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*Research Article*

# Experimental Bleaching of a Reef-Building Coral Using a Simplified Recirculating Laboratory Exposure System

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Determining stressor-response relationships in reef building corals continues to be a critical research need due to global declines in coral reef ecosystems and projected declines for the future. A simplified recirculating coral exposure system was coupled to a solar simulator to allow laboratory testing of a diversity of species and morphologies of reef building corals under ecologically relevant conditions of temperature and solar radiation. Combinations of lamps and attenuating filters allowed for assignment of solar radiation treatments in experimental bleaching studies. Three bleaching experiments were performed using the reef building coral, *Pocillopora damicornis*, to assess the reproducibility of system performance and coral responses under control and stress conditions. Experiments showed consistent temperature- and solar radiation dependent-changes in pigment, numbers of symbiotic algae, photosystem II quantum yield, and tissue loss during exposure and recovery. The laboratory exposure system is recommended for use in experimental bleaching studies with reef building corals.

## 1. Introduction

Coral reef ecosystems have declined throughout the world over the last 30 years, and declines are projected to continue in the future from climate change, increasing human uses, sedimentation, nutrients, pollutants, and other stressors [1]. Many species of reef-building (Scleractinian) corals are particularly sensitive to small increases in temperature because they live near their upper threshold for temperature. Large-scale coral bleaching events leading to massive coral deaths have been linked to episodic water temperature increases. Numerous factors influence the degree and extent of temperature-induced coral bleaching [2, 3]. More recently, solar radiation has been demonstrated to exacerbate coral bleaching, but the specific interaction with temperature is complex and can be species and location specific [4–6]. Intensity and spectrum of incident solar radiation, attenuation, other environmental conditions, acclimatization, and the algal composition and species of the coral have been associated with altering bleaching susceptibility in coral reef ecosystems. Determining stressor-response relationships in reef-building corals remains a critical component of understanding global change and water quality impacts on coral reef ecosystems [2].

Development of stressor-response models for coral has been challenging because of the diversity of species, location-specific responses, and uncertain causal linkages [6]. Various approaches have been applied to quantifying stressor-response relationships, including outdoor systems [7] and *in situ* exposures [8]. There have been relatively few laboratory studies of stressor impacts on reef-building corals because of the difficulty in maintaining and testing healthy specimens of scleractinians under controlled and environmentally realistic conditions [9, 10]. The majority of laboratory research on reef-building corals have used flow-through systems that require either proximity to a coral reef or large quantities of artificial seawater [11–14]. Fewer studies have used controlled solar radiation exposures to quantify bleaching thresholds and the interaction between temperature and solar radiation.

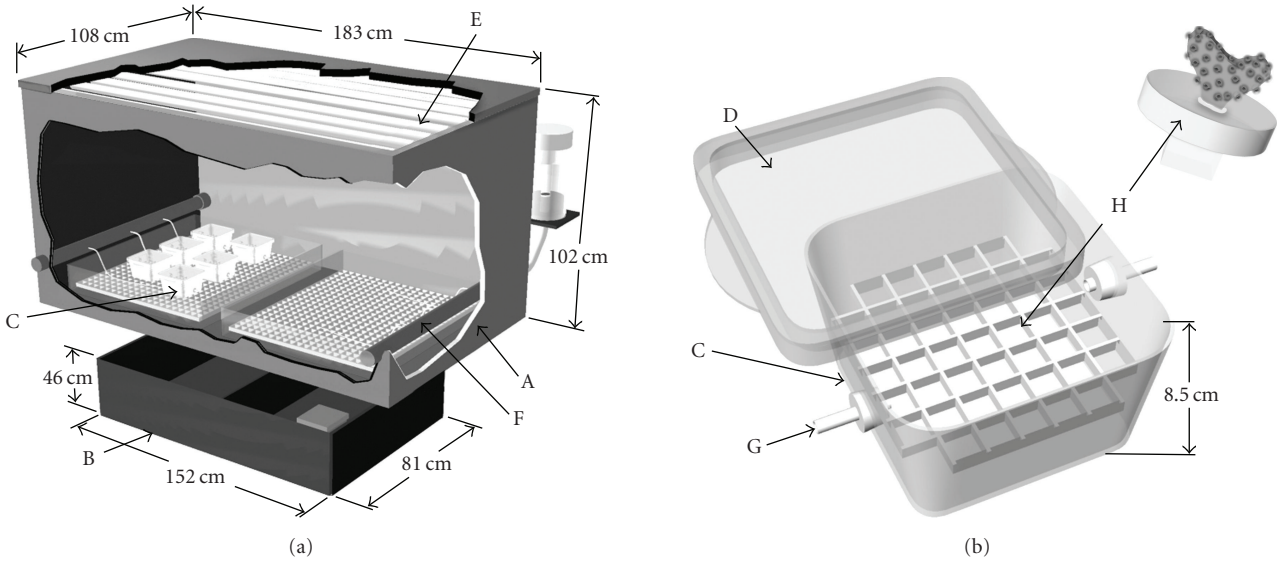


Figure 1: Schematic of coral exposure system. A: exposure chamber, B: sump, C: test vessel (6 of 18 shown), D: UV filters, E: bulbs, F: heater box; G: water delivery lines, H: influent line, and I: coral, base, and pedestal. Dimensions are shown on figure.

A simple recirculating experimental system was developed to allow determination of stressor-response relationships in reef-building corals under controlled and ecologically relevant conditions. This system was designed to accommodate laboratory testing of a diversity of species and morphologies of reef-building corals under controlled conditions of temperature and solar radiation for periods up to 15 days. Three bleaching experiments were performed using the model reef-building coral, *Pocillopora damicornis,* to assess the reproducibility of system performance and coral responses under control and stress conditions. *P. damicornis* is a species that has been frequently used in experimental determinations of photosynthetic impairment and host bleaching responses, and sensitivity to chemical stressors [9, 15–17].

## 2. Material and Methods

*2.1. Laboratory Experimental System.* The laboratory exposure system was designed to provide reproducible temperature and solar radiation treatments under recirculating conditions for a diversity of species and morphological types of scleractinian corals. The two major components (Figure 1) were a water recirculation system and a solar exposure system adapted from Little and Fabacher [18]. The water recirculation system consists of a 152 × 81 × 46 cm divided fiberglass sump (2.54 cm × 33 cm) located beneath the exposure chamber. Temperatures are maintained by two chillers with digital controllers with independent control of each side of the sump (Figure 1). The exposure system was filled with a 1 : 1 ratio of culture water and filtered natural sea water. Water level was maintained approximately 5 cm above the partition to allow for mixing between the sumps while maintaining separate temperature regimes. The exposure system was simplified from culture systems as it did not contain any biological or mechanical filtration typically associated with reef aquaria. Due to the lack of filtration, corals were not fed during experimentation.

The solar exposure chamber was a 183 × 108 × 102 cm enclosure lined with specular aluminum with separately controlled lamps allowing an adjustable photoperiod and ramping of solar radiation exposure during the light cycle. Lighting was provided by a complex of 16.5 cm metal halide (three Coralife; 175 watt; 12-hour photoperiod), 1.8 m fluorescent (ten VHO: General Electric, 165 watt; 10 hours photoperiod), and UVA (8 Houvalite, 100 watt; National Biological Corporation; 8 hours photoperiod) lamps. Corals were tested in clear, plastic flow through test chambers (1.2 L square Rubbermaid containers) randomly positioned within the exposure system (Figure 1). Solar radiation treatments were assigned by placing light attenuating plastic covers (Acrylite OP4, Memphis Net and Twine 63.5 mm black standard duty mesh, or New York Wire window screen) over each test vessels that were randomly positioned within the exposure system.

*2.2. Coral Specimens. P. damicornis* were obtained from an aquaculture facility (ORA-farms; August 2005) and propagated at the U.S. EPA Coral Research Laboratory (Gulf Breeze, FL) until tested. Coral were cultured in recirculating systems under controlled conditions (e.g., 26 ± 1°C, 36 ± 1‰, 20± W/m$^2$ visible, 1± W/m$^2$ UVA, 0 W/m$^2$ UVB). One week prior to testing, coral specimens were cut (about 1.3 cm height) from the cultured colonies with bone cutters and mounted on clear Plexiglas pedestals (Figure 1). Water quality parameters were tested as described below.

*2.3. Exposure Regime.* Corals were exposed in a two (temperature regime) × three (solar radiation treatments) factorial

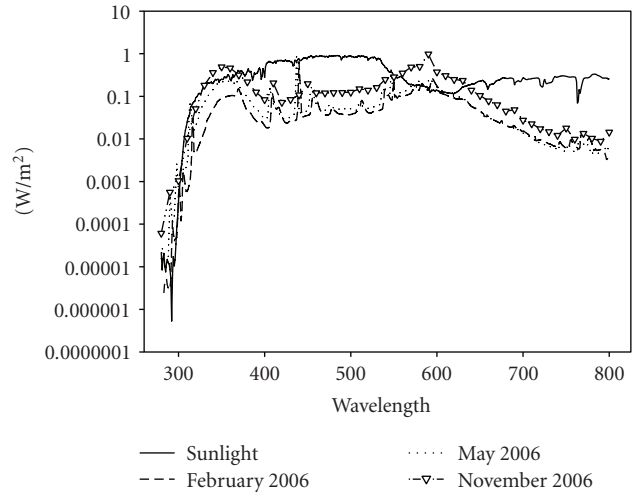


Figure 2: Solar spectral irradiance (intensity, W/m$^2$) in natural sunlight compared to irradiance spectra in three experiments with *P. damicornis*.

Table 1: Intensity (W/m$^2$) and daily dose (W·hr/m$^2$) of visible, ultraviolet A (UVA) and ultraviolet B (UVB) solar radiation in low, medium, and high solar radiation treatments.

| Solar radiation | Low | | Medium | | High | |
|---|---|---|---|---|---|---|
| | W/m$^2$ | W·hr/m$^2$ | W/m$^2$ | W·hr/m$^2$ | W/m$^2$ | W·hr/m$^2$ |
| UVB (280–320 nm) | 0.13 | 1.01 | 0.30 | 2.37 | 0.63 | 5.04 |
| UVA (320–400 nm) | 4.82 | 38.55 | 10.74 | 85.92 | 22.8 | 182.76 |
| Visible (400–700 nm) | 14.10 | 155.05 | 30.70 | 337.74 | 64.6 | 710.83 |

design, with three replicate test vessels (Figure 1) per each of the six treatment combinations for a total of 18 test vessels. The temperature regime was either a constant 26°C (control) or a 26 to 30°C (stressor) temperature increase (2°C/d ramp) that encompassed optimal and stressful temperatures reported for Scleractinian corals [19]. Three coral specimens were placed horizontally about 4 cm from the water surface in each replicate test vessel. Test vessel temperature was monitored continuously using a temperature data logger (Model TBI32-05 + 37, Onset, Bourne, MA, USA). Water quality parameters were tested prior to and following each experiment. Ammonia, nitrate, nitrite, and phosphate levels were measured using a HACH colorimeter (DR/890, Loveland, CO, USA). Calcium levels were measured periodically with a Pinpoint Calcium Monitor. Salinity was measured twice daily using a handheld YSI-63 meter and was maintained by daily additions of deionized water. Calcium concentration of approximately 350 mg/L CaCO3 was maintained by daily additions of 400–800 mL/d calcium hydroxide ($Ca(OH)_2$) (Kalkwasser) to the sump.

Filters covering the test vessels provided three solar radiation treatments that simulated near high (near surface), medium, and low intensity environments in coral reefs [20], with the low light treatment approximating culture conditions (Table 1). The exposure regime consisted of a 12-hour photoperiod with a light regime of 12 hr/d halide, 10 hr/d fluorescent, and 8 h/d UVA. The intensity (W/m$^2$) of ultraviolet B (UVB) (280–320 nm), ultraviolet A (UVA) (320–340 nm), and visible light were measured at 1 nm intervals with a spectroradiometer (OL 752 Optronics Lab., Orlando, FL, USA) at the end of the exposure period and used to determine solar radiation dosimetry (Figure 2, Table 1). Solar radiation levels were also measured within the test chambers prior to test initiation and at end of exposure using a Macam broad wavelength radiometer (Macam Photometric, Scotland, United Kingdom) to ensure consistency throughout the experimental system. Coral specimens ($n = 6$) were sampled immediately prior to the start of experimental exposure to determine levels of chlorophyll *a* concentration and zooxanthellae density in unstressed corals. The experimental bleaching protocol was repeated three times (9, 13, and 15 d exposures) over a 9-month period.

*2.4. Fluorometric Monitoring.* Pulse amplitude modulation (PAM) fluorometry was used to quantify chlorophyll fluorescence within corals as a measure of photosystem II efficiency as quantum yield [21]. Quantum yield was measured every other day for 10 days. Measurements were then taken daily for the next seven days to determine the exact day for termination. Corals were dark adapted for 30 minutes. A DIVING-PAM (Heinz-Walz, Effeltrich, Germany) was used to quantify initial fluorescence (F), maximum fluorescence (Fm), and quantum yield (Fv/Fm = (Fm−F)/Fm, where Fv is the difference in fluorescence between F and Fm). Measurements were taken *in situ* by placing the fiber optic

probe approximately 2 mm above the surface of the coral. Yields below 0.3 were assigned a value of one-half the operational limit of quantitation (0.15).

*2.5. Bleaching Endpoints.* Zooxanthellae and chlorophyll *a* concentrations were determined from the tissue blastate produced using the water pick method [22]. Each blastate was homogenized using a glass-glass tissue grinder, placed on ice, vortexed, and then 50 $\mu$L aliquots were serially diluted in 96-well plates. Each well received 10 $\mu$L iodine/potassium iodide (KI) (Lugols) solution, and then was refrigerated until zooxanthellae were enumerated using an inverted scope. A 1 mL subsample of blastate was maintained at 4°C and analyzed for pigment concentrations by high performance liquid chromatography according to Rogers and Marcovich [23]. The coral skeleton was dried, and the total number of calyxes was enumerated under a dissecting scope. The number of polyps was determined from the total number of calyxes counted after blasting minus the number of empty calyxes counted prior to blasting. Bleaching endpoints were computed as number of zooxanthellae and concentration of chlorophyll *a* normalized by the number of polyps in the coral specimen.

*2.6. Recovery Assessment.* A coral from each treatment replicate ($n = 3$) was transferred from the experimental system and maintained under culture conditions for an 8-week recovery period. PAM fluorometry measurements were performed every two days for the first 4 weeks and then once per week for the remaining 4 weeks. The condition of each coral specimen was monitored weekly during recovery and scored according to a semiquantitative index based on the severity of bleaching (0: no bleaching or tissue loss; 6: >75% surface area bleached or lost).

Photogrammetry was used to determine the percentage coverage of live tissue and dead tissue/bare skeleton on each specimen at the end of the recovery period. Three side-view (60° arc intervals of rotation) and one overhead view digital photographs were taken of each specimen along with a millimeter scale for reference. Photo editing software (PhotoShop; Adobe Systems Inc, San Jose, CA, USA) was used to create black and white, two-dimensional masks from the silhouettes of live tissue and dead tissue/bare skeleton on each photograph, respectively. The areas ($mm^2$) of each mask were determined using Image-J analysis software (http://rsb.info.nih.gov/ij/) calibrated using the millimeter scale images for each view [24]. The percentage of live tissue was determined from the ratio of live surface area to total surface area on each specimen.

*2.7. Statistics.* Zooxanthellae and chlorophyll *a* concentrations were log transformed to meet normality assumptions. Differences between solar radiation and temperature treatments for zooxanthellae, chlorophyll *a* concentrations, and percent tissue loss were tested using two-way ANOVAs and Tukey multiple comparisons with Minitab 15 software (Minitab Inc, State College, PA, USA). A repeated measures analysis was used to compare temperature and light differences between quantum yield values over time for both exposure and recovery periods using the *R* statistical computing software with the lm procedure for linear models (http://www.r-project.org/).

## 3. Results

*3.1. Performance of Experimental System.* The system provided controlled and reproducible light regimes of visible, UVA, and UVB that simulated three levels of sunlight exposure in corals (Figure 2, Table 1). These three levels approximated the daily solar radiation dose in shallow (5–10 m) (high treatment), mid (10–20 m) (medium treatment), and deeper (20–25 m) (low treatment) coral colonies (e.g., Figure 2 [20]). The system also demonstrated reproducible control of constant (26°C) and ramping temperature (26 to 31.5°C) regimes over 9- to 15-day experimental bleaching periods (Figure 3). Exposure temperatures were most variable in high treatment groups, with a maximum of 0.5°C increases during daily solar radiation treatments. The recirculating system required daily additions of deionized water and calcium to maintain salinity levels of $36 \pm 1$‰, and Ca of $350 \pm 50$ mg/L. Water quality parameters for phosphate, ammonia, nitrate, and nitrite were within recommended levels [25] and the end of the experiment and nearly identical to pretreatment measurements.

*3.2. Fluorometric Monitoring.* Quantum yields in *P. damicornis* were consistent across the three experiments, with a maximum value of approximately 0.7 and declines to below 0.4 under temperature and solar radiation stress (Figure 3). Quantum yields in the control treatment (26°C; low solar radiation) ranged from 0.6 to 0.8 and were comparable to average yields in *P. damicornis* specimens prior to experimental exposure. Quantum yields declined in both the medium and high solar radiation treatments in the 30°C regime ($P = .0002$) (Figure 4).

*3.3. Bleaching Endpoints.* Concentrations of zooxanthellae and chlorophyll *a* in *P. damicornis* showed significant treatment related decreases after the 15-day exposure to elevated temperature and solar radiation. Coral exposed in the 30°C regime exhibited significantly reduced zooxanthellae numbers in all three solar radiation treatments ($P = .016$), but no reductions at 26°C. Chlorophyll *a* concentrations were significantly reduced in coral exposed in the 30°C regime to medium, and high solar radiation treatments ($P < .0001$), whereas at 26°C pigment concentrations were only reduced in the high solar radiation treatment (Figure 5). There was no significant interaction between temperature and solar radiation treatments on zooxanthellae number ($P = .71$) and chlorophyll *a* concentrations ($P = .47$).

*3.4. Recovery Assessment.* Corals exposed at 26°C in low, medium and high solar radiation treatments all showed recovery of quantum yields to unstressed conditions (>0.75) within 40 days under culture conditions (Figure 4). In contrast, quantum yields did not recover in coral exposed

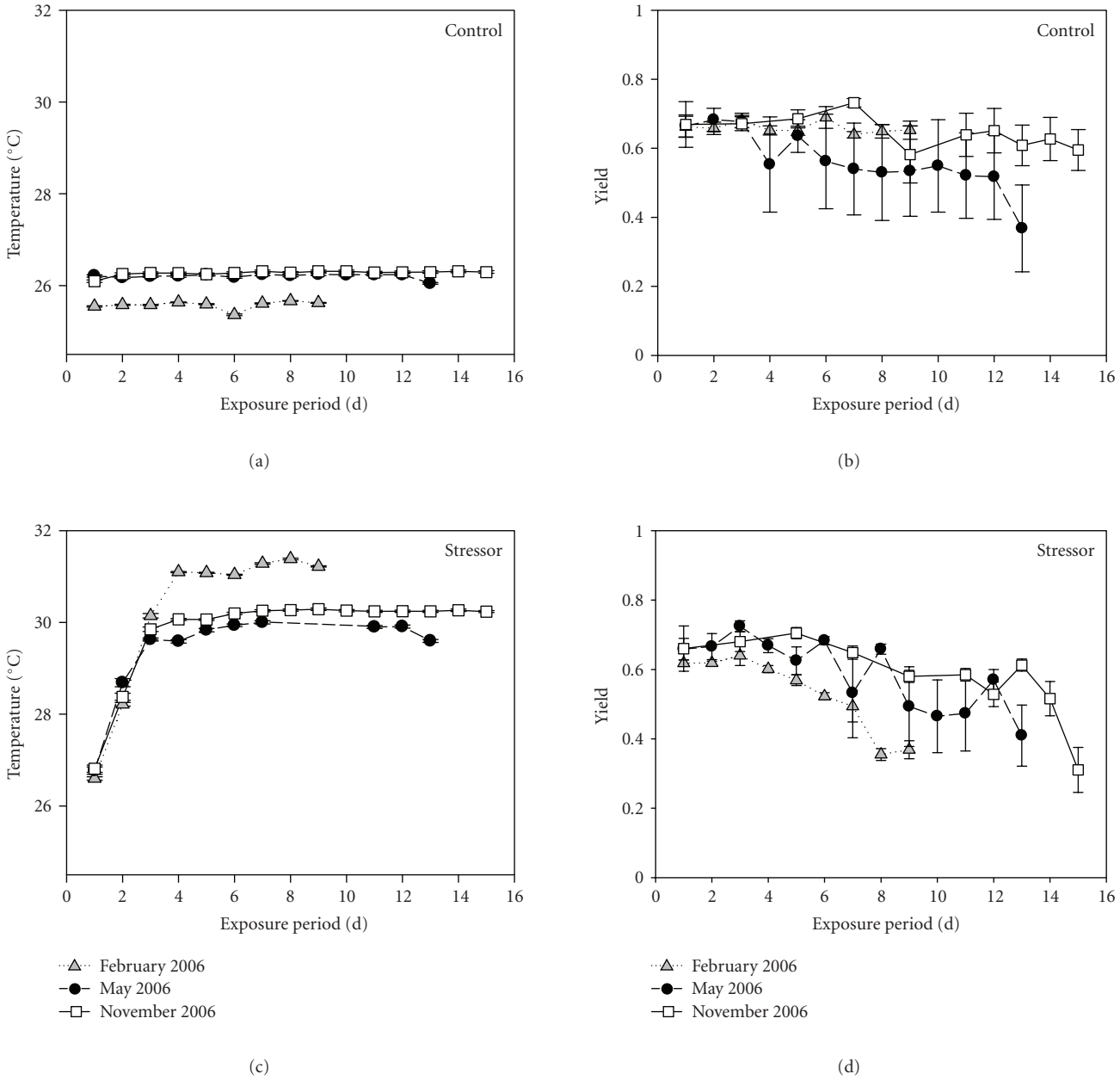


Figure 3: Comparison of temperature and quantum yield responses in *P. damicornis* from three experiments ($n = 3$ in each) under two temperature and light regimes: controls: 25.5–26°C, low solar radiation; stressor: high temperature ramp, high solar radiation.

at 30°C at high solar radiation, with significant declines below detection limits over time ($P = .0028$) (Figure 4). Consistent with yield measurements, visual assessment of corals indicated that the severity of bleaching was greatest and required longer recovery times in the 30°C regime. Visual assessment also indicated greater severity of bleaching and longer recovery with increasing solar radiation treatment in both 26°C and 30°C temperature regimes. Tissue loss and coral death were treatment related. Coral in all three replicates of the high temperature and solar radiation treatment group died within 43 days of recovery. The percentage of live tissue on corals from the 26°C regime was significantly greater ($P < .001$) than corals from the 30°C regime (Figure 6). No significant interactions occurred between temperature and light for percent of live tissue (ANOVA, $P = .396$).

## 4. Discussion

A simplified recirculating coral exposure system was coupled to a solar simulator [18] to allow controlled laboratory testing of reef-building corals under ecologically relevant and controlled conditions of temperature and solar radiation. The solar exposure component provided reproducible and

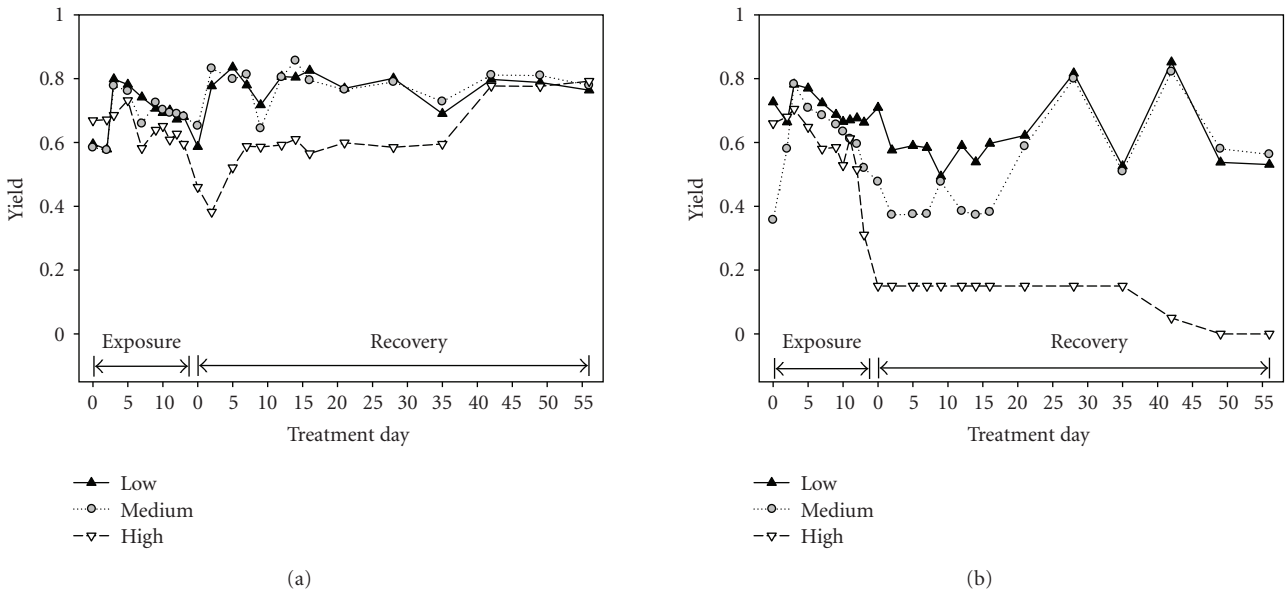


Figure 4: Comparison of quantum yield responses in *P. damicornis* in a single experiment (November 2006) under two temperature regimes (a): constant 26°C; (b): 26 to 30°C ramp) and three solar radiation treatments (low, medium, high) during experimental bleaching and recovery ($n = 3$).

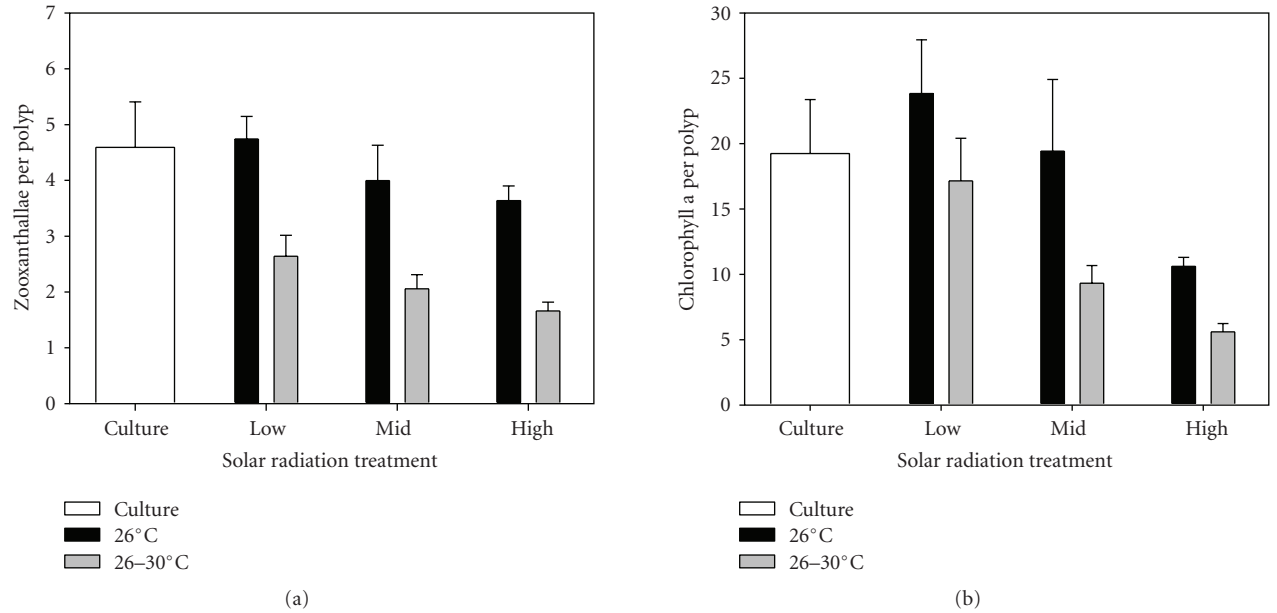


Figure 5: Number of Zooxanthellae and pigment concentrations in *P. damicornis* following temperature and solar radiation treatments ($n = 3$). (a) zooxanthellae/polyp; (b) chlorophyll *a*/polyp.

ecologically relevant levels of visible and ultraviolet radiation that approximated shallow, mid, and deeper depths of coral reefs [20]. The recirculating system may be of value to those without access to coral reef quality water and in experimental bleaching studies requiring controllable solar radiation dosimetry. The system provides an alternative to *in situ* and outdoor systems typically used in bleaching studies. However, there are limitations to all recirculating systems, particularly those for reef-building corals because of the narrow ranges of environmental conditions generally required by corals. Despite the design elements to minimize evaporative water loss, the system required daily manual additions of deionized water and calcium. The system performed well in repeated experimental studies with *P. damicornis* and is adaptable to a diversity of specimens of reef-building coral species of various morphologies (e.g., branching, massive) using a base and pedestal system with either vertical or horizontal orientations. Exposure

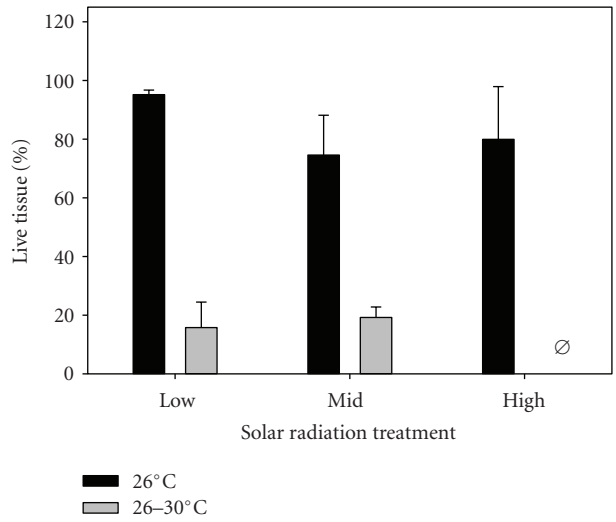


Figure 6: Percent live surface area in *P. damicornis* ($n = 3$) following experimental bleaching under two temperature regimes black square: constant 26°C; grey square: 26 to 30°C ramp) and three solar radiation treatments (low, medium, high). ∅: all dead; no live tissue.

temperatures were well controlled with most variability in high treatment groups. This range in variability (0.5°C) was within the diurnal variation of water temperatures within coral reef systems [26].

Experimental bleaching results using the model reef-building coral, *P. damicornis*, showed reproducible temperature- and solar radiation-dependent changes in quantum yields, and related time-dependent changes in pigment, zooxanthellae, and tissue loss during exposure and recovery. The quantum yields in the control treatment were comparable to *P. damicornis* specimens maintained in culture, at the initiation of the experiment, and those reported by others [16]. Zooxanthellae and chlorophyll *a* levels showed significant decreases at higher temperatures and exhibited significantly greater reductions with increasing solar radiation treatments. These results were consistent with reports that moderate to high solar radiation exacerbates bleaching in *P. damicornis* and other species [5, 6, 11, 13, 19, 27, 28]. Additionally, solar radiation representative of shallow reef conditions reduced *P. damicornis* chlorophyll *a* concentrations under acclimated (26°C) thermal conditions, which has not been previously reported for this species. In contrast, the low light treatment exhibited elevated chlorophyll a concentrations which has been previously reported for deeper water corals [4].

Overall, *P. damicornis* recovered from all experimental bleaching treatments except for corals exposed to both high temperature and high solar radiation. Visual assessment of corals indicated that the severity of bleaching was greatest, required longer recovery times, and showed significantly greater percent tissue loss under conditions of high bleaching stress. These results confirm reports that the severity of bleaching can determine subsequent recovery in scleractinian corals [3, 11, 19, 29]. Additional research is needed to link short-term bleaching endpoints with longer-term coral growth and recovery. Developing stressor-response models for coral remains difficult because of the complexity in species-specific sensitivity to solar radiation and temperature [6]. The laboratory exposure system described here is recommended for performing controlled experimental bleaching studies with multiple species of reef-building corals.

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## References

[1] T. P. Hughes, A. H. Baird, D. R. Bellwood et al., "Climate change, human impacts, and the resilience of coral reefs," *Science*, vol. 301, no. 5635, pp. 929–933, 2003.

[2] W. K. Fitt, B. E. Brown, M. E. Warner, and R. P. Dunne, "Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals," *Coral Reefs*, vol. 20, no. 1, pp. 51–65, 2001.

[3] R. J. Jones, "Coral bleaching, bleaching-induced mortality, and the adaptive significance of the bleaching response," *Marine Biology*, vol. 154, no. 1, pp. 65–80, 2008.

[4] M. P. Lesser and J. H. Farrell, "Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress," *Coral Reefs*, vol. 23, no. 3, pp. 367–377, 2004.

[5] S. H. Yee and M. G. Barron, "Predicting coral bleaching in response to environmental stressors using 8 years of global-scale data," *Environmental Monitoring and Assessment*, vol. 161, pp. 423–438, 2010.

[6] S. H. Yee, D. L. Santavy, and M. G. Barron, "Comparison of combined taxa and species specific models of environment influences on coral bleaching in the Florida Keys," *Ecological Modeling*, vol. 218, pp. 162–174, 2008.

[7] R. Bhagooli and I. Yakovleva, "Differential bleaching susceptibility and mortality patterns among four corals in response to thermal stress," *Symbiosis*, vol. 37, no. 1–3, pp. 121–136, 2004.

[8] A. G. Grottoli-Everett and L. B. Kuffner, "Uneven bleaching within colonies of the Hawaiian coral *Montipora verrucosa*," in *Ultraviolet Radiation and Coral Reefs*, D. Gulko and P. L. Jokiel, Eds., pp. 115–120, University of Hawaii, Honolulu, Hawaii, USA, 1995.

[9] C. L. Mitchelmore, E. A. Verde, and V. M. Weis, "Uptake and partitioning of copper and cadmium in the coral *Pocillopora damicornis*," *Aquatic Toxicology*, vol. 85, no. 1, pp. 48–56, 2007.

[10] S. Shafir, J. Van Rijn, and B. Rinkevich, "The use of coral nubbins in coral reef ecotoxicology testing," *Biomolecular Engineering*, vol. 20, no. 4–6, pp. 401–406, 2003.

[11] C. Hueerkamp, P. W. Glynn, L. D'Croz, J. L. Maté, and S. B. Colley, "Bleaching and recovery of five eastern pacific corals in an El Niño-related temperature experiment," *Bulletin of Marine Science*, vol. 69, no. 1, pp. 215–236, 2001.

[12] O. Siebeck, "Experimental investigation of UV tolerance in hermatypic corals (Scleractinian)," *Marine Ecological Progress Series*, vol. 43, pp. 95–103, 1988.

[13] J. Stimson, "The annual cycle of density of zooxanthellae in the tissues of field and laboratory-held *Pocillopora damicornis* (Linnaeus)," *Journal of Experimental Marine Biology and Ecology*, vol. 214, no. 1-2, pp. 35–48, 1997.

[14] J. H. Torregiani and M. P. Lesser, "The effects of short-term exposures to ultraviolet radiation in the Hawaiian Coral *Montipora verrucosa*," *Journal of Experimental Marine Biology and Ecology*, vol. 340, no. 2, pp. 194–203, 2007.

[15] R. Hill, A. W. D. Larkum, C. Frankart, M. Kühl, and P. J. Ralph, "Loss of functional photosystem ii reaction centres in zooxanthellae of corals exposed to bleaching conditions: using fluorescence rise kinetics," *Photosynthesis Research*, vol. 82, no. 1, pp. 59–72, 2004.

[16] P. J. Ralph, R. Gademann, A. W. D. Larkum, and M. Kühl, "Spatial heterogeneity in active chlorophyll fluorescence and PSII activity of coral tissues," *Marine Biology*, vol. 141, no. 4, pp. 639–646, 2002.

[17] C. Schlöder and L. D'Croz, "Responses of massive and branching coral species to the combined effects of water temperature and nitrate enrichment," *Journal of Experimental Marine Biology and Ecology*, vol. 313, no. 2, pp. 255–268, 2004.

[18] E. E. Little and D. E. Fabacher, "Exposure of freshwater fish to simulated solar UVB radiation," in *Techniques in Aquatic Toxicology*, G. K. Ostrander, Ed., pp. 141–158, Lewis, Boca Raton, Fla, USA, 1996.

[19] P. L. Jokiel and S. L. Coles, "Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature," *Coral Reefs*, vol. 8, no. 4, pp. 155–162, 1990.

[20] M. G. Barron, D. N. Vivian, S. H. Yee, and D. L. Santavy, "Methods to estimate solar radiation dosimetry in coral reefs using remote sensed, modeled, and *in situ* data," *Environmental Monitoring and Assessment*, vol. 151, no. 1–4, pp. 445–455, 2009.

[21] B. Genty, J. M. Briantais, and N. R. Baker, "The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence," *Biochimica et Biophyscia Acta*, vol. 990, pp. 87–92, 1989.

[22] R. E. Johannes and W. J. Wiebe, "Method for determination of coral tissue biomass and composition," *Limnology and Oceanography*, vol. 15, no. 5, pp. 822–824, 1970.

[23] J. E. Rogers and D. Marcovich, "A simple method for the extraction and quantification of photopigments from *Symbiodinium* spp," *Journal of Experimental Marine Biology and Ecology*, vol. 353, no. 2, pp. 191–197, 2007.

[24] M. D. Abràmoff, P. J. Magalhães, and S. J. Ram, "Image processing with imageJ," *Biophotonics International*, vol. 11, no. 7, pp. 36–41, 2004.

[25] E. H. Borneman, "Water chemistry," in *Aquarium Corals: Selection, Husbandry, and Natural History*, pp. 343–357, T.F.H Publications, Neptune City, NJ, USA, 2001.

[26] J. J. Leichter, B. Helmuth, and A. M. Fischer, "Variation beneath the surface: quantifying complex thermal environments on coral reefs in the Caribbean, Bahamas and Florida," *Journal of Marine Research*, vol. 64, no. 4, pp. 563–588, 2006.

[27] K. R. N. Anthony, S. R. Connolly, and O. Hoegh-Guldberg, "Bleaching, energetics, and coral mortality risk: effects of temperature, light, and sediment regime," *Limnology and Oceanography*, vol. 52, no. 2, pp. 716–726, 2007.

[28] L. D'Croz and J. L. Maté, "Experimental responses to elevated water temperature in genotypes of the reef coral Pocillopora damicornis from upwelling and non-upwelling environments in Panama," *Coral Reefs*, vol. 23, no. 4, pp. 473–483, 2004.

[29] B. E. Brown and Suharsono, "Damage and recovery of coral reefs affected by El Niño related seawater warming in the Thousand Islands, Indonesia," *Coral Reefs*, vol. 8, no. 4, pp. 163–170, 1990.