The Journal of Experimental Biology 208, 843-848
Published by The Company of Biologists 2005
doi:10.1242/jeb.01496

UV incites diverse levels of DNA breaks in different cellular compartments of a branching coral species

Rinkevich Baruch*, Nanthawan Avishai and Claudette Rabinowitz

National Institute of Oceanography, Israel Oceanographic and Limnological Research, Tel-Shikmona, PO Box 8030, Haifa 31080, Israel

*Author for correspondence (e-mail: buki@ocean.org.il)

Accepted 12 January 2005

Summary

This study evaluates *in vitro* the effects of UVB irradiation on three cellular compartments of a shallow water coral species. Coral tissues were dissociated by Ca^{2+}-Mg^{2+}-free artificial seawater. Cell suspensions were divided into the major cellular compartments (animal cells, algal cells, holobiont entities) by sucrose gradient and then by detergent treatments. Cell fractions were irradiated by UVB lamp (4.05, 8.1 and 12.2 kJ m^{-2}) and subjected to the comet assay. UVB radiation, at levels that induced a moderate DNA breakage to the non-symbiotic coral and algal cell compartments, caused dramatic increase in DNA breakage to the holobiont entities. After a 1 h repair period, DNA breakage levels in the algal and animal cell fractions were augmented as compared with a reduction in DNA breakage in the holobiont fraction. This discordancy in DNA breakage between the three cellular compartments reveals that the holobiont cell fraction is more vulnerable to increased natural UV irradiation and associated anthropogenic genotoxic impacts, providing another possible explanation for recent increase in worldwide coral bleaching events.

Key words: comet assay, coral, DNA breakage, DNA repair, free radicals, UV radiation.

Introduction

The predominant type of DNA damage caused by exposure to UVB radiation is the formation of dimers between adjacent pyrimidines (cyclobutane pyrimidine dimers and pyrimidine-pyrimidone 6–4 photoproducts) that are removed by the versatile nucleotide excision repair mechanism (Mullenders and Berneburg, 2001). In addition, UV can induce oxidative DNA-base damage in the form of 7,8-dihydro-8-oxoguanine, independently of absorption of radiation in DNA itself (Kvam and Tyrrell, 1997). Without repair, these DNA lesions are lethal to cells because they deform the DNA helix, thereby interfering with replication and transcription.

Solar radiation (including ultraviolet radiation, UVR, at 280–400 nm wave length) alone, or in combination with other factors, such as increased seawater temperature, has been cited as a major cause of coral bleaching in field and laboratory manipulations (Siebeck, 1988; Glynn, 1993; Shick et al., 1996; Brown, 1997; Hoegh-Guldberg, 1999; Anderson et al., 2001; Wissmann, 2003; Lesser and Farrell, 2004). However, attempts to correlate coral bleaching with specific wave bands (UVR *vs* photosynthetically active radiation, PAR, at 400–700 nm) proved to be insubstantial. For example, Gleason and Wellington (1993) reported results from field experiments, showing that, irrespective of the water temperature, the between-depths coral transplants bleached in response to UVR increase. However, their results have since been questioned (Dunne, 1994) because their experimental design had not taken into account the slight differences in PAR between treatments, nor the possibility of an interactive effect between PAR and UVR. Considerable numbers of investigations were further directed towards the ecological and physiological consequences of solar irradiation on coral reef photoautotrophic and other epifaunal organisms (Jokiel, 1980; Glynn, 1993; Brown et al., 1995; Fitt and Warner, 1995; Le Tissier and Brown, 1996; Shick et al., 1996; Brown, 1997; Hoegh-Guldberg, 1999; Warner et al., 1999; Lesser, 2000; Lesser and Farrell, 2004). Therefore, while a fair amount is known about the cellular processes that lead to loss of algal cells from coral tissue during bleaching (Lesser et al., 1990; Gates et al., 1992; Brown et al., 1995; Le Tissier and Brown, 1996; Warner et al., 1999; Sawyer and Muscatine, 2001; and literature therein), very little is known about the impacts of UVB radiation on the DNA level in hermatypic corals and the possible alignment of elevated DNA damages with coral bleaching. Only a single study observed, in solar simulation, the increase of thymine dimers in *Porites* colonies exposed to artificial solar irradiance (Anderson et al., 2001) and another study (Lesser and Farrell, 2004) has evaluated the formation of cyclobutane pyrimidine dimmers following exposure of coral colonies to UVR.

To determine the potential genotoxic impact of UVB radiation on coral–algal symbiosis, we evaluated by *in vitro* experiments the DNA damage inflicted on the three major

cellular compartments of this association, on the non-symbiotic fractions of coral and algal cells and the symbion-entity (the holobiont, the *in hospite* unit). The coral species used in this study is the Indo-Pacific, shallow water branching form, *Stylophora pistillata*. UV-induced DNA damage and DNA repair levels were evaluated by the single cell gel electrophoresis assay, also known as the comet assay, one of the most reliable and sensitive methods for evaluating DNA damage induced in individual cells by various agents (Mitchelmore and Chipman, 1998; Avishai et al., 2003; Reinhardt et al., 2003).

Materials and methods

Cell preparation

Small *Stylophora pistillata* (Esper 1797) colonies were collected from shallow water (<10 m) coral reefs at Eilat (Red Sea, Israel) and transported in insulated containers to the laboratory where they were held for several months in a temperature controlled (25°C) flowing seawater system, illuminated by the combination of three different fluorescent tubes: Cool White, Floura and Blue-Blue (Osram, Germany; 12 h:12 h L:D regimen). Branch samples were first rinsed three times (20 min each) in sterile seawater (SSW; 0.22 μm, supplemented with 0.1% gentamycin from stock solution at 50 mg ml^{-1}), and then placed for 10 min (room temperature) in 6 cm Petri dished containing Ca^{2+}-Mg^{2+}-free artificial seawater (ASW) with ethylene diamine tetracetic acid (EDTA). This solution was prepared as the following: Na_2HSO_4 7 mmol l^{-1}, $NaHCO_3$ 0.2 mmol l^{-1}, Tris HCl 20 mmol l^{-1}, KCl 10 mmol l^{-1}, NaCl 540 mmol l^{-1}, EDTA 20 mmol l^{-1}. pH was corrected to 8.2. Cell dissociation was achieved by aspirating Ca^{2+}-Mg^{2+}-free ASW+EDTA throughout the branch. Cell suspensions were collected every 5 min, diluted with sterile seawater and centrifuged twice (550 and 2400 ***g***, 10 min each, at room temperature). Pellets were resuspended in SSW and cells were then separated on a density sucrose gradient in 15 ml centrifuge tubes (N=4). Three sucrose solutions (2.5, 15 and 60%) were prepared with SSW. Cell suspensions were loaded onto the surface of the gradient (10^7 cells in 2 ml SSW). The tubes were then centrifuged (1250 ***g*** for 10 min at 20°C) and two fractions were collected according to their density: (a) 3.75 ml from the top, a fraction that contained mainly coral cells and only few algal cells; and (b) 6.0 ml from the top of the remaining gradient, that contained mainly free algal cells, holobionts and few host cells. Each fraction was rinsed (×3) in SSW to remove all sucrose traces [viability >90% using trypan blue exclusion test and the viability probe 5-(and-6) carboxyfluorescein diacetate 5(6)-CFDA]. Fraction 'b' was equally divided into two tubes. One was then re-suspended with 0.1% Triton X-100 in SSW, incubated for 5 min and then washed (×3) in SSW at 3100 ***g***. This procedure provided a pellet of intact algal cells and digested host cells in the supernatant that was discarded (Tom et al., 1999).

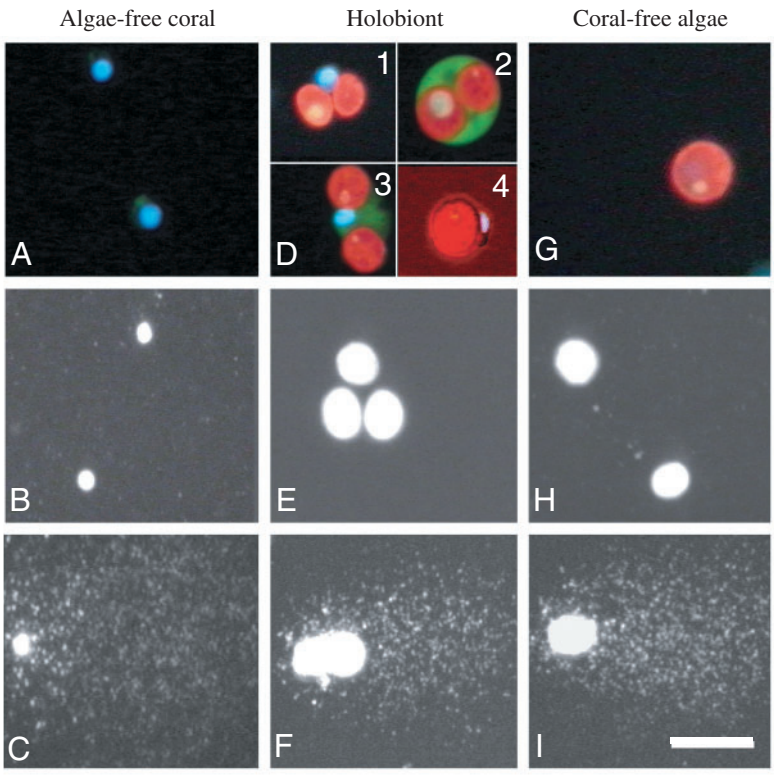

Fig. 1. Fluorescence emitted by algal-free coral nuclei (A–C), in hospite nuclei and cells (D–F) and coral-free algal nuclei and cells (G–I). A, D, G, DAPI (blue) for nuclei, chlorophyll auto fluorescent (red) for algal cells and 6-CFDA (6-carboxylfluorescein diacetate; green) for animal cells' cytoplasm. The holobiont state reveal coral cells with a single algal cell (D, no. 4), two algal cells in a single unit (D, numbers 1–3); three algal cells in a single unit (E) and sometimes more (C.R., unpublished). B, E, H ethidium bromide fluorescent intact nuclei; C, F, I comets, stained by ethidium bromide. DAPI was observed with Olympus filters U-MNU (excitation 360–370 nm); 6-CFDA labeling was observed with Olympus filter U-MW1B (excitation 460–490 nm). Scale bar (fitted all figures A–I, 10 μm).

UV irradiation

In each experiment, cells were divided into three 24-well plates (TPP, Trasadinger, Switzerland), suspended in culture medium made of 2.5% Dulbecco modified Eagle medium (DMEM) in 2× artificial seawater supplemented with 3% heat inactivated fetal calf serum (HI-FCS), 0.01 mol l^{-1} HEPES, 2 mmol l^{-1} glutamine and 1% of antibiotic cocktail (10 U ml^{-1} penicillin G, 10 mg ml^{-1} streptomycin, 25 μg ml^{-1} amphotericin B). 2×ASW was prepared as the following: NaCl 13.67 g, KCl 0.412 g, $CaCl_2$ $2H_2O$ 0.721 g, $MgSO_4$ $7H_2O$ 5.57 g and $MgCl_2$ $6H_2O$ 3.05 g were added to 250 ml tissue culture grade water. Cultures were incubated overnight (20 h) in the dark in a humidified incubator (5% CO_2), at 20°C. Irradiation was performed in the dark by a UVB lamp (VL-6M, 16 W tube, a peak wavelength at 312 nm, power 12 W; Vilber Lourmat, Marne La Vallée, France) for 15, 30 and 45 min, respectively

(equivalent to 4.05, 8.1 and 12.2 kJ m^{-2}; irradiation was measured by a sensor, radiometer CUV3; Kipp and Zonen, Delft, Holland). The spectral power distribution of the broadband lamp was determined by measuring the integrated spectral irradiance, since the sensor used does not record total lamp output. Lamp/specimen geometry was kept identical in all experiments. After irradiation, cells were collected by centrifugation (2400 ***g***) at room temperature and loaded onto glass slides for the comet assay. Repair experiments were left in the dark for 1 h after irradiation.

The comet assay

In this assay, 10 µl of cell suspensions ($2–10\times10^5$ cells) were embedded in 90 µl of 0.65% low-melting agarose (Amresco, Solon, OH, USA) layered on a Star-frost microscope slide, pre-coated with 0.65% normal melting agarose. After 10 min of solidification on ice, a third layer containing 120 µl of 0.65% low-melting agarose was placed on top and left on ice for an additional 10–15 min until solidification. The cells were then lysed by immersing the slides for 1 h in a freshly prepared lysis solution (2.5 mol l^{-1} NaCl, 100 mmol l^{-1} EDTA, 10 mmol l^{-1} Tris, 1% Triton X-100, 10% DMSO, pH 10.0) at 4°C. After lysis, the slides were washed three times (5 min each) in cold double distilled water and placed, for 20 min, in a horizontal gel electrophoresis apparatus containing freshly prepared electrophoresis buffer (1.0 mmol l^{-1} EDTA, 300 mmol l^{-1} NaOH, pH 13.0) to allow DNA unwinding. Electrophoresis was done at 20 V (a starting current of 300 mA) for 20 min. Thereafter, the slides were neutralized with three washes (5 min each) of 0.4 mol l^{-1} Tris, pH 7.5, dehydrated with ethanol, dried, stained with 65 µl of 20 µg ml^{-1} ethidium bromide solution and viewed under a fluorescent microscope using a U-MNG filter (Olympus, Hamburg, Germany). All steps were performed in the dark to prevent additional DNA damage. The analysis was done on 400× magnification images. The cell images were projected onto a high resolution Heper-HAD™ (Sony, Tokyo, Japan) CCD camera [8 bits (Applitec, Holon, Israel; LIS-700)] and analyzed with Viscomet image analysis software using the MV Delta frame grabber (Matrix Vision, Oppenweiler, Germany). DNA damage in each treatment was measured in duplicates of 50 cells each, using two highly informative parameters (Avishai et al., 2003): tail extent (sum of all distances of each horizontal scan line from the first signal pixel to the last signal pixel divided by number of scan lines) and tail extent moment (tail length × percentage tail DNA). Controls (algal-free cells, animal cells, holobionts) were handled as experimental cells, but without UVR treatment.

Results

Three major cellular compartments of the symbiotic association, host (animal) cells, host-free algal cells and holobiont (symbion-entities, *in hospite* state) were exposed to three doses of UVB at 4.05, 8.1 and 12.2 kJ m^{-2}. Four different experiments (in duplicates) were conducted on cells obtained from two *S. pistillata* colonies. Each experiment was performed separately. When examining undamaged nuclei (Fig. 1A,B,D,E,G,H) stained with ethidium bromide (Fig. 1B,E,H), *S. pistillata* nuclei (Fig. 1A,B) were clearly distinct by being smaller (1.8–2.5 µm, *N*=1600) than algal nuclei (Fig. 1G,H; 4.8–7.1 µm; *N*=1600) and holobiont-entities (Fig. 1D,E; 4.9–7.2 µm; *N*=1600), in which the chlorophyll fluorescence contributed to the readings (Fig. 1E,F,H,I). Comets developed in all three subsets of animal–algal cells (Fig. 1C,F,I). However, when examining comets produced by the holobiont-entities (Fig. 1F), it was impossible to distinguish between the contribution of the two partners, and these comets might have been produced by either the holobiont-entity or by the DNA of a single partner. Since algal cells occur exclusively within the endodermal cells, it is also possible that the algal-free cell fractions are predominantly ectodermal cells and the results obtained may reveal their

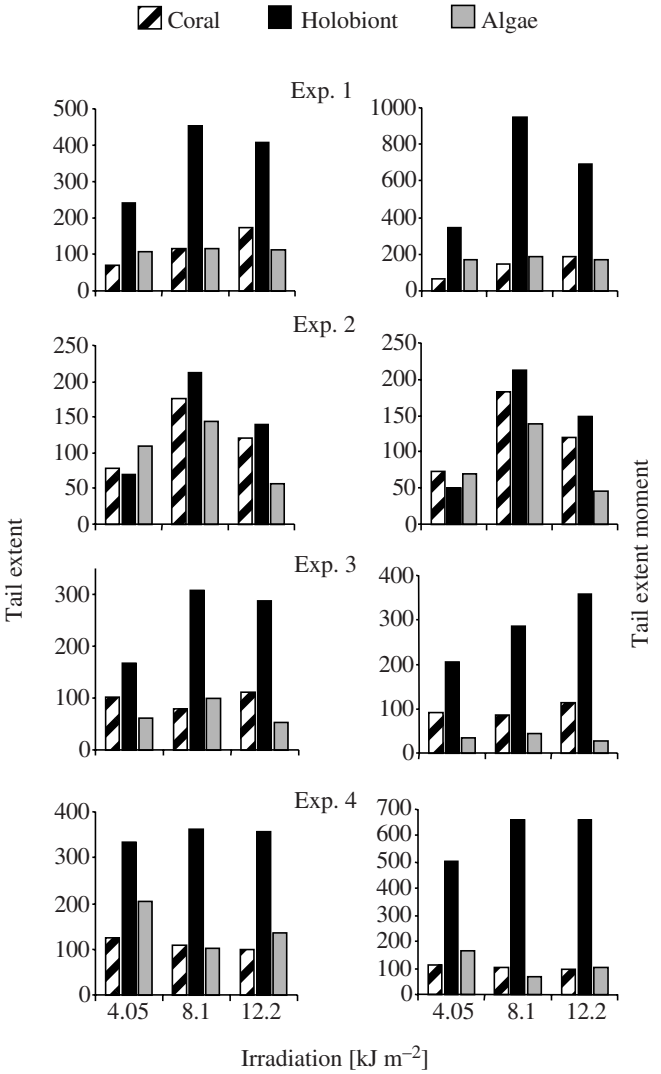

Fig. 2. UV irradiated coral, algal and holobiont cell fractions. Percentage of increased genotoxicity as compared with the corresponding controls. Responses to three different doses are depicted for the parameters tail extent and tail extent moment.

specific sensitivity to UVR as compared with the cells from the endodermal layer.

Analysis of parameters studied (Fig. 2 depicts values for tail extent and tail extent moment) revealed clearly that under the three UVB doses used, the holobiont-entities of the tested colonies were significantly more sensitive to the irradiation than the other two cell types ($P<0.05$, using Duncan test; SPSS 10.0 for windows). Thus, not only was an increased DNA breakage distinct to this cell compartment (i.e. comet assay measurement of tail extent moment values, up to 5.0, 9.5 and 6.9 times higher than controls and for tail extent analyses up to 3.4, 4.6 and 4.1 times higher values than controls in the 4.05, 8.1 and 12.2 kJ m^{-2} UVB doses, respectively; Fig. 2), but also DNA breakage levels in the holobiont fractions exceeded the levels observed for the other two compartments, the animal cells and the animal-free algal cell fractions, in most cases ($P<0.05$, Duncan test; up to 5.9, 9.9 and 13.4 times higher for 4.05, 8.1 and 12.2 kJ m^{-2} doses, respectively; Table 1). Results of the lowest dose, 4.05 kJ m^{-2} in experiment no. 2, were different. The holobiont entity is, therefore, more sensitive to the genotoxic impacts of UVB irradiation than the other cell compartments, the algal-free animal cells and algal cells.

The damages observed may represent the combination of directly induced strand breaks, of alkaline-labile lesions and endonucleolythic incisions at the sites of base damage (mainly pyrimidine dimmers). Thus, not only the apparent levels of induced damage but also the efficiency and speed of base and nucleotide excision repair during the irradiation (which may differ between the compartments) may affect the results. Three other sets of irradiation experiments (30 min; 8.1 kJ m^{-2}) further revealed DNA repair patterns by comparing DNA breaks levels immediately after irradiation with those recorded after 1 h (under dark conditions) repair periods, in an attempt to elucidate possible different repair patterns in the three coral cellular compartments. As before (Fig. 2), the results (Fig. 3) documented a distinctive immediate increase in DNA damage levels in the holobiont cell compartment ($P<0.05$, Duncan test; up to 2.4 and 60.0 times higher than that recorded in the animal and algal cell fractions, respectively). One hour following the irradiation, in most of the animal and the algal-free cell fractions, a presumed active nucleotide and base excision repair mechanisms were reflected by increased DNA break levels (up to 4.7 for the animal cell compartment and up to twice for the algal cell compartment as compared with levels measured immediately after irradiation; Fig. 3). Conversely, 1 h after irradiation, the holobiont fraction demonstrated, in

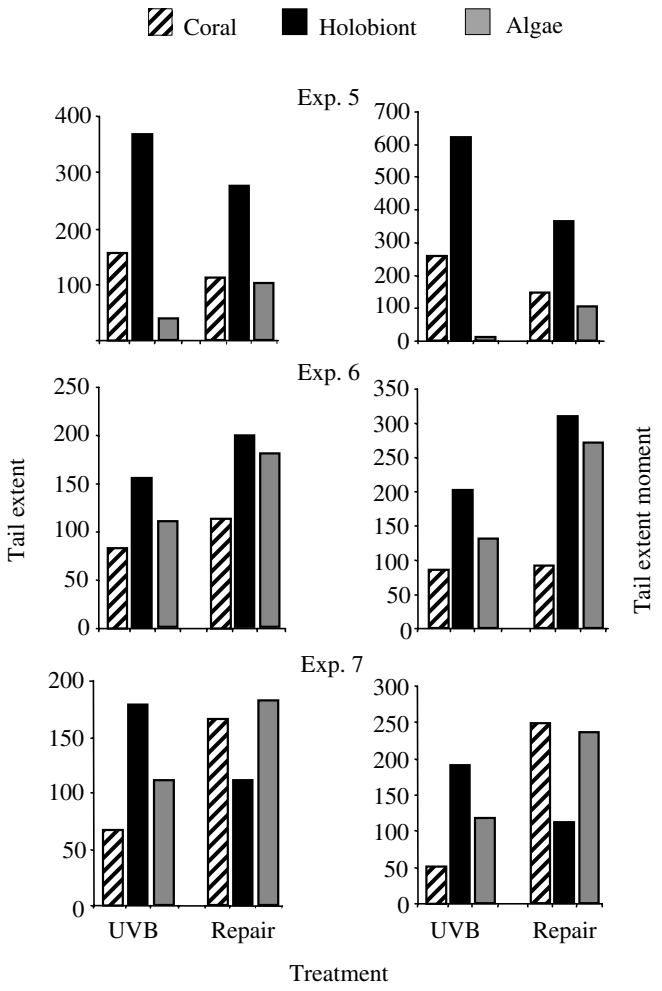

Fig. 3. UV irradiated (8.1 kJ m^{-2}) coral, algal and holobiont cell fractions: percentage of increased genotoxicity (immediately and 1 h following irradiation) as compared with the corresponding controls.

Table 1. *Fold increase to DNA breakage at the holobiont-entity as compared with the symbiotic-free host cells and host-free algal cells*

		Increased genotoxicity in the holobiont *vs* dose (kJ m^{-2})					
		4.05 kJ m^{-2}		8.1 kJ m^{-2}		12.2 kJ m^{-2}	
Experiment no.	Genotoxic parameter	Host	Free zoox.	Host	Free zoox.	Host	Free zoox.
1	Tail extent	3.38	2.18	3.93	3.97	2.33	3.56
	Tail extent moment	5.34	1.98	6.39	5.09	3.70	3.97
2	Tail extent	0.89	0.63	1.19	1.46	1.18	2.42
	Tail extent moment	0.69	0.74	1.16	1.53	1.23	3.27
3	Tail extent	1.64	2.77	3.92	3.13	2.59	5.56
	Tail extent moment	2.20	5.94	3.32	6.63	3.22	13.43
4	Tail extent	2.65	1.63	3.37	3.59	3.66	2.60
	Tail extent moment	4.38	3.06	6.46	9.90	6.85	6.25

two out of the three experiments, a reduction in DNA breakage levels as compared with the levels recorded following the irradiation (down to 50%; Fig. 3). Two additional sets of experiments (data not shown) revealed similar results, namely, a 1 h post irradiation decrease in the holobiont DNA breaks values as compared with an elevated DNA breakage in most samples of the other cell compartments. The above results may suggest, therefore, that only in the holobiont fraction a rapid process of repair develops simultaneously to irradiation, without or with only minimal lag periods. This makes this cell fraction more vulnerable to synergistic impacts UV radiation and simultaneously activated other genotoxic agents (see below), a possibility that should be evaluated by additional sets of experiments on each individual cellular compartment.

The role of UVR in bleaching seems to generate many disputes (Brown, 1997; Anderson et al., 2001; Douglas, 2003). Some studies (Helbling et al., 2001) maintain that natural levels of UVB are not sufficient to be acknowledged as the major contributor to bleaching. Consequently, the genotoxic impact of UVR, which was particularly addressed in phytoplanktonic organisms (Gieskes and Buma, 1997; Helbling et al., 2001) as a consequence of the formation of cyclobutane pyrimidine dimers (Mullenders and Berneburg, 2001), was not implicated as a notable subject for bleaching in symbiotic cnidarians. However, the discovery of the importance of solar UVR as a factor affecting the biology of coral reefs is fairly recent (Jokiel, 1980; Gleason and Wellington, 1993). Most studies concentrated on the impacts of physiological and biochemical parameters, such as coral calcification, reproduction, amounts of UV-absorbing compounds in coral tissues, body mass and photosynthesis capacities (Siebeck, 1988; Gleason and Wellington, 1993; Glynn, 1993; Dunne, 1994; Brown et al., 1995; Le Tissier and Brown, 1996; Shick et al., 1996; Brown, 1997; Hoegh-Guldberg, 1999; Westholt et al., 1999; Wissmann, 2003). Furthermore, several studies (Lesser et al., 1990; Dykens et al., 1992; Lesser, 1996; Lesser and Farrell, 2004; Takahashi et al., 2004) considered the impacts of reactive oxygen species (ROS) produced by UVR and the inherent susceptibility of symbiotic cnidarians to oxidative stress, as playing major roles in coral bleaching. It is possible, therefore, that the holobiont susceptibility to UVB radiation, as demonstrated in this study, reflects a synergistic breakage of DNA strand augmented by the formation of dimers between adjacent pyrimidines (Anderson et al., 2001), the ROS damage (Kvam and Tyrrell, 1997; Lesser and Farrell, 2004) and fast repair mechanisms. Synergism between solar radiation (that includes UVB radiation) and other environmental stressors, like temperature, may also coalesce to produce stressful conditions (Lesser et al., 1990; Glynn et al., 1992; Brown et al., 1995; Wissmann, 2003; Lesser and Farrell, 2004), including an increase in DNA damage.

This study reveals that natural levels of UVB radiation induce unalike DNA breaks in different coral cells, a phenomenon that is followed by different DNA repair rates. It is well documented that, in clear tropical seawater, UVR penetrates to ecologically important depths (Gleason and Wellington, 1993; Shick et al., 1996; Brown, 1997). Coral colonies at 1 m depth may receive up to 98% of surface UVB radiation (Gattuso et al., 1991). One may also consider changes in UVR underwater attenuation, which may be influenced by climate changes (Gleason and Wellington, 1993; Brown, 1997). It is possible that short-term increases in UVR intensity, under extremely calm (doldrums) clear water column conditions (Gleason and Wellington, 1993) may contribute to bleaching in reef corals as a result of increased DNA damage, specifically to the symbiont-entity. It is not clear yet as to what extent the expression of antioxidant enzymes, one of the defense mechanisms of symbiotic cnidarians against ROS (Richier et al., 2003), will be able to efficiently cope with this elevated DNA damage, or what would be the consequences of faster repair mechanisms. Anyhow, it is intriguing to find that the intimate coral–algal symbiotic unit is strictly hypersensitive to UVB radiation, a point that should be considered when discussing global changes and synergism between several factors (Lesser et al., 1990; Gleason and Wellington, 1993; Glynn, 1993; Brown et al., 1995; Le Tissier and Brown, 1996; Shick et al., 1996; Brown, 1997; Hoegh-Guldberg, 1999) that can jointly result in massive bleaching of coral reefs worldwide. Such consideration would help establishing the causative relationship between UVR and apparent coral bleaching events based upon mechanistic rather than on correlative information. Coral bleaching, then, is a considerably more complicated mechanism phenomenon than portrayed in earlier studies (Hoegh-Guldberg, 1999). Although there is as yet no experimented evidence to the claim that coral bleaching is associated with DNA damage, the results of the present study directly document the possible vulnerability of the holobiont entity to elevated levels of DNA damages. This new approach of evaluating DNA damages may contribute to our understanding and predicting the fate of coral reefs under different scenarios of global change.

Supported by BARD (IS-3319-02R), the US-Israel Binational Agricultural Research and Development Fund. Thanks are due to B. Laster for carefully reading the manuscript and R. P. Dunne for valuable critical remarks.

References

Anderson, S., Zepp, R., Machula, J., Santavy, D., Hansen, L. and Mueller, E. (2001). Indicators of UV exposure in corals and their relevance to global climate change and coral bleaching. *Hum. Ecol. Risk Assess.* **7**, 1271-1282.

Avishai, N., Rabinowitz, C. and Rinkevich, B. (2003). Use of the comet assay for studying environmental genotoxicity: Comparisons between visual and image analyses. *Environ. Mol. Mutagen.* **42**, 155-165.

Brown, B. E. (1997). Coral bleaching: causes and consequences. *Coral Reefs* **16** (Suppl. 1), S129-S138.

Brown, B. E., Le Tissier, M. D. A. and Bythell, J. C. (1995). Mechanisms of bleaching deduced from histological studies of reef corals sampled during a natural bleaching event. *Mar. Biol.* **122**, 655-663.

Douglas, A. E. (2003). Coral bleaching-how and why? *Mar. Pollut. Bull.* **46,** 385-392.

Dunne, R. P. (1994). Radiation and coral bleaching. *Nature* **368**, 697.

Dykens, J. A., Shick, J. M., Benoit, C., Buettner, G. R. and Winston G.

W. (1992). Oxygen radical production in the sea anemone Anthopleura elegantissima and its endosymbiotic algae. *J. Exp. Biol*. **168**, 219-241.
Fitt, W. K. and Warner, M. E. (1995). Bleaching patterns of four species of Caribbean reef corals. *Biol. Bull.* **189**, 298-307.
Gates, R. D., Baghdasarian, G. and Muscatine, L. (1992). Temperature stress causes host cell detachment in symbiotic cnidarians: implications for coral bleaching. *Biol. Bull*. **182**, 324-332.
Gattuso, J.-P., Pichon, M. and Joubert, J. (1991). Physiology and taxonomy of scleractinian corals: a case study in the genus *Stylophora*. *Coral Reefs* **9**, 173-182.
Gieskes, W. W. C. and Buma, A. G. J. (1997). UV damage to plant life in a photobiologically dynamic environment: the case of marine phytoplankton. *Plant Ecol.* **128,** 17-25.
Gleason, D. F. and Wellington, G. M. (1993). Ultraviolet radiation and coral bleaching. *Nature* **365**, 836-838.
Glynn, P. W. (1993). Coral reef bleaching: ecological perspectives. *Coral Reefs* **12**, 1-17.
Glynn, P. W., Imai, R., Sakai, K., Nakano, Y. and Yamazato, K. (1992). Experimental responses of Okinawan (Ryukyu Islands, Japan) reef corals to high sea temperature and UV radiation. *Proc. 7th Int. Coral Reef Symp*. **1**, 27-37.
Helbling, E. W., Buma, A. G. J., de Boer, M. K. and Villafañe, V. E. (2001). *In situ* impact of solar ultraviolet radiation on photosynthesis and DNA in temperate marine phytoplankton. *Mar. Ecol. Prog. Ser*. **211**, 43-44.
Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshw. Res*. **50**, 839-866.
Jokiel, P. L. (1980). Solar ultraviolet radiation and coral reef epifauna. *Science* **207**, 1069-1071.
Kvam, E. and Tyrrell, R. M. (1997). Induction of oxidative DNA base damage in human skin cells by UV and near visible radiation. *Carcinogenesis* **18**, 2379-2384.
Lesser, M. P. (1996). Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. *Limnol. Oceanogr*. **41**, 271-283.
Lesser, M. P. (2000). Depth-dependent photoacclimatization to solar ultraviolet radiation in the Caribbean coral *Montastraea faveolata*. *Mar. Ecol. Prog. Ser*. **192**, 137-151.
Lesser, M. P. and Farrell, J. H. (2004). Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. *Coral Reefs* **23**, 367-377.
Lesser, M. P., Stochaj, W. R., Tapley, D. W. and Shick, J. M. (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.
Le Tissier, M. D. A. and Brown, B. E. (1996). Dynamics of solar bleaching in the intertidal reef coral *Goniastrea aspera* at Ko Phuket, Thailand. *Mar. Ecol. Prog. Ser*. **136**, 235-244.
Mitchelmore, C. L. and Chipman, J. K. (1998). DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring. *Mut. Res*. **399**, 135-147.
Mullenders, L. H. F. and Berneburg, M. (2001). Photoimmunology and nucleotide excision repair: impact of transcription coupled and global genome excision repair**.** *J. Photochem. Photobiol. B: Biol*. **65**, 97-100.
Reinhardt, P., Cybulski, M., McNamee, J. P., McLean, J. R., Gorman, W. and Deslauriers, Y. (2003). Protection from solar simulated radiation-induced DNA damage in cultured human fibroblasts by three commercially available sunscreens. *Can. J. Physiol. Pharmacol*. **81**, 690-695.
Richier, S., Merle, P.-L., Furla, P., Pigozzi, D., Sola, F. and Allemand, D. (2003). Characterization of superoxide dismutases in anoxia- and hyperoxia-tolerant symbiotic cnidarians. *Biochim. Biophys. Acta* **1621**, 84-91.
Sawyer, S. J. and Muscatine, L. (2001). Cellular mechanisms underlying temperature-induced bleaching in the tropical sea anemone *Aiptasia pulchella*. *J. Exp. Biol*. **204**, 3443-3456.
Shick, J. M., Lesser, M. P. and Jokiel, P. L. (1996). Ultraviolet radiation and coral stress. *Global Change Biol.* **2**, 527-545.
Siebeck, O. (1988). Experimental investigation of UV tolerance in hermatypic corals (Scleractinia). *Mar. Ecol. Prog. Ser*. **43**, 95-103.
Takahashi, S., Nakamura, T., Sakamizu, M., van Woesik, R. and Yamasaki, H. (2004). Repair machinery of symbiotic photosynthesis as a primary target of heat stress for reef building corals. *Plant Cell Physiol*. **45**, 251-255.
Tom, M., Douek, J., Yankelevich, I., Bosch, T. C. G. and Rinkevich, B. (1999). Molecular characterization of the first heat shock protein 70 from a reef coral. *Biochem. Biophys. Res. Commun*. **262**, 103-108.
Warner, M. E., Fitt, W. K. and Schmidt, G. W. (1999). Damage to photosystem II in symbiotic dinoflagellates: A determinant of coral bleaching. *Proc. Natl. Acad. Sci. USA* **96**, 8007-8012.
Westholt, R., Kestler, P., Sicken, O. and Westheide, W. (1999). Influence of sublethal long-term UV-irradiation on body mass, reproduction and behaviour of north European *Actinia equina* (Anthozoa: Actiniaria). *J. Mar. Biol. Ass.* **79**, 415-424.
Wissmann, S. M. (2003). The effects of elevated ultraviolet B radiation and elevated water temperature on the loss of zooxanthellae from *Aiptasia pallida*. *Trans. Kansas Acad. Sci.* **106**, 92-98.