger peak were classified as its 'shoulders' and thus eliminated (Dunbar et al. 2001). Only peaks over a threshold of 50 fluorescence units and with the heights contributing at least 1% to the integrated peak height were used for analysis. Terminal fragments <35 bp and >500 bp were excluded from the analysis to avoid detection of primers and uncertainties of size determination.

Phylogenetic ribotypes were assigned to the same T-RFs by 2 methods. (1) We used the TAP-TRFLP Java Applet program (retrievable from the RPD II Website at http://rdp8.cme.msu.edu/html/TAP-trflp.html#program), which performs a simulated restriction digestion of the submitted sequences in the prokaryotic 16S rRNA database. (2) We used 100 marine bacterial isolates from the Hong Kong University of Science and Technology (HKUST) culture collection and assigned these to the T-RFs; we digested 16S rRNA of each bacterial isolate and performed their T-RFLP analysis (described above) to determine the length of T-RFs.

A Bray-Curtis similarity matrix based on the total number of T-RFs observed in all samples with the presence (denoted as 1) or absence (denoted as 0) of specific T-RFs in individual samples was constructed. This matrix was used for the construction of a multi-dimensional scaling (MDS) plot to show the similarities between microbial communities using the PRIMER 3.1 software (Plymouth Marine Laboratory). Since each T-RF represents a unique ribotype, as a measure of species diversity the Shannon-*H′* diversity index and Margalef's species richness (Warwick & Clarke 1995, Clarke & Gorley 2001) were calculated using PRIMER 3.1 software (Plymouth Marine Laboratory).

Analysis of macrofouling communities. The density and species composition of macrofouling species that settled inside the Petri dishes were analyzed under a dissecting microscope. The number of individuals of each species was counted, and these values were then converted to densities (cm^{-2}) for each species. In the case of *Ulva* (previously *Enteromorpha*) sp., the area (cm^2) occupied by algae was measured using the Java image processing software Image J (http://rsbweb.nih.gov/ij/). The area occupied by algae was then converted to percentage of algal cover. As a measure of species diversity, the Shannon-*H′* diversity index, Margalef's species richness *d*, and Pielou's evenness *J′* (Warwick & Clarke 1995, Clarke & Gorley 2001) were calculated using PRIMER 3.1 software (Plymouth Marine Laboratory). We compared the densities of individual invertebrate species and the area covered by *Ulva* sp., as well as species diversity, richness, and evenness of communities among the 3 light treatments, using repeated measures ANOVA (Zar 1996). Individually, for each sampling date, post hoc multiple comparisons were performed using the Tukey HSD test at a significance level of $\alpha = 0.05$. The normality assumption was verified with the Shapiro-Wilk test (Shapiro & Wilk 1965). The effects of UVR and PAR on community structure were analyzed using analysis of similarity (ANOSIM) and similarity percentage (SIMPER) procedures (PRIMER 3.1 software, Plymouth Marine Laboratory), which are based on MDS of the Bray-Curtis dissimilarity index (Warwick & Clarke 1995).

Size measurements of *Hydroides elegans*. To measure the size of the polychaete *H. elegans*, 6 Petri dishes from each treatment were collected and returned to the laboratory. Digital images of the Petri dishes collected on Days 7, 14, and 30 were taken with a digital camera attached to a dissecting microscope. These were converted to 8-bit black and white images, and their background intensity was adjusted to enhance contrast. Thirty worms were haphazardly selected from each Petri dish, and the tube surface area (mm^2) of each individual was measured using the Image J computer image analysis software (http://rsbweb.nih.gov/ij/). The mean tube surface area of *H. elegans* in each light treatment was calculated, the normality assumption was verified using the Shapiro-Wilk test (Shapiro & Wilk 1965), and the mean surface areas were compared among treatments using repeated measures ANOVA (Zar 1996). Post hoc multiple comparisons were performed using the Tukey HSD test at a significance level of $\alpha = 0.05$.

RESULTS

Microbial community analysis

Densities of bacteria and diatoms differed significantly among the 3 light treatments (Fig. 2). ANOVA tests revealed that light regime and age of the biofilm had significant effects individually ($F = 20.4$, df = 2, $p < 0.0001$ and $F = 31.7$, df = 2, $p < 0.0001$, respectively) on the density of bacteria, as well as a significant interaction ($F = 6.1$, df = 4, $p = 0.004$). After 7 d, bacterial densities were lowest on the panels exposed to PAR+UVR (full spectrum treatment; Fig. 2A). By Day 14, however, bacterial densities were similar in all treatments. Diatom densities were also affected by light regime, age of the biofilm, and the interaction of these factors (ANOVAs: $F = 63.07$, df = 2, $p < 0.0001$; $F = 12.03$, df = 2, $p < 0.0001$; and $F = 4.01$, df = 4, $p = 0.023$, respectively). The lowest densities of diatoms were consistently found in the minimal light treatment, and the highest densities were recorded in the PAR treatment (Fig. 2B).

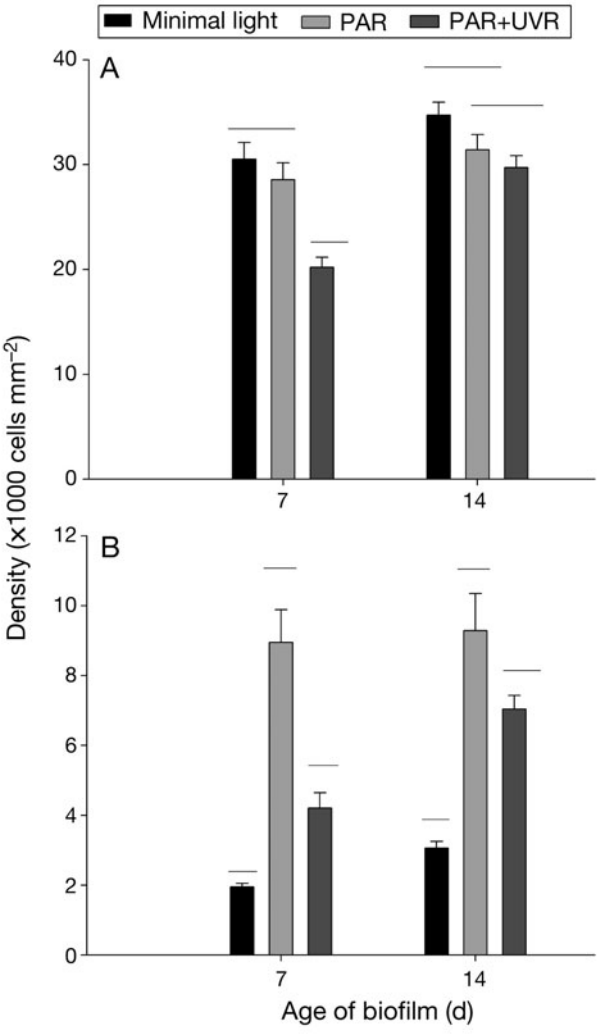

Fig. 2. Densities (×1000 cells mm^{-2}) of (A) bacteria and (B) diatoms on Petri dishes. Values are mean + SD (n = 3). Means not joined by a horizontal line differ significantly (ANOVA, HSD: $p < 0.05$)

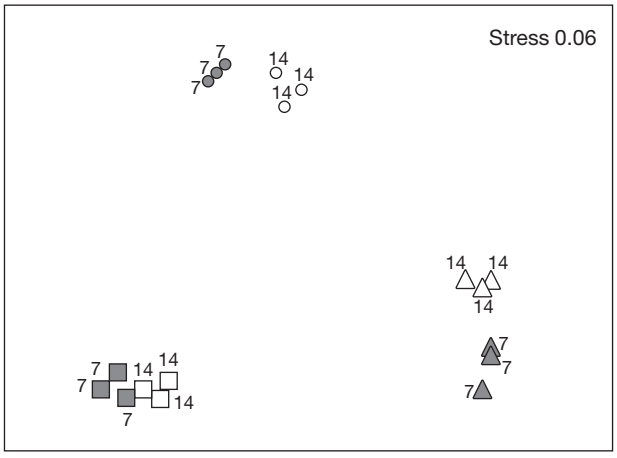

Fig. 3. Multidimensional scaling (MDS) of the similarity matrix of the terminal restriction fragments (T-RFs) of bacterial community DNA samples developed under different light regimes (triangles: minimal light; circles: PAR only; squares: UV-PAR) after 7 d (grey) and 14 d (white) of the experiment. Experimental communities were replicated 3 times

T-RFLP analysis revealed that bacterial communities developing under different light conditions were highly different from each other, while communities forming on replicate dishes of a same light treatment did not differ much over the 14 d period (Fig. 3). Bacterial communities under different light treatments could be distinguished based on differences in certain T-RFs or ribotypes (Table 1). After 7 d, the highest number of bacterial ribotypes (29) was found in biofilms exposed to the full light spectrum (PAR+UVR). These biofilms had the highest diversity and ribotype richness. After 14 d, however, the number of bacterial ribotypes was similar in all light treatments (14 to 18), while the biofilms in PAR and minimal light treatments had the highest diversity and richness (Table 1). Some bacterial strains occurred only under particular light and UV conditions throughout the experiment. For example, the presence of T-RFs at 79, 90, 281, and 361 bp were specific to the full spectrum treatments; T-RFs at 148, 183, 207–208, 328, and 370 bp were only present in the biofilms developed under minimal light conditions; and T-RFs at 220, 246, 295, 304, and 335 bp were found only in the PAR treatments throughout the experiment. In contrast, T-RF at 92 bp was present in all treatments (Table 1). Some bacterial ribotypes were present only at a particular time point of the experiment. For example, T-RF at 58 bp was found only in the minimal light treatment after 14 d, while T-RF at 80 bp was observed only in the minimal light treatment after 7 d.

Analysis of T-RFs by the TAP-TRFLP program provides preliminary information on the identities of ribotypes within a bacterial community (Table 1). In all treatments, the most abundant ribotype was represented by T-RFs of 92 bp in length, which was putatively identified as belonging to the *Plactomycetes* (mostly *Pirellula* spp.). *Alphaproteobacteria* (mostly *Sphingomonas* spp.), *Betaproteobacteria* (mostly *Thiobacillus* spp.), and *Firmicutes* (mostly *Eubacteria* spp.) were specific to the full spectrum treatments, while the *Bacteroidetes*/*Chlorobi* group (mostly *Porphyromonas* spp.) and *Actinobacteria* (mostly *Streptomyces* spp. and *Mycobacterium* spp.) occurred only in the minimal light treatment. It was difficult to identity bacterial ribotypes in the PAR treatments. Most of the T-RFs did not match isolates from the HKUST culture collection (Table 1), which might suggest that these were uncultivable bacteria. In some cases, identities of the T-RFs were different for both methods used (T-RFs

Table 1. Terminal restriction fragment length polymorphism (T-RFLP) profiles of bacterial communities developed on Petri dishes exposed to different light regimes (minimal light, PAR, and full spectrum treatments) for 7 and 14 d. Presence and fragment size (base pairs, bp) of individual terminal restriction fragments (T-RFs) are denoted as follows — 3: present in 3 out of 3 replicates, 2: present in 2 out of 3 replicates. Cases of presence in only 1 out of 3 replicates are omitted. Diversity *H′* and species richness *d* of microfouling communities reported as mean (SE) (n = 3). HKUST: Hong Kong University of Science and Technology

T-RF (bp)	Minimal light-7d	PAR-7d	Full spectrum-7d	Minimal light-14d	PAR-14d	Full spectrum-14d	Identity according to TAP-TRFLP	Identity according to HKUST collection
50	3	.	.	.	.	2	*Chloroflexus* sp.	Unknown
52	.	.	3	.	.	.	Unknown	Unknown
58	.	.	.	2	.	.	*Aminobacterium* sp.	Unknown
64	2	3	.	3	3	.	Unknown	Unknown
67	.	.	3	.	.	.	*Brevibacterium* sp.	Unknown
69	.	2	.	.	2	.	*Arthrobacter* sp.	Unknown
70	3	.	.	.	.	.	Unknown	Unknown
75	.	.	.	.	2	.	*Fibrobacter* sp.	Unknown
79	.	.	2	.	.	3	Unknown	Unknown
80	3	.	.	.	.	.	*Lactobacillus* sp.	Unknown
81	.	.	2	.	.	.	*Streptococcus* sp.	Unknown
88	.	.	3	.	.	.	Unknown	Unknown
90	.	.	2	.	.	3	*Sphingomonas* sp.	Unknown
91	.	2	2	.	.	.	*Cytophaga* sp., *Pseudomonas* sp.	Unknown
92	2	3	3	3	3	2	*Pirellula* sp.	Unknown
102	3	.	.	.	.	.	Unknown	Unknown
104	.	.	2	.	.	.	Unknown	Unknown
108	.	3	.	.	2	.	Unknown	Unknown
125	3	.	3	.	.	2	*Deinococcus* sp.	Unknown
126	.	.	.	3	3	.	*Leptospira* sp., *Planctomycetes* sp.	Unknown
127	.	.	3	.	.	2	*Desulfobotulus* sp.	Unknown
128	.	2	.	.	.	.	*Mycobacterium* sp.	*Pseudolateromonas* sp.
130	.	.	.	.	3	.	Unknown	Unknown
132	.	.	2	.	.	.	Unknown	Unknown
134	.	.	3	.	.	2	*Bastochloris* sp.	Unknown
148	3	.	.	3	.	.	Unknown	Unknown
156	.	.	2	3	.	.	Unknown	Unknown
158	2	2	.	.	3	.	*Bastochloris* sp.	Unknown
167							Unknown	Unknown
180	.	.	3	.	.	2	*Sphingomonas* sp.	Unknown
182	.	3	.	.	3	.	*Frankia* sp.	Unknown
183	3	.	.	3	.	.	*Porphyromonas* sp.	Unknown
184	.	3	3	.	.	.	Unknown	Unknown
185	.	2	.	.	.	.	Unknown	Unknown
205	.	.	.	.	2	.	*Acetobacter* sp., *Rhodococcus* sp., *Streptosporangium* sp.	Unknown
206	.	.	3	.	.	3	*Mycobacterium* sp.	Unknown
207	2	.	.	2	.	.	*Streptomyces* sp.	*Vibrio* sp., *Idiomarina* sp.
208	2	.	.	2	.	.	*Mycobacterium* sp.	*Brevibacterium* sp.
218	.	2	3	.	.	2	Unknown	Unknown
219	3	.	.	.	.	.	Unknown	Unknown
220	.	2	.	.	3	.	Unknown	Unknown
230	2	.	.	3	.	.	Unknown	Unknown
246	.	3	.	.	3	.	*Chlorella* sp.	*Deleya* sp., *Halomonas* sp.
247	.	.	.	.	3	.	Unknown	Unknown
266	.	.	.	3	.	.	*Rhodobium* sp.	Unknown
269	3	.	.	.	.	.	Unknown	Unknown
281	.	.	2	.	.	3	*Thiobacillus* sp.	Unknown
292	.	.	2	.	.	.	*Mycoplasma* sp.	Unknown
295	.	3	.	.	3	.	Unknown	Unknown
296	.	.	.	3	.	.	*Treponema* sp., *Fibrobacter* sp.	Unknown
299	3	.	3	.	.	.	*Clostridium* sp., *Bacillus* sp., *Sphingomonas* sp.	Unknown

Table 1 (continued)

T-RF (bp)	Minimal light-7d	PAR-7d	Full spectrum-7d	Minimal light-14d	PAR-14d	Full spectrum-14d	Identity according to TAP-TRFLP	Identity according to HKUST collection
303	.	.	2	.	.	.	*Bacillus* sp.,	*Kocuria* sp., *Bacillus* sp.
304	.	3	.	.	3	.	*Mycoplasma* sp.	*Micrococcus* sp.
312	.	.	3	.	.	.	*Pseudomonas* sp., *Azoarcus* sp.	*Pseudoalteromonas* sp.
328	2	.	.	3	.	.	Unknown	Unknown
335	.	2	.	.	2	.	Unknown	Unknown
336	.	.	3	.	.	.	*Verucomicrobium* sp.	Unknown
361	.	.	2	.	.	3	*Eubacterium* sp.	Unknown
362	.	3	.	.	.	.	*Mycoplasma* sp.	Unknown
365	.	.	.	.	3	.	*Cytophaga* sp., *Fusobacterium* sp.	Unknown
370	3	.	.	3	.	.	*Bacillus* sp., *Flavobacterium* sp.	Unknown
371	.	.	2	.	2	2	*Corynebacterium* sp., *Bacillus* sp., *Microbacterium* sp.	*Bacillus* sp.
374	.	.	2	.	.	.	Unknown	Unknown
423	.	.	.	3	.	.	*Achromatium* sp.	*Stenotrophomonas* sp.
432	.	.	3	.	.	2	*Rhizobium* sp.	Unknown
445	.	.	3	.	.	.	Unknown	Unknown
Total no. of T-RFs	18	17	29	15	18	14		
Diversity	2.88 (0.06)	2.83 (0.14)	3.32 (0.13)	2.99 (0.05)	3.08 (0.02)	2.65 (0.13)		
Species richness	5.80 (0.28)	5.71 (0.55)	8.15 (0.83)	6.34 (0.23)	6.72 (0.08)	5.0 (0.44)		

of 128, 207–208, and 423 bp in length), while in other cases (T-RFs of 303, 371, and 312 bp in length), both methods resulted in similar bacterial identity. Differences between predicted and actual cut sites for both methods can be explained by the fact that the length of T-RFs predicted by the TAP-TRFLP program is often bigger than actual fragment length obtained by restriction of isolates or clones (Lueders & Friedrich 2003). This can lead to possible misidentification of the fragments by the program.

Analysis of macrofouling communities

In total, 13 macrofouling species were found on the Petri dishes. These included the green alga (Chlorophyta: Ulotrichales) *Ulva* (*Enteromorpha*) sp., hydroid polyps (Cnidaria: Hydrozoa) *Obelia* sp. and an unidentified Leptomedusa, the polychaetes (Annelida: Polychaeta) *Hydroides elegans* and *Spirorbis foraminosus*, the barnacles (Crustacea: Cirripedia) *Balanus amphitrite* and *B. trigonus*, the bivalves (Mollusca: Bivalvia) *Perna viridis* and *Anomia chinensis*, the bryozoans (Bryozoa: Cheilostomata) *Schizoporella unicornis* and an unidentified bryozoan, and the tunicates (Tunicata: Styelidae) *Botryllus* sp. and an unidentified tunicate. During the experiments, the alga *Ulva* sp., the tube worm *H. elegans*, and the green mussel *P. viridis* dominated on all plates, but in different proportions.

The mean overall density of invertebrates remained at ~2 to 5 ind. cm^{-2} throughout the 30 d experimental period, except in the minimal light conditions treatment in which densities were 2 to 7 times higher (Fig. 4A). The minimal light treatment was characterized by significantly higher (HSD, ANOVA: $p < 0.001$) densities of macrofoulers on Days 14 and 30 than in the 2 other treatments. Algal cover increased during the experiment from 0% at the beginning of the experiment to 19.4% (PAR treatment) or 80.0% (full spectrum treatment) on Day 30. Surprisingly, *Ulva* sp. cover became highest in the full spectrum treatment and significantly lower in the PAR treatment (Fig. 4B). Light treatment, age of the community, and the interaction of these 2 factors each had a significant and strong influence on the density of invertebrates and the surface area covered by *Ulva* sp. on the dishes (Table 2).

The biofouling communities that formed under the 3 light treatments differed significantly (Table 2) throughout this 30 d experiment in terms of their diversity, species richness, and evenness. All 3 measures of community structure were significantly lower in the full spectrum (PAR+UVR) treatment than in the other 2

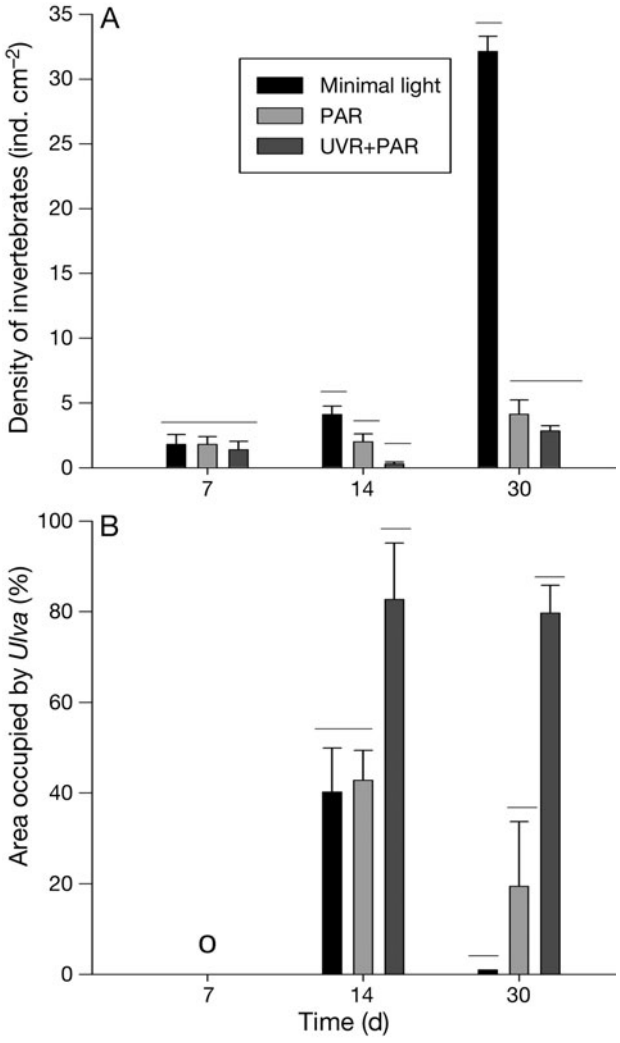

Fig. 4. (A) Density of invertebrate foulers (ind. cm^{-2}) on the dishes and (B) percent of algal cover occupied by the green alga *Ulva* sp. during the experiments. Values are mean +SD (n = 6). Means not joined by a horizontal line differ significantly (ANOVA, HSD: $p < 0.05$)

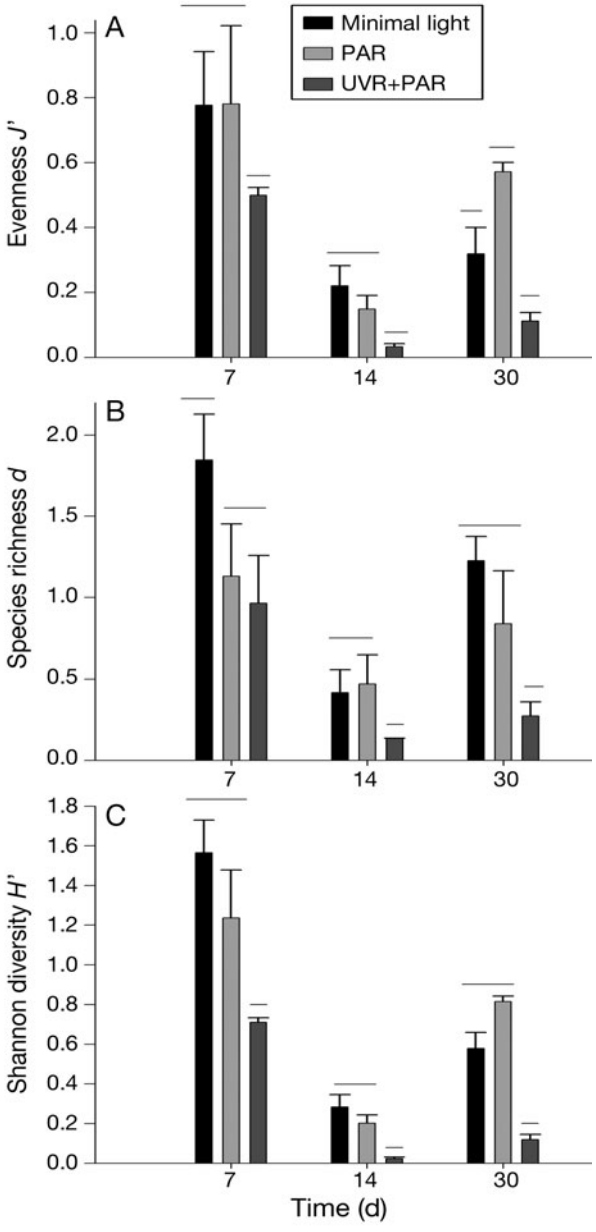

Fig. 5. (A) Pielou's evenness, (B) Margalef species richness, and (C) Shannon diversity index of macrofouling communities developed on the dishes during the field experiment under different light and UV regimes. Data are expressed as mean + SD of 6 replicates. Means not joined by a horizontal line differ significantly (ANOVA, HSD: $p < 0.05$)

treatments (Fig. 5). *Obelia* sp., *Perna viridis*, and *Hydroides elegans* dominated in the PAR and minimal light treatments. Communities developing in the minimal light and PAR treatments, however, generally had similar levels of diversity, species richness, and evenness. These 3 measures of community structure were

Table 2. Repeated measures ANOVA on the effect of 3 light treatments on the density of invertebrates, area occupied by algae, diversity H', species richness d, and evenness J' of macrofouling communities during the 30 d field experiment

Source	df	Density		Area		Diversity		Richness		Evenness	
		F	p	*F*	p	*F*	p	*F*	p	*F*	p
Light *L*	2	39.30	<0.0001	176.82	<0.0001	56.94	<0.0001	44.06	<0.0001	63.18	<0.0001
Time *T*	2	23.13	<0.0001	316.00	<0.0001	178.43	<0.0001	83.93	<0.0001	217.90	<0.0001
$L \times T$	4	6.58	0.0002	56.16	<0.0001	9.19	<0.0001	6.40	0.0004	9.43	0.0001

also strongly affected by the age of the community, being highest on Day 7, and by the interaction of light treatment and age (Table 2).

Cluster analysis revealed that the macrofouling communities developing under different light conditions formed separate clusters (Fig. 6). In most cases, the community that formed in the minimal light treatment was highly different from the communities that developed in the other 2 treatments. The community developing in the full spectrum treatment became distinct from that in the PAR treatment by Day 14.

An analysis of counts for each species, using the ANOSIM procedure, revealed that species composition differed significantly among light treatments (Table 3). The ANOSIM R-statistic, which is a useful tool to evaluate differences between communities (Clarke & Gorley 2001), was higher than 0.63 at each time interval. The high R-statistic values for the communities in our experiments indicate that ANOSIM was able to discriminate the macrofouling communities that developed under different light conditions. The analysis of percent similarity among communities, using the SIMPER procedure, revealed that the tubeworm *Hydroides elegans*, the clam *Perna viridis*, and the alga *Ulva* sp. were the main species responsible for dissimilarities among communities throughout the study (Table 3). By Day 14 and onwards, due to the

Table 3. Comparisons among macrofouling communities in species composition (using analysis of similarities, ANOSIM, and similarity percentage, SIMPER) exposed to 3 light treatments for 7, 14, and 30 d. ANOSIM R-statistic, which varies from −1 to +1, was used for evaluation differences between communities. Significant values of R have $p < 0.05$. SIMPER was used to assess the contribution of each species for an observed difference between communities

Species responsible for differences in communities	Contribution (%)
After 7 d	ANOSIM R = 0.637, p = 0.001
Hydroides elegans	28.30
Obelia sp.	18.41
Unidentified *Hydrozoa*	17.35
Perna viridis	12.29
Bugula sp.	9.67
Anomia chinesis	4.32
Other species	9.66
After 14 d	ANOSIM R = 0.786, p = 0.001
Ulva (*Enteromorpha*) sp.	54.49
Hydroides elegans	24.64
Perna viridis	5.59
Other species	15.28
After 30 d	ANOSIM R = 0.921, p = 0.001
Ulva (*Enteromorpha*) sp.	79.08
Hydroides elegans	4.77
Perna viridis	2.27
Other species	13.88

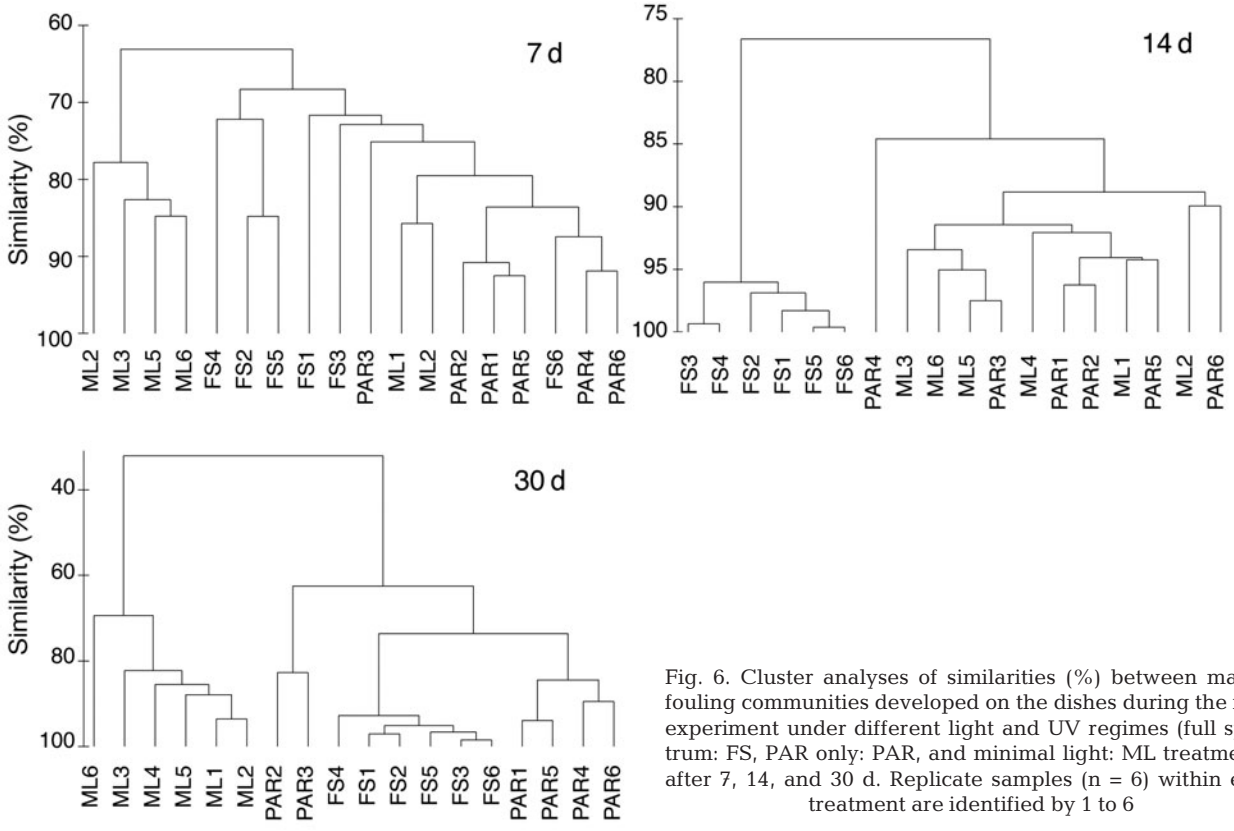

Fig. 6. Cluster analyses of similarities (%) between macrofouling communities developed on the dishes during the field experiment under different light and UV regimes (full spectrum: FS, PAR only: PAR, and minimal light: ML treatments) after 7, 14, and 30 d. Replicate samples (n = 6) within each treatment are identified by 1 to 6

rapid growth of the green alga *Ulva* sp. in the full spectrum treatment, this species accounted for more than 50% of the dissimilarities among communities.

Size of *Hydroides elegans*

The average area of *H. elegans* tubes was strongly influenced by the number of days elapsed since the start of the experiment (ANOVA: $F = 95.5$, df = 2, $p < 0.0001$), the light treatment (ANOVA: $F = 40.3$, df = 2, $p < 0.0001$), and the interaction of these factors (ANOVA: $F = 10.2$, df = 4, $p < 0.0001$). Significant differences were apparent by Day 14 of the experiment (Fig. 7). *H. elegans* tubes were largest in the minimal light and PAR treatments. By Day 30, the average surface area of *H. elegans* tubes in the PAR treatment and under minimal light conditions was 1.9 times and 3.2 times larger, respectively, than that in the full spectrum treatment.

DISCUSSION

Our study revealed that solar UVR and PAR have a significant effect on the development of tropical micro- and macrofouling communities. Bacterial densities were lowest in the full spectrum treatment (PAR+ UVR), while diatom densities were lowest in the minimal light treatment and the full spectrum treatment. These results suggest the density of diatoms in a biofilm is positively affected by PAR (within the range of ambient values), which diatoms need for photosynthesis, but also by an inhibitory effect of UVR on diatom growth. The mechanisms responsible for the observed differences in bacterial densities, as well as differences in bacterial community composition, were partly revealed by our use of a culture-independent technique, T-RFLP (Dunbar et al. 2001). To our knowledge, this is the first time this technique has been used to investigate the effects of solar UVR and PAR on the development of natural microbial communities. T-RFLP analysis revealed that unique bacterial ribotypes were associated with particular treatments. While we cannot accurately identify bacterial strains by the TAP-TRFLP program due to possible shifts in predicted fragment lengths, our data suggest that some strains did not grow in the presence of UVR (Table 1), probably due to a high sensitivity of these strains to the harmful effects of UVR on bacterial DNA, extracellular enzymes, organic matter uptake, and general metabolic activity (Lyons et al. 1998, Wulff et al. 1999, Sommaruga & Buma 2000, Hung et al. 2005b, Hernández et al. 2006). Nevertheless, by Day 7 of our experiment, the highest ribotype diversity and richness were found in microbial biofilms that had developed in the full light treatment (PAR+UVR). These communities undoubtedly became dominated by bacterial strains that were resistant to UVR and were thus capable of growing under these conditions, possibly due to specific regulatory and DNA repair genes (Qiu et al. 2004) and absence of bacterial grazers (Marangoni et al. 2004). UVR therefore restricted the overall rate of growth of the bacterial community and also altered its composition, virtually eliminating putative UVR-sensitive strains and favoring UVR-resistant strains. Ambient PAR did not influence total bacterial densities but did alter the composition of the bacterial community.

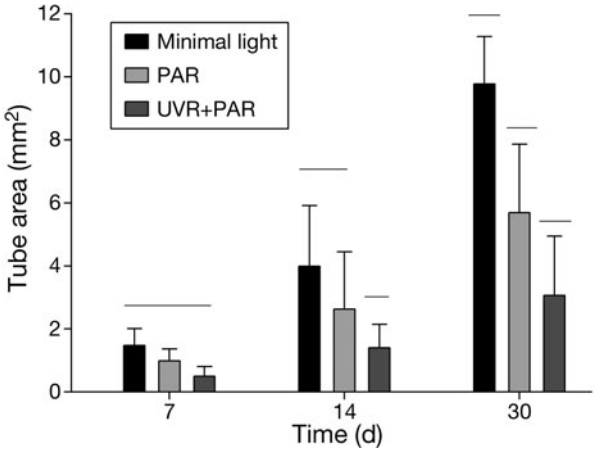

Fig. 7. *Hydroides elegans.* Mean + SD (n = 6) area (mm^2) of polychaete tubes observed under different light and UV regimes. Means not joined by a horizontal line differ significantly (ANOVA, HSD: $p < 0.05$)

Given the changes in microbial densities and composition that occurred between Days 7 and 14 of our study, it is possible that these parameters continued to change beyond Day 14. Although other investigators (Webster et al. 2004) have been successfully analyzing old and dense tropical biofilms, we were unable to count microorganisms beyond Day 14 due to the increased layering of bacteria and diatoms on the surfaces of the Petri dishes. Additionally, the presence of PCR reaction inhibitors and DNA-degrading enzymes in the biofilms prevented the amplification of bacterial DNA and subsequent T-RFLP analysis of microbial communities and requires using different methods to optimize DNA extraction from these types of samples in future experiments.

The species composition of the shallow subtidal macrofouling community was also affected by light conditions over the 30 d of this study. In most cases, communities exposed to UVR had lower species richness, Shannon diversity, and evenness than communities developing in the absence of UVR (i.e. minimal light and PAR treatments); a finding that is consistent

with other reports for macrofouling communities in Canadian (Lotze et al. 2002) and Hong Kong (Dobretsov et al. 2005) waters. PAR had little effect on the overall density or diversity of invertebrate species, but the presence of PAR in comparison to the low light treatment did alter the species composition of the macrofouling community, favoring *Ulva* sp. and restricting the abundance of *Hydroides elegans* and *Obelia* sp. This effect of PAR on the development of shallow macrofouling communities has not been demonstrated previously.

Several mechanisms may be responsible for the observed effects of UVR and PAR on macrofouling community development. (1) UVR can directly influence the colonization process by decreasing the survival and settlement of invertebrate larvae and algal spores and by inhibiting the development of settlers (Chalker-Scott et al. 1992, Bingham & Reitzel 2000, Wiencke et al. 2000, Kuffner 2001, Chiang et al. 2003, Hoag 2003, Bonaventura et al. 2005, Dobretsov et al. 2005, Ban et al. 2007, Nahon et al. 2009). Some species and their larvae can survive exposure to high levels of UVR (Damkaer & Dey 1982, Santas et al. 1998b) and PAR (Glasby 1999, Chiu et al. 2007) better than others, thus influencing the abundance of each species in the community. Our findings demonstrate that *Ulva* sp. predominately grows under high levels of PAR and UVR, while invertebrate species such as *Hydroides elegans*, *Obelia* sp., and *Perna viridis* prefer low light conditions. (2) UVR and PAR may affect the composition of a biofouling community by altering the abundance of predators of these organisms or by moderating the pressure from competitors (Lotze et al. 2002, Boeing et al. 2004). It is possible that ambient UVR conditions favored *Ulva* sp. in competing for space with invertebrate species. (3) UVR and PAR may also have an indirect effect on the development of the macrofouling community by altering the composition of microbial communities (Santas et al. 1998b, Abboudi et al. 2008, this study) which, in turn, can influence propagule attachment on the biofilms (Hung et al. 2005a,b, this study). For example, biofilms developed in the dark and with low abundance of diatoms significantly reduced recruitment of *Crepudula onix* (Chiu et al. 2007). The present study revealed that surfaces available to propagules for settlement do indeed differ in terms of biofilm community composition and density, and thus cues to the propagules, depending on the light conditions to which the surfaces were exposed. This is an important finding, as the development of the biofouling community can be mediated through the production of chemical cues by the microbial community of biofilms (see review by Qian et al. 2007).

Since *Hydroides elegans* was present in all treatments, we analyzed the effect of light treatments on the growth of this polychaete. *H. elegans* achieved a greater rate of growth under minimal light conditions than in the presence of ambient PAR and UVR. The slower growth of *H. elegans* when exposed to PAR and UVR likely resulted from an increase in the amount of energy expended to repair UVR damage to DNA, proteins, and receptors (Hoag 2003, Lesser et al. 2003). Additionally, changes in biofouling communities, such as intensive growth of *Ulva* sp. under UVR and PAR conditions, might increase competition between species, thus leading to low polychaete growth rate.

In conclusion, the findings of this study suggest that UVR and PAR modify both the micro- and macrofouling communities by affecting their species composition, density, heterogeneity, and richness. These parameters were low in the presence of UVR and, in contrast, high in the low light and PAR treatments. These differences among treatments can partly be explained by direct effects on the growth and survival of a small number of dominant macrofouling species, such as *Hydroides elegans*, *Ulva* sp., and *Perna viridis.* In addition, UVR and PAR can indirectly influence macrofouling community development by influencing competition intensity, as well as the composition of microbial communities that serve as cues for settlement and metamorphosis by competent invertebrate larvae and algal spores.

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