Marine Biology (1995) 123: 693–703

D. F. Gleason · G. M. Wellington

Variation in UVB sensitivity of planula larvae of the coral *Agaricia agaricites* along a depth gradient

Received: 19 June 1995/Accepted: 23 June 1995

Abstract The effects of natural intensities of ultraviolet A (UVA, 320 to 400 nm) and B (UVB, 280 to 320 nm) radiation on planktonic planula larvae of the reef-building coral *Agaricia agaricites* (Linnaeus) were investigated through field experiments. Survival, chlorophyll concentrations, and solubilized protein concentrations were determined for larvae spawned from colonies at 3 and 24 m depth and subjected to one of three light regimes at 3, 10, or 24 m depth for 72 h: PAR (photosynthetically active radiation, 400 to 700 nm) only, PAR + UVA, or PAR + UVA + UVB. At 3 m depth, larvae in the PAR + UVA + UVB treatment showed lower survivorship than larvae exposed to either PAR alone or PAR + UVA. Within the PAR + UVA + UVB treatment at 3 m depth, larvae from colonies at 24 m depth suffered higher mortality than those from 3 m. Differences in survivorship between larvae originating from 3 and 24 m depth corresponded with tissue concentrations of UVB-protective mycosporine-like amino acids: larvae from 3 m had higher concentrations of mycosporine-glycine ($\lambda_{max} = 310$ nm) and palythine ($\lambda_{max} = 320$ nm) than those from 24 m depth. Chlorophyll concentrations were inversely correlated with PAR intensities, but variation in this parameter did not appear to be detrimental. These results show that sensitivity to high intensities of UVB radiation may affect survival of *A. agaricites* larvae in shallow reef-waters.

Introduction

The vitality of coral reefs is dependent on successful recruitment of hermatypic coral larvae within and between reefs. For most reef-building corals, whether broadcast spawners or brooders, recruitment is accomplished by means of planktonic larvae (see Harrison and Wallace 1990 for review). Planktonic larval duration varies among coral species and ranges from minutes to months (Richmond 1987; Harrison and Wallace 1990; Carlon and Olson 1993). Factors affecting survivorship of hermatypic coral planulae while in the plankton ultimately have an impact on reef growth and sustainability. Many factors, such as predation (Carlon and Olson 1993) and variation in water-current patterns (Simpson et al. 1993), can induce mortality in dispersing planulae. One environmental parameter that has not received attention as an agent of mortality in planktonic coral larvae is ultraviolet (UV) radiation.

The intensities of UVA (320 to 400 nm) and UVB (280 to 320 nm) radiation reaching the earth's surface in tropical regions are the highest found world-wide due to the relative thinness of the ozone layer near the equator and the low zenith angle of the sun (Baker et al. 1980). On average, high intensities of UV light penetrate to considerable depths on coral reefs because of the substantial clarity of surrounding waters (Jerlov 1968; Fleischmann 1989; Wellington and Gleason in preparation). Intensities of UV radiation present at depths from the surface to 25 m on tropical coral reefs are of ecological and physiological relevance. For example, UV radiation may contribute to coral bleaching events (Goenaga et al. 1989; Lesser et al. 1990; Gleason and Wellington 1993), set limits on distributions of adult reef-building corals and other sessile benthic organisms (Jokiel 1980; Wood 1987; Gleason 1993), and inhibit photosynthesis in benthic algae and phytoplankton (Jokiel and York 1984; Hebling et al. 1992; Larkum and Wood 1993; Behrenfeld et al. 1993).

Given the potential for UV radiation to cause biological damage in reef-building corals and other sessile benthic organisms, it is not surprising that tropical

Communicated by N.H. Marcus, Tallahassee

D.F. Gleason (✉) · G.M. Wellington
University of Houston, Program in Evolutionary Biology and Ecology, Department of Biology, Houston, Texas 77204-5513, USA

marine species have evolved several means of mitigating its effects (Jokiel 1980; Siebeck 1988; Lesser and Shick 1989). One UV-protective mechanism common not only in coral reef organisms, but in sessile benthic organisms throughout the world's oceans, is the presence of mycosporine-like amino acids (MAAs) within tissues (Dunlap and Chalker 1986; Karentz et al. 1991; and many others). These free amino acids have absorbance maxima ranging from 310 to 360 nm and have been shown to provide protection from biologically damaging UV radiation by acting as sunscreens (Garcia-Pichel et al. 1993) and as antioxidants that counteract toxic effects of active oxygen species resulting from UV radiation exposure (Dunlap and Yamamoto personal communication). Concentrations of MAAs within coral tissues are inversely correlated with depth, presumably as a response to changes in UV radiation intensities that occur along depth gradients (Dunlap et al. 1986; Gleason and Wellington 1993). Whether or not these depth-related MAA patterns occur in coral planulae spawned from adults inhabiting different UV light regimes has never been investigated.

Despite growing evidence that UV light is of biological significance in tropical waters, its effects on critically important larval stages of sessile coral reef organisms has not been adequately investigated. In this study we used manipulative field experiments to determine if natural intensities of UV radiation can negatively affect planula larvae of the brooding coral *Agaricia agaricites* (Linnaeus) and if there is a relationship between UV sensitivity and MAA concentrations. Adult *A. agaricites* are common on Caribbean coral reefs, occur at depths ranging from < 3 to > 30 m, and are important reef-builders. *A. agaricites* larvae possess intracellular algal symbionts (i.e., zooxanthellae) that require sunlight for photosynthetic processes and may be important as a source of fixed carbon for larvae during dispersal (Richmond 1982). In the presence of suitable substratum, only a few (23%) larvae settle within the first 24 h after release (Carlon and Olson 1993), and most settle between 72 and 96 h (authors' personal observations). Thus, current evidence indicates that *A. agaricites* larvae may be exposed to high intensities of UV radiation for several days while in the water column.

Materials and methods

Experimental manipulations

To determine if UV radiation negatively affects *Agaricia agaricites* (Linnaeus) larvae, we exposed planulae from this species to a range of UV light intensities. Planulae were collected from adult colonies at 3 and 24 m depth at Pickles (25°59'N; 80°23'W) and Conch (24°57'N; 80°27'W) reefs, respectively. Both sites are adjacent to Plantation Key, Florida, USA. Sexually mature colonies of *A. agaricites* were removed from the reef with a chisel and hammer. These colonies were kept submerged and were shielded from sunlight during transport from the reef site to the marine laboratory on Key Largo. Upon return to the laboratory, colonies were placed in 2-liter buckets containing sea water (one colony per bucket) that were situated in an open-air, covered laboratory. This arrangement allowed adults to experience natural light/dark cycles without associated exposure to high intensities of UV radiation. Water temperatures were kept between 26 and 29 °C by floating the buckets in a tank supplied with continuously flowing sea water.

Planulae were usually released at night within 2 to 3 d of collection. The following morning, larvae were segregated by depth of origin and groups of 12 haphazardly selected larvae from the same depth of origin were placed in individual 1.5 ml polypropylene microcentrifuge tubes containing sea water. Tubes with their enclosed larvae were kept in the dark during transport to the study site. *Agaricia agaricites* larvae were transferred to silica tubes (4.5 cm i.d. × 30 cm length) at the study site: one set of 12 larvae per tube. After larval addition, each tube was sealed at its ends with 150 µm Nitex® nylon mesh. Larval transfer was completed in a shaded area of the research vessel to avoid premature exposure to high intensities of UV radiation. In addition to the larvae added to the tubes, five planulae per colony for five of the colonies from each depth were immediately frozen at −20 °C for subsequent MAA analysis (see subsection "UV light-absorbing compounds" below).

Silica tubes containing *Agaricia agaricites* larvae were attached to polyvinyl chloride (PVC) racks positioned ≃ 15 cm above the reef substratum at 3, 10 or 24 m depth on Conch Reef. Stainless steel threaded rods (0.7 × 22 cm) cemented 5 cm deep into the bottom secured these racks to the reef. Larvae from both 3 and 24 m depth were exposed to ambient light at 3, 10, and 24 m depth. At each depth there were four replicates per larval depth of origin of the following UV light treatments: (1) PAR, UVA, and UVB present (silica tube alone); (2) PAR and UVA present (silica tube shaded with clear polyester film); and (3) PAR only (silica tube shaded with 0.6 cm thick acrylic cover). The silica (Vycor, Corning Inc.) was ≃ 93% transparent to UV and 94% transparent to visible light. The polyester film (Mylar, DuPont Co.) was virtually opaque to UVB radiation between 280 and 313 nm (i.e., < 1% transmission), was from 2 to 39% transparent to UVB from 313 to 320 nm, transmitted from 39% at 320 nm to 93% at 400 nm of UVA radiation, and was from 93 to 96% transparent to PAR. The acrylic (OP-2, Cyro Industries) blocked virtually all of the UVA (< 1% transmission up to 390 nm) and UVB (< 1% transmission) radiation, was 16 to 94% transparent in the 400 to 440 nm PAR range, and 94 to 97% transparent to wavelengths > 440 nm.

Experimental manipulations were conducted between 30 June and 10 July 1993. UV and visible light intensities were monitored during experiments with three LiCor LI-1800UW scanning spectroradiometers. One radiometer was placed at each experimental depth and programmed to scan from 300 to 700 nm in 2 nm increments between 07:00 and 18:00 hrs US Eastern Standard Time (EST). To ensure that comparisons of light intensity could be made across depths, all radiometers were calibrated to a 200 W tungsten halogen lamp (LiCor model 1800-02 optical calibrator) prior to deployment. Mean daily UV radiation doses and maximum dose rates for the 3 d intervals were determined with and without corrections for wavelength-specific biological effectiveness. Calculations for biologically effective dose rates between 300 and 340 nm were based on the analytical representation of Green and Miller (1975).

Survivorship, chlorophyll concentrations and protein levels

The number of living and dead larvae in each tube was determined 72 h after deployment. Assays for chlorophyll and protein concentrations were conducted on survivors to identify sublethal effects of PAR exposure and UV light. Survivors in each tube (1 to 12 larvae) were pooled, and chlorophyll was extracted with two successive treatments of 0.5 ml acetone at 4 °C for 24 h. Pigment concentrations were extrapolated from spectrophotometric measures of

Table 1 Total daily doses and maximum dose rates of photosynthetically active radiation (400 to 700 nm), ultraviolet A (UVA, 320 to 400 nm), and ultraviolet B (UVB, 300 to 320 nm) received by *Agaricia agaricites* larvae in PAR + UVA + UVB treatments btween 07:00 and 18:00 hrs (US Eastern Standard Time) at three depths on Conch Reef. Values are means (± SE) for 3 d experimental period

Depth of origin (m)	Incubation depth (m)	Total dose d^{-1} ($J\,m^{-2} \times 10^4$)			Maximum dose rates ($W\,m^{-2}$)		
		PAR	UVA	UVB	PAR	UVA	UVB
3	3	662.92 (32.52)	85.92 (4.29)	3.42 (0.27)	274.42 (12.24)	36.16 (2.00)	1.62 (0.19)
24	3	671.38 (15.78)	77.18 (5.64)	2.66 (0.30)	259.31 (11.33)	31.12 (3.22)	1.19 (0.15)
3	10	319.19 (40.95)	39.15 (7.98)	0.62 (0.18)	132.50 (20.29)	16.56 (3.75)	0.28 (0.10)
24	10	284.30 (79.75)	34.60 (11.92)	0.50 (0.17)	101.90 (34.76)	13.35 (5.36)	0.22 (0.09)
3	24	157.35 (13.42)	14.41 (2.51)	0.06 (0.02)	67.14 (7.33)	6.16 (1.33)	0.03 (0.01)
24	24	151.41 (15.94)	11.96 (3.02)	0.05 (0.01)	62.82 (8.12)	5.14 (1.31)	0.02 (0.01)

chlorophylls *a* and c_2 at 663 and 630 nm (Jeffrey and Humphrey 1975), with total chlorophyll expressed as μg $larva^{-1}$.

Protein assays were conducted on the same samples used for chlorophyll extractions. Larval proteins were solubilized by placing survivors from a single silica tube in 0.6 ml 0.12 *N* NaOH and heating the sample for 60 min at 90 °C in a shaker bath. Solubilized protein was subsequently quantified by spectrophotometry (595 nm) with Coomassie Brilliant Blue G-250 (Bio-Rad Laboratories, Inc.) according to Bradford (1976). Bovine gamma globulin was used as the protein standard, with final concentrations expressed as μg $larva^{-1}$.

UV light-absorbing compounds

Ultraviolet light-absorbing MAAs were extracted from larvae of ten colonies: 5 from 3 m and 5 from 24 m depth. Five larvae from each adult were pooled and immersed in a 1 ml solution of 80% MeOH in H_2O at 4 °C. After 24 h, samples were centrifuged at 14000 rpm for 5 min. Presence of UV-absorbing compounds within each sample was initially confirmed by scanning the supernatant from 290 to 400 nm on a Milton-Roy Spectronic 3000 Array diode-array spectrophotometer.

MAAs were separated by reverse-phase isocratic high-pressure liquid chromatography (HPLC). Sample preparation followed the protocols of Dunlap and Chalker (1986), except that the low volume of the larval samples allowed us to process them using one-half as many Waters C-18 Sep Paks (Waters Chromatography Division, Millipore Corporation, Milford, Massachusetts). Briefly, the solution obtained from the extraction outlined above was diluted with 9 ml of 80% MeOH in distilled H_2O. Non-polar photosynthetic pigments were removed by filtration through one Waters C-18 Sep Pak. The Sep Pak was washed with 10 ml of distilled water and the solutions were combined. Organic solvents were removed by placing the solution in a desiccator under vacuum. After 24 h, the remaining liquid was passed through a single Sep Pak that was subsequently rinsed with 10 ml of distilled water. Aqueous solutions were combined and lyophilized. Dry samples were stored at −20 °C prior to analysis.

MAAs were separated on a Brownlee RP-8 column (Spheri-5, 4.6 mm × 10 cm long) protected with an RP-8 guard column (Spheri-5, 4.6 mm × 3 cm long). Dried samples were resuspended in 0.5 ml of mobile phase and 2 to 10 μl subsamples were injected into the column. The mobile phase consisted of 0.1% acetic acid, 15% methanol, and 84.9% water at a flow rate of 0.8 ml min^{-1}. Identification of MAAs was by spectrophotometric peak confirmation on a Milton-Roy Spectronic 3000 Array diode-array spectrophotometer and by co-chromatography with standard compounds provided by W.C. Dunlap (Australian Institute of Marine Science).

Standard compounds were prepared from crude methanolic extracts of organisms previously characterized for MAA content: mycosporine-glycine from the colonial ascidian *Lissoclinum patella* (Dunlap and Yamamoto, personal communication), and palythine from lens tissue of the coral trout *Plectropomus leopardus* (Dunlap et al. 1989). Methanol used to extract standard compounds was removed under reduced pressure, residues were dissolved in H_2O, and liphophilic pigments were removed on Waters C-18 Sep Pak cartridges. Lyophilized standards were stored at −20 °C and, when required, dissolved in the mobile phase and chromatographed.

Quantification of MAAs was based on peak-area calibration of chromatographed standards (Dunlap personal communication). Concentrations of aqueous standards were determined by UV spectrophotometry using published molar extinction coefficients (ε) at wavelengths of maximum absorbance: $\varepsilon_{310} = 28100$ for mycosporine-glycine (Ito and Hirata 1977; Dunlap and Chalker 1986) and $\varepsilon_{320} = 36200$ for palythine (Takano et al. 1978). MAA concentrations were standardized to picomoles per μg protein. Protein assays were conducted after extraction of MAAs according to protocols given in the previous subsection. Extraction efficiencies for each MAA were determined using procedures outlined by Dunlap and Chalker (1986).

Results

Experimental manipulations

UVB intensities showed greater differences along the experimental depth gradient than either PAR or UVA (Table 1). For both total daily dose and maximum dose rate, levels of UVB radiation were an order of magnitude higher on average at 10 m than 24 m depth, and ≃ 5.5-fold higher at 3 m than 10 m depth. Total daily doses and maximum dose rates for UVA and PAR were ≃ 2.7 and 1.9-fold higher, respectively, at 10 m depth compared to 24 m depth. Both UVA and PAR showed ≃ 2.2-fold greater intensities, for both total daily dose and maximum dose rate, at 3 m than at 10 m depth.

Larval exposure experiments were run back-to-back rather than simultaneously because of limitations imposed by manpower and supplies. This design resulted in slight, but statistically non-significant, differences in UV and visible light environments for larvae subjected to similar treatments at the same depth (Table 1). Differences in the light environment were most marked in the UVB portion of the light spectrum for larvae incubated at 3 m depth. Specifically, *Agaricia agaricites*

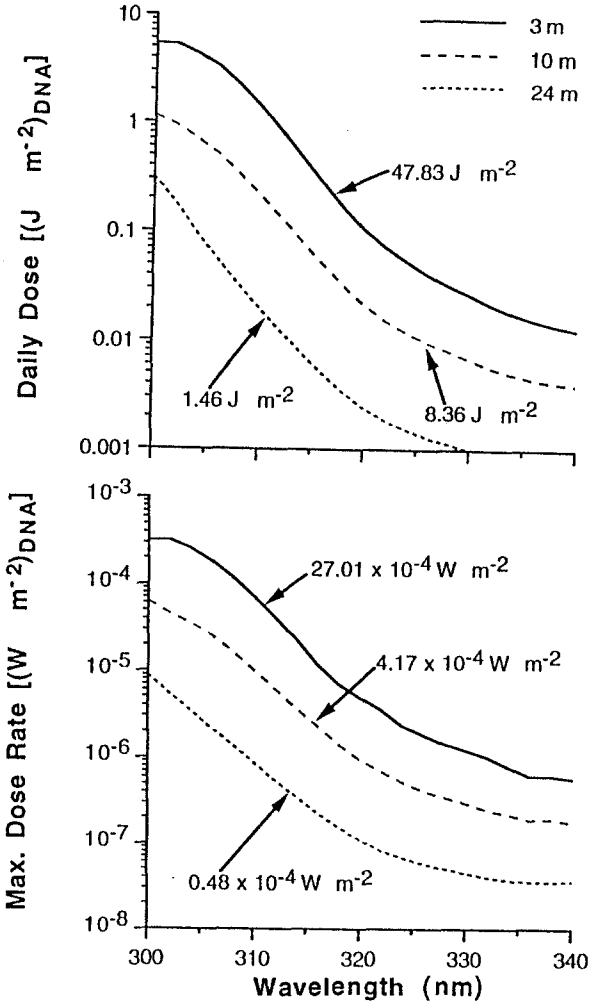

Fig. 1 Mean daily total and mean maximum DNA-weighted UV-radiation doses at 3, 10, and 24 m depth during survivorship experiments of larval *Agaricia agaricites*. Each curve reflects mean of 3 d calculated for experimental period at each depth when highest ultraviolet B (UVB) radiation intensities occurred (see Table 1). Integrated values represent area under UVB portion of curve (300 to 320 nm). Biological effectiveness weightings are based on DNA action spectrum of Setlow (1974) and are normalized to 1 at 265 nm

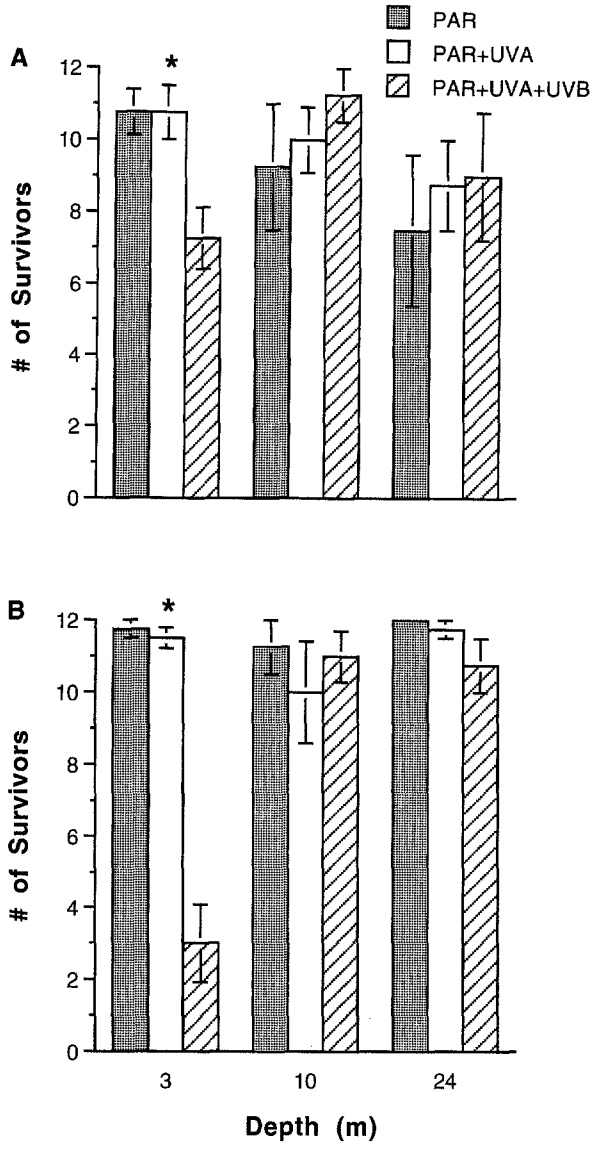

Fig. 2 *Agaricia agaricites*. Mean ($\pm$ SE) number of surviving planulae after 72 h exposure to three different light regimes at 3, 10, and 24 m depth [*PAR* photosynthetically active radiation (400 to 700 nm); *UVA* ultraviolet A (320 to 400 nm); *UVB* ultraviolet B (280 to 320 nm)]. Larvae were obtained from adult colonies collected at either 3 m (**A**) or 24 m (**B**) depth (*Asterisk above bar* significant difference ($P < 0.04$) between groups at a single depth, as determined by nonparametric Kruskal–Wallis one-way analysis of variance). Sample size was 4 silica tubes per treatment with initial densities of 12 larvae per tube

larvae obtained from adults at 3 m depth and relocated to 3 m experienced a UVB light environment that was 1.29 fold-higher than larvae from 24 m depth placed at 3 m.

Biologically effective (i.e., DNA-weighted) daily doses and maximum dose rates were inversely correlated with wavelength (Fig. 1). Measurable quantities of biologically effective UV radiation, while small, were present even at 24 m depth. Maximum DNA-weighted dose rates at all depths were usually sustained between 12:00 and 13:00 hrs EST. Integration of these maxima over 60 min resulted in biologically effective UV radiation doses of 9.72, 1.50, and 0.17 $J\,m^{-2}$ at 3, 10, and 24 m depth, respectively. Thus, at 3, 10, and 24 m depth, respectively, larvae received 20.3, 17.9, and 11.6% of the total biologically effective daily dose at the maximum dose rate.

Survivorship, chlorophyll concentrations and protein levels

The number of larvae surviving to 72 h did not vary across light treatments for larvae originating from 3 and 24 m depth and placed at 10 or 24 m depth (Fig. 2). Larvae from 24 m showed high survivorship (83 to 100%) in all treatments at these two depths, whereas larvae from 3 m exhibited slightly lower survivorship (63 to 75%) in all light treatments at 24 m

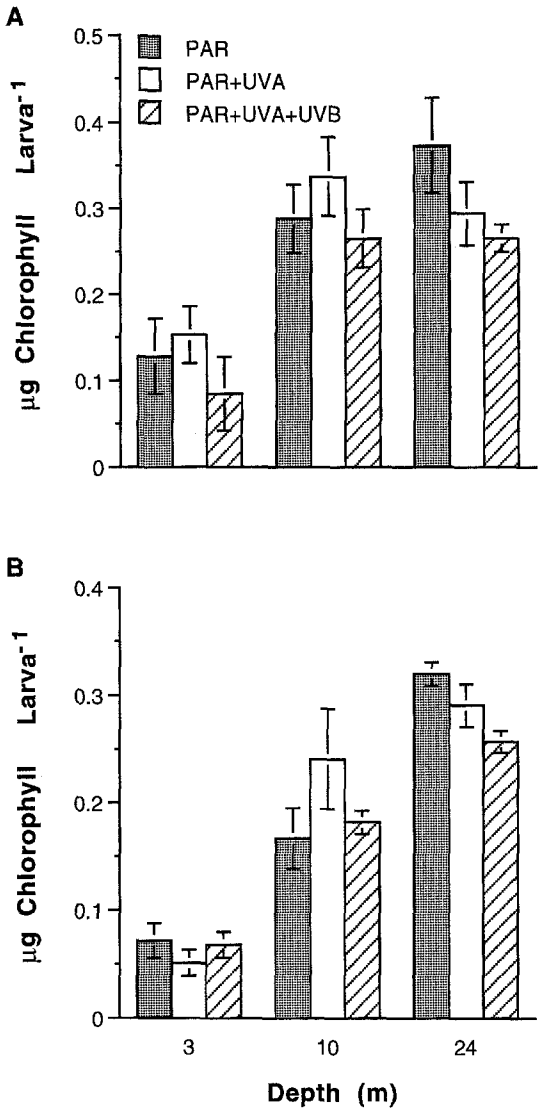

Fig. 3 *Agaricia agaricites.* Mean (± SE) concentrations of chlorophyll in planulae surviving exposure to three different light regimes at 3, 10, and 24 m depth for 72 h. Larvae were obtained from adult colonies collected at either 3 m (**A**) or 24 m (**B**) depth. Sample size was 4 silica tubes per treatment, except in PAR + UVA + UVB treatment at 3 m depth in **A**, where one sample was lost and $n = 3$. Final densities were 1 to 12 larvae per tube. Initial sample of larvae from four randomly selected adults at both 3 and 24 m depth contained chlorophyll concentrations of 0.27 (± 0.12 SE) and 0.29 (± 0.06) µg per larva, respectively

depth (Fig. 2). At 3 m depth, the inclusion of UVB radiation resulted in significantly increased mortality for larvae originating from both 3 and 24 m depth (Fig. 2). The magnitude of mortality in PAR + UVA + UVB treatments at 3 m depth was significantly greater for larvae originating from 24 m depth than for those from 3 m depth (Mann–Whitney U-test, $U_s = 15$, $P = 0.043$). In all cases, deceased larvae appeared as opaque, immobile tissue masses devoid of cilia and zooxanthellae.

Table 2 *Agaricia agaricites.* Results of two-way ANOVA for total chlorophyll concentrations in planula larvae exposed to three different light regimes (PAR only, PAR + UVA, or PAR + UVA + UVB) at 3, 10, and 24 m depth for 72 h. Larvae were obtained from adult colonies collected at either 3 or 24 m depth. Results based on Systat's general linear-model procedure (Wilkinson 1990)

Source of variation	*df*	Mean square	*F*	*P*
3 m origin				
Depth	2	0.1230	19.54	< 0.001
Light	2	0.0122	1.93	0.165
Depth × light	4	0.0047	0.74	0.570
Error	26	0.0063		
24 m origin				
Depth	2	0.1543	80.54	< 0.001
Light	2	0.0020	1.03	0.369
Depth × light	4	0.0043	2.24	0.091
Error	27	0.0019		

Many surviving larvae in shallow-water treatments became paler in color during experiments. Differences in pigment concentrations across treatments were quantitatively confirmed through chlorophyll analyses (Fig. 3). A two-way analysis of variance indicated a significant effect on chlorophyll concentrations due to depth only (Table 2). Specifically, increasing PAR intensities resulted in lower concentrations of total chlorophyll for larvae obtained from either 3 or 24 m depth. In contrast to survivorship, neither UVA nor UVB radiation had an effect on chlorophyll concentrations. Despite reductions in total chlorophyll, most bleached larvae appeared otherwise healthy and exhibited mobility similar to that observed in normally pigmented larvae.

Effects of the treatments on protein concentrations in surviving larvae were differential with respect to larval depth of origin (Fig. 4). While a two-way analysis of variance indicated no significant effects due to depth or light treatment in larvae originating from 24 m depth, larvae from 3 m displayed a significant light-treatment effect (Table 3). Larvae from 3 m depth generally contained lower concentrations of protein, especially at the 3 and 10 m treatment depths, when exposed to the full qualitative light regime (i.e., PAR + UVA + UVB) than when subjected to a photic environment excluding UVB radiation (Fig. 4).

UV light-absorbing compounds

Spectrophotometric scans of MeOH extracts of *Agaricia agaricites* planulae from both 3 and 24 m depth revealed a shoulder between 310 and 320 nm, indicating the presence of qualitatively similar MAAs (Fig. 5A). Differences in the heights of these peaks indicated that larvae from the shallower depth were

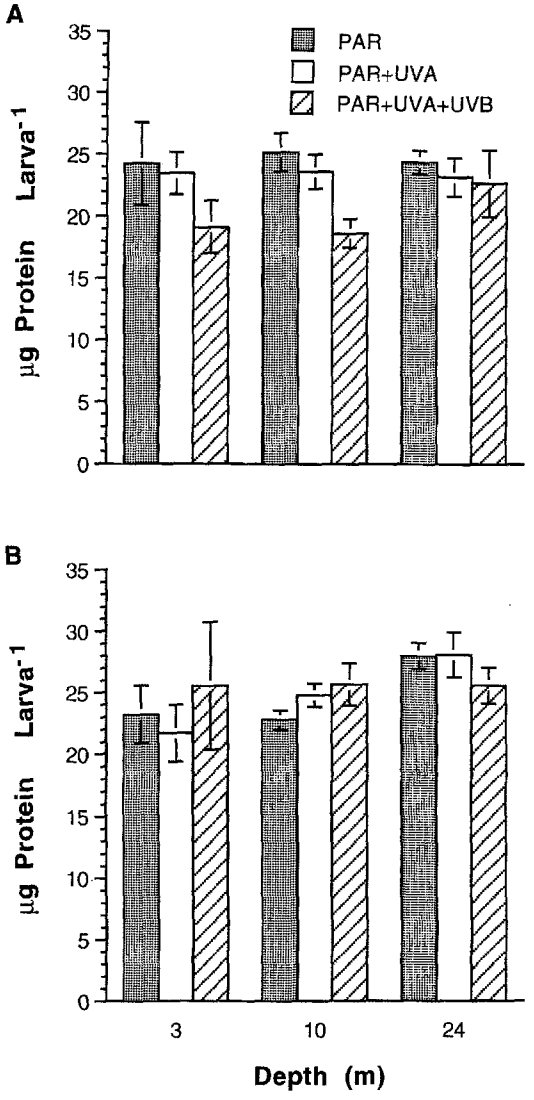

Fig. 4 *Agaricia agaricites.* Mean (± SE) concentrations of protein in planulae surviving exposure to three different light regimes at 3, 10, and 24 m depth for 72 h. Experimental details as in legend to Fig. 3. Initial sample of larvae from four randomly selected adults at both 3 and 24 m depth contained protein concentrations of 25.0 (± 1.85 SE) and 21.0 (± 3.30) µg per larva, respectively

Table 3 *Agaricia agaricites.* Results of two-way ANOVA for protein concentrations in planula larvae exposed to three different light regimes (PAR only, PAR + UVA, or PAR + UVA + UVB) at 3, 10, and 24 m depth for 72 h. Further details as in legend to Table 2

Source of variation	*df*	Mean square	*F*	*P*
3 m origin				
Depth	2	4.56	0.30	0.740
Light	2	59.40	3.97	0.031
Depth × light	4	8.06	0.54	0.708
Error	26	14.95		
24 m origin				
Depth	2	45.51	2.09	0.143
Light	2	3.00	0.14	0.872
Depth × light	4	14.38	0.66	0.624
Error	27	21.75		

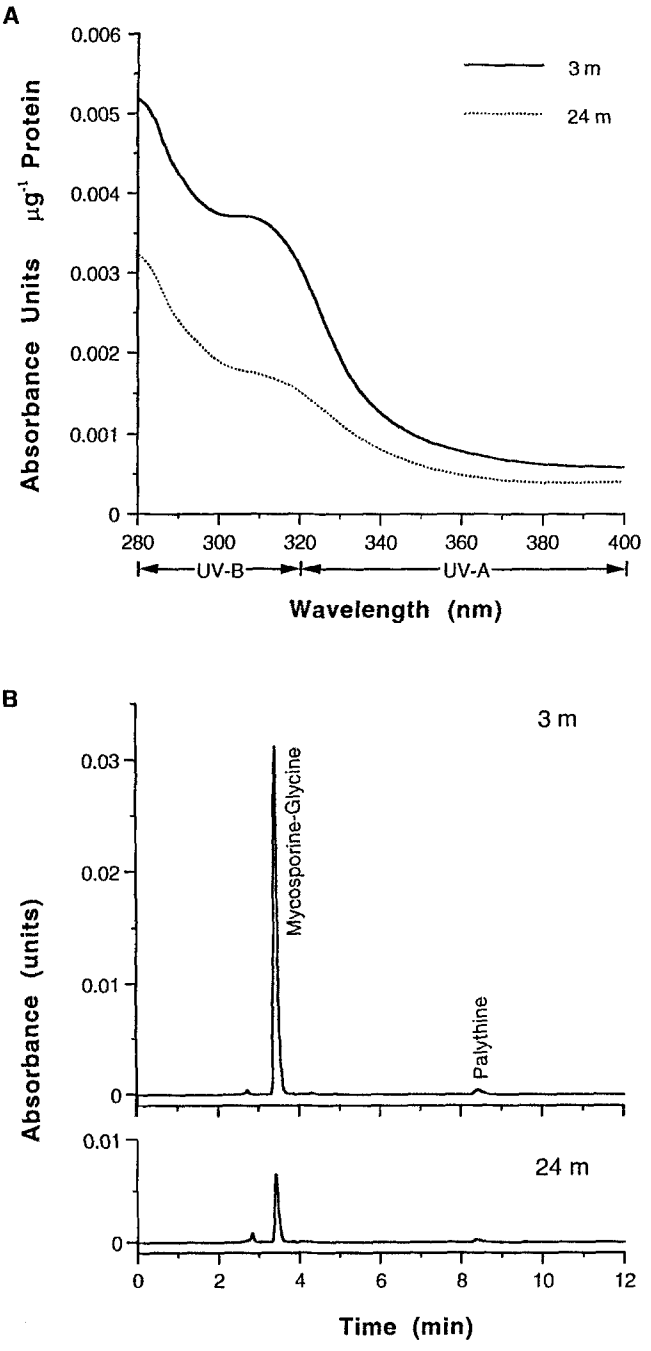

Fig. 5 *Agaricia agaricites.* Mycosporine-like amino acids (MAAs) in larvae collected from 3 and 24 m depth. **A** Spectrophotometric scans of 80% MeOH extracts of planula larval tissues; each curve represents mean of 5 larval samples. **B** Representative high-performance liquid chromatography (HPLC) chromatograms showing two mycosporine-like amino acids present in coral larvae; planulae from 3 m had significantly higher concentrations of both mycosporine-glycine and palythine

released from adult colonies with higher concentrations of MAAs.

HPLC analyses demonstrated that the shoulder observed in spectrophotometric scans resulted from the presence of two MAAs (Fig. 5B): mycosporine-glycine

Table 4 *Agaricia agaricites.* Mean (± SE) concentrations of mycosporine-like amino acids (MAAs) extracted from planula larvae. Individual samples consisted of at least 5 larvae from single adult colony extracted together. Sample size represents groups of larvae from 5 randomly selected colonies at each depth

Depth (m)	Mycosporine-like amino acids (pmol μg^{-1} protein)	
	mycosporine-glycine	palythine
3	132.04 (24.69)	1.54 (0.30)
24	43.60 (7.99)	0.65 (0.15)
Student's *t*-value	3.41	2.68
Significance	$P = 0.009$	$P = 0.03$

(λ_{max} = 310 nm) and palythine (λ_{max} = 320 nm). Extraction efficiencies for both MAAs were > 97%, and larvae from the shallower reef zone harbored significantly higher concentrations of these compounds (Table 4). Increases of 3.0- and 2.4-fold in larval mycosporine-glycine and palythine concentrations, respectively, between 24 and 3 m depth, were associated with a change in UVB intensity of ≃ 56-fold.

Discussion

Our field experiments conducted using natural sunlight showed that high intensities of both PAR and UV radiation induce changes in *Agaricia agaricites* planulae within 72 h. The former causes significant reductions in chlorophyll concentrations while the latter lowers survivorship by as much as 74%. The portion of the UV radiation spectrum that induces mortality is in the UVB range (280 to 320 nm), because high survivorship was observed in treatments where PAR alone or PAR + UVA were present. Sensitivity to UVB radiation varies with larval depth of origin and internal concentrations of MAAs. Larvae originating from 3 m depth exhibited significantly higher survivorship when exposed to PAR + UVA + UVB than larvae from 24 m, and also displayed higher concentrations of both mycosporine-glycine and palythine. Combined, these data indicate that no single-action spectrum or solar-effectiveness spectrum may be general enough, even within a single species, to predict the response of coral larvae to UVB radiation.

Survivorship, chlorophyll concentrations and protein levels

In addition to the low survivorship observed in PAR + UVA + UVB treatments at 3 m depth, survivorship was slightly reduced in all light treatments at 24 m depth containing larvae originating from 3 m (Fig. 2A). Since larval absence was also categorized as mortality, these reduced numbers could have been a consequence of either death resulting from inability of zooxanthellae to cope with the lower PAR levels present at 24 m depth, or higher than average escape rates from around the edges of the Nitex mesh used to seal the ends of the silica tubes. Two pieces of information strongly implicate larval escape as the explanation for these lower than average larval numbers. First, we tested for effects of low PAR by enclosing silica tubes containing 12 larvae each in white polyvinyl chloride (PVC) pipe and deploying these tubes at 24 m depth for 72 h. Even though the PVC was opaque to both UV radiation and PAR, survivorship of larvae was high (mean = 11.0 ± 1.0 SE, $n = 4$ silica tubes). Second, chlorophyll concentrations were similar between larvae from 3 and 24 m depth placed at 24 m, indicating that photopigments in both sets of larvae may have acclimated to the lower PAR intensities.

The effects that PAR had on larval chlorophyll concentrations is interesting when considered in the context of studies linking coral bleaching to UV radiation. Whereas we have previously shown that adult colonies of *Montastrea annularis* bleach in response to increased intensities of UV radiation (Gleason and Wellington 1993), the present study demonstrated that reductions in chlorophyll concentrations in *Agaricia agaricites* planulae occur under high levels of PAR (Fig. 3). Whether or not these chlorophyll reductions represented decreases in the number of zooxanthellae per larva or lower concentrations of chlorophyll per zooxanthella is not known, since we were unable to make accurate counts of zooxanthellae within larvae. Given that most bleached planulae appeared otherwise healthy and exhibited mobility similar to that observed in unbleached larvae, these PAR-induced changes in chlorophyll concentrations did not seem to have a negative impact. Perhaps these changes in chlorophyll concentrations represented a photoacclimatory response to enhanced PAR similar to that observed in many adult corals (Falkowski and Dubinsky 1981; Thinh 1991).

Whether or not patterns observed for larval protein concentrations were of biological relevance is equivocal. Larvae from 3 m depth in the PAR + UVA + UVB treatment at 3 and 10 m depth had the lowest protein concentrations, indicating a UVB effect, whereas larvae from 24 m depth showed no differences across depths or treatments (Fig. 4). Presence of a UV-radiation effect is counter to previous studies that have assayed for changes in protein concentrations in coral reef anthozoans exposed to enhanced levels of UV light (Lesser et al. 1990; Gleason 1993; Gleason and Wellington 1993).

Role of MAAs in UV protection

In the PAR + UVA + UVB treatment at 3 m depth, *Agaricia agaricites* larvae originating from 3 m displayed

higher rates of survivorship than those from 24 m, even though the former were exposed to UVB doses that were 1.29-fold higher (Table 1, Fig. 2). The greater resistance to UV radiation exhibited by larvae collected from shallower water may be a product of the high concentrations of MAAs in their tissues (Table 4). Recent studies with cyanobacteria recorded a MAA sunscreen factor of 0.3 (MAAs prevented 3 out of 10 photons from hitting cytoplasmic targets), and found that cells with high concentrations of MAAs are $\simeq 25\%$ more resistant to UV radiation centered at 320 nm (Garcia-Pichel et al. 1993).

In addition to their direct sunscreening action, Dunlap and Yamamoto (personal communication) have shown that MAAs may act as antioxidants to lessen cellular damage resulting from UV-induced production of active oxygen species. The MAA these researchers found to be most effective as an antioxidant was mycosporine-glycine – the predominant MAA present in *Agaricia agaricites* larvae. Thus, MAAs may furnish *A. agaricites* larvae from shallow water with protection from biologically damaging UVB radiation through both the direct sunscreening activity of mycosporine-glycine ($\lambda_{max} = 320$ nm) and palythine ($\lambda_{max} = 320$ nm) and the antioxidant properties of mycosporine-glycine.

Comparisons with temperate studies

Intensities of UVB radiation reaching the earth's surface in equatorial regions are substantially higher than those present at greater latitudes (Baker et al. 1980). If UVB radiation has been a selective force in the evolutionary ecology of planktonic larvae and other zooplankton, it is reasonable to predict that near-surface marine zooplankton exclusive to tropical waters should be more resistant to UVB radiation than those restricted to temperate zones. Comparisons between studies require that both total dose and dose rate be considered because reciprocity (i.e., effects as a function of dose, regardless of dose rate) does not always apply in investigations of UVB radiation effects on biological systems (Damkaer et al. 1981; Hunter et al. 1981; Cullen and Lesser 1991).

Comparison of our results with those conducted on zooplankton at higher latitudes is complicated by the fact that investigations in temperate waters have computed UVB doses in the range from 290 to 320 nm (Hunter et al. 1981; Karanas et al. 1981) or 285 to 315 (Damkaer et al. 1981), whereas we were limited to wavebands between 300 and 320 nm. At wavelengths below 300 nm, the amount of solar radiation reaching the earth at northern tropical latitudes decreases rapidly while the attenuation coefficients for these wavelengths in water increase sharply (Smith and Baker 1979). As such, having a lower bound of 300 nm did not appear to result in gross underestimates of UVB doses in our experiments. Extrapolating our UV-light measurements below 300 nm showed that even at 3 m depth these wavelengths probably contributed much less than 1% to both the integrated DNA-weighted daily dose and the maximum dose rate.

Even though our UV light estimates were mildly conservative, DNA-weighted daily doses at 3 m depth (Fig. 1) were nearly double those needed to induce mortality of temperate populations of the copepod *Acartia clausii* after only 1 d of exposure (Karanas et al. 1981). Furthermore, total UVB doses causing mortality of *Agaricia agaricites* larvae at 3 m depth over 72 h (143.5 $J\,m^{-2}$, Fig. 1) far exceeded the 85 $J\,m^{-2}$ threshold required for mortality (LD_{50}) in temperate populations of shrimp larvae (Damkaer et al. 1981). In contrast, daily UVB intensities causing 40 and 75% mortality of *A. agaricites* larvae from 3 and 24 m depth, respectively, over 3 d, would have to be sustained for roughly 12 d to reach the LD_{50} for northern anchovy (Hunter et al. 1981). Thus, in terms of total dose, planula larvae of *A. agaricites* appear to be less affected by high intensities of UVB radiation than several species of temperate zooplankton, but are equally sensitive, or more so, than larvae of at least one temperate fish species.

Investigations conducted on temperate zooplankton have usually used artificial light sources to enhance UVB intensities (Hunter et al. 1979; Karanas et al. 1979; Damkaer et al. 1980, 1981; Karanas et al. 1981). Using artificial lights has led to unrealistic dose rates, because neither diurnal variation in UV intensities resulting from changes in sun angle nor the solar spectrum is easily mimicked. As such, direct comparisons between our results under natural solar energy and those of earlier studies are problematic. For example, in three species of temperate shrimp larvae, Damkaer et al. (1981) determined that a DNA-weighted dose rate of at least 0.0020 $W\,m^{-2}$ sustained for 3 h per day is necessary for UVB-induced damage to outpace biological repair mechanisms. While the maximum DNA-weighted dose rate we observed at 3 m depth (0.0027 $W\,m^{-2}$) exceeded this value, *Agaricia agaricites* larvae received this dose for only 1 h per day when the sun was at its zenith. This maximum was bounded on either side by irradiation at lower dose rates, as the sun followed its normal daily track across the sky. How temperate shrimp larvae would be affected by such a light environment is unclear. More studies conducted under natural UV and PAR light conditions are needed before valid comparisons of dose-rate thresholds can be made for temperate and tropical zooplankton.

Implications for larval mortality in the plankton

While our results show that intensities of UV radiation occurring as deep as 3 m below the ocean surface on coral reefs can cause mortality of *Agaricia agaricites* larvae, caution should be exercised in extrapolating

them to measures of population-level larval mortality. Several mechanisms acting alone or in concert under natural conditions may mitigate UV-induced mortality.

First, *Agaricia agaricites* planulae may settle cryptically at night within the first few hours after release. This strategy would enable larvae to avoid damage resulting from exposure to near-surface levels of UVB radiation. Data we and other researchers have collected on *A. agaricites* indicate that the planktonic phase is on the order of 3 to 4 d and are not consistent with this hypothesis (Carlon and Olson 1993).

Second, *Agaricia agaricites* larvae may be able to sense high intensities of UV radiation and actively avoid UVB exposure while in the water column. Both avoidance of UV radiation stress and predation have been suggested as evolutionary explanations for diel vertical migrations in zooplankton and planktonic echinoderm larvae (Damkaer 1982; Pennington and Emlet 1986). If ciliated planula larvae of *A. agaricites* possess photoreceptors that are operative in the UVB and can adequately regulate their depth within the water column, highly actinic UV radiation might be avoided. Alternatively, larvae of this species may evade high UVB intensities if they possess PAR photoreceptors. In general, UVB intensities are positively correlated with PAR, and negative phototaxis in response to high levels of PAR would also result in avoidance of UVB radiation. To date, evidence for diel vertical migrations in planulae of reef-building corals has only been obtained for *Pocillopora damicornis* in Hawaii (Hodgson 1985). While Hodgson's study indicates the potential for planulae to undergo diel vertical migrations, investigations using other species are needed before the generality of this behavior can be suggested.

Third, even if the above two mechanisms are not operating, vertical mixing of the water column could potentially reduce exposure of planktonic organisms to UVB radiation. Mixing may be particularly relevant if *Agaricia agaricites* larvae lack, or only have very minimal, ability to regulate their depth within the water column. Mathematical treatments of this concept based on passive suspended particles indicate that vertical mixing can change the exposure of planktonic organisms to UVB radiation by several orders of magnitude (Smith and Baker 1982) – similar to the differences in UVB intensities we observed between 3 and 24 m depth (Table 1). Furthermore, recent empirical studies in the Florida Keys indicate that interacting oceanic currents trap passive planktonic larvae between the shallow coastal boundary and stable thermocline, and that most zooplankton occur between 10 and 50 m depth (Lee et al. 1992). While this earlier study did not consider abundances of coral planulae, it is interesting to note that the shallowest portion of this depth range is below that where UVB-induced mortality of *A. agaricites* larvae occurred.

Conclusions

Many studies in temperate environments have shown that zooplankton and drifting eggs and larvae of marine fish can be irreversibly damaged or killed by high levels of UVB radiation (see Worrest 1982; Worrest and Häder 1989 for reviews). Our studies with larvae of the tropical reef-building coral *Agaricia agaricites* corroborate and strengthen the ecological and evolutionary implications of these earlier findings by showing that propagules from a species that has evolved in a marine environment constantly exposed to high intensities of UV radiation are susceptible to its effects.

If *Agaricia agaricites* larvae are able to detect and actively avoid high intensities of UVB radiation, then our data indicate that larvae spawned from colonies in both shallow and deeper reef areas probably disperse at depths > 3 m during the day and avoid settling in open habitats in shallow waters. To overcome the constraints imposed on settlement by UVB radiation, we hypothesize that adult colonies of *A. agaricites* in shallow zones became established there by initially settling in cryptic habitats and then growing out of these protected areas as concentrations of MAAs within tissues and other photoprotective mechanisms developed. Support for this hypothesis has been provided by a study showing that *A. agaricites* larval recruits progressively shift their settlement orientation from heavily-shaded horizontal undersurfaces to more light-exposed vertical surfaces with increasing depth (Rogers et al. 1984). Now that we have shown that coral larvae can be negatively affected by natural intensities of UVB radiation, assessments of planula distributions in the plankton, along with determinations of the relative limits predation versus UV susceptibility places on settlement patterns, are needed to fully understand the role that UVB radiation plays in coral recruitment.

Acknowledgements This study benefitted from the able field assistance of A. Hulbert, N. Hulbert, T. Potts, J. Schmerfeld, J. Villinski, and D. Ward. MAA standards were graciously provided by W. Dunlap. Many thanks are due to S. Miller and the staff of the National Undersea Research Center's Florida Keys Program for providing exceptional logistical support. For valuable comments on an earlier version of this manuscript we thank L. Gutierrez, M. Hill, J. Villinski, T. Wilcox and two anonymous reviewers. Financial support was furnished by the National Science Foundation (OCE 9301750) and National Oceanic and Atmospheric Administration's National Undersea Research Center at the University of North Carolina at Wilmington (UNCW 9226).

References

Baker K, Smith RC, Green AES (1980) Middle ultraviolet reaching the ocean surface. Photochem Photobiol 32: 367–374

Behrenfeld M, Hardy J, Gucinski H, Hanneman A, Lee II H, Wones A (1993) Effects of ultraviolet-B radiation on primary production along latitudinal transects in the South Pacific ocean. Mar envirl Res 35: 349–363

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt Biochem 72: 248–254

Carlon DB, Olson RR (1993) Larval dispersal distance as an explanation for adult spatial pattern in two Caribbean reef corals. J exp mar Biol Ecol 173: 247–263

Cullen JJ, Lesser MP (1991) Inhibition of photosynthesis by ultraviolet radiation as a function of dose and dosage rate: results for a marine diatom. Mar Biol 111: 183–190

Damkaer DM (1982) Possible influences of solar UV radiation in the evolution of marine zooplankton. In: Calkins J (ed) The role of solar ultraviolet in marine ecosystems. Plenum Press, New York, pp 701–706

Damkaer DM, Dey DB, Heron GA (1981) Dose/dose rate responses of shrimp larvae to UV-B radiation. Oecologia 48: 178–182

Damkaer DM, Dey DB, Heron GA, Prentice EF (1980) Effects of UV-B radiation on near-surface zooplankton of Puget Sound. Oecologia 44: 149–158

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

Dunlap WC, Chalker BE, Oliver JK (1986) Bathymetric adaptations of reef-building corals at Davies Reef, Great Barrier Reef, Australia. 3. UV-B absorbing compounds. J exp mar Biol Ecol 104: 239–248

Dunlap WC, Williams DM, Chalker BE, Banaszak AT (1989) Biochemical photoadaptation in vision: UV-absorbing pigments in fish eye tissues. Comp Biochem Physiol 93B: 601–607

Falkowski PG, Dubinsky Z (1981) Light-shade adaptation of *Stylophora pistillata*, a hermatypic coral from the Gulf of Eilat. Nature, Lond 289: 172–174

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

Garcia-Pichel F, Wingard CE, Castenholz RW (1993) Evidence regarding the UV sunscreen role of a mycosporine-like compound in the cyanobacterium *Gleocapsa* sp. Appl envirl Microbiol 59: 170–176

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

Gleason DF, Wellington GM (1993) Ultraviolet radiation and coral bleaching. Nature, Lond 365: 836–838

Goenaga C, Vicente VP, Armstrong RA (1989) Bleaching induced mortalities in reef corals from La Parguera, Puerto Rico: a precursor of change in the community structure of coral reefs. Caribb J Sci 25: 59–65

Green AES, Miller JH (1975) Measures of biologically effective radiation in the 280–340 nm region. In: Impacts of climatic change on the biosphere. US Department of Transportation, Washington, DC (CIAP Monogr No. 5)

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky, Z (ed) Ecosystems of the world. No. 25. Elsevier, New York, pp 133–207

Helbling EW, Villafañe V, Ferrario M, Holm-Hansen O (1992) Impact of natural ultraviolet radiation on rates of photosynthesis and on specific marine phytoplankton species. Mar Ecol Prog Ser 80: 89–100

Hodgson G (1985) Abundance and distribution of planktonic coral larvae in Kaneohe Bay, Oahu, Hawaii. Mar Ecol Prog Ser 26: 61–71

Hunter JR, Kaupp SE, Taylor JH (1981) Effects of solar and artificial ultraviolet-B radiation on larval northern anchovy, *Engraulis mordax*. Photochem Photobiol 34: 477–486

Hunter JR, Taylor JH, Moser HG (1979) Effect of ultraviolet irradiation on eggs and larvae of the northern anchovy, *Engraulis mordax*, and the Pacific mackerel, *Scomber japonicus*, during the embryonic stage. Photochem Photobiol 29: 325–338

Ito S, Hirata Y (1977) Isolation and structure of a mycosporine from the zoanthid *Palythoa tuberculosa*. Tetrahedron Lett 28: 2429–2430

Jeffrey SW, Humphrey G (1975) New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pfl 167: 191–194

Jerlov NG (1968) Optical oceanography. Elsevier, New York

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

Jokiel PL, York RH (1984) Importance of ultraviolet radiation in photoinhibition of microalgal growth. Limnol Oceanogr 29: 192–199

Karanas JJ, Van Dyke H, Worrest RC (1979) Midultraviolet (UV-B) sensitivity of *Acartia clausii* Giesbrecht (Copepoda). Limnol Oceanogr 24: 1104–1116

Karanas JJ, Worrest RC, Van Dyke H (1981) Impact of UV-B radiation on the fecundity of the copepod *Acartia clausii*. Mar Biol 65: 125–133

Karentz D, McEuen FS, Land MC, Dunlap WC (1991) Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. Mar Biol 108: 157–166

Larkum AWD, Wood WF (1993) The effect of UV-B radiation on photosynthesis and respiration of phytoplankton, benthic macroalgae and seagrasses. Photo Res 36: 17–23

Lee TN, Rooth C, Williams E, McGowan M, Szmant AF, Clarke ME (1992) Influence of Florida Current, gyres and wind-driven circulation on transport of larvae and recruitment in the Florida Keys coral reefs. Contin Shelf Res 12: 971–1002

Lesser MP, Shick JM (1989) Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of *Aiptasia pallida*: primary production, photoinhibition, and enzymic defenses against oxygen toxicity. Mar Biol 102: 243–255

Lesser MP, Stochaj WR, Tapley DW, Schick JM (1990) Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. Coral Reefs 8: 225–232

Pennington JT, Emlet RB (1986) Ontogenetic and diel vertical migration of planktonic echinoid larva, *Dendraster excentricus* (Eschscholtz): occurrence, causes, and probable consequences. J exp mar Biol Ecol 104: 69–95

Richmond RH (1982) Energetic considerations in the dispersal of *Pocillopora damicornis* (Linnaeus) planulae. Proc 4th int coral Reef Symp 2: 153–156 [Gomez ED et al. (eds) Antenne Museum-EPHE, Moorea, French Polynesia]

Richmond RH (1987) Energetics, competency, and long-distance dispersal of planula larvae of the coral *Pocillopora damicornis*. Mar Biol 93: 527–533

Rogers CS, Fitz III HC, 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

Setlow RB (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. Proc natn Acad Sci USA 71: 3363–3366

Siebeck O (1988) Experimental investigation of UV tolerance in hermatypic corals (Scleractinia). Mar Ecol Prog Ser 43: 95–103

Simpson CJ, Cary JL, Masini RJ (1993) Destruction of corals and other reef animals by coral spawn slicks on Ningaloo Reef, Western Australia. Coral Reefs 12: 185–191

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

Smith RC, Baker KS (1982) Assessment of the influence of enhanced UV-B on marine primary productivity. In: Calkins, J (ed) The role of solar ultraviolet in marine ecosystems. Plenum Press, New York, pp 509–537

Takano S, Uemura D, Hirata Y (1978) Isolation and structure of a new amino acid, palythine, from the zoanthid *Palythoa tuberculosa*. Tetrahedron Lett 26: 2299–2300

Thinh LV (1991) Photo-adaptation in two species of *Acropora* grown under controlled conditions. Photosynthetica 25: 365–371

Wilkinson L (1990) SYSTAT: the system for statistics. SYSTAT, Inc., Evanston, Illinois, USA

Wood WF (1987) Effect of solar ultra-violet radiation on the kelp *Ecklonia radiata*. Mar Biol 96: 143–150

Worrest RC (1982) Review of literature concerning the impact of UV-B radiation upon marine organisms. In: Calkins J (ed) The role of solar ultraviolet in marine ecosystems. Plenum Press, New York, pp 429–457

Worrest RC, Häder DP (1989) Effects of stratospheric ozone depletion on marine organisms. Envir Conserv 16: 261–263