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# Do effects of ultraviolet radiation on microbial films have indirect effects on larval attachment of the barnacle *Balanus amphitrite*?

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## Abstract

We have examined the indirect effects of UV-A and UV-B on cypris attachment of the barnacle *Balanus amphitrite* Darwin through their effects on microbial films. Specifically, we tested the hypothesis that both UV-A and UV-B radiation can indirectly affect the larval attachment of barnacles by altering the microbial film bioactivity. Microbial films were developed from mid-intertidal region (~1 m above Mean Low Water Level) for 6 days and subjected to ambient levels of ultraviolet radiation. Response of cyprids to untreated and UV-treated microbial films was investigated using double-dish still water choice bioassay. Results showed that both UV-A and UV-B caused a decrease in the percentage of respiring bacterial cells in microbial films and this effect increased with UV energy. With the same UV energy, UV-B caused a greater decrease in respiring bacterial cells than UV-A. However, despite strong UV radiation, the bioactivities of microbial films (i.e., stimulation of cypris attachment) remain unchanged. Results of this study suggest that increased UV radiation, which might occur due to ozone depletion, may not significantly affect the barnacle recruitment by means of affecting the inductive larval attachment cues of microbial films. © 2005 Elsevier B.V. All rights reserved.

**Keywords:** *Balanus amphitrite*; Barnacle settlement; Cypris attachment; Microbial films; Ultraviolet radiation

## 1. Introduction

Planktonic larvae of several benthic marine invertebrates attach and metamorphose to surfaces mainly in response to biological characteristics of the

surfaces, e.g., microbial films, and presence of conspecific adults (e.g., Olivier et al., 2000; Jeffery, 2002). Hard substrata in the marine environment are usually covered by microorganisms such as bacteria, microalgae, and fungi, collectively called as “microbial films”. Microbial films are utilized as an attachment (settlement) cue by larvae of a broad range of sessile marine invertebrates (reviewed by Mitchell and Maki, 1988; Pawlik, 1992; Wicczorek and Todd, 1998;

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Holmstrom and Kjelleberg, 2000; Maki et al., 2000; Steinberg et al., 2001). For example, larvae of intertidal barnacle species, *Balanus amphitrite*, can identify the tidal height by detecting variation of microbial film components along the intertidal zone (Strathmann et al., 1981; Thomason et al., 1998; Miron et al., 1999; Qian et al., 2003). Microbial films therefore play an important role in larval habitat selection (or attachment) process; any factor that can alter microbial films may indirectly affect larval attachment and thus, recruitment.

Most previous laboratory-based studies of larval attachment responses to microbial films have focused on bacterial cell density (e.g., Maki et al., 1988). For example, a highly inductive bacterial species was effective in inducing larval settlement of *Hydroides elegans* at much lower densities than weakly or non-inductive bacteria (Huang and Hadfield, 2003). Recent studies showed that bacterial community structure of microbial films also plays significant role in determining the attractiveness of microbial films to barnacle larvae (Qian et al., 2003; Thiyagarajan et al., in press). The properties (such as quality and quantity) of microbial films, however, are influenced by a number of environmental factors that may indirectly affect larval attachment through their influence on microbial films. Recent studies from our laboratory demonstrated that water temperature and salinity affect the composition of microbial films, subsequently influencing the larval metamorphosis (Lau et al., 2005). In addition, the nature of the substratum could also affect microbial films and in turn influence larval attachment (e.g., O'Connor and Richardson, 1996; Maki et al., 2000; Faimali et al., 2004). Another factor that can affect microbial films is solar ultraviolet radiation (UVR), however, the effect of UVR on microbial films and the resulting consequences for larval attachment have not been addressed in previous investigations.

Ultraviolet irradiation can be classified as UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (<280 nm) radiation. UV-C does not reach the earth's surface as it is absorbed by the ozone layer. In water, UV-A and UV-B are absorbed by suspended particles and dissolved compounds. However, UV-B can penetrate to significant depths in clear marine water (Smith et al., 1992; Kirt, 1994) and UV-A can even penetrate further than UV-B

(Conde et al., 2000). The impact of UV-B radiation on global ecosystems has been a great concern since the discovery of the depletion of the ozone layer, particularly over Antarctica (e.g., Farmen et al., 1985; Hofmann, 1996) and the Arctic (e.g., Müller et al., 1997). The resulting enhanced UV-B radiation has long been recognized as a significant factor that can affect pelagic and shallow water benthic communities (e.g., Mundy and Babcock, 1998; Santas et al., 1998; Lotze et al., 2002; Molis et al., 2003). For example, when larvae of reef coral *Pocillopora damicornis* are exposed to high UV radiation, their attachment and metamorphosis were significantly inhibited (Kuffner, 2001).

UV radiation can possibly affect the larval attachment and metamorphosis in two ways. First, UVR may directly affect the larva. For example, UV-B damages eyes of *B. amphitrite* larvae and impairs their attachment success (Chiang et al., 2003). Second, production of surface-bound larval settlement cues by microorganisms found in microbial films, might be altered by UVR. Microbial films are well known to provide settlement cues that induce or inhibit larval settlement of benthic invertebrates, especially barnacles (e.g., Qian et al., 2003; Lau et al., 2005; Thiyagarajan et al., in press). The present study therefore examines indirect effects of UV-A and UV-B on the cypris attachment and metamorphosis of the barnacle *B. amphitrite* through their effects on microbial films. Specifically, we examined: (1) the effects of UV-A and UV-B on the viability of bacterial cells in microbial films; and (2) the attachment response of *B. amphitrite* cyprids to UV-A and UV-B treated microbial films using natural microbial films, inductive monospecies bacterial films, and inhibitive monospecies bacterial films.

## 2. Materials and methods

### 2.1. Monospecies bacterial films

Monospecies bacterial films were formed on polystyrene dishes (#1006, Falcon, USA) for cypris attachment bioassays according to Lau and Qian (1997). Two bacterial strains were used in this study: one strain isolated from the intertidal zone (not yet identified) induces cypris attachment of *B. amphitrite*

(unpublished data) while the other strain, *Micrococcus* sp. strain designation UST950701006, isolated from subtidal zone inhibits the cypris attachment of *B. amphitrite* (Lau et al., 2003b). Stocks of these two bacterial strains were obtained from the Hong Kong Marine Bacteria Culture Collection at the Hong Kong University of Science and Technology. To prepare the bacterial cultures, 2 ml stock of each strain was inoculated to individual culture flasks containing 500 ml sterile nutrient broth (0.3% (w/v) yeast extract, 0.5% (w/v) peptone; Oxoid, UK) prepared with 0.22- $\mu\text{m}$  filtered seawater (FSW). Cultures were grown to stationary phase for 48 h (12:12-h light–dark) at 30 °C with aeration. Bacterial cells were washed in autoclaved FSW with 2 cycles of centrifugation (6000 g for 10 min). Cell pellets were resuspended in autoclaved FSW. Polystyrene dishes were filled with 5 ml of bacterial suspension and incubated at room temperature (ca. 24 °C) for 3 h under visible light. After incubation, dishes were dip-rinsed 10 times in autoclaved FSW to remove loosely attached bacteria. Bacteria remaining on the dish surface were regarded as attached bacteria.

## 2.2. Natural microbial films

Natural microbial films were developed on polystyrene Petri dishes (FALCON, #1006). Dishes were placed in a nylon mesh bag (mesh size:110  $\mu\text{m}$ ) in order to prevent the attachment of invertebrate larvae. The bag was submerged for 6 days in mid-intertidal height (~ 1 m above Mean Low Water Level) in Port Shelter (22°19'N, 114°16'E), a bay in the eastern Hong Kong waters. The dishes were then transported to the laboratory in seawater from the field. Prior to the use in the bioassays, the dishes were dip-rinsed 10 times in autoclaved FSW to remove loosely attached bacteria.

## 2.3. UV-A and UV-B exposure experiments

UV-A and UV-B treatments were performed in a UV chamber at 25 °C. Artificial UV-A and UV-B irradiations were carried out using UV-emitting fluorescent lamps (UV-B VILBER-LOURMAT T-8 M with peak irradiance at 302 nm; UV-A VILBER-LOURMAT T-8 L with peak irradiance at 365 nm). We used irradiance levels of  $2.0 \pm 0.2 \text{ W m}^{-2}$  (Low) and  $4.0 \pm 0.2 \text{ W m}^{-2}$

(High) for both UV-A and UV-B treatments. These irradiance levels were selected as in Hong Kong, UV-B irradiance level ranges from 1–2  $\text{W m}^{-2}$  (Dobretsov et al., in press) and peaks at 4  $\text{W m}^{-2}$  during midday in summer (Chiang et al., 2003). A broadband spectroradiometer (DRC-100X, Spectroline, Westbury, New York, U.S.A.) was used to measure UV irradiance levels. Monospecies bacterial films and natural microbial films were illuminated for different durations to obtain different dosages of UV energy for each level of irradiance (Table 1). During this procedure, the microbial films were immersed under a thin layer of autoclaved FSW to avoid desiccation. The positive and negative controls consisted of the microbial films that were not exposed to UVR and unfiltered sterile polystyrene dishes, respectively.

## 2.4. Enumeration of bacterial density

Both viable and total bacteria in monospecies bacterial films were examined immediately after the UVR treatment according to Lau et al. (2003a). Bacterial films were stained with 6 mM 5-cyano-2,3-ditolyl tetrazolium chloride (CTC, Polysciences, USA) in FSW and incubated for 4 h at 28 °C. Bacterial films were counterstained with 0.5  $\text{mg ml}^{-1}$  4,6-diamidino-2-phenylindole (DAPI, Fluka Chemie, Switzerland) for 15 min after a brief rinse with FSW. Cell numbers were counted at a magnification of 1000 $\times$  in five haphazardly chosen fields of view. Three to six dish replicates were used for each treatment. All bacterial cells appeared blue (DAPI stain) under UV light. Only viable bacterial cells appeared red under UV light due to the deposition of insoluble formazan (reduced CTC) by cellular respiration (Haglund et al., 2002).

After the larval attachment bioassays, the total density of bacteria in the films was determined by epifluorescent microscopy (Zeiss Axiophot fluores-

Table 1

The table below shows the time (in minutes) required to obtain the UV-B and UV-A dose used in this study under two irradiance levels

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

cence microscope;  $\lambda_{\text{ex}}=359$  nm,  $\lambda_{\text{em}}=441$  nm) by staining with DAPI

### 2.5. *Balanus amphitrite* larval culture

Adult *B. amphitrite* Darwin were collected from the intertidal zone in Hong Kong (22°19'N, 114°16'E). Nauplii larvae, obtained from >100 adults, were reared to cypris stage using *Chaetoceros gracilis* Schutt as food according to Thiyagarajan et al. (2003). Newly transformed cyprids were harvested from the cultures and were used immediately for bioassays.

### 2.6. *Balanus amphitrite* larval attachment bioassays

The attachment response of cyprids to microbial films treated with UV-A or UV-B was investigated in still-water choice assays (or double dish bioassay) according to Harder et al. (2001). Briefly, the cyprids were placed within two polystyrene dishes that are joined and sealed using parafilm, called attachment vessels. One dish is coated with a UV-treated film and the other is an unfilmed dish. Our positive controls consisted of one dish containing an untreated microbial film, paired with an unfilmed dish. The negative controls consisted of two unfilmed dishes. For each treatment, there were 6 replicates, each receiving 50–60 cyprids (the attachment was independent of cypris density within this range) in autoclaved FSW. All paired dishes were placed vertically in a tray and incubated for 24 h at 28 °C under a 15 h light and 9 h dark photoperiod (visible light only). The dishes were separated after 24 h, and the number of both attached and metamorphosed individuals on each dish was counted under a dissecting microscope.

### 2.7. Statistical analysis

All percentage and count data were subject to angular and log transformation, respectively, before statistical analysis. One-way ANOVA followed by Tukey's HSD-test as a post-hoc test was used to analyze the respiring and total bacterial densities among UVR treated and untreated microbial films. Attachment response of cyprids to UVR treated and untreated microbial films in attachment vessels was compared to the null hypothesis of 50:50 distribution of attached cyprids on either side of the vessel using

replicated *G*-tests for the goodness of fit (Zar, 1999). The *G* value was calculated as a measure of heterogeneity among replicate vessels within experiment. Homogenous data sets were pooled and corresponding *G* values were transformed by Williams' correction (Zar, 1999).

## 3. Results

### 3.1. Enumeration of bacterial densities

After monospecies inductive bacteria were exposed to UV-B, total bacterial densities showed no signifi-

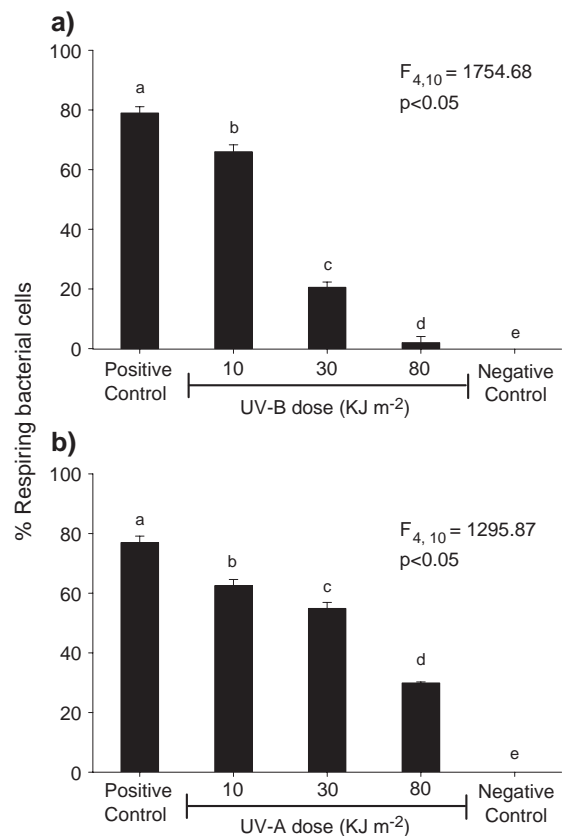


Fig. 1. The percentage of respiring cells in monospecies bacterial films (inductive) after being exposed to (a) UV-B ( $4 \text{ Wm}^{-2}$ ), (b) UV-A ( $4 \text{ Wm}^{-2}$ ); "Positive control" represents bacterial film without UV radiation; "Negative control" represents a sterile Petri dish. Data are expressed as mean + 1 S.E. of three replicates. Data that are significantly different at  $\alpha=0.05$  in Tukey's test are indicated by different letters above the bars.

cant differences among UV doses ( $F_{3,8}=2.76$ ,  $p>0.05$ ). No bacteria were present in the negative control (unfilmed dishes). However, the percent respiring bacterial cells differed significantly among treatments ( $F_{4,10}=1754.68$ ,  $p<0.05$ ), decreasing to 2% at the highest dose (Fig. 1a). Similarly, after exposure to UV-A, total bacterial densities showed no significant differences among UV doses ( $F_{3,8}=3.47$ ,  $p>0.05$ ). No bacteria were present in the negative control (unfilmed dishes). However, the percent respiring bacterial cells differed significantly among treatments ( $F_{4,10}=1295.87$ ,  $p<0.05$ ), decreasing to 30% at the highest dose (Fig. 1b). Like inductive bacterial films, when monospecies inhibitive bacterial films were exposed to both UV-A and UV-B, the percent respiring bacterial cells decreased significantly (data not shown).

After the larval attachment bioassays, total bacterial densities were not significantly different among UV doses (ANOVA,  $p>0.05$ ). Bacterial densities did differ significantly, however, between biofilmed and unfilmed dishes (ANOVA,  $p<0.05$ ). Bacterial densities on filmed surfaces were as follows: natural microbial films:  $16.56\text{--}25.84 \times 10^3 \text{ mm}^{-2}$ ; monospecies inductive bacterial films:  $35.84\text{--}45.92 \times 10^3 \text{ mm}^{-2}$ ; monospecies inhibitive bacterial films:  $154.72\text{--}177.2 \times 10^3 \text{ mm}^{-2}$ . Densities on unfilmed surfaces after the 24 h assays were as

follows: paired with natural microbial films:  $1.8\text{--}3.42 \times 10^3 \text{ mm}^{-2}$ ; paired with monospecies inductive bacterial films:  $3.74\text{--}5.44 \times 10^3 \text{ mm}^{-2}$ ; paired with monospecies inhibitive bacterial films:  $10.56\text{--}19.84 \times 10^3 \text{ mm}^{-2}$ .

### 3.2. Larval attachment on UV treated natural microbial films

For both UV-A and UV-B treated natural microbial films, cyprids attached preferentially to biofilmed surfaces and strongly avoided unfilmed dishes regardless of UV irradiance level or dose (Table 2, Figs. 2 and 3). In paired control dishes, cyprids showed no preference and attached with equal frequency to both unfilmed dishes (Table 2, Figs. 2 and 3).

### 3.3. Larval attachment on UV treated monospecies inductive bacterial films

For both UV-A and UV-B treated monospecies inductive bacterial films, cyprids attached preferentially to biofilmed surfaces and strongly avoided unfilmed dishes regardless of UV irradiance level or dose (Table 3, Figs. 4a,b and 5a,b). In the paired control dishes, cyprids showed no preference and attached with equal frequency to both unfilmed dishes (Table 3, Figs. 4a,b and 5a,b). In addition, cyprids

Table 2

Results of log-likelihood ratio analysis used to test preference between filmed (BF) and unfilmed (PS-C) polystyrene dishes offered (in test and control dishes) to cyprids

Irradiance level	Treatment	UV-B on natural microbial films		UV-A on natural microbial films	
		Gadj	$p$ ( $\nu$ )	Gadj	$p$ ( $\nu$ )
$4\text{Wm}^{-2}$	NUV-BF/PS-C	240.51	< <b>0.001</b> (1)	117.62	< <b>0.001</b> (1)
	UV10-BF/PS-C	210.05	< <b>0.001</b> (1)	119.04	< <b>0.001</b> (1)
	UV30-BF/PS-C	219.44	< <b>0.001</b> (1)	137.72	< <b>0.001</b> (1)
	UV80-BF/PS-C	252.69	< <b>0.001</b> (1)	115.39	< <b>0.001</b> (1)
	PS-C/PS-C	0.85	>0.25 (1)	0.69	>0.25 (1)
$2\text{Wm}^{-2}$	NUV-BF/PS-C	83.47	< <b>0.001</b> (1)	108.41	< <b>0.001</b> (1)
	UV10-BF/PS-C	89.78	< <b>0.001</b> (1)	89.55	< <b>0.001</b> (1)
	UV30-BF/PS-C	126.91	< <b>0.001</b> (1)	94.99	< <b>0.001</b> (1)
	UV80-BF/PS-C	56.43	< <b>0.001</b> (1)	83.69	< <b>0.001</b> (1)
	PS-C/PS-C	2.05	>0.10 (1)	0.78	>0.25 (1)

Microbial films were treated with different UV doses (UV10, UV30, UV80  $\times 10^3 \text{ J m}^{-2}$ ) and one was untreated (NUV).  $p$ -values are given with the degree of freedom ( $\nu$ ) in parentheses. The critical value for log-likelihood test is  $G_{\text{adj}}(0.05, 1)=3.841$ . Gadj:  $G$  adjusted by Williams' correction. Significant values are showed in bold. The significant log-likelihood  $G$ -values indicate that cyprids preferentially attached on one side of the paired dishes.



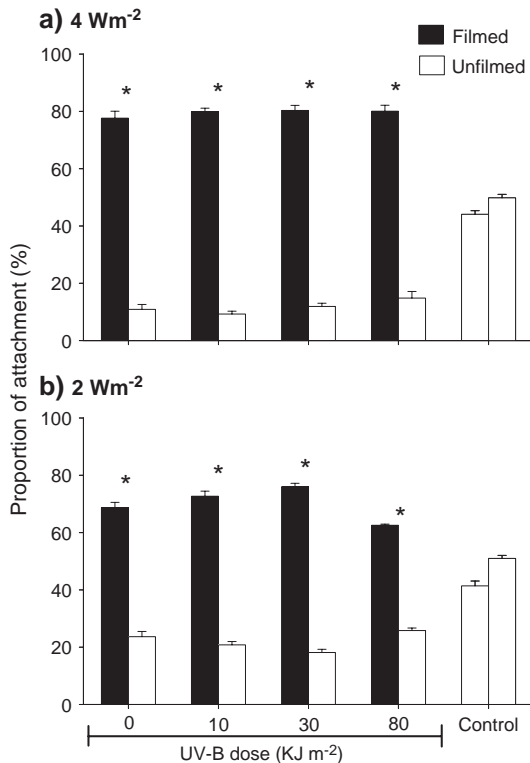


Fig. 2. Double-dish bioassay: attachment response of *Balanus amphitrite* cyprids to filmed (natural microbial films treated with UV-B dose ranged from 0 to 80 kJ m<sup>-2</sup>) or unfilmed surfaces in the paired dishes under (a) 4 Wm<sup>-2</sup>, (b) 2 Wm<sup>-2</sup>. The control treatment had unfilmed surfaces on both sides. Data are expressed as mean + 1 S.E. of six replicates. \* indicates with significant difference in log-likelihood ratio analysis.

showed no preference between a biofilm treated with the highest UV dose and an untreated biofilm (Table 3, Figs. 4c and 5c).

### 3.4. Larval attachment on UV treated monospecies inhibitive bacterial films

For both UV-A and UV-B treated monospecies inhibitive bacterial films, cyprids attached preferentially to unfilmed surfaces and strongly avoided biofilmed dishes regardless of UV irradiance level or dose (Table 3, Figs. 6a,b and 7a,b). In the paired control dishes, cyprids showed no preference and attached with equal frequency to both unfilmed dishes (Table 3, Figs. 6a,b and 7a,b). In addition, cyprids showed no preference between a biofilm treated with

the highest UV dose and an untreated biofilm (Table 3, Figs. 6c and 7c).

## 4. Discussion

As expected, exposure to either UV-A or UV-B resulted in a decrease in the percentage of respiring bacterial cells and this effect increased with increasing UV energy. The viability of bacterial films was confirmed by epifluorescent microscopy using a redox dye CTC with the monospecies films. Other studies have also found that the percentage survival of bacteria decreases in a dose-dependent manner with UV-B radiation (e.g., Joux et al., 1999) and with UV-C radiation (e.g., Lau et al., 2003a). Our results

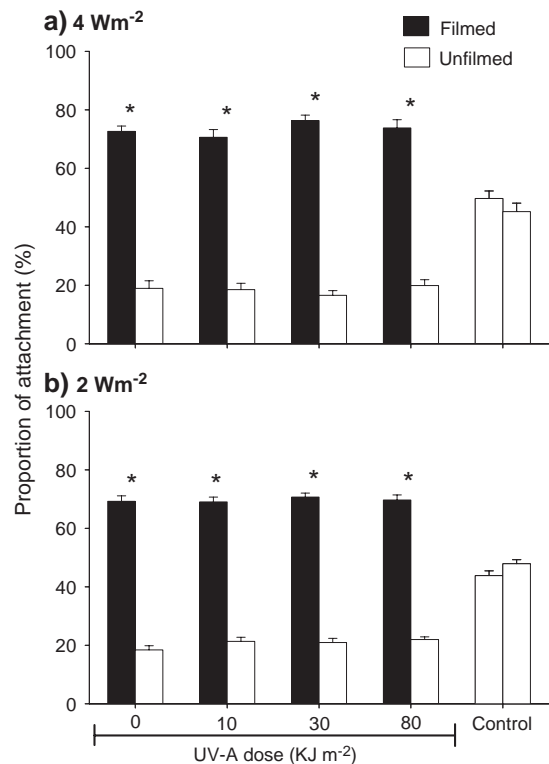


Fig. 3. Double-dish bioassay: attachment response of *Balanus amphitrite* cyprids to filmed (natural microbial films treated with UV-A dose ranged from 0 to 80 kJ m<sup>-2</sup>) or unfilmed surfaces in the paired dishes under (a) 4 Wm<sup>-2</sup>, (b) 2 Wm<sup>-2</sup>. The control treatment had unfilmed surfaces on both sides. Data are expressed as mean + 1 S.E. of six replicates. \* indicates with significant difference in log-likelihood ratio analysis.

Table 3

Results of log-likelihood ratio analysis used to test preference between filmed (BF) and unfilmed (PS-C) polystyrene dishes offered (in test and control dishes) to cyprids

Irradiance level	Treatment	UV-B on monospecies inductive bacterial film		UV-A on monospecies inductive bacterial film		UV-B on monospecies inhibitive bacterial film		UV-A on monospecies inhibitive bacterial film	
		Gadj	<i>p</i> ( <i>v</i> )	Gadj	<i>p</i> ( <i>v</i> )	Gadj	<i>p</i> ( <i>v</i> )	Gadj	<i>p</i> ( <i>v</i> )
4Wm <sup>-2</sup>	NUV-BF/PS-C	122.41	<0.001 (1)	115.92	<0.001 (1)	48.84	<0.001 (1)	49.63	<0.001 (1)
	UV10-BF/PS-C	107.78	<0.001 (1)	134.96	<0.001 (1)	36.63	<0.001 (1)	66.00	<0.001 (1)
	UV30-BF/PS-C	130.80	<0.001 (1)	114.41	<0.001 (1)	35.26	<0.001 (1)	52.15	<0.001 (1)
	UV80-BF/PS-C	115.39	<0.001 (1)	108.19	<0.001 (1)	48.29	<0.001 (1)	53.76	<0.001 (1)
	PS-C/PS-C	1.02	>0.25 (1)	0.17	>0.50 (1)	0.39	>0.50 (1)	1.08	>0.25 (1)
	NUV-BF/UV80-BF	0.64	>0.25 (1)	0.08	>0.75 (1)	0.11	>0.50 (1)	0.30	>0.50 (1)
2Wm <sup>-2</sup>	NUV-BF/PS-C	117.78	<0.001 (1)	105.47	<0.001 (1)	40.73	<0.001 (1)	59.25	<0.001 (1)
	UV10-BF/PS-C	119.14	<0.001 (1)	79.30	<0.001 (1)	36.63	<0.001 (1)	64.08	<0.001 (1)
	UV30-BF/PS-C	97.76	<0.001 (1)	83.76	<0.001 (1)	48.41	<0.001 (1)	65.60	<0.001 (1)
	UV80-BF/PS-C	80.97	<0.001 (1)	101.41	<0.001 (1)	49.88	<0.001 (1)	59.47	<0.001 (1)
	PS-C/PS-C	0.74	>0.25 (1)	0.37	>0.50 (1)	0.06	>0.75 (1)	1.09	>0.25 (1)
	NUV-BF/UV80-BF	0.11	>0.50 (1)	0.53	>0.25 (1)	0.11	>0.50 (1)	1.05	>0.25 (1)

Microbial films were treated with different UV doses (UV10, UV30, UV80 × 10<sup>3</sup> J m<sup>-2</sup>) and one was untreated (NUV). *p*-values are given with the degree of freedom (*v*) in parentheses. The critical value for log-likelihood test is  $G_{adj(0.05, 1)} = 3.841$ . Gadj: *G* adjusted by Williams' correction. Significant values are showed in bold. The significant log-likelihood *G*-values indicate that cyprids preferentially attached on one side of the paired dishes.

showed that, with the same UV energy, UV-B caused a greater loss of respiring bacterial cells than UV-A. Number of respiring bacterial cells in natural microbial films was also observed using a redox dye CTC, however, due to the presence of other microbial components such as diatoms, we found difficulties in enumeration of bacterial cells (data not shown). But our observation showed that the exposure to either UV-A or UV-B substantially reduced the number of respiring bacterial cells and this effect increased with increasing UV energy.

In this study, the substantial reduction in densities of viable bacteria on dish surfaces (i.e., microbial films) caused by exposure to UV-A or UV-B did not result in a change in cypris attachment preferences. Cyprids attached and metamorphosed in response to microbial films regardless of whether the bacterial cells in the film are alive or dead, suggesting that bacterial metabolic activity is not required for the film to have an inductive or inhibitive effect. This finding was further demonstrated in the assays in which cyprids were offered an untreated filmed surface and a film treated with the highest dose of UVR. The microbial films treated with the highest dose of UVR contained the lowest density of respiring bacterial cells (UV-B:  $0.83 \times 10^3$  mm<sup>-2</sup>; UV-A:  $11.89 \times 10^3$  mm<sup>-2</sup>), while the untreated films

contained the highest ( $31$  to  $32 \times 10^3$  cells mm<sup>-2</sup>). Cyprids showed no preference between the UV treated film and untreated film. This finding probably reflects the situation in the field: the intertidal zone is exposed to high levels of UVR, which may well kill the bacteria on most surfaces. *Balanus amphitrite* nevertheless use the microbial films as a cue that identifies an appropriate location for attachment, regardless of whether the bacterial cells in the microbial films are alive or dead.

UV-B can damage microbial DNA, causing a decrease in the density of respiring bacterial cells (e.g., Helbling et al., 1995; Jeffrey et al., 1996; Joux et al., 1999). As for UV-A, in Antarctic pelagic bacterial communities, ambient UV-A can kill more bacteria than UV-B (Marguet and Helbling, 1994; Helbling et al., 1995). Similar results were found in temperate pelagic bacterial communities: UV-A caused a greater loss in cellular viability than UV-B (Sommaruga et al., 1997). UV-A damages the DNA replication process through photodynamic reaction, which also causes a decrease in the density of respiring bacterial cells (Helbling et al., 1995). In our experiments, however, UV-B had a greater effect on the percentage of respiring cells than UV-A, which may be due to the irradiance levels used. Our treatments used the same irradiance levels and doses for UV-A and UV-B in

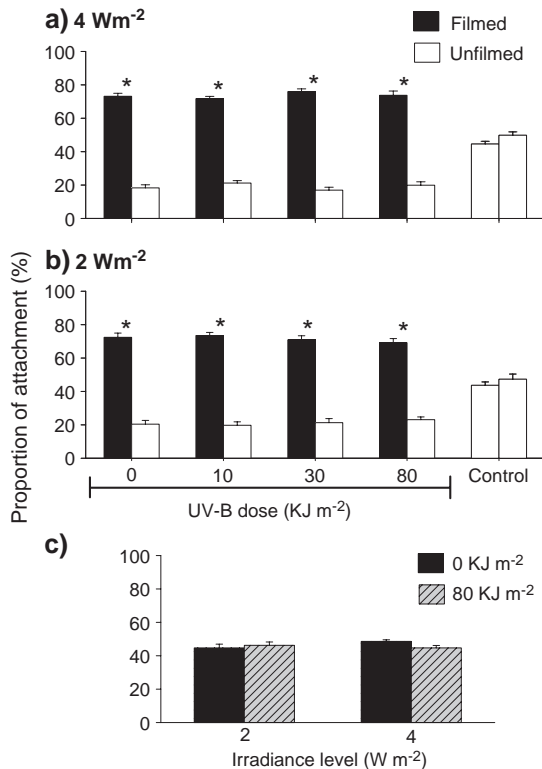


Fig. 4. Double-dish bioassay: attachment response of *Balanus amphitrite* cyprids to filmed (monospecies inductive bacterial films treated with UV-B dose ranged from 0 to 80 kJ m<sup>-2</sup>) or unfilmed surfaces in the paired dishes under (a) 4 Wm<sup>-2</sup>, (b) 2 Wm<sup>-2</sup>, and (c) response to two filmed surfaces (monospecies inductive bacterial films treated with UV-B dose 0 and 80 kJ m<sup>-2</sup>, respectively) in the paired dishes. The control treatment had unfilmed surfaces on both sides. Data are expressed as mean+1 S.E. of six replicates. \* indicates with significant difference in log-likelihood ratio analysis.

order to compare the cellular viability between UV-A and UV-B treatments under otherwise similar conditions. Natural UV-A irradiance levels, however, are 10 times higher than in our experiments (Dobretsov et al., in press). Therefore, the UV-A dose reaching marine bacteria would be much higher than UV-B for a same period of exposure.

Other limitation is that the double dish bioassay has the disadvantage of allowing bacterial contamination of the unfilmed surfaces within a 24 h attachment bioassay period. This contamination occurred as a result of bacteria introduced with the cyprids or from the bacterial film on the opposite dish. Contamination was modest, however, with bacterial

densities on the unfilmed surfaces being 6% to 17% of that on the filmed surfaces. However, there were similar amounts of cyprid attachment on the two unfilmed surfaces in paired control dishes, indicating that cyprid attachment preferences for either filmed or unfilmed surfaces were not due to uncontrolled external stimuli in the larval settlement bioassay experiment.

Previous studies have found that bacterial cell surface components (exopolymer components) were responsible for the induction of larval attachment in several species of marine invertebrates, such as the polychaete *Janua brasiliensis* (Kirchman et al., 1982; Maki and Mitchell, 1985), the barnacle *B. amphitrite*

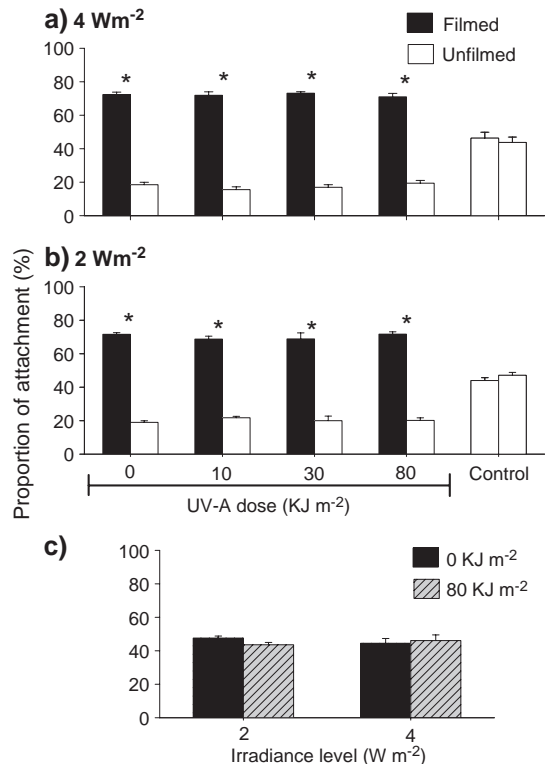


Fig. 5. Double-dish bioassay: attachment response of *Balanus amphitrite* cyprids to filmed (monospecies inductive bacterial films treated with UV-A dose ranged from 0 to 80 kJ m<sup>-2</sup>) or unfilmed surfaces in the paired dishes under (a) 4 Wm<sup>-2</sup>, (b) 2 Wm<sup>-2</sup>, and (c) response to two filmed surfaces (monospecies inductive bacterial films treated with UV-A dose 0 and 80 kJ m<sup>-2</sup>, respectively) in the paired dishes. The control treatment had unfilmed surfaces on both sides. Data are expressed as mean+1 S.E. of six replicates. \* indicates with significant difference in log-likelihood ratio analysis.



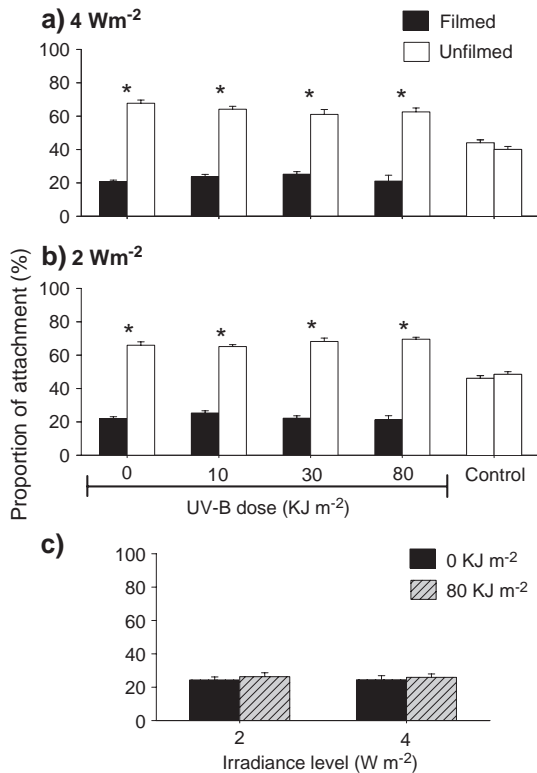


Fig. 6. Double-dish bioassay: attachment response of *Balanus amphitrite* cyprids to filmed (monospecies inhibitive bacterial films treated with UV-B dose ranged from 0 to 80 kJ m<sup>-2</sup>) or unfilmed surfaces in the paired dishes under (a) 4 Wm<sup>-2</sup>, (b) 2 Wm<sup>-2</sup>, and (c) response to two filmed surfaces (monospecies inhibitive bacterial films treated with UV-B dose 0 and 80 kJ m<sup>-2</sup>, respectively) in the paired dishes. The control treatment had unfilmed surfaces on both sides. Data are expressed as mean + 1 S.E. of six replicates. \* indicates with significant difference in log-likelihood ratio analysis.

(Maki et al., 1990), and the tunicate *Ciona intestinalis* (Szewzyk et al., 1991). The inductive cues for larval attachment remained active even when the bacteria were killed. For example, larvae of the polychaete *J. brasiliensis* settle on both live bacterial films and films treated with formaldehyde or antibiotics (Kirchman et al., 1982). Similarly, another example demonstrated that the inhibitive bacterial strains retained their inhibitory activity after the exposure to UV-C (Lau et al., 2003b). Our study further reveals that UV-A and UV-B also kill bacteria in microbial films, but neither UV-A nor UV-B alters the inductive or inhibitory properties of a microbial film. The extracellular polysaccharides on the bacterial cell surface,

which resist UV radiation, may be involved in the signaling of larval settlement (Lau et al., 2003a). However, the agents used to kill bacteria in the other studies cited above do not occur naturally, whereas both UV-A and UV-B are present in intertidal and shallow subtidal habitats.

Our findings contrast with recent studies showing that the inductive effect of one bacterial strain on larval attachment in *H. elegans* is dependent on bacterial viability (Unabia and Hadfield, 1999; Lau and Qian, 2001). These two different findings indicate that bacteria–larval interactions are complex, as larvae of different species respond differently to microbial films. Apart from the bacteria–larval interaction, the

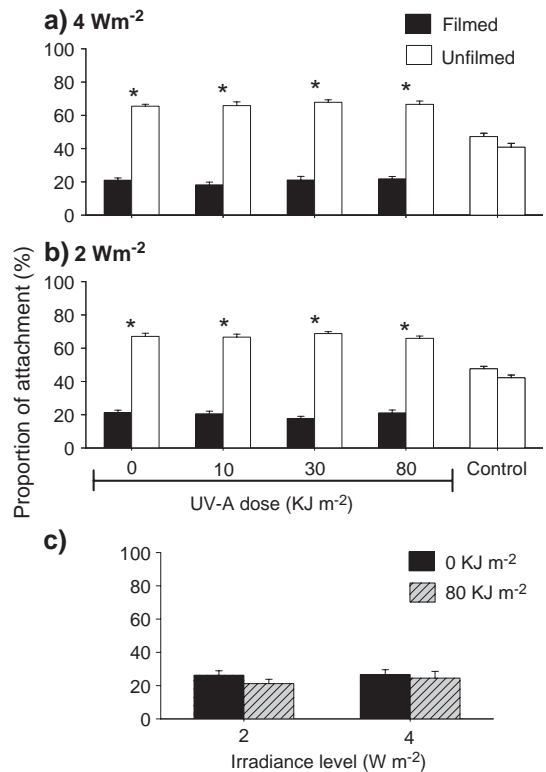


Fig. 7. Double-dish bioassay: attachment response of *Balanus amphitrite* cyprids to filmed (monospecies inhibitive bacterial films treated with UV-A dose ranged from 0 to 80 kJ m<sup>-2</sup>) or unfilmed surfaces in the paired dishes under (a) 4 Wm<sup>-2</sup>, (b) 2 Wm<sup>-2</sup>, and (c) response to two filmed surfaces (monospecies inhibitive bacterial films treated with UV-A dose 0 and 80 kJ m<sup>-2</sup>, respectively) in the paired dishes. The control treatment had unfilmed surfaces on both sides. Data are expressed as mean + 1 S.E. of six replicates. \* indicates with significant difference in log-likelihood ratio analysis.

interaction between bacteria viability and UV energy is also complex. Different bacterial species may respond differently to the same UV energy (Joux et al., 1999).

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## References

- Chiang, W.L., Au, D.W.T., Yu, P.K.N., Wu, R.S.S., 2003. UV-B damages eyes of barnacle larvae and impairs their photo-responses and settlement success. *Environ. Sci. Technol.* 37, 1089–1092.
- Conde, D., Aubriot, L., Sommaruga, R., 2000. Changes in UV penetration associated with marine intrusions and freshwater discharge in a shallow coastal lagoon of the southern Atlantic Ocean. *Mar. Ecol. Prog. Ser.* 207, 19–31.
- Dobretsov, S.V., Qian, P.Y., Wahl, M., in press. Effect of solar ultraviolet radiation on the formation of shallow, early successional biofouling communities in Hong Kong. *Mar. Ecol. Prog. Ser.*
- Faimali, M., Garaventa, F., Terlizzi, A., Chiantore, M., Cattaneo-Vietti, R., 2004. The interplay of substrate nature and biofilm formation in regulating *Balanus amphitrite* Darwin, 1854 larval settlement. *J. Exp. Mar. Biol. Ecol.* 306, 37–50.
- Farmen, J.C., Gardiner, B.G., Shanklin, J.D., 1985. Large losses of total ozone in Antarctica reveal seasonal CLOx/NOx interaction. *Nature* 315, 207–210.
- Haglund, A.L., Tömbölm, E., Boström, B., Tranvik, L., 2002. Large differences in the fraction of active bacteria in plankton, sediments, and biofilm. *Microb. Ecol.* 43, 232–241.
- Harder, T., Thiyagarajan, V., Qian, P.Y., 2001. Effect of cyprid age on the settlement of *Balanus amphitrite* Darwin in response to natural biofilms. *Biofouling* 17, 211–219.
- Helbling, E.W., Marguet, E.R., Villafañe, V.E., Holm-Hansen, O., 1995. Bacterioplankton viability in Antarctic waters as affected by solar ultraviolet radiation. *Mar. Ecol. Prog. Ser.* 126, 293–298.
- Hofmann, D.J., 1996. The 1996 Antarctic ozone hole. *Nature* 383, 129.
- Holmstrom, C., Kjelleberg, S., 2000. Bacterial interactions with marine fouling organisms. In: Evans, L.V. (Ed.), *Biofilms: Recent Advances in Their Study and Control*. Harwood Academic Publisher, Amsterdam, pp. 101–115.
- Huang, S., Hadfield, M.G., 2003. Composition and density of bacterial biofilms determine larval settlement of the polychaete *Hydroides elegans*. *Mar. Ecol. Prog. Ser.* 260, 161–172.
- Jeffery, C.J., 2002. New settlers and recruits do not enhance settlement of a gregarious intertidal barnacle in New South Wales. *J. Exp. Mar. Biol. Ecol.* 275, 131–146.
- Jeffrey, W.H., Pledger, R.J., Aas, P., Hager, S., Coffin, R.B., Haven, R.V., Mitchell, D.L., 1996. Diel and depth profiles of DNA photodamage in bacterioplankton exposed to ambient solar ultraviolet radiation. *Mar. Ecol. Prog. Ser.* 137, 283–291.
- Joux, F., Jeffrey, W.H., Lebaron, P., Mitchell, D.L., 1999. Marine bacterial isolates display diverse responses to UV-B radiation. *Appl. Environ. Microbiol.* 65, 3820–3827.
- Kirchman, D., Graham, S., Reish, D., Mitchell, R., 1982. Bacteria induce settlement and metamorphosis of *Janua* (Dexiospira) *brasilienis* Grube (Polychaeta: Spirorbidae). *J. Exp. Mar. Biol. Ecol.* 56, 153–163.
- Kirt, J.T.O., 1994. Optics of UV-B radiation in natural waters. *Arch. Hydrobiol. Beih.* 43, 1–16.
- Kuffner, I.B., 2001. Effects of ultraviolet (UV) radiation on larval settlement of the reef coral *Pocillopora damicornis*. *Mar. Ecol. Prog. Ser.* 217, 251–261.
- Lau, S.C.K., Qian, P.Y., 1997. Phlorotannins and related compounds as larval settlement inhibitors of the tube-building polychaete *Hydroides elegans*. *Mar. Ecol. Prog. Ser.* 159, 219–227.
- Lau, S.C.K., Qian, P.Y., 2001. Larval settlement in the serpulid polychaete *Hydroides elegans* in response to bacterial films: an investigation of the nature of putative larval settlement cue. *Mar. Biol.* 138, 321–328.
- Lau, S.C.K., Harder, T., Qian, P.Y., 2003. Induction of larval settlement in the serpulid polychaete *Hydroides elegans* (Haswell): role of bacterial extracellular polymers. *Biofouling* 19, 197–204.
- Lau, S.C.K., Thiyagarajan, V., Qian, P.Y., 2003. The bioactivity of bacterial isolates in Hong Kong waters for the inhibition of barnacle (*Balanus amphitrite* Darwin) settlement. *J. Exp. Mar. Biol. Ecol.* 282, 43–60.
- Lau, S.C.K., Thiyagarajan, V., Cheung, S.C.K., Qian, P.Y., 2005. Roles of bacterial community composition in biofilms as a mediator for larval settlement of three marine invertebrates. *Aquat. Microb. Ecol.* 38, 41–51.
- Lotze, H.K., Worm, B., Molis, M., Wahl, M., 2002. Effects of UV radiation and consumers on recruitment and succession of a marine macrobenthic community. *Mar. Ecol. Prog. Ser.* 243, 57–66.
- Maki, J.S., Mitchell, R., 1985. Involvement of lectins in the settlement and metamorphosis of marine invertebrate larvae. *Bull. Mar. Sci.* 37, 675–683.
- Maki, J.S., Rittschof, D., Costlow, J.D., Mitchell, R., 1988. Inhibition of attachment of larval barnacles, *Balanus amphitrite*, by bacterial surface films. *Mar. Biol.* 97, 199–206.
- Maki, J.S., Rittschof, D., Samuelsson, M.O., Szewzyk, U., Yule, A.B., Kjelleberg, S., Costlow, J.D., Mitchell, R., 1990. Effect of marine bacteria and their exopolymers on the attachment of barnacle cypris larvae. *Bull. Mar. Sci.* 46, 499–511.

- Maki, J.S., Ding, L., Stokes, J., Kavouras, J.H., Rittschof, D., 2000. Substratum/Bacterial interactions and larval attachment: films and exopolysaccharides of *Halomonas marina* (ATCC 25347) and their effect on barnacle cyprid larvae, *Balanus amphitrite* Darwin. *Biofouling* 16, 159–170.
- Marguet, E.R., Helbling, E.W., 1994. Effects of solar radiation on viability of two strains of Antarctic bacteria. *Antarc. J. U.S.* 29, 5.
- Miron, G., Boudreau, B., Bourget, E., 1999. Intertidal barnacle distribution: a case study using a multiple working hypothesis. *Mar. Ecol. Prog. Ser.* 189, 205–219.
- Mitchell, R., Maki, J.S., 1988. Microbial surface films and their influence on larval settlement and metamorphosis in the marine environment. In: Thompson, M., Sarojini, R., Nagabhushanam, R. (Eds.), *Marine Biodeterioration: Advanced Techniques Applicable to the Indian Ocean*. Oxford and IBH Publishing, New Delhi, India, pp. 489–497.
- Molis, M., Lenz, M., Wahl, M., 2003. Radiation effects along a UV-B gradient on species composition and diversity of a shallow-water macrobenthic community in the western Baltic. *Mar. Ecol. Prog. Ser.* 263, 113–125.
- Müller, R., Crutzen, P.J., Groob, J.U., Brühl, C., Russel III, J.M., Gernandt, H., McKenna, D.S., Tuck, A.F., 1997. Several chemical ozone loss in the Arctic during the winter of 1995–96. *Nature* 389, 709–712.
- Mundy, C.N., Babcock, R.C., 1998. Role of light intensity and spectral quality in coral settlement: implications for depth-dependent settlement? *J. Exp. Mar. Biol. Ecol.* 223, 235–255.
- O'Connor, N.J., Richardson, D.L., 1996. Effects of bacterial films on attachment of barnacle (*Balanus improvisus* Darwin) larvae: laboratory and field studies. *J. Exp. Mar. Biol. Ecol.* 206, 69–81.
- Olivier, F., Tremblay, R., Bourget, E., Rittschof, D., 2000. Barnacle settlement: field experiments on the influence of larval supply, tidal level, biofilm quality and age on *Balanus amphitrite* cyprids. *Mar. Ecol. Prog. Ser.* 199, 185–204.
- Pawlik, J.R., 1992. Chemical ecology of the settlement of benthic marine invertebrates. *Oceanogr. Mar. Biol. Annu. Rev.* 30, 273–335.
- Qian, P.Y., Thiyagarajan, V., Lau, S.C.K., Cheung, S.C.K., 2003. Relationship between bacterial community profile in biofilm and attachment of the acorn barnacle *Balanus amphitrite*. *Aquat. Microb. Ecol.* 33, 225–237.
- Santas, R., Korda, A., Lianou, Ch., Santas, Ph., 1998. Community responses to UV radiation: I. Enhanced UVB effects on biomass and community structure of filamentous algal assemblages growing in a coral reef mesocosm. *Mar. Biol.* 131, 153–162.
- Smith, R.C., Prezelin, B.B., Baker, K.S., Bidigare, R.R., Boucher, N.P., Coley, T., Karentz, D., MacIntyre, S., Matlick, H.A., Menzies, D., Ondrusek, M.E., Wan, Z., Waters, K.J., 1992. Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. *Science* 255, 952–959.
- Sommaruga, R., Obermosterer, I., Herndl, G.J., Psenner, R., 1997. Inhibitory effect of solar radiation on Thymidine and Leucine incorporation by freshwater and marine bacterioplankton. *Appl. Environ. Microbiol.* 63, 4178–4184.
- Steinberg, P.D., de Nys, D., Kjelleberg, S., 2001. Chemical mediation of surface colonization. In: McClintock, J.B., Baker, B.J. (Eds.), *Marine Chemical Ecology*. CRC Press, Boca Raton, Florida, pp. 355–387.
- Strathmann, R.R., Branscomb, E.S., Vedder, K., 1981. Fatal errors in set as a cost of dispersal and the influence of intertidal flora on set of barnacles. *Oecologia* 48, 13–18.
- Szewzyk, U., Holmström, C., Wrangstadh, M., Samuelsson, M.O., Maki, J.S., Kjelleberg, S., 1991. Relevance of the exopolysaccharide of marine *Pseudomonas* sp. strain S9 for the attachment of *Ciona intestinalis* larvae. *Mar. Ecol. Prog. Ser.* 75, 259–265.
- Thiyagarajan, V., Harder, T., Qiu, J.W., Qian, P.Y., 2003. Energy content at metamorphosis and growth rate of the early juvenile barnacle *Balanus amphitrite*. *Mar. Biol.* 143, 543–554.
- Thiyagarajan, V., Lau, S.C.K., Cheung, S.C.K., Qian, P.Y., in press. Cypris habitat selection facilitated by microbial biofilms influences the vertical distribution of subtidal barnacle *Balanus trigonus*. *Microb. Ecol.*
- Thomason, R.C., Norton, T.A., Hawkins, S.J., 1998. The influence of epilithic microbial films on the settlement of *Semibalanus balanoides* cyprids—a comparison between laboratory and field experiments. *Hydrobiologia* 375, 203–216.
- Unabia, C.R.C., Hadfield, M.G., 1999. Role of bacteria in larval settlement and metamorphosis of the polychaete *Hydroides elegans*. *Mar. Biol.* 133, 55–64.
- Wieczorek, S.K., Todd, C.D., 1998. Inhibition and facilitation of settlement of epifaunal marine invertebrate larvae by microbial biofilm cues. *Biofouling* 12, 81–118.
- Zar, J.H., 1999. *Biostatistical Analysis*, 4th ed. Prentice Hall, Englewood Cliffs, NJ.