

## Algal Symbionts Increase DNA Damage in Coral Planulae Exposed to Sunlight

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**Badrun Nesa, Andrew H Baird, Saki Harii, Irina Yakovleva, and Michio Hidaka (2012)** Algal symbionts increase DNA damage in coral planulae exposed to sunlight. *Zoological Studies* 51(1): 12-17. To test the hypothesis that algal symbionts make coral larvae more susceptible to high photosynthetically active radiation (PAR) and ultraviolet radiation (UVR), symbiotic and non-symbiotic planulae of *Acropora tenuis* were exposed to natural sunlight (high PAR and UVR) at an ambient temperature of approximately 27°C for 4 d. DNA damage to host cells was detected using a comet assay (single-cell gel electrophoresis). Coral cells from symbiotic planulae had longer comet tails than those from non-symbiotic planulae, indicating that cells in symbiotic larvae had more DNA damage than those in non-symbiotic larvae. This result suggests that symbiotic algae are a source of oxidative stress in larvae under conditions at the ocean surface.  
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Reef-building corals contain endosymbiotic dinoflagellates (zooxanthellae) within their gastrodermal cells. Various types of stress can cause corals to lose symbionts leading to coral bleaching, which if prolonged can cause the death of a colony (Brown 1997). While sea surface temperature anomalies appear to be the main cause of mass bleaching episodes, field and laboratory studies have revealed that solar radiation also plays an important role in coral bleaching, either alone or in synergy with high temperatures (see review by Baird et al. 2009a).

Planulae of broadcast-spawning corals have an obligate planktonic duration of 2-4 d before they are competent to settle (Fadlallah 1983), and planulae of species with large eggs require up to 48 h before they become motile (Babcock and Heyward 1986). In addition, coral planulae

are positively buoyant due to the high proportion of lipids in their tissues (Harii et al. 2007). Consequently, some coral propagules may be exposed to high solar radiation at the surface of the ocean for at least a day following spawning.

Previous work demonstrated that planulae are adversely affected by high temperatures (Edmunds et al. 2001 2005). In particular, algal symbionts in larvae exposed to high temperatures appear to be a source of oxidative stress, inducing higher levels of antioxidant activity and causing lipid breakdown and increased mortality (Yakovleva et al. 2009). Similarly, ultraviolet (UV) radiation (UVR) can have a deleterious effect on coral larvae by reducing survivorship (Gleason and Wellington 1995) and rates of recruitment (Gleason et al. 2006). It is possible that algal symbionts make coral larvae more susceptible to high photosynthetically active

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radiation (PAR) and UVR. Herein, we tested the hypothesis that algal symbionts increase DNA damage in planula larvae under conditions that prevail on the ocean surface (high PAR and UVR) using a single-cell gel electrophoresis assay known as a comet assay.

## MATERIALS AND METHODS

### Collection and maintenance of coral larvae

Colonies of *Acropora tenuis* were collected from a reef at Ishigaki I., Okinawa, and transferred to the Sesoko Station of the Tropical Biosphere Research Center, Univ. of the Ryukyus, Japan prior to spawning in May 2008. The corals were maintained in running seawater prior to spawning. Four colonies spawned on 25 May 2008. After spawning, egg and sperm bundles from the 4 colonies were cultured following Babcock et al. (2003) and kept in 2-L plastic containers in 0.22- $\mu\text{m}$ -filtered seawater (FSW) at densities of 1 larva/ml. The water was replaced daily.

### Inoculation of larvae with symbionts

*Symbiodinium* cells for use in inoculating larvae were isolated from parent *A. tenuis* colonies. Coral tissue was removed from the skeleton using a WaterPik (Teledyne, WP-70J, Fort Collins, CO, USA). Tissue suspensions were filtered through a nylon mesh (180  $\mu\text{m}$ ) and then concentrated using a centrifuge at 2400 rpm for 4 min. The algal pellets were resuspended in FSW, filtered (40  $\mu\text{m}$ ), and then rinsed twice with FSW using a centrifuge at 1800-2000 rpm for 4 min. Densities of algal cells were estimated using a hemocytometer.

To infect larvae of *A. tenuis* with the homologous symbionts, 3000 larvae (8 d old) were transferred to each of 3 plastic containers containing 400 ml of 0.2- $\mu\text{m}$ -FSW with symbionts at densities of  $4.8 \times 10^4$  cells/ml and homogenized *Artemia* sp. After 4 h, 1 L of FSW was added to each of the containers to dilute the symbiont concentration, and larvae were kept in this condition overnight. The next day, larvae were washed with FSW and placed in 400 ml of 0.2- $\mu\text{m}$ -FSW. Non-symbiotic larvae were treated similarly but without inoculation. Both symbiotic and non-symbiotic larvae were maintained in the laboratory for 1 wk at room temperature (25-27°C). Water was changed on days when larvae were sampled to estimate the density of symbionts. Twelve to

17 larvae were sampled on days 1, 3, 5, and 7, and the number of larvae with symbionts and the number of symbionts per larvae were enumerated under a fluorescence microscope (Nikon, Microphot-FXA, Tokyo, Japan). The average proportion of larvae infected over this period (1-7 d after inoculation) was  $96\% \pm 2.0\%$  (mean  $\pm$  standard error,  $n = 4$ ), and the average number of symbionts per larva on day 7 was  $56.3 \pm 10.3$  ( $n = 11$ ).

### Exposure to solar radiation

Symbiotic and non-symbiotic larvae of *A. tenuis* were exposed to the sun for 4 d on 9-12 June 2008. Six 2-L plastic containers each with 1 L of FSW were prepared. The containers were square with a bottom area of 170  $\text{cm}^2$  and a water depth of approximately 5.5 cm. Symbiotic larvae were placed in each of 3 containers with non-symbiotic larvae in each of the remaining 3 containers. The containers were held in an open-air outdoor aquarium with running seawater to maintain temperatures close to those on the ocean surface (26-28°C). Daily changes in PAR ( $\lambda = 400$ -700 nm), UV A radiation (UVAR,  $\lambda = 315$ -400 nm) and UV B radiation (UVBR,  $\lambda = 280$ -315 nm) were monitored in air every daylight hour throughout the experiment using a light meter (LI-250A, LI-COR, Lincoln, NE, USA) and a UV meter (UV203 Ultraviolet Radiometer, Macam Photometrics Ltd, Livingston, Scotland). Mean ( $\pm$  standard error; SE) daily unweighted doses of UVAR, UVBR, and PAR during the 4-d experimental period were  $374.1 \pm 124.7$ ,  $43.9 \pm 14.6$ , and  $28.8 \pm 11.3$   $\text{mol}/\text{m}^2/\text{d}$ , respectively. The biologically effective UVBR weighted by the DNA-damage action spectrum (Setlow 1974) varied 0.41-0.98  $\text{kJ}/\text{m}^2/\text{d}$ . Mean ( $\pm$  SE) daily temperatures in the aquaria ranged from  $26.44 \pm 0.35^\circ\text{C}$  on day 4 to  $27.06 \pm 1.82^\circ\text{C}$  on day 2.

### Comet assay

A comet assay kit (Trevigen, Gaithersburg, MD, USA) was used to detect possible DNA damage in coral and symbiont cells. At the end of the experimental period, two planulae from each of the 3 replicate containers from each treatment were placed into 1 ml of ice-cold 0.01 M phosphate-buffered saline (PBS) containing 20 mM ethylenediaminetetraacetic acid (EDTA), and cells were dissociated by pipetting for 2 min. Dissociated cells were collected by centrifugation

(6000 rpm, 1 min) and mixed with 300  $\mu$ l of 0.01 M PBS containing 20 mM EDTA. The cell suspension (at 50  $\mu$ l) was mixed with 500  $\mu$ l of low-melting-point agarose. This mixture (at 75  $\mu$ l) was applied to a comet slide and spread using a pipette tip. Slides were placed in a refrigerator at 4°C for 20 min. Slides were then immersed in lysis solution containing 1% sodium lauryl sarcosinate for 30 min at 4°C. Slides were immersed in an alkaline (pH 13) solution containing 200 mM EDTA at room temperature for 20 min. Slides were then placed on a horizontal electrophoresis apparatus. TBE electrophoresis buffer was added to the electrophoresis tray to cover the slides. Electrophoresis was conducted at 20-22 V and 0.01 mA for 10 min at room temperature. After that, slides were immersed in 70% ethanol for 5 min. DNA staining was performed by adding 50  $\mu$ l diluted SYBR Green I to each circle of slides.

Samples were visualized and photographed with a fluorescence microscope (OPTIPHOT-2, Nikon, Tokyo, Japan) at 500x using a digital camera (Nikon Digital Sight DS-LI). Tail lengths of comets derived from coral cells were measured using Image J (vers. 1.40) software (Research Service Branch, National Institute of Health, USA). The comet tail length is a good estimate of DNA damage in cells (Lee and Steinert 2003). Numbers of replicates were 3 and 2 for the aposymbiotic and symbiotic planulae, respectively. At least 50 comets were measured in each of the replicate assays.

## RESULTS

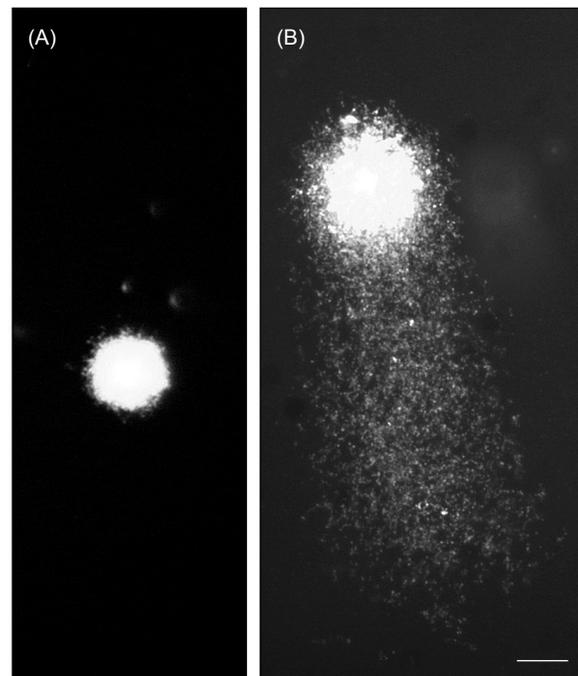
The comet assay revealed remarkable differences in host cell nuclei between symbiotic and non-symbiotic larvae. After 4 d of exposure to solar radiation, host cell nuclei of non-symbiotic planulae produced comets with no or only a short tail. In contrast, host nuclei of symbiotic planulae formed comets with long tails (Figs. 1, 2). The average comet tail length was 15-times longer for symbiotic planula samples than for non-symbiotic ones (Fig. 3).

Comets derived from coral cells could be distinguished from those derived from algal symbionts. The symbionts on the comet slide possessed intact cell walls in most cases and did not show a typical comet shape (Fig. 4). We could not detect DNA damage of algal symbionts in planulae exposed to solar radiation for 4 d using the present comet assay method.

## DISCUSSION

The present results clearly show that symbionts increased DNA damage in host cells when exposed to environmental conditions that prevail at the ocean surface. Rinkevich et al. (2005) also demonstrated that isolated coral cells containing algal symbionts suffer greater DNA damage than coral cells without symbionts or algal cells under UVR. Similarly, recent studies using aggregates of dissociated coral cells also showed that symbionts increase DNA damage and shorten the survival time of coral cell aggregates under thermal stress (Nesa and Hidaka 2009a b).

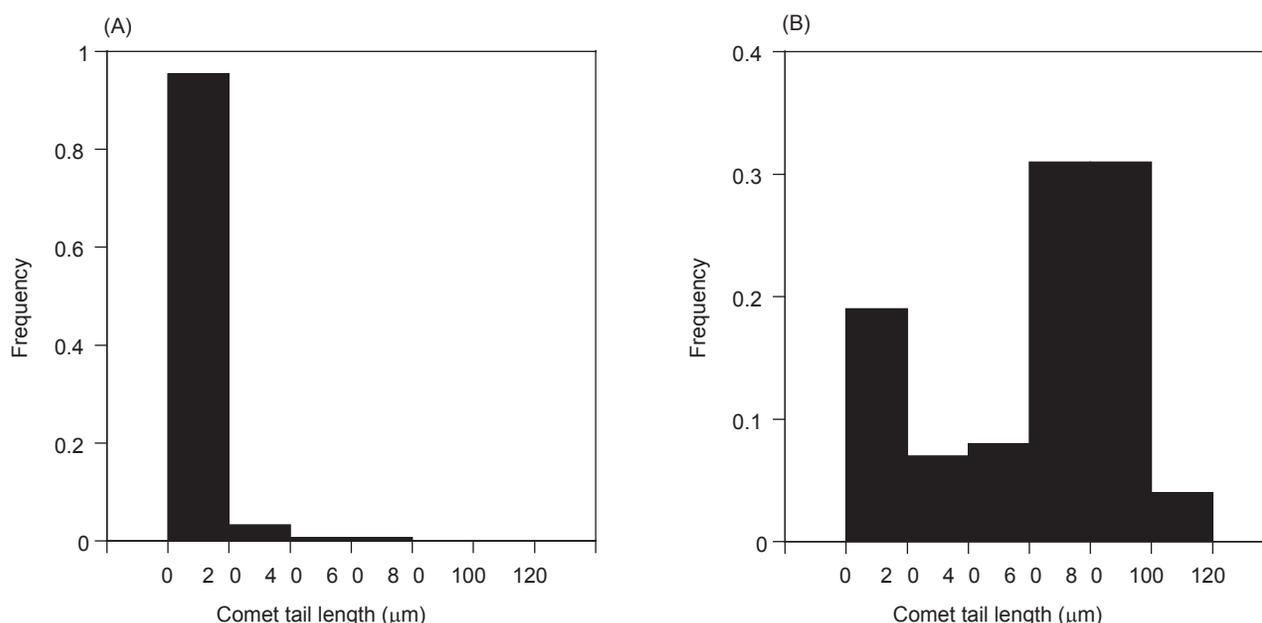
Levels of the DNA-weighted UVBR dose (0.41-0.98 kJ/m<sup>2</sup>/d) seen in the present study can inhibit growth of some phytoplankton, indicating UV-induced DNA damage (Buma et al. 1996 2000). It is likely that UVR is responsible for directly damaging DNA and also causing indirect damage to host tissues by increasing the production of reactive oxygen species (ROS) and oxidative stress (Lesser et al. 1990, Downs et al. 2002). DNA damage in symbiotic planulae might be due to the production of ROS in coral cells with algal symbionts (Yakovleva et al. 2009). Symbiotic



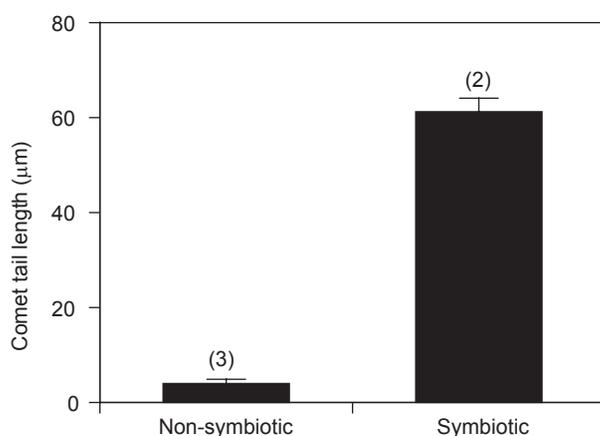
**Fig. 1.** Fluorescence photomicrographs of nuclei of coral cells from the comet assay of non-symbiotic (A) and symbiotic planulae (B) of *Acropora tenuis* after 4 d of exposure to solar radiation. Scale bar = 10  $\mu$ m.

cnidarians generally have large quantities of O<sub>2</sub> within their host tissues due to algal photosynthesis (Dykens and Shick 1982), and UVR enhances photosynthetically generated hyperoxia to produce ROS in host tissues (Dykens et al. 1992). ROS produced during algal photosynthesis diffuse to the host cytosol (Downs et al. 2002, Tchernov et al. 2004).

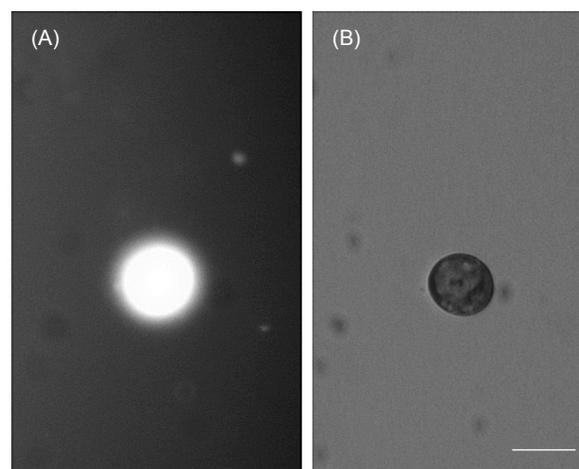
In the present study, 8-d-old planulae were used for inoculation of symbiotic algae and were allowed to establish a stable symbiosis for 1 wk until being used in the stress experiment. Thus the symbiotic planulae were 15 d old when exposed to natural sunlight. The point in coral life history at which the next generation becomes infected by symbionts remains an open question. Early reports suggested that infection



**Fig. 2.** Histograms of comet tail lengths of coral cells in non-symbiotic (A) and symbiotic (B) planulae of *Acropora tenuis* exposed to solar radiation for 4 d. Data from 3 (non-symbiotic) and 2 (symbiotic) replicated assays were separately pooled. Numbers of comets measured were 150 and 100 for the non-symbiotic and symbiotic conditions, respectively.



**Fig. 3.** Comet tail length of coral cells of symbiotic and non-symbiotic *Acropora tenuis* larvae exposed to solar radiation for 4 d. Mean  $\pm$  S.D. The number in the parenthesis is the number of replicated assays.



**Fig. 4.** Photomicrographs of zooxanthella cells on comet slides of an *Acropora tenuis* larva exposed to solar radiation for 4 d. (A) Fluorescence microscopic and (B) light microscopic images. Scale bar = 10 μm.

occurred following metamorphosis (Babcock and Heyward 1986); however, more-recent research demonstrated that larvae can acquire symbionts. For example, larvae of *A. tenuis* kept in 100- $\mu$ m-FSW contained zooxanthellae 37 days after gamete release (Nishikawa et al. 2003). Similarly, most planulae of *A. monticulosa* cultured in the presence of sediment acquired zooxanthellae 9 d after spawning (Adams et al. 2009). Inoculation experiments with homologous symbionts showed that *A. digitifera* and *A. tenuis* larvae first acquire zooxanthellae 6 and 5 d, respectively, after fertilization, and the number of zooxanthellae per planula increased thereafter (Harii et al. 2009). Therefore, our experimental conditions are not unrealistic, and the infection of planulae with symbionts is not in itself harmful.

The exact timing of acquisition of symbionts by *Acropora* planulae in the field will have to wait for further research, and it is not known whether larvae regularly acquire zooxanthellae during the planktonic phase. However, it is likely that *Acropora* planulae can disperse from the parent colony before they take up symbionts. This strategy might render *Acropora* corals a geologically widespread, dominant reef builder. On the other hand, some corals produce zooxanthellate eggs, and most brooder corals release planulae containing zooxanthellae (Harrison and Wallace 1990, Baird et al. 2009b). How corals with a vertical mode of symbiont transmission avoid UV damage or tolerate ROS production during development and the planktonic phase at the ocean surface is a challenging subject for future research. Since concentrations of UV-protective mycosporine-like amino acids (MAAs) are positively correlated with survival of planula larvae (Gleason and Wellington 1995, Wellington and Fitt 2003), MAAs might play an important role in the survival of zooxanthellate planulae during the planktonic phase.

The present experimental approach using symbiotic and non-symbiotic *Acropora* planulae demonstrated that symbiotic algae can be a burden to coral planulae during the obligate planktonic phase of propagules of many broadcast-spawning species. We hypothesize that this may explain the high proportion of broadcast spawning species that lack symbionts in the eggs.

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