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Effect of ultraviolet radiation on biofilms and subsequent larval settlement of *Hydroides elegans*

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ABSTRACT: This study investigated the effect of UV-A and B (UVR) on larval settlement of the polychaete *Hydroides elegans* through their influence on biofilms. Multispecies biofilms were treated with 3 doses of UVR (10, 30 and 80 KJ m^{-2}) at 2 environmentally realistic irradiance levels (4 and 2 W m^{-2}) in the laboratory, and their ability to induce the settlement of *H. elegans* larvae was then examined in both laboratory and field conditions. For the field experiment, only 10 and 80 KJ m^{-2} doses under 4 W m^{-2} were used. In addition, this study evaluated the effects of UVR on monospecies bacterial biofilms and then their ability to induce larval settlement in the laboratory. Results demonstrated that the ability of multispecies biofilms to trigger larval settlement could be compromised as a result of enhanced UVR exposure. Although larval settlement on multispecies biofilms treated with the lowest UV dose (at both irradiance levels) was at the same level as that of the control, the exposure of biofilms to the highest dose significantly reduced their larval settlement triggering ability. Furthermore, UVR treatments decreased the percentage of metabolically active bacterial cells in monospecies biofilms; the effect increased with increasing UV dose. Larval settlement response to monospecies biofilms decreased with increasing UV dose, suggesting that the bacterial metabolic activity in biofilms is essential for the biofilms to have an inductive effect on larval settlement. This study suggests that enhanced UVR, which might occur due to ozone depletion, may have a significant effect on the larval settlement of *H. elegans* by affecting a biofilm's inductive cues.

KEY WORDS: *Hydroides elegans* · Larval settlement · Biofilms · Ultraviolet radiation

INTRODUCTION

For sessile benthic invertebrates with a planktonic larval stage, recruitment success is dependent on larval settlement in suitable habitat (reviewed by Pawlik 1992). Larvae of several organisms facilitate this habitat selection process by responding to a variety of surface-associated physical and biological cues. Of these cues, biofilms, which are the assemblages of microorganisms and organic molecules, play a key role in the larval habitat selection process (reviewed by Holmstrom & Kjelleberg 2000, Maki et al. 2000, Hadfield & Paul 2001, Steinberg et al. 2001). For example, barnacle larvae distinguish between biofilms of varying composition and preferentially settle on biofilms characteristic of their adult habitat, suggesting that microorganisms therein serve as a potential indicator of substrata for larvae seeking a suitable habitat (e.g. Strathmann et al. 1981, Miron et al. 1999, Olivier et al. 2000, Qian et al. 2003, Thiyagarajan et al. in press). Response of larvae to biofilms is thus of great interest to marine ecologists.

Biofilms are ubiquitous on solid substrata in marine environments. The abundance and composition of microorganisms in biofilms, however, are critically controlled by surrounding environments (Hudon & Bourget 1981, Anderson 1995, Kavouras & Maki 2003, Faimali et al. 2004). Many physical and biological factors potentially contribute to such changes in microbial abundance and composition, e.g. tidal height, salinity, temperature, substratum type and illumination. These changes in biofilms may affect the ability of biofilms to

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induce larval settlement. Consequently, biofilms play a key role in determining the recruitment success of invertebrates. The precise interactions among environmental conditions, biofilm dynamics and larval settlement, however, have been poorly investigated (Lau et al. 2005).

Among all the potential environmental factors, ultraviolet radiation (UVR, 280 to 400 nm) is considered one of the most important variables in marine ecosystems. Although the ozone layer in the atmosphere filters out most of the biologically destructive UVR, depletion of ozone due to anthropogenic pollution results in an increase in short-wavelength UVR that reaches the earth's surface (Smith et al. 1992). This enhanced UVR causes significant unfavorable effects in marine ecosystems, as both UV-B (280 to 315 nm) and UV-A (315 to 400 nm) can penetrate to significant depths (Kirt 1994, Conde et al. 2000). For example, previous studies documented the negative effects of UVR on phytoplankton (e.g. Santas et al. 1998, Helbling et al. 2001 and references therein), bacterioplankton (e.g. Helbling et al. 1995, Jeffrey et al. 1996, Kaiser & Herndl 1997, Gustavson et al. 2000), zooplankton (e.g. Chiang et al. 2003) and the marine community as whole (e.g. Lotze et al. 2002, Molis & Wahl 2004, Dobretsov et al. 2005). Importantly, UVR has a detrimental effect on the settlement of coral larvae (Baker 1995, Mundy & Babcock 1998, Kuffner 2001). On the other hand, it is unclear how the effect of larval settlement cues (i.e. biofilms) interacts with the impinging UVR and, consequently, determines larval settlement success (Hung et al. 2005). It was speculated that UVR might affect the bacterial films that are necessary to trigger larval settlement (Kuffner 2001).

Like many sessile marine invertebrates, the life cycle of the serpulid polychaete *Hydroides elegans* includes a planktonic larval phase followed by juvenile and adult benthic phases. The transition process between larval and juvenile phases, generally known as 'larval settlement', occurs after a larva has become competent in responding to cues. It is well known that biofilms are one of the key natural inductive cues for *H. elegans* larvae (e.g. Lau et al. 2005). The larva of *H. elegans* is an excellent model to study the mechanisms underlying settlement cue detection during the habitat selection process (reviewed by Qian 1999). This species is also particularly suitable for examining the relationships among environmental conditions, biofilm dynamics and larval settlement (Lam et al. 2005).

The role of UVR on biofilms, and consequently on the larval settlement, has as yet not been broadly investigated. In this study, we exposed sublittoral multispecies biofilms to UVR and then examined the films' ability to induce the settlement of *Hydroides elegans* larvae under both laboratory and field conditions. In addition, we also evaluated the adverse effects of UVR on monospecies bacterial biofilms and then examined their ability to induce the settlement of *H. elegans* larvae in the laboratory.

MATERIALS AND METHODS

Multispecies biofilms. Multispecies biofilms were developed on polystyrene Petri dishes (#1006, Falcon). Dishes without lids were placed in a nylon mesh bag (mesh pore size = 110 µm) to prevent the attachment of larvae during film development. The bag was submerged for 6 d in the subtidal zone (~3 m below mean low water level) in eastern Hong Kong waters (22° 19′ N, 114° 16′ E). Dishes were then retrieved and transported to the laboratory in seawater. Before UV treatments, dishes were dip-rinsed 10 times in autoclaved 0.22 µm filtered seawater (FSW) to remove loosely attached bacteria. Generally, these films contained a large number of bacteria (~10^4 to 10^6 cells mm^{-2}) and a small number of diatoms (~10^1 to 10^2 cells mm^{-2}).

Monospecies bacterial biofilms. Monospecies bacterial biofilms were formed on polystyrene Petri dishes (#1006, Falcon) according to Maki et al. (1988). One strain isolated from the subtidal zone (not yet identified) inducing larval settlement of *Hydroides elegans* was used in this study. Briefly, 2 ml of the bacterial stock was inoculated into sterile culture broth (with 0.3% yeast extract and 0.5% peptone) and grown at 30°C for 48 h to the stationary phase. Suspended bacteria were washed in FSW. Polystyrene dishes were filled with 4 ml of washed bacterial suspension and incubated for 3 h at 24°C. Dishes were then dip-rinsed 10 times in FSW to remove loosely attached bacteria. Bacteria remaining on the dish surface were regarded as an attached bacterial film. Our preliminary study showed that films of density (~10^4 cells mm^{-2}) induced the settlement of *H. elegans* larvae.

Ultraviolet radiation (UVR) exposure experiments. UVR exposure experiments were performed in a UV chamber at 25°C according to Hung et al. (2005). Artificial UV-A and UV-B radiation was generated using UV-emitting fluorescent lamps (UV-B VILBER-LOURMAT T-8M with peak irradiance at 302 nm; UV-A VILBER-LOURMAT T-8L with peak irradiance at 365 nm). In Hong Kong, UV-B irradiance level ranges from 1 to 2 W m^{-2} during summer months (Dobretsov et al. 2005) and peaks at 4 W m^{-2} during midday in summer (Chiang et al. 2003). Consequently, a low irradiance of 2.0 ± 0.2 W m^{-2} and a high irradiance of 4.0 ± 0.2 W m^{-2} for both UV-A and UV-B exposures were used. A broadband spectroradiometer (DRC-100X, Spectroline) was used to measure the actual level of

Table 1. Time (min) required to obtain the UV-B and UV-A dose used in this study under the 2 irradiance levels

UV irradiance ($W m^{-2}$)	Dosage ($\times 10^3$ J m^{-2}) 0	10	30	80
2	0	83	250	667
4	0	42	125	333

UV irradiance during exposure. Biofilms were illuminated for different durations to obtain a range of environmentally realistic dosages of UV energy under each level of irradiance (Table 1). Biofilms were immersed under a thin layer of FSW to avoid desiccation during UV exposures.

Bacterial community analysis in multispecies biofilms. Before and after the field larval settlement bioassay, the bacterial community structures in multispecies biofilms were analyzed by T-RFLP according to Qian et al. (2003) to understand the extent to which the native bacterial community of biofilms was altered at the end of the larval bioassay. Briefly, the bacterial community DNA of biofilm samples (n = 3) was extracted according to Zhou et al. (1996). The 16S rRNA genes (rDNA) of bacteria were amplified by PCR using the universal primers 968F and 1346R (Lau et al. 2005). Fluorescently labeled PCR products were digested with 20 U *Msp* I. The digested amplicons were mixed with an internal size standard (ET-550, Amersham). This mixture was denatured at 95°C and immediately chilled on ice before electrophoresis on a MegaBace genetic analyzer (Amersham). The length of the fluorescently labeled terminal-restriction fragments (T-RFs) was determined by comparison with internal standards using fragment profiler software (Amersham). The T-RFs patterns of different samples were analyzed by visual comparison of the electropherograms.

Enumeration of bacterial abundance in monospecies bacterial biofilms. The abundance of metabolically active and total bacteria in biofilms was examined immediately after UV exposure according to Lau et al. (2003a). Only biofilms that had been treated under 4 W m^{-2} were examined. Briefly, biofilms were covered with 6 mM 5-cyano-2, 3-ditolyl tetrazolium chloride (CTC, Polysciences) in FSW and incubated for 4 h. Biofilms were counterstained with 0.5 mg ml^{-1} 4, 6-diamidino-2-phenylindole (DAPI, Fluka Chemie) for 15 min. After a brief rinse with FSW, bacterial abundance was determined at a magnification of 1000× in 5 randomly chosen fields of view. Three replicate dishes were used for each treatment. In the same field of view, total bacterial cells appeared blue (DAPI stain) under blue fluorescent light, with only metabolically active bacterial cells appearing red under green light due to the deposition of insoluble formazan (reduced CTC) by cellular respiration (Haglund et al. 2002).

***Hydroides elegans* larval culture.** Larvae of *H. elegans* were reared to competent stage according to Qiu & Qian (1997). The competency of larvae was determined according to Qian & Pechenik (1998). When most of the larvae in the culture were competent, it was gently filtered through a 110 µm nylon mesh. The larvae retained on the mesh were immediately used for larval settlement bioassays.

Expt 1: Effect of UV-treated multispecies biofilms on larval settlement. The aim of this experiment was to test the extent to which UV radiation affects biofilms and, subsequently, the larval settlement of *Hydroides elegans* under both laboratory and field conditions.

Laboratory bioassay: This experiment was performed using a stillwater bioassay (i.e. single-dish bioassay) according to Unabia & Hadfield (1999). There were 3 treatments for each radiation (UV-B and A) and irradiance levels (2 and 4 W m^{-2}): UV-10 (biofilms treated with UV dose 10 KJ m^{-2}), UV-30 (biofilms treated with UV dose 30 KJ m^{-2}), and UV-80 (biofilms treated with UV dose 80 KJ m^{-2}). Untreated biofilms served as positive control, while initially clean dishes served as negative control. The positive controls were treated exactly in the same way as those under the highest UV dose but UVR was blocked with a filter (opaque acrylic sheet). Three replicate dishes, each having 20 competent larvae and 4.5 ml of FSW, were used in each treatment and control. All the dishes were kept at 28°C under darkness. The percentage of larval settlement was scored after 24 h. This experiment was repeated 3 times between June and September 2004 for UV-B. However, experiments with UV-A were carried out only once.

Field bioassay: Unlike in the laboratory bioassay, only one radiation (UV-B) and irradiance level (4 W m^{-2}) was used (Table 2). Two UV-B treatments (10 KJ m^{-2} and 80 KJ m^{-2}) and 2 controls (positive and negative) were included. In addition, formalin-treated biofilms prepared according to Lau et al. (2003b) were also used to investigate the response of larvae to biofilms composed of 100% dead bacteria. Formalin-treated biofilms were dip-rinsed 10 times in FSW before the bioassay. The settlement response of larvae to biofilms was investigated on site near Victoria Harbor, Hong Kong. Dishes were stuck to square mounting frames. Each frame held 25 dishes in 5 × 5 arrays according to an orthogonal Latin-square design, leaving 3 cm between adjacent dishes. Untreated biofilms served as positive control, while initially clean dishes served as negative control. Each of the 5 treatment dishes (2 UV-B treatments, a formalin treatment and 2 controls) appeared exactly once in each of the 5 rows and in each of the 5 columns. This experiment was re-

Table 2. Protocol for determining the effect of UV-treated biofilms (multispecies and monospecies) on larval settlement under field and laboratory conditions. For UV-B and UV-A exposure, 2 irradiance levels were used (4 W m^{-2} and 2 W m^{-2}). Three treatments and 2 controls were included under each irradiance level. Biofilm analysis was performed in some treatments. UV-10 = biofilms treated with UV-B dose 10 KJ m^{-2}, UV-30 = biofilms treated with UV-B dose 30 KJ m^{-2}, UV-80 = biofilms treated with UV-B dose 80 KJ m^{-2}, formalin = formalin-treated biofilms, positive control = untreated biofilms, negative control = initially clean dish, NP = not performed, NA = not analyzed

Type of biofilm	Type of bioassay	UV treatments				Biofilm analysis	
		UV-B		UV-A			
		4 W m^{-2}	2 W m^{-2}	4 W m^{-2}	2 W m^{-2}	Bacterial community (T-RFLP)	Metabolically active bacterial count
Multispecies (Expt 1)	Field	Positive control UV-10 UV-80 Formalin Negative control	NP	NP	NP	Yes	NA
	Lab	Positive control UV-10 UV-30 UV-80 Negative control	Positive control UV-10 UV-30 UV-80 Negative control	Positive control UV-10 UV-30 UV-80 Negative control	Positive control UV-10 UV-30 UV-80 Negative control	NA	NA
Monospecies (Expt 2)	Lab	Positive Control UV-10 UV-30 UV-80 Negative control	Positive Control UV-10 UV-30 UV-80 Negative control	Positive Control UV-10 UV-30 UV-80 Negative control	Positive control UV-10 UV-30 UV-80 Negative control	NA	Only biofilms treated under 4 W m^{-2} were analyzed

peated 3 times (Repeats 1 to 3) between June and September 2004. Each repeat consisted of 3 replicate frames, which were retrieved after a 24 h period of immersion in the subtidal zone. The number of settled *Hydroides elegans* larvae on each treatment dish was recorded.

Expt 2: Effect of UV-treated monospecies bacterial biofilms on larval settlement. The objective of this experiment was to examine the extent to which UV radiation affects the metabolic activity of bacterial cells in biofilms and, subsequently, their ability to trigger the settlement of *Hydroides elegans* larvae under controlled laboratory conditions. All 3 treatments per radiation (UV-B and -A) and irradiance level (2 and 4 W m^{-2}) and the positive and negative controls used in the laboratory bioassay of Expt 1 were also used in this experiment. The experiment was repeated 3 times (Repeats 1 to 3) between June and September 2004. Three replicate dishes, each having 20 competent larvae and 4.5 ml of FSW, were used in each treatment and control. The larval bioassay was conducted according to the procedure described in Expt 1.

Statistical analysis. All percentage and count data were subjected to angular and log (x + 1) transformation, respectively (Zar 1999). After transformation, data were checked for normality with the Shapiro–Wilk's test and homogeneity of variance with Cochran's C-test. In Expt 1, the results of the laboratory bioassay were analyzed using 1-way ANOVA and Tukey's multiple comparison test. For the field larval settlement bioassay, the numbers of settled larvae were analyzed with a replicated Latin-square ANOVA (Grassle et al. 1992). The effects of row, column, treatment (fixed factor), and replicate frames (random factor) on larval settlement were analyzed for each experimental repeats. If the effects of row, column, and treatment on larval settlement were not consistent among replicate frames, the number of settled larvae in each replicate frame was analyzed using 1-way Latin-square ANOVA. If a significant effect was detected, Tukey's multiple comparison test was used to determine differences among the controls and treatments. In Expt 2, 1-way ANOVA and Tukey's multiple comparison test were used to analyze the percent settlement as well as abundance of bacterial cells in biofilms.

RESULTS

Expt 1: Effect of UV-treated multispecies biofilms on larval settlement

Laboratory bioassay

At both irradiance levels (2 and 4 W m^{-2}) of UV-B radiation, larval settlement on the films treated with the highest dose (UV-80 KJ m^{-2}) and in the negative control was generally very low (<25%) (Fig. 1). However, the percent settlement on the films treated with low and medium UV doses and on positive control

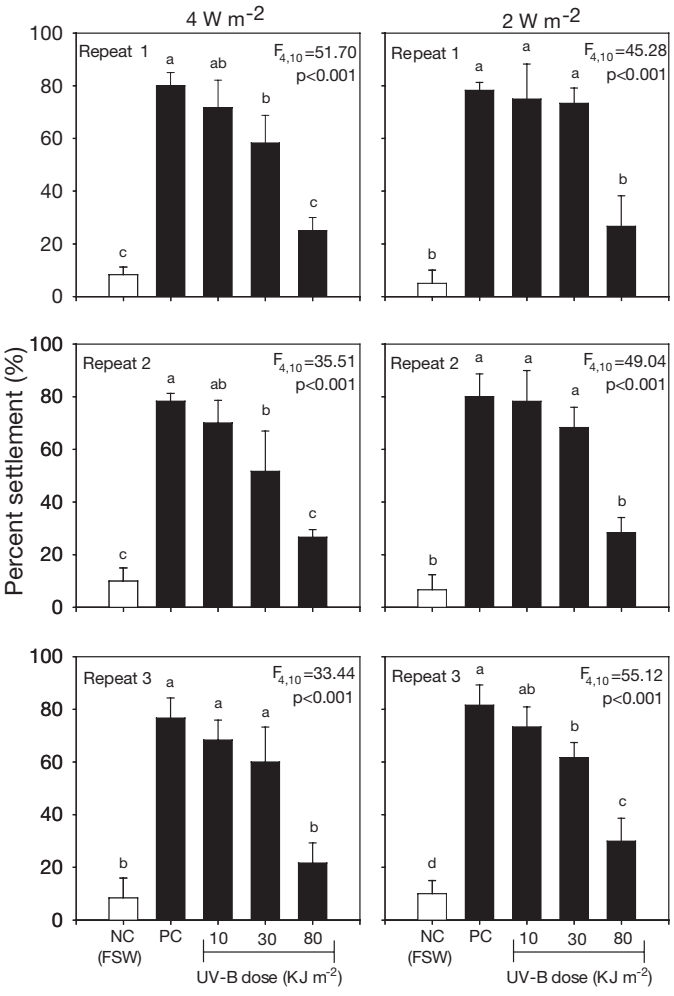

Fig. 1. *Hydroides elegans.* Single-dish bioassay: percent settlement of larvae in response to multispecies biofilms (treated with UV-B dose ranging from 10 to 80 KJ m^{-2}), positive control (PC, untreated biofilms) and negative control (NC, initially clean dish); left column for 4 W m^{-2} and right column for 2 W m^{-2}. Three repeats were performed. Data expressed as mean + 1 SD of 3 replicates. Data that are significantly different at $\alpha = 0.05$ in Tukey's test are indicated by different letters above the bars

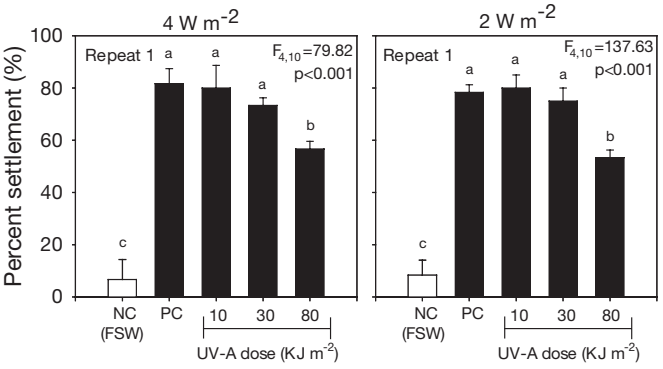

Fig. 2. *Hydroides elegans.* Single-dish bioassay: percent settlement of larvae in response to multispecies biofilms (treated with UV-A dose ranging from 10 to 80 KJ m^{-2}), positive control (PC, untreated biofilms), and negative control (NC, initially clean dish); left column for 4 W m^{-2} and right column for 2 W m^{-2}. Data expressed as mean + 1 SD of 3 replicates. Data that are significantly different at $\alpha = 0.05$ in Tukey's test are indicated by different letters above the bars

films were 2 to 3 times higher (60 to 80%) than on the films treated with the highest dose. In contrast to UV-B, the percent settlement on the films treated with the highest UV-A dose (at both irradiance levels) was significantly higher (>50%) than in the negative control (Fig. 2).

Field bioassay

The actual numbers of larvae settled on the UV-B-treated films (10 and 80 KJ m^{-2}), formalin-treated films, untreated biofilms (positive control) and initially clean dishes (negative control) in 9 frames (3 replicates per frame × 3 repeats) are shown in Fig. 3. In general, similar numbers of larvae settled on the films treated with the highest UV-B dose and formalin-treated films, which were significantly higher than in the negative control but lower than on the films treated with the lowest UV-B dose and the positive control. The number of settled larvae differed significantly among the treatments and controls; however, those differences were not consistent among the replicate frames in all 3 repeats. Therefore, differences in mean larval settlement among the treatments were examined in each frame (Fig. 4). In 8 of the 9 frames, fewer larvae settled in the negative control than in other treatments. In all 9 frames, the mean number of larvae settled on the films treated with the lowest UV-B dose was not different than that in the positive control. In 8 of the 9 frames, the mean number of larvae settled on the films treated with the highest UV-B dose was not different than that in formalin-treated films. These differences in larval settlement among the treatments cannot be accounted for by the arrangement of dishes in the frame because neither row nor column effects were significant in 2 of the 3 experiments (Table 3).

Analysis of bacterial community in biofilms before and after bioassay

Data of T-RFLP analysis on the bacterial community profile can be interpreted as 'semiquantitative' according to the number of T-RFs in each sample (i.e. number of distinguishable bacterial types), as well as qualitatively according to the position of T-RFs (i.e. occur-

rence of unique bacterial types). Most of the T-RFs observed in the biofilms of positive controls (e.g. Peaks 1–7 in Fig. 5A) were also found in the films exposed to UV-B (Fig. 5B). For the positive control, it was not possible to detect any T-RFs that exclusively appeared after 24 h immersion in the subtidal zone of the field bioassay. Alternatively, none of the T-RFs that had been present in the positive control films were exclusively lost during the 24 h bioassay period (Fig. 5A,C). This trend was also observed for UV-B-treated biofilms, although the intensity of some T-RFs (e.g. Peaks 2, 6, 7, a, and b in Fig. 5B) was marginally reduced after the larval bioassay. During the bioassay period, only a few T-RFs were found in the films of the negative control (e.g. Peaks 1 and 5 in Fig. 5E).

Table 3. Results of Latin-square design ANOVA of larval settlement data in Expt 1. Four factors were considered: Row (5 levels: column 1 to 5), Column (5 levels: row 1 to 5) and Treatment (5 levels: negative control, positive control, UV-10, UV-80, formalin) were used as fixed factors, and experimental repeats (3 levels: Repeats 1 to 3) were used as random factors. All interactions were assumed to be not significant. Data were log ($x + 1$) transformed to meet ANOVA assumptions. $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; $^{ns}p > 0.05$

Factor	df	Repeat 1		Repeat 2		Repeat 3	
		MS	*F*	MS	*F*	MS	*F*
Row	4	0.003	1.25^{ns}	0.007	1.9^{ns}	0.06	7.60^{***}
Column	4	0.003	1.02^{ns}	0.002	0.67^{ns}	0.004	0.52^{ns}
Treatment	4	0.86	343.09^{***}	0.9	243.31^{***}	1.1	144.01^{***}
Replicate frame	2	0.17	67.7^{***}	0.02	4.56^{*}	0.06	7.70^{**}
Error	60	0.15		0.004		0.008	

Expt 2: Effect of UV-treated monospecies bacterial biofilms on larval settlement

Laboratory bioassay: At both irradiance levels (2 and 4 W m^{-2}) of UV-B radiation, larval settlement on the films treated with any UV-B dose was significantly lower than in the positive control films (Fig. 6). Larval settlement on the films treated with the highest UV-B was not different than in the negative control, i.e. only a few larvae settled on these films (<20%). However, the percent settlement on the films treated with the lowest and medium UV-B doses and on the positive control films were significantly higher (30 to 80%) than on the films treated with the highest dose. In contrast to UV-B, the percent settlement on the films treated with the highest UV-A dose (at both irradiance levels) was significantly higher (>30%) than in the negative control (Fig. 7). Also, larval settlement on the films treated with the lowest and medium UV-A dose were more or less the same as in the positive control (Fig. 7).

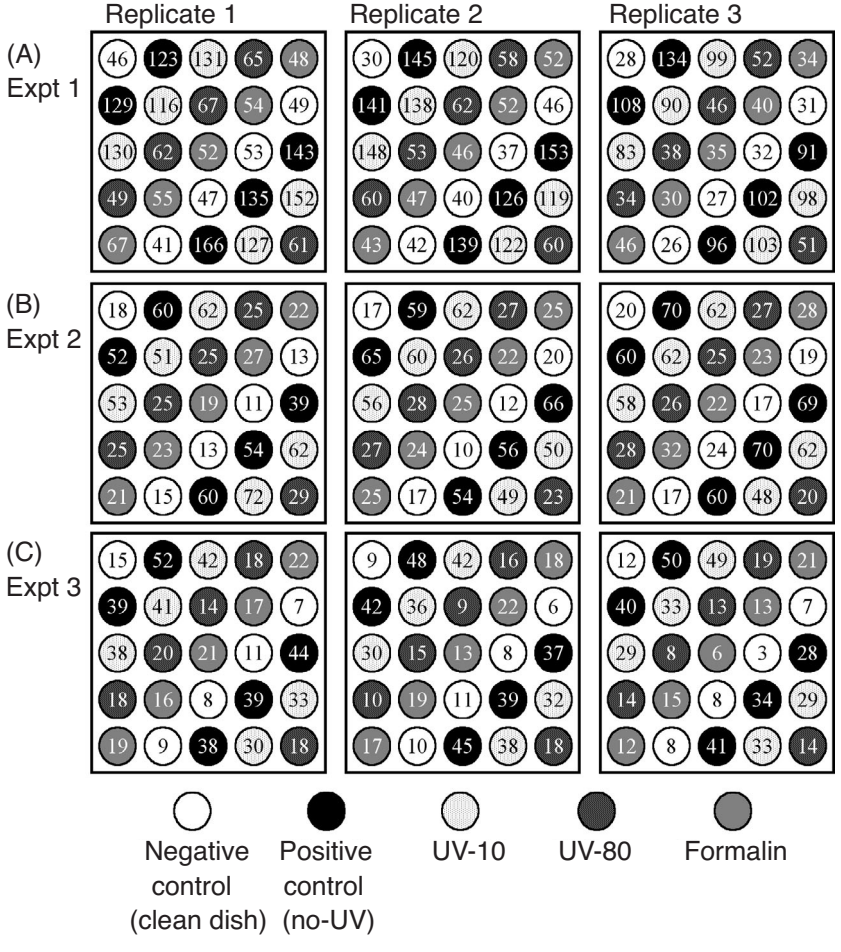

Fig. 3. Number of larvae settled in field multiple-choice bioassay. Treatments were arranged in 5 × 5 orthogonal Latin-square designs

Enumeration of bacterial abundance in monospecies bacterial biofilms

After exposure to UV-B or UV-A, regardless of the dose, the percentage of metabolically active bacterial cells in biofilms was significantly reduced compared to the positive control (Fig. 8A,B). At both UV-B and A irradiation, the percentage of metabolically

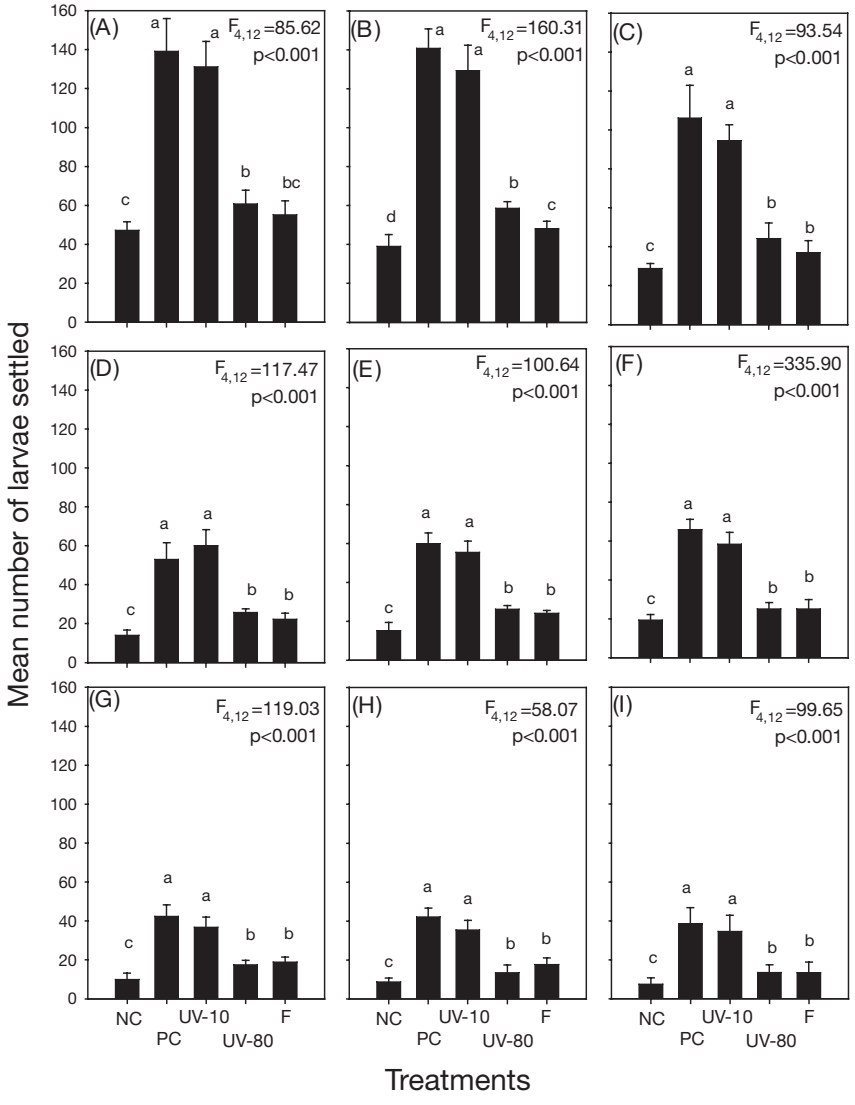

Fig. 4. *Hydroides elegans.* Larval settlement in Expts 1 (A to C: replicates 1 to 3, respectively), 2 (D to F: replicates 1 to 3, respectively), and 3 (G to I: replicates 1 to 3, respectively). Data are expressed as mean +1 SD of 5 replicates. Data that are significantly different at $\alpha = 0.05$ in Tukey's test are indicated by different letters above the bars. Positive control: PC, untreated biofilms; negative control: NC, initially clean dish; UV-10: biofilms treated with UV-B 10 KJ m^{-2}; UV-80: biofilms treated with UV-B 80 KJ m^{-2}; F: biofilms treated with formalin

active bacterial cells differed significantly among UV treatments, decreasing to about 2% at the highest UV-B dose (Fig. 8A) and to about 30% at the highest UV-A dose (Fig. 8B).

DISCUSSION

This study demonstrated that the ability of natural multispecies biofilms to trigger the settlement of *Hydroides elegans* larvae can be interrupted as a result of enhanced UVR exposure. Although larval settlement on the biofilms treated with the lowest UV dose (10 KJ m^{-2}) was at the same level as that of the positive control, exposure of biofilms to the highest dose (80 KJ m^{-2}) significantly reduced their larval settlement triggering ability in both laboratory and field conditions.

It has long been known that biofilms are one of the major sources of settlement cues for the larvae of *Hydroides elegans*. Although natural multispecies biofilms induce the settlement of this species (Lau et al. 2005), monospecies biofilms can have an inductive, neutral or inhibitive effect on settlement (Unabia & Hadfield 1999, Lau & Qian 2001, Lau et al. 2002, 2003a, Huang & Hadfield 2003, Lee & Qian 2003). All these results were obtained from laboratory experiments. This study provides additional evidence to support the general consensus that multispecies biofilms trigger the larval settlement of *H. elegans*. The interesting part of our results, however, is the larval settlement response to the UV-treated biofilms, which in general decreased with increasing UV dose. The field experiments showed a clear larval preference to the untreated multispecies biofilms over the biofilms treated with the highest UV-B dose. A similar negative effect of UVR (e.g. UV-B and UV-A) on biofilms and subsequent larval settlement was also observed in the laboratory experiments, irrespective of irradiance level (4 and 2 W m^{-2}). Thus, this work offers some insight that UVR may affect larval settlement by altering biofilms and possibly settlement cues.

How does the high-dose UVR alter the larval settlement triggering ability of the multispecies biofilms to larval settlement? We have no immediate explanation for this question. Multispecies biofilms are the assemblages of microorganisms (such as bacteria, diatoms, cyanobacteria and fungi) and organic molecules. According to previous studies, UVR can either have deleterious effects on any one of these biofilm components or simultaneously affect all those components (reviewed by Vincent & Neal 2000). UVR can potentially impact the photosynthetic machinery of micro-

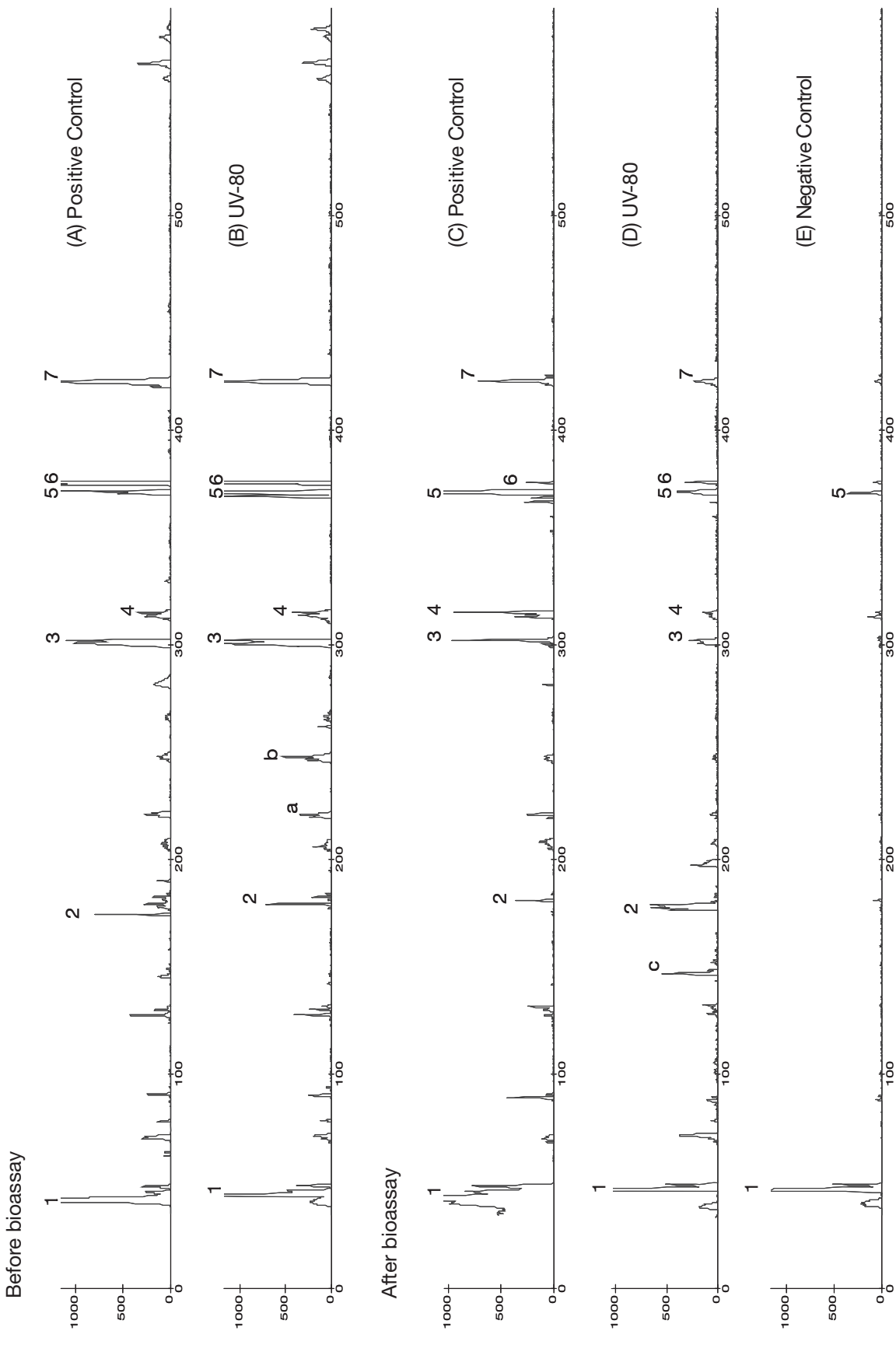

Fig. 5. T-RFLP profiles of (A,C) untreated (positive control) biofilms (A) before and (C) after settlement bioassay, (B,D) UV-B (80 KJ m^{-2}) treated biofilms (B) before and (D) after settlement bioassay and (E) initially clean dish (negative control) after settlement bioassay. Corresponding peaks in different treatments are indicated by the same number

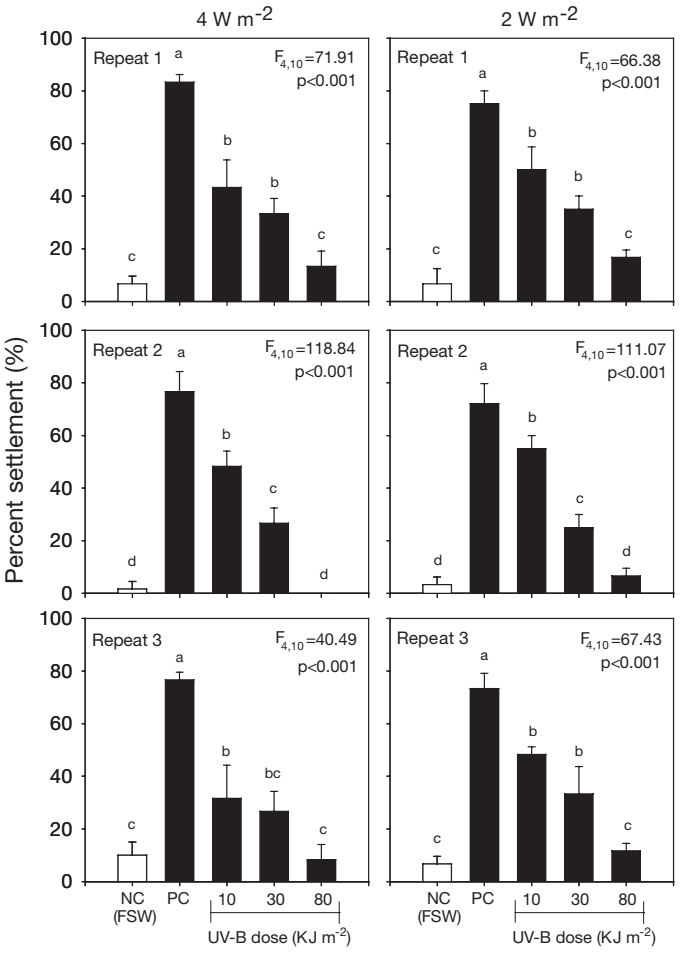

Fig. 6. *Hydroides elegans.* Single-dish bioassay: percent settlement of larvae in response to monospecies bacterial biofilms (treated with UV-B dose ranging from 10 to 80 KJ m^{-2}), positive control (PC, untreated biofilms) and negative control (NC, initially clean dish). See Fig. 1 for details

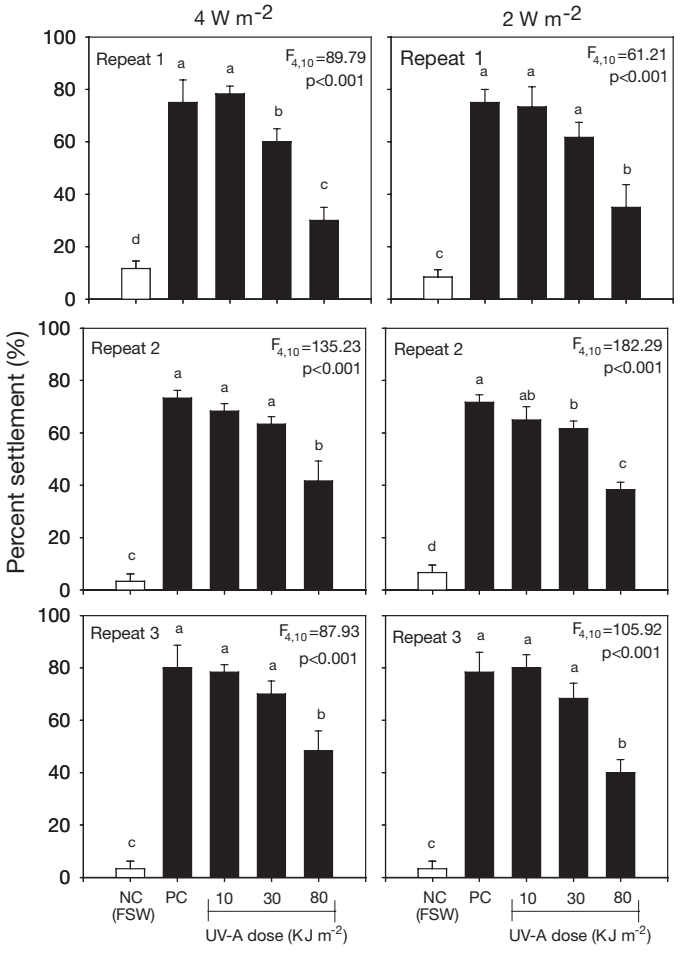

Fig. 7. *Hydroides elegans.* Single-dish bioassay: percent settlement of larvae in response to monospecies bacterial biofilms (treated with UV-A dose ranging from 10 to 80 KJ m^{-2}), positive control (PC, untreated biofilms) and negative control (NC, initially clean dish). See Fig. 1 for details

algae (Roleda et al. 2004 and references therein) and as a result affect both growth and viability of diatoms and cyanobacteria held in the biofilms. In addition, UVR can also damage genetic materials of microorganisms as a consequence of the formation of nucleotide dimers (Jeffrey et al. 1996, Kaiser & Herndl 1997, Joux et al. 1999). Thus, UVR may have multiple impacts on biofilms and in turn indirectly affect larval settlement.

Alternatively, the effect of UVR on biofilms and then on larval settlement may be mediated through the production of chemical cues from metabolically active bacterial cells in biofilms. We recently showed that environmentally realistic UVR (i.e. UV-A dose ≥ 16.2 KJ m^{-2} and UV-B dose ≥ 5.4 KJ m^{-2}) could impair the mitochondrial function of sea urchin sperm (Lu & Wu 2005). Since metabolism is closely related to mitochondrial function, this may strengthen the argument that UVR may affect the production of metabolites. In this study, we have tested this hypothesis using monospecies biofilms (Expt 2). The number of metabolically active bacterial cells in biofilms decreased with increasing UV dose; this relationship was more pronounced in UV-B than in UV-A treatments (i.e. UV-B caused a greater reduction in the number of active bacterial cells than UV-A at the same energy level). Our treatments used the same irradiance levels and doses for both UV-B and A treatments in order to compare the cellular metabolic activity between UV-B and UV-A treatments under otherwise similar conditions. However, under natural conditions in Hong Kong, the irradiance levels of UV-A can be 10 times higher than in our experiments (Dobretsov et al. 2005). The larval settlement response to monospecies biofilms decreased with decreasing numbers of metabolically active bacterial cells in biofilms, suggesting that bacte-

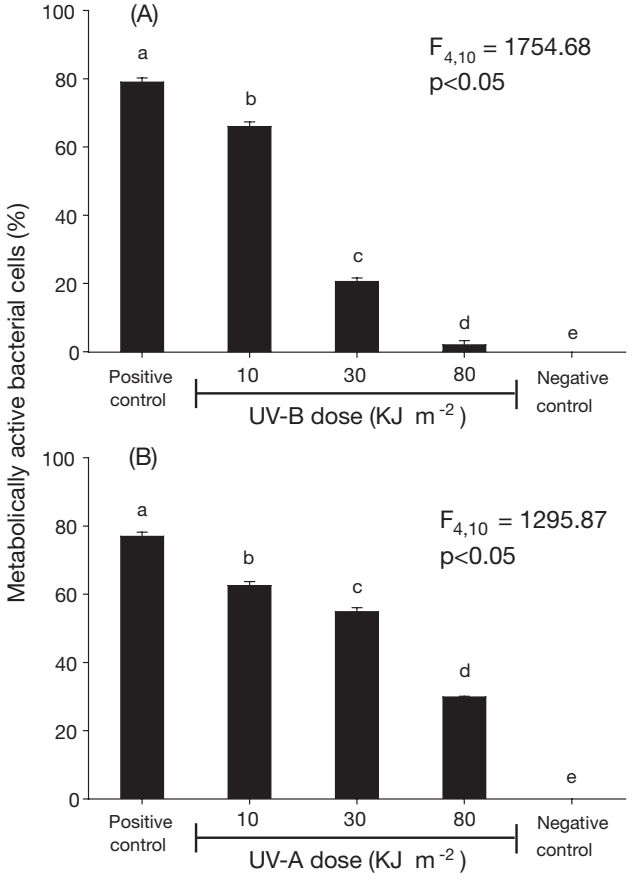

Fig. 8. Percentage of metabolically active cells in monospecies bacterial films after treatment with (A) UV-B (4 W m^{-2}), (B) UV-A (4 W m^{-2}); positive control = bacterial film without UV treatment, negative control = initially clean dish. Data are expressed as mean + 1 SD of 3 replicates. Data that are significantly different at α = 0.05 in Tukey's test are indicated by different letters above the bars

rial metabolic activity is required for the biofilms to have an inductive effect. This was further supported by our field experiments in which fewer larvae settled on the formalin-treated multispecies biofilms than in the positive control. This observation corroborates previous studies reporting a positive correlation between the number of metabolically active bacterial cells in biofilms and percentage of larval settlement of *Hydroides elegans* (Unabia & Hadfield 1999, Lau & Qian 2001, Lau et al. 2003a). In previous investigations, the number of metabolically active bacterial cells in biofilms was modulated using either formalin or highly destructive UV-C (<280 nm) irradiation. Our experiments, however, used naturally occurring UV-B and UV-A radiation.

On the other hand, the present results contrast with our previous study on the effect of UVR on biofilms and the subsequent settlement of an intertidal barnacle *Balanus amphitrite* (Hung et al. 2005). UVR (at the same levels used in this study) significantly reduced the number of metabolically active bacterial cells in biofilms; however, it did not affect the larval settlement of barnacles. Previous studies suggested that bacterial cell surface components (exopolymer components) may be involved in the induction of larval settlement in barnacle *B. amphitrite* (Maki et al. 1990). The extracellular polysaccharides on the bacterial cell surface, which resist UV radiation, might have been involved in the signaling of larval settlement in barnacles (Lau et al. 2003b). These 2 opposing results indicate that biofilms and larval interactions are complex and highly species specific, as larvae of different species respond differently to chemical cues derived from biofilms. Also, the interaction between bacterial metabolic activity and UVR is important. UVR may shift the population of bacteria as different bacterial species may respond differently to the same UV energy (Marguet & Helbling 1994, Joux et al. 1999).

One recent study showed the shade preference of settling larvae; communities exposed to UVR had lower species richness than communities not exposed to UVR (Dobretsov et al. 2005). Although some species can avoid UVR by settling in shade regions, there is growing evidence that invertebrate communities in coastal waters are to some extent adversely affected by enhanced UVR, either due to the impairment of settling larvae (e.g. Chiang et al. 2003) or alteration of larval settlement behavior (e.g. Kuffner 2001). It is argued that increases in UVR due to ozone thinning may override the larval ability to detect or avoid UVR and can have a significant impact on their subsequent performance (Peachey 2005). As evidenced by this study, UVR has the potential to alter settlement cues that trigger the metamorphosis of at least the larvae of *Hydroides elegans*. Also, it has been reported that bacteria are particularly susceptible to UV radiation, unlike other organisms such as phytoplankton (Jeffrey et al. 1996), because of their small size and large surface-to-volume ratio. Due to escalating ozone thinning, therefore, the impact of UVR on larval settlement cannot completely be ruled out. However, the effects of UVR on marine organisms are dependent on latitude. Annual UVR flux decreases with increasing distance from the equator (Diffey 1991). For instance, average UV-B exposure at the equator is over a thousand times higher than in the polar seas. Therefore, marine organisms in tropical and subtropical waters are at higher risk than those in higher latitudes.

Overall, evidence presented in this paper suggests that enhanced UVR could influence the larval settlement success of *Hydroides elegans* through its adverse effects on one of the natural larval settlement cues, multispecies biofilms.

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