Effects of ultraviolet (UV) radiation on larval settlement of the reef coral *Pocillopora damicornis*

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ABSTRACT: Sedimentation, predation and visible light are cited as causal factors to explain the preferential recruitment of coral larvae to shaded habitats in shallow water. Here I examine the effects of ultraviolet radiation (UVR, 280 to 400 nm) on settlement of *Pocillopora damicornis* (Linnaeus) larvae, and the role of UV-absorbing compounds, mycosporine-like amino acids (MAAs), in 3 field experiments. Larvae were obtained from adults of 4 origins: shallow (<0.5 m), deep (2 to 3 m), acclimated under UV-transparent (UVT) filters, and under UV-opaque (UVO) filters. Larvae were then exposed to UVT or UVO conditions in specially designed larval settlement chambers placed on the reef at 0.5 m depth. UVR had a negative effect on total settlement. However, UVR did not significantly increase mortality. High performance liquid chromatography analyses revealed significantly higher concentrations of MAAs in adults incubated in UVT conditions compared to those in UVO, and in shallow larvae compared to deep larvae. Despite the differences in MAA concentrations, larval origin did not significantly affect survival or settlement. Lack of an 'origin' effect suggests that either MAAs may not be important to the larval ecology of this species, or that the deep and UVO larvae had at least the minimum amount of MAAs required for UVR protection. Regardless of MAAs, these experiments indicate that larvae may delay settling to the substrate when UVR levels are high (behavioral response), or that UVR is inhibiting the settlement process in some way (physiological response).

KEY WORDS: Mycosporine-like amino acids · Coral recruitment · UV radiation · Coral planulae

INTRODUCTION

Coral reefs are deteriorating at an alarming rate and on a global scale (Hoegh-Guldberg 1999, Hughes & Connell 1999). The success of management efforts to protect and rehabilitate reefs depends upon a clear understanding of coral reproductive ecology (Hughes et al. 1999, Hughes et al. 2000). Reef rejuvenation may be dependent upon the establishment of juvenile corals, which in turn may depend upon adult coral fecundity, fertilization success, larval transport, settlement (successful metamorphosis) and recruitment (survival of settlers into the population). In this study I examine the effects of a frequently ignored variable, ultraviolet radiation (UVR, 280 to 400 nm), on coral settlement.

Factors known to negatively affect coral settlement and/or recruitment include sedimentation (Hodgson 1990, Babcock & Davies 1991, Gilmour 1999), high biomass of the fouling community (Birkeland 1977), eutrophication (Tomascik 1991), and solar irradiance in the visible region (Maida et al. 1994, Babcock & Mundy 1996). Numerous studies report that coral larvae preferentially recruit to the vertical or underside surfaces of settlement tiles placed at less than 10 m depth (Birkeland et al. 1981, Rogers et al. 1984, Sato 1985, Wallace 1985, Tomascik 1991, Maida et al. 1994, 1995, Babcock & Mundy 1996), invoking some of the above-mentioned factors to explain this pattern. Often

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ignored in settlement and recruitment studies, UVR is an important environmental factor that can have profound effects on aquatic organisms (Calkins & Thordardottir 1980, Holm-Hansen et al. 1993). Only in the past few decades has UVR been considered important in structuring coral reef communities (Jokiel 1980), probably because of the misconception that UVR is absorbed rapidly in the water column. Oligotrophic waters are highly transparent to UVR, and UVR penetration is common to depths of 20 m (Jerlov 1950, Smith & Baker 1979, Fleischmann 1989, Dunne & Brown 1996).

Mycosporine-like amino acids (MAAs), putative sunscreen molecules found in the tissues of many marine organisms (see Dunlap & Shick 1998 for review), accu-

mulate in the tissues of adult corals in response to UVR exposure (Gleason 1993, Kinzie 1993). A photoprotective role has been ascribed to MAAs based upon the absorbance properties of the molecules (λ_{max} ranging from 309 to 360 nm) and studies correlating MAA concentration to UVR exposure (see Dunlap & Shick 1998 for review). UV-B radiation (280 to 320 nm) has been shown to negatively affect the survivorship of *Agaricia agaricites* larvae that have low MAA concentrations; larvae originating from depths of 24 m had higher mortality as a result of UV-B radiation than those from 3 m depth (Gleason & Wellington 1995). This difference was attributed to the 3-fold higher concentration of MAAs in the larvae from 3 m depth compared to those from 24 m depth.
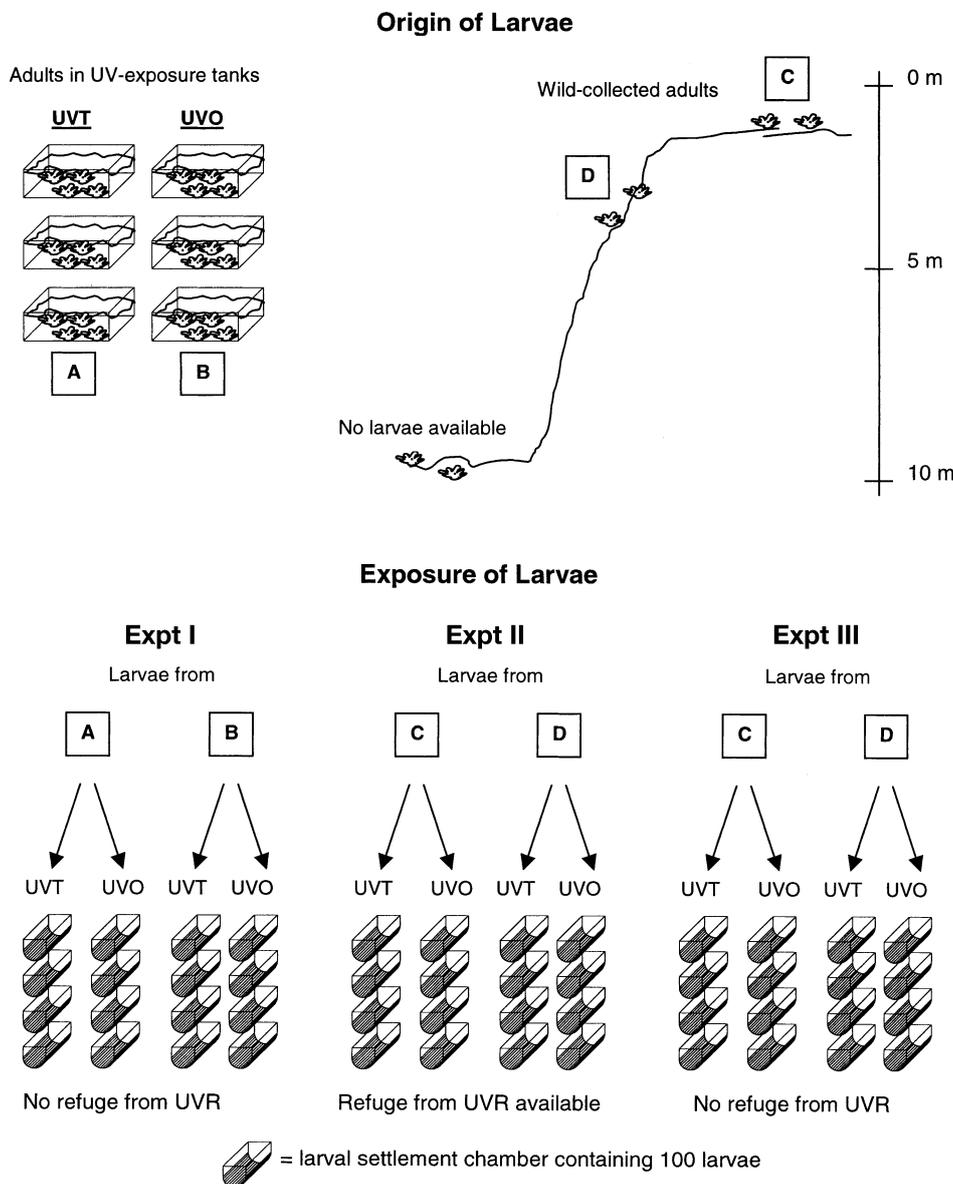
Fig. 1. Schematic representation of the experimental design. Larvae were obtained from adults (A) collected from 0.5 m and acclimated under UV-transparent (UVT) Plexiglas for 51 d, (B) collected from 0.5 m and acclimated under UV-opaque (UVO) Plexiglas for 51 d, (C) collected fresh from 0.5 m, and (D) collected fresh from 2 to 3 m. During larval exposure in Expts I to III, settlement chambers (UVT and UVO) were filled with 100 larvae each and placed on the reef flat at 0.5 m for 1 wk

The aim of this study was to examine the effects of UVR on survivorship and settlement of the Indo-Pacific reef coral *Pocillopora damicornis* (Linnaeus), and to determine if MAA concentration in the larvae affects survivorship and settlement. It has been shown that the concentration of MAAs in corals can be experimentally reduced by screening UVR from the corals' environment (Kinzie 1993). Further, MAA concentrations within coral tissues are inversely correlated with the depth at which the coral is growing (Dunlap et al. 1986, Banaszak et al. 1998). Thus, larvae with differing amounts of MAAs were obtained from adults that had been experimentally acclimated to very low UVR and ambient UVR exposure, and from adults naturally occurring along a depth gradient in the field. The larvae were then tested for their sensitivity to UVR in 3 factorial field experiments. I hypothesized that UVR would have negative effects on larvae from deep adults and adults acclimated in the absence of UVR because these larvae would be expected to have lower concentrations of MAAs.

MATERIALS AND METHODS

Kaneohe Bay environment. The study was conducted in Kaneohe Bay, Hawaii (21° 29' N, 157° 50' W) during the summer of 1998. Kaneohe Bay is characterized by shallow (<0.5 m) patch reefs scattered throughout the bay, with live coral growth reaching a depth of about 10 m, below which soft-sediments accumulate in the depressions between reefs. The water column is fairly turbid with vertical attenuation coefficients for photosynthetically active radiation (PAR) of $K_d(PAR) = 0.28$, and for UVR from 300 to 400 nm, $K_d(UVR) = 0.50$ (Banaszak et al. 1998). Using these values, the depth at which 10% of the sub-surface irradiance remains is 8.3 m for PAR and only 4.6 m for UVR.

Collection and maintenance of corals. *UV-exposure tank adults:* Colonies of *Pocillopora damicornis* were collected from 4 patch reefs in Kaneohe Bay, Hawaii, on May 23, 1998. All of the colonies were taken from the reef flat at a depth of 0.5 m. Twenty-four colonies were transported to the Hawaii Institute of Marine Biology (HIMB) where 1 from each location was placed in each of 6 outdoor aquaria supplied with continuously flowing seawater. Three aquaria were covered with UV-transparent (UVT, Fig. 1A) Plexiglas filters and 3 with UV-opaque (UVO; Fig. 1B) (described in Jokiel & York 1982). The UVO Plexiglas removed 98% of surface UVR from the UVO acclimation tanks (measured with instrument described below). Previous research has shown that corals adjust MAA levels in response to changes in UVR within 3 to 6 wk (Scelfo 1986, Kinzie 1993, Kuffner 2001). Thus, adult corals

were acclimated to the UVR treatments for 7 wk (51 d) before larvae were obtained for experimentation.

Wild-collected adults: On September 9, 1998, 6 colonies were collected from a depth of <0.5 m on the reef flat (Fig. 1C), and 6 from a depth of 2 to 3 m (Fig. 1D). Originally, the 'deep' colonies were collected from 10 m, but these colonies did not release larvae. There were no colonies found in the depth range between 3 and 10 m. Colonies were transported back to HIMB and placed individually in planulae collection containers described by Jokiel et al. (1985). Briefly, each coral was placed in a pitcher supplied with running seawater that overflowed into a mesh cup in which the larvae would collect.

Larvae from the UVT/UVO acclimation tank adults were used in Expt I, and larvae from the deep and shallow colonies were used in Expts II and III (Fig. 1).

Larval settlement chambers. Larval settlement chambers were constructed of 15.2 cm long halves of 15.2 cm diam. transparent polyvinylchloride (PVC) tubes with a plate of either UV-transparent (UVT) or UV-opaque (UVO) Plexiglas attached to the top (Fig. 2). The transparent PVC tubing was of the same optical quality as the UVT filters (transparent to 95% of ambient UVR and 98% of visible light). Mesh of 183 m was adhered with a glue gun to the sides of the chambers. This sized mesh allowed maximum water circulation while keeping the larvae from escaping. The bottoms of the chambers were secured to 30×30 cm terracotta tiles via 10×10 cm Velcro™ patches, providing secure platforms for the chambers when placed on the reef. A 5 mm porthole was drilled into the side of each chamber so that the larvae could be gently released inside using a large-tipped pipette. The porthole was then covered with vinyl electrical tape. A biologically condi-
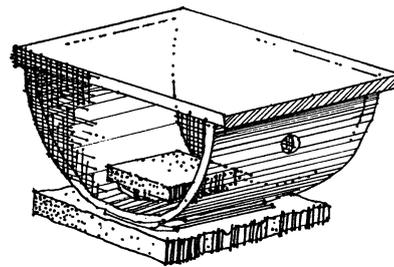
Fig. 2. Line drawing of a larval settlement chamber. The chambers consisted of a half-pipe section of clear Plexiglas tubing topped with either UV-transparent or UV-opaque Plexiglas. Each chamber contained a biologically conditioned terracotta tile held in place with plasticine. Nylon mesh, fixed to the sides with hot glue, had an opening size of 183 μm. After the conditioned tile was sealed within the chamber, larvae were loaded through the porthole, after which the hole was covered with vinyl electrical tape

tioned terracotta settlement tile approximately 10 ×
10 × 1 cm was placed in each chamber and attached
with plastic clay ('plasticine'). During Expts I and III,
the space underneath the tile was entirely filled with
plasticine so that there was no refuge from UVR.
During Expt II, refuge space was left available to the
larvae.

To confirm the UVR treatments, measurements of
the spectral irradiance reaching the inside of the
chambers at 0.5 m depth on the reef were taken with a
Li-Cor LI-1800UW scanning spectroradiometer (Li-Cor,
Lincoln, NB, USA). One of each type of chamber (UVO
and UVT) was modified to fit over the sensor in order to
measure the amount and quality of irradiance falling
on the settlement tile of an intact chamber while
underwater. The 2-pi sensor was fitted so that it sat at
the bottom of the chamber where the recruitment tile
would sit; thus, scattered UVR reaching the inside of
the chamber through the mesh and UVT sides was
included in these measurements.

Planula larvae. In Hawaii, *Pocillopora damicornis*
releases brooded planula larvae for several days fol-
lowing the full moon (Jokiel & York 1982). On the day
of the full moon, corals were moved from the UVR
acclimation tanks or directly from the field to individ-
ual larvae collection containers. Larvae were released
mainly during the day, and were collected from the
containers the following morning. All larvae from
adults which had undergone the same treatment were
pooled. All colonies released larvae, so different geno-
types were most likely represented in each treatment.
Larvae were counted under a dissecting microscope
and placed into glass scintillation vials until they were
transferred to the settlement chambers. One hundred
larvae were placed in each of the 48 chambers (Fig. 1).
The chambers were placed at a protected reef site in
the lee of Coconut Island, Kaneohe Bay, Hawaii, at
0.5 m depth for 1 wk. Sediment was cleaned from the
tops of the chambers each morning.

Scoring of settlement tiles. At the end of 1 wk, the
chambers were transported in seawater back to HIMB
and temporarily placed in flowing seawater until they
were examined within 12 h. A settler was defined as a
larva that had undergone metamorphosis with evi-
dence of skeletal calcification. Larvae attached by
sticky threads to the substrate were not considered
settlers, as many will re-enter the water column after
this tenuous stage of attachment. Larvae were scored
according to settlement location: tile top, tile sides, tile
underside (available in Expt II only), mesh sides,
chamber surface exposed to direct irradiance, and
chamber surface shaded from direct irradiance. Later,
these categories were merged into 2 groups for Expts I
and III: those exposed to direct sun (tile top plus ex-
posed chamber surface) and those in cryptic or shaded

locations (tile sides, mesh, tile bottom and shaded
chamber surface). For Expt II, a third category was
added: larvae in shaded locations underneath the tiles.
The water inside the chamber was poured through a
183 μm mesh sieve to count the larvae still swimming
within the chamber. Total survival was calculated by
adding the number of settlers and the number of swim-
ming larvae.

Experimental design. Three experiments were per-
formed following the collection of larvae in July and
September 1998 (Fig 1). Each experiment was of facto-
rial design, crossing larval origin and UVR exposure.
There were 4 independent replicates of each treatment
combination (i.e., 16 chambers for each experiment).

The purpose of Expt I was to examine the UVR sen-
sitivity of planulae from adult colonies incubated in
ambient UVR (UVT) and in the absence of UV (UVO).
The spaces under the settlement tiles were filled with
plasticine so that the larvae could not escape direct
UVR. The planulae were collected form adult colonies
on July 13, and placed in chambers on the reef from
July 15 to 22. The settlement tiles had been biologi-
cally conditioned for 30 d in a shaded, flowing seawa-
ter tank and were uniformly covered by spots of crus-
tose coralline algae (CCA) and turf algae.

The purpose of Expt II was to compare the UVR sen-
sitivity of planulae obtained from shallow (<0.5 m)
and deep (2 to 3 m) adults. The spaces under the set-
tlement tiles were available to the larvae to determine
if the location of settlement (direct sun, indirect sun
and shade) is affected by the presence of UVR. Larvae
were collected from adult colonies on September 10,
and placed in chambers placed on the reef from Sep-
tember 11 to 18. The settlement tiles had been biologi-
cally conditioned for 80 d in an outdoor sea table in the
shade. Patches of CCA were distributed preferentially
on 1 face of each tile. Thus, the CCA-covered surfaces
were all faced downward so that the orientation was
consistent in all chambers across all treatments. CCA
cover on the undersides of the tile was patchy, but
equally distributed among treatments.

Expt III also tested the UVR sensitivity of larvae from
shallow and deep, but the spaces under the tiles were
filled so that the larvae could not avoid UVR. Larvae
released on September 10 by the same colonies used
in Expt II were placed in chambers on the reef from
September 12 to 19. The settlement tiles had been con-
ditioned for 81 d under the same conditions mentioned
above. CCA was patchily distributed over all sides of
the tiles.

**High Performance Liquid Chromatography (HPLC)
of MAAs.** When larvae were counted for placement
into the settlement chambers, subsamples of the larval
pool were taken for analysis of MAA concentration.
Five batches of 20 larvae were taken from the UV-

exposure tank adults (UVT and UVO), and 5 batches of 10 larvae were taken from the wild-collected (shallow and deep) adults, and immediately frozen at –50°C. On May 21 to 22, 1999, MAAs were extracted from the frozen larvae in 100 μl HPLC-grade methanol, and sonicated for 15 min on ice. The methanol extracts were centrifuged for 3 min to remove particles, the supernatant drawn off and transferred to a polypropylene, low-volume insert placed inside an autosampler vial for HPLC analysis.

Also at the time of planula release, adult tissue samples were taken from 3 colonies of each larval origin (deep, shallow, UVO, UVT) for analysis of MAAs. The MAAs were extracted with 20 ml HPLC-grade methanol, and sonicated for 12 min on ice. The supernatant was then centrifuged for 2 min to remove particles, and analyzed with HPLC.

MAA analyses were performed by slightly modifying the procedures described in Dunlap & Chalker (1986) and Shick et al. (1992). Using a Shimadzu HPLC system, MAAs were separated by reverse-phase isocratic HPLC on a Brownlee RP-8 column (25 cm, 5 μm) protected with a RP-8 guard, in an aqueous mobile phase of 0.1% acetic acid and 40% methanol. The flow rate was 0.8 ml min^{-1}, and the detection of peaks was by UV absorbance at 313 and 340 nm. Identities of peaks were confirmed by co-chromatography with authentic, quantitative standards prepared by the author under the guidance of W. C. Dunlap (Australia Institute of Marine Science). Peaks were integrated using EZ-Chrom software, and quantification of the individual MAAs was accomplished by calibration with quantitative standards. MAA concentrations were normalized to either number of larvae or to gram dry skeletal weight for adult corals.

Statistical analysis. Model I (fixed factor) 2-way ANOVA was performed for each of the 3 experiments using SAS (version 6.12) or Statistix (version 2.0). The models included the 2 factors, UV treatment and origin of the larvae, plus the interaction term. The suitability of the ANOVA model was evaluated by examining normal probability plots and plots of residuals versus fitted values. When percentages fall between 30 and 70%, it is generally not necessary to arcsine transform proportional data (Sokal & Rohlf 1981); thus, total survival and total settlement data were not transformed. Proportional data examining settlement in certain locations were arcsine transformed (Neter et al. 1990).

RESULTS

Larval settlement chambers

Spectral irradiance measurements comparing a UVT and a UVO chamber revealed that approximately 91% of the irradiance in the UV portion (300 to 400 nm) measured in the UVT chamber was eliminated in the UVO chamber (Fig. 3). The 9% of the UVR measured inside the UVO chamber was mainly scattered radiation reaching the bottom of the chamber through the UV-transparent Plexiglas and mesh sides, for when the sensor was held 1 cm below the UVO-Plexiglas, 98% of the UVR was eliminated. The jagged appearance of the spectral scan is a result of the focusing of light by wavelets passing over the chamber that is a real phenomenon experienced by organisms in shallow coral reef environments.

General patterns of larval settlement

Of the 1600 larvae placed in the chambers during each experiment, 56.2% settled in the first experiment, 56.6% in the second experiment, and 32.4% in the third experiment (Table 1). Coral larvae mainly settled on the biologically conditioned tiles, but they also settled on the plastic chamber bottoms, sides and roofs. In the first experiment, 69.6% of the settlers settled on the tiles, and 27.6% settled on the plastic. In the second experiment, the only experiment wherein the undersides of the tiles were available for settlement, 90.2% settled on the tiles, and only 9.5% settled on the plas-
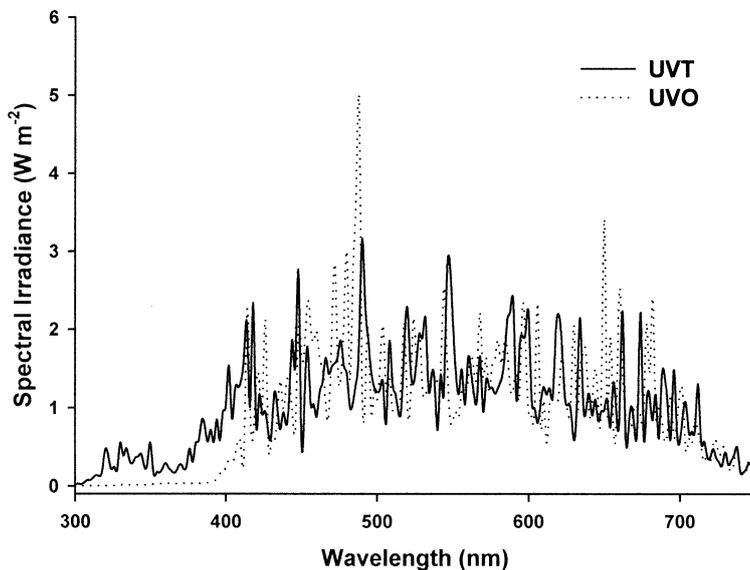
Fig. 3. Spectral irradiance scans inside of an UV-transparent (UVT) and an UV-opaque (UVO) larval settlement chamber placed at 0.5 m depth on the reef. Approximately 91% of the UVR (integrated 300 to 400 nm) measured in the UVT chamber was screened out in the UVO chamber

Table 1. *Pocillopora damicornis*. Settlement location of 100 larvae per settlement chamber. Values are means (SE) followed by percentage of total settlement where applicable. Settlers in direct sun included those on the top of the tile and the bottom of the plastic chamber; settlers in indirect sun included those on the mesh, sides of the tile, and the side of the plastic chamber. In some instances percentages do not add up to 100% due to rounding error and the occasional dislodged settler not being designated to a location. There were 4 replicates per treatment in all cases except where marked (•) n = 3. UVO = UV-opaque, UVT = UV-transparent, na = not available

Origin and UV treatment	Total settlement	Settlers in direct sun	Settlers in indirect sun	Settlers under tile
Expt I				
UVO in UVT	51.3 (4.9)	20.5 (1.6) – 40%	30.8 (5.1) – 60%	na
UVT in UVT	44.5 (4.1)	18.3 (6.4) – 41%	26.3 (6.2) – 59%	na
UVO in UVO	66.0 (5.4)	31.8 (2.6) – 48%	34.3 (7.3) – 52%	na
UVT in UVO	63.0 (5.4)	35.3 (3.7) – 56%	27.8 (7.6) – 44%	na
Expt II				
Deep in UVT•	41.0 (10.0)	11.3 (5.9) – 28%	26.3 (8.7) – 64%	3.3 (1.9) – 8%
Shallow in UVT	48.3 (4.4)	8.5 (2.9) – 18%	25.0 (2.7) – 52%	14.8 (7.6) – 31%
Deep in UVO	63.8 (2.4)	9.0 (0.7) – 14%	46.8 (3.5) – 73%	7.8 (3.1) – 12%
Shallow in UVO•	73.3 (3.4)	3.0 (1.5) – 4%	31.7 (5.2) – 43%	38.0 (9.6) – 52%
Expt III				
Deep in UVT	27.0 (3.4)	5.0 (2.0) – 19%	22.0 (4.1) – 81%	na
Shallow in UVT	24.3 (3.4)	3.3 (1.7) – 14%	21.0 (8.3) – 86%	na
Deep in UVO	40.3 (8.8)	16.8 (3.3) – 42%	23.5 (5.8) – 58%	na
Shallow in UVO	38.3 (5.0)	6.5 (1.3) – 17%	31.8 (4.9) – 83%	na

tic. In the third experiment, 79.8% settled on the tile and 19.5% settled on the plastic. A negligible number of larvae settled on the mesh and the plasticine.

Expt I
UVT/UVO larvae, no refuge from UVR

Survival of larvae averaged 69.6% across all treatments (Fig. 4A). In 1 replicate chamber, there was an infestation of juvenile gastropods, which had grazed the live tissues from the skeletons by the time of analysis. Because the mortality occurred after settlement, empty skeletons and live settlers were combined for this replicate in estimating total settlement.

There was a significant negative effect of UVR on settlement (p < 0.0059, Fig. 4B, Table 2). UVR appears to have had a slight negative effect on survival, but this was not statistically significant (p < 0.0655, Fig. 4A, Table 2). Larval origin did not significantly affect either larval survival or settlement (Fig. 4A,B).

There was no effect of either origin or treatment on the proportion of settlers located in spaces exposed to direct sunlight (Tables 1 & 2).

Expt II
Shallow/Deep larvae, refuge from UVR available

Total survival of larvae was high in all treatments, averaging 69% (Fig. 4C), except in 2 of the chambers in which very few survivors were found. A replicate from the deep/UVT and 1 replicate from shallow/UVO treat-

ment were almost entirely killed by an unknown cause. These data were removed from the analysis on the basis that something other than treatment effects caused the death of all the larvae before settlement occurred.

Total settlement was negatively affected by UVR (p < 0.0012, Fig. 4D, Tables 1 & 2), and was not affected by the origin of the larvae. For total survival, there was a significant interaction between the effects of UVR and larval origin (p < 0.0137, Fig. 4C, Table 2). Due to the presence of the interaction, 2-sample *t*-tests were performed for the deep and the shallow larvae separately. UVR was not important in determining survival for the deep larvae (t = 0.97, df = 5, p < 0.38). The shallow larvae had lower mortality in the UVO chambers than in the UVT chambers (t = 3.12, df = 5, p < 0.026).

The undersides of the tiles were accessible to the larvae in this experiment. Despite having shade available, most of the larvae settled in indirect sunlight on the sides of the tiles and plastic chamber (Table 1). The exception to this pattern was the shallow larvae in the UVO chambers: 52% of settlers were found on the underside of the tile. A possible confounding effect in this experiment could have been the patchiness in CCA on the tiles. There was more CCA on the undersides of the tiles than there was on the tops of the tiles; however, the area of CCA was generally equal among treatments. Larvae may have been encouraged to settle on the undersides of the tiles, but this extra encouragement was probably close to equal among treatments although it was not quantified. As a result, caution should be used when interpreting these re-
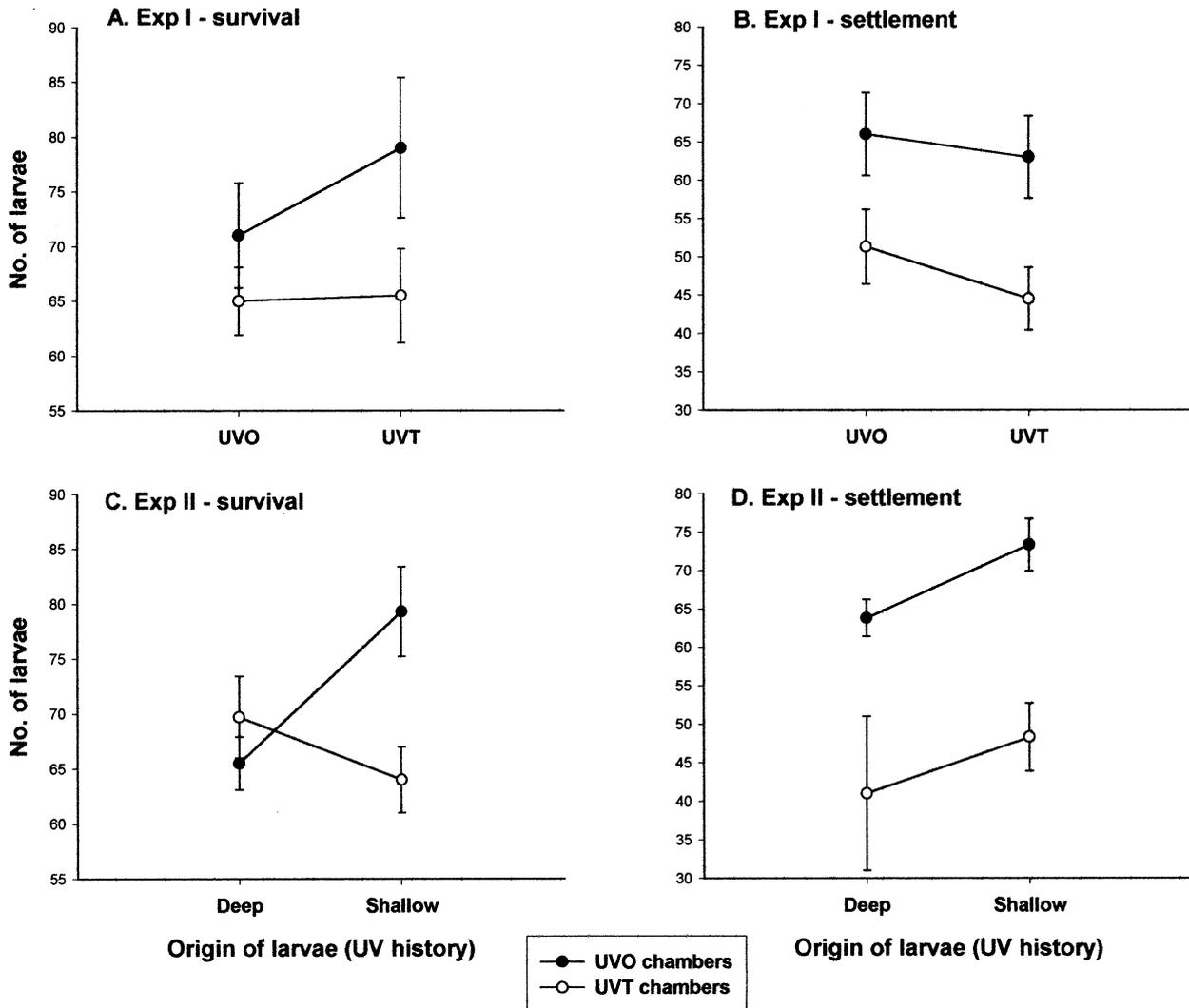
Fig. 4. *Pocillopora damicornis.* Mean (± SE) total survival and total settlement of larvae in Expts I and II. (A,B) Larvae were obtained from UV-transparent (UVT) and UV-opaque (UVO) acclimated adults. (C,D) Larvae were obtained from shallow (<0.5 m) and deep (2 to 3 m) depths. There were 4 chambers per treatment (n = 4) except in (C) n = 3 for deep in UVT and shallow in UVO

sults. Statistical analysis revealed that a higher proportion of larvae settled in direct sun when UV was present, regardless of their origin (UVR p < 0.05; Table 2). Further, a higher proportion of deep larvae settled in indirect sun than shallow larvae (origin p < 0.05), and a higher proportion of shallow larvae settled in shade than deep larvae (origin p < 0.025), regardless of UVR treatment (Table 2).

Expt III
Shallow/Deep larvae, no refuge from UVR

In this experiment, survival was universally low, averaging 26%. However, it appeared that mortality ensued after settlement had occurred because fully formed skeletons were abundant on the tiles. Live settlers and empty skeletons were combined for the estimation of total settlement. Since low survival was probably due to some water column event common to all replicates within the experiment and not a result of the treatment effects, it was not statistically analyzed. Recruitment may have been affected by the unknown cause of death as well, evidenced by the lower recruitment rates in this experiment compared to the other two (Table 1). Therefore, conclusions should be drawn from these data with caution.

Settlement was negatively affected by the presence of UVR (p < 0.0315; Table 2). The proportion of settlers located in direct sun was affected by larval origin (p < 0.05; Table 2), with deep larvae showing a preference

for directly lit places. The proportion of settlers found directly exposed to sunlight also seemed to be affected by UVR, with more settlement in direct sun in the absence of UVR (UVO treatment) than in its presence (UVT treatment), although this effect was marginally significant ($p < 0.06$; Table 2).

Table 2. *Pocillopora damicornis*. Two-way ANOVA of survival, settlement and settlement location of larvae from adult colonies originating from UVT or UVO incubation (Expt I) and shallow or deep (Expts II and III) exposed to either UVT or UVO conditions. P-values < 0.05 in bold

	df	*F*-value	p-value
Expt I			
Survival			
Origin	1, 12	0.78	0.394
UVR	1, 12	4.11	0.066
Origin × UVR	1, 12	0.61	0.451
Settlement			
Origin	1, 12	0.96	0.346
UVR	1, 12	11.17	**0.0059**
Origin × UVR	1, 12	0.14	0.713
% of settlers in sun[a]			
Origin	1, 12	0.13	0.722
UVR	1, 12	2.40	0.148
Origin × UVR	1, 12	0.25	0.626
Expt II[b]			
Survival			
Origin	1, 10	1.56	0.240
UVR	1, 10	2.92	0.118
Origin × UVR	1, 10	8.90	**0.014**
Settlement			
Origin	1, 10	2.49	0.145
UVR	1, 10	20.1	**0.0012**
Origin × UVR	1, 10	0.05	0.831
% settlers in sun[a]			
Origin	1, 10	3.85	<0.10
UVR	1, 10	5.30	**<0.05**
Origin × UVR	1, 10	0.15	>0.10
% settlers in indirect sun[a]			
Origin	1, 10	5.43	**<0.05**
UVR	1, 10	0.004	>0.10
Origin × UVR	1, 10	1.16	>0.10
% settlers in shade[a]			
Origin	1, 10	7.68	**<0.025**
UVR	1, 10	1.89	>0.10
Origin × UVR	1, 10	0.86	>0.10
Expt III			
Settlement			
Origin	1, 12	0.18	0.679
UVR	1, 12	5.92	**0.032**
Origin × UVR	1, 12	0.00	0.948
% of settlers in sun[a]			
Origin	1, 12	4.74	**0.050**
UVR	1, 12	4.25	0.062
Origin × UVR	1, 12	0.79	0.392

[a] Arsine transformed; [b] MS adjusted for unequal sample sizes

HPLC analysis of mycosporine-like amino acids

The larvae of *Pocillopora damicornis* had the same MAAs present in their tissues as the adult corals. There were 6 MAAs identified in the extractions: mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythene, and one unknown compound. Adult corals incubated in the presence of UVR had 2-fold higher concentrations of identified MAAs than those in the absence of UVR ($p < 0.015$; Table 3). A significant difference in total identified MAA concentration was not detected in the adults from <0.5 m depth and those from 2 to 3 m depth ($p < 0.253$; Table 3). However, the mean concentration of the shallow adults was double that of the deep adults.

Larvae originating from shallow corals had significantly higher concentrations of identified MAAs than larvae from depth ($p < 0.0058$; Table 3). Unfortunately, the extractions of the UVO and UVT larvae were too dilute because less material was available for extraction, and measurements were too close to detection limits to quantify.

DISCUSSION

The results of all 3 experiments show that UVR had a negative effect on the settlement of *Pocillopora damicornis* larvae. The presence of UVR within the chamber resulted in a 26 to 35 % reduction in larval settlement, regardless of the presence or absence of refuge spaces from UVR. There was no effect of larval origin on settlement as hypothesized. Larvae from adults in shallow water and from adults incubated in UVR did not have higher survivorship or settlement in the UVT treatment than those from deep water or incubated in the absence of UVR; in fact, only shallow larvae in Expt II showed UVR sensitivity. This was surprising considering that larvae from shallow colonies had significantly greater concentrations of MAAs than larvae from deep colonies. These results indicate that either MAAs may not provide photoprotection in this species, or that the reduced concentrations of MAAs tested here were adequate for UVR protection. Regardless of MAAs, the results of these experiments indicate that larvae may avoid or delay recruiting to the substrate when UVR levels are high (behavioral response), or that UVR is inhibiting the settlement process in some way (physiological response).

Role of mycosporine-like amino acids

The results of this study differed somewhat from previous research investigating the photoprotective role

Table 3. *Pocillopora damicornis*. Results of HPLC analysis for larvae and adult colonies, with accompanying 2-sample *t*-tests for differences due to origins of the adults. Values for identified mycosporine-like amino acids (MAAs) are means (SE). UVT = UV-transparent. UVO = UV-opaque

Pocillopora damicornis	Total identified MAAs (nmol g^{-1} skeleton or larva^{-1})	Two-sample *t*-test	
Adults (n = 3)			
UVT	163.8 (15.3)	*t*-value	4.09
UVO	80.2 (13.6)	p-value (df = 4)	< 0.015
Shallow	240.0 (76.5)	*t*-value^a	1.57
Deep	118.5 (13.0)	p-value^a (df = 2.1)	<0.253
Larvae (n = 6)			
Shallow	0.0501 (0.0099)	*t*-value^a	2.81
Deep	0.0207 (0.0032)	p-value^a (df = 6)	<0.031
^{a}*t*-tests adjusted for unequal variance using Satherwaite's approximation reported by SAS (Littell et al. 1991)			

of MAAs in coral larvae. Gleason & Wellington (1995) found that larvae collected from 3 m and 24 m were both sensitive to UVB radiation compared to controls, but larvae from 24 m were more so. The depth of collection (or artificial UVR-acclimation of adults) was not particularly important in determining survivorship in my study. The only significant effect was in Expt II where shallow larvae had higher survival in UVT than in UVO conditions. When the MAA concentrations for *Agaricia agaricites* planulae originating at 3 m reported by Gleason & Wellington (1995) are expressed in nmol larva^{-1} (using their reported value of 25 µg protein larva^{-1}), their values are 2 orders of magnitude higher than those found here. It is possible that *Pocillopora damicornis* may not supply the brooded larvae with MAAs to the extent that *A. agaricites* does. In addition, the differences in depth that these researchers were working with were much more pronounced than mine: I was unable to obtain larvae from corals collected at 10 m, and there were no colonies found between 10 and 3 m.

MAAs were only reduced by half after more than 7 wk of being in very low UVR (Table 3). Also, MAAs from the deepest larvae-producing colonies that I could find still had half the amount of MAAs found in colonies on the reef flat (Table 3). Thus, corals may retain a base level of MAAs in the absence of a prominent UVR cue. A base level of MAA production would be advantageous in a dynamic light environment, especially for organisms that release gametes or free-swimming larvae into the water column where UVR levels could be higher than those experienced by the parent.

Other researchers have sought to provide evidence for the adaptive role of MAAs in larval ecology. Adams & Shick (1996) demonstrated that cleavage delay caused by UVR in the green sea urchin *Strongylocentrotus droebachiensis* is reduced in eggs containing high concentrations of MAAs in the presence of UVR. However, a similar study on the eggs of the sea hare *Aplysia dactylomela* failed to demonstrate a photoprotective role of MAAs (Carefoot et al. 1998). They found no effect of diet (MAA-rich or MAA-poor) on developmental rates or larval size at hatching, and results regarding hatching success were equivocal. Other studies have shown that MAAs are concentrated in the ovaries of the sea cucumber *Holothuria atra* (Bandaranayake & Des Rocher 1999) and associated with sexual maturity in the sponge *Dysidea herbacea* (Bandaranayake et al. 1997), and suggest that MAAs may perform roles associated with the reproductive cycle in these species rather than UVR protection.

Other coral settlement/recruitment studies

Baker (1995) demonstrated inhibitory effects of UVR on the settlement of *Pocillopora damicornis* larvae in an outdoor laboratory experiment. Larvae in petri dishes exposed to photosynthetically active radiation (PAR) alone had higher percent settlement compared to those in PAR + UV-A and PAR + UV-A + UV-B. Similar to my results, his study did not reveal any UVR treatment effects on larval survival, nor an effect of larval origin (larvae were obtained from adults acclimated to PAR, PAR + UV-A and PAR + UV-A + UV-B) on survival or settlement.

Mundy & Babcock (1998) demonstrated that larvae of different coral species respond differently to spectral quality and light intensity. In 5 of the 6 species tested, larvae remained in the water column as opposed to settling in high intensity light treatments of certain spectral qualities. These observations are consistent with the results of my study. In fact, UVR could have been responsible for their findings, as the spectra reported for the filters used in the treatments reported to inhibit settlement allowed transmittance in the UV region.

A number of factors, such as sedimentation, algal growth, predation by grazers, and visible light intensity, have been put forward to explain the phenomenon that coral larvae recruit to shaded microhabitats in shallow water (Birkeland et al. 1981, Sato 1985, Tomascik 1991, Maida et al. 1994, 1995, Babcock & Mundy 1996). So far, no one has invoked UVR to explain this pattern. My results suggest that *Pocillopora damicornis* larvae may use UVR as an anti-settlement cue in shallow water. It is important to distinguish between factors affecting settlement (larval substrate choice and metamorphosis) and recruitment (survival of settlers until being counted by an observer) (Keough &

Downes 1982). Exposure to UVR would be positively correlated with exposure to sedimentation, filamentous algal growth, and predators, all of which have been invoked to explain recruitment patterns. Concluding that these factors control recruitment patterns could be erroneous due to the unknown effects of larval microhabitat choice during settlement when only recruitment and not settlement has been measured.

Babcock & Davies (1991) specifically tested the hypothesis that sediments affect the location of settlement by experimentally manipulating sediment loads in tanks with coral larvae. Their results showed that high sedimentation resulted in fewer settlers on the upper surfaces of settlement tiles, but sedimentation had no effect on total settlement. My results suggest a negative effect of UVR on total settlement, but little effect on settlement location. In fact, the only statistically significant pattern of UVR determining settlement location was that proportionally more larvae settled in direct sunlight when UVR was present (Expt II), which is quite counterintuitive. These results seem to indicate that if the UVR effect on total settlement is behavioral, it is happening early on at the 'settle vs do not settle' stage, and not during the 'where' stage of microhabitat selection. A physiological delay in the settlement process is also quite possible. It would have been interesting to extend the length of these experiments to see if the larvae still swimming in the UVT treatments would have eventually settled.

Mechanism of UV detection

Members of the Phylum Cnidaria are at the tissue level of organization and lack a centralized nervous system. Hyman (1940) notes that some medusoid stages of Class Hydrozoa and Class Scyphozoa possess ocelli, pigment-filled sensory cells that function as eyespots. Hyman states that members of the phylum lacking ocelli that respond to light must perceive light intensity via the general sensory epithelium. Coral larvae have been long recognized to be phototactic, positively so at first release and then negatively so thereafter until settlement (Edmondson 1929, Lewis 1974). It is unknown whether coral larvae are capable of perceiving UVR. Detection of UVR could be indirect. Perhaps UVR affects the bacterial film or the chemical composition of CCA that is necessary to trigger larva metamorphosis in many species of coral (Morse et al. 1988, 1994, Heyward & Negri 1999).

Implications for coral reproductive ecology

This study demonstrated a link between the presence of ambient UVR and reduced settlement of *Pocil-*

lopora damicornis larvae. My results illustrate the significance of UVR as an important physical parameter that affects the ecology of *P. damicornis*. Even if the presence of UV does not directly cause harm to the larvae themselves, my results suggest corals may have evolved to use UVR as a negative cue to metamorphosis during the pre-settlement stage of life. Jokiel & York (1982) have shown that ambient UVR negatively affects adult coral growth while increasing planula production in *P. damicornis*. Environmental pressures may have selected for allocation of energy toward production of planulae when UVR is particularly high, as well as for an avoidance response of high UVR at the larval stage. Future studies to ascertain if the larvae of other species of coral exhibit a similar response to ambient UVR would be instructive.

LITERATURE CITED

Adams NL, Shick JM (1996) Mycosporine-like amino acids provide protection against ultraviolet radiation in eggs of the green sea urchin *Strongylocentrotus droebachiensis*. J Photochem Photobiol 64:149–158

Babcock R, Davies P (1991) Effects of sedimentation on settlement of *Acropora millipora*. Coral Reefs 9:205–208

Babcock R, Mundy C (1996) Coral recruitment: consequences of settlement choice for early growth and survivorship in two scleractinians. J Exp Mar Biol Ecol 206:179–201

Baker A (1995) Solar UV-A inhibition of planulae larvae in the reef-building coral *Pocillopora damicornis*. In: Gulko D, Jokiel PL (eds) Ultraviolet radiation and coral reefs. Hawaii Institute of Marine Biology Tech Rep 41. Sea Grant, Honolulu, p 149–163

Banaszak AT, Lesser MP, Kuffner IB, Ondrusek M (1998) Relationship between ultraviolet (UV) radiation and mycosporine-like amino acids (MAAs) in marine organisms. Bull Mar Sci 63:617–628

Bandaranayake WM, Des Rocher A (1999) Role of secondary metabolites and pigments in the epidermal tissues, ripe ovaries, viscera, gut contents and diet of the sea cucumber *Holothuria atra*. Mar Biol 133:163–169

Bandaranayake WM, Bourne DJ, Sim RG (1997) Chemical composition during maturing and spawning of the sponge *Dysidea herbacea* (Porifera: Demospongiae). Comp Biochem Physiol 118B:851–859

Birkeland C (1977) The importance of rate of biomass accu-

mulation in early successional stages of benthic communities to the survival of coral recruits. 3rd Int Coral Reef Symp 1:15–21

Birkeland C, Rowley D, Randall R (1981) Coral recruitment patterns at Guam. Proc 4th Int Coral Reef Symp 2:339–344

Calkins J, Thordardottir T (1980) The ecological significance of solar UV radiation on aquatic organisms. Nature 283: 563–566

Carefoot TH, Harris M, Taylor BE, Donovan D, Karentz D (1998) Mycosporine-like amino acids: possible UV protection in eggs of the sea hare *Aplysia dactylomela*. Mar Biol 130:389–396

Dunlap WC, Chalker BE (1986) Identification and quantitation of near-UV absorbing compounds (S-320) in a hermatypic scleractinian. Coral Reefs 5:155–159

Dunlap WC, Shick JM (1998) Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: a biochemical and environmental perspective. J Phycol 34: 418–430

Dunlap WC, Chalker BE, Oliver JK (1986) Bathymetric adaptations of reef-building corals at Davies Reef, Great Barrier Reef, Australia. III. UV-B absorbing compounds. J Exp Mar Biol Ecol 104:239–248

Dunne RP, Brown BE (1996) Penetration of solar UVB radiation in shallow tropical waters and its potential biological effects on coral reefs; results from the central Indian Ocean and Andaman Sea. Mar Ecol Prog Ser 144:109–118

Edmondson CH (1929) Growth of Hawaiian corals, Bull Bernice P. Bishop Museum, Vol 58. Bernice P. Bishop Museum, Honolulu

Fleischmann EM (1989) The measurement and penetration of ultraviolet radiation into tropical marine water. Limnol Oceanogr 34:1623–1629

Gilmour J (1999) Experimental investigation into the effects of suspended sediment on fertilisation, larval survival and settlement in a scleractinian coral. Mar Biol 135:451–462

Gleason DF (1993) Differential effects of ultraviolet radiation on green and brown morphs of the Caribbean coral *Porites asteroides*. Limnol Oceanogr 38:1452–1463

Gleason DF, Wellington GM (1995) Variation in UVB sensitivity of planula larvae of the coral *Agaricia agaricites* along a depth gradient. Mar Biol 123:693–703

Heyward AJ, Negri AP (1999) Natural inducers for coral larvae metamorphosis. Coral Reefs 18:273–279

Hodgson G (1990) Sediment and the settlement of larvae of the reef coral *Pocillopora damicornis*. Coral Reefs 9:41–43

Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. Mar Freshw Res 50:839–866

Holm-Hansen O, Lubin D, Helbling EW (1993) Ultraviolet radiation and its effects on organisms in aquatic environments. In: Young AR, Bjorn LO, Moan J, Nultsch W (eds) Environmental UV photobiology. Plenum Press, New York, p 379–425

Hughes TP, Connell JH (1999) Multiple stressors on coral reefs: a long term perspective. Limnol Oceanogr 44: 932–940

Hughes TP, Baird AH, Dinsdale EA, Moltschaniwkyj NA, Pratchett MS, Tanner JE, Willis BL (1999) Patterns of recruitment and abundance of corals along the Great Barrier Reef. Nature 397:59–63

Hughes TP, Baird AH, Dinsdale EA, Moltschaniwskyj NA, Pratchett MS, Tanner JE, Willis BL (2000) Supply-side ecology works both ways: the link between benthic adults, fecundity, and larval recruits. Ecology 81:2241–2249

Hyman LH (1940) The invertebrates: Protozoa through Ctenophora. McGraw-Hill, New York

Jerlov NG (1950) Ultraviolet radiation in the sea. Nature 116: 111–112

Jokiel PL (1980) Solar ultraviolet radiation and coral reef epifauna. Science 207:1069–1071

Jokiel PL, York RH (1982) Solar ultraviolet photobiology of the reef coral *Pocillopora damicornis* and symbiotic zooxanthellae. Bull Mar Sci 32:301–315

Jokiel PL, Ito RY, Liu PM (1985) Night irradiance and synchronization of lunar release of planula larvae in the reef coral *Pocillopora damicornis*. Mar Biol 88:167–174

Keough MJ, Downes BJ (1982) Recruitment of marine invertebrates: the role of actice larval choices and early mortality. Oecologia 54:348–352

Kinzie RA III (1993) Effects of ambient levels of solar ultraviolet radiation on zooxanthellae and photosynthesis of the reef coral *Montipora verrucosa*. Mar Biol 116:319–327

Kuffner IB (2001) Effects of ultraviolet radiation and water motion on the reef coral *Porites compressa* Dana: a flume experiment. Mar Biol 138:467–476

Lewis JB (1974) The settlement behavior of planulae larvae of the hermatypic coral *Favia fragum* (Esper). J Exp Mar Biol Ecol 15:165–172

Littell RC, Freund RJ, Spector PC (1991) SAS system for linear models. SAS Institute Inc., Cary

Maida M, Coll JC, Sammarco PW (1994) Shedding new light on scleractinian coral recruitment. J Exp Mar Biol Ecol 180:189–202

Maida M, Sammarco PW, Coll JC (1995) Effects of soft corals on scleractinian coral recruitment. I: Directional allelopathy and inhibition of settlement. Mar Ecol Prog Ser 121:191–202

Morse DE, Hooker N, Morse ANC, Jensen RA (1988) Control of larval metamorphosis and recruitment in sympatric agariciid corals. J Exp Mar Biol Ecol 116:193–217

Morse DE, Morse ANC, Raimondi PT, Hooker N (1994) Morphogen-based chemical flypaper for *Agaricia humilis* coral larvae. Biol Bull 186:172–181

Mundy CN, Babcock RC (1998) Role of light intensity and spectral quality in coral settlement: implications for depth-dependent settlement. J Exp Mar Biol Ecol 223:235–255

Neter J, Wasserman W, Kutner MH (1990) Applied Linear Statistical Models: regression, analysis of variance, and experimental designs. Richard D. Irwin, Inc., Burr Ridge

Rogers CS, Fitz HCI, Gilnack M, Beets J, Hardin J (1984) Scleractinian coral recruitment patterns at Salt River submarine canyon, St. Croix, U.S. Virgin Islands. Coral Reefs 3:69–76

Sato M (1985) Mortality and growth of juvenile coral *Pocillopora damicornis* (Linnaeus). Coral Reefs 4:27–33

Scelfo GM (1986) Relationship between solar radiation and pigmentation of the coral *Montipora verrucosa* and its zooxanthellae. In: Jokiel PL, Richmond RH, Rogers RA (eds) Coral reef population biology: Tech Report 37. Hawaii Institute of Marine Biology, Honolulu, p 440–451

Shick JM, Dunlap WC, Chalker BE, Banaszak AT, Rosenzweig TK (1992) Survey of ultraviolet radiation-absorbing mycosporine-like amino acids in organs of coral reef holothuroids. Mar Ecol Prog Ser 90:139–148

Smith RC, Baker KS (1979) Penetration of UV-B and biologically effective dose-rates in natural waters. Photochem Photobiol 29:311–323

Sokal RR, Rohlf FJ (1981) Biometry, 2nd edn. WH Freeman & Co., New York

Tomascik T (1991) Settlement patterns of Caribbean scleractinian corals on artificial substrata along a eutrophication gradient, Barbados, West Indies. Mar Ecol Prog Ser 77: 261–269

Wallace CC (1985) Four years of juvenile coral recruitment to five reef front sites. 5th Int Coral Reef Symp 4:385–390