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Ultraviolet radiation: helpful or harmful to growth of cultured zooxanthellae?

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ABSTRACT: Typical *in vitro* culturing techniques of microalgae employ the use of fluorescent lighting, which produces ultraviolet (UV) radiation. Zooxanthellae cultures from the sea anemone *Aiptasia pulchella* were used to test the hypothesis that UV produced by fluorescent bulbs used in laboratory culturing may bedetrimental to their growth. Aliquots of forty milliliters of diluted culture were placed under a lighting system that consisted of four 30 watt Westinghouse brand "Cool White" fluorescent bulbs on a 12:12 hour light:dark photoperiod. This system provided an total irradiance of 18.5 W $m^{-2}s^{-1}$ (0.0467 W $m^{-2}s^{-1}$ at 300 - 320 nm (UV-B), 0.498 W $m^{-2}s^{-1}$ at 320 - 400 nm (UV-A) and 18.0 W $m^{-2}s^{-1}$ at 400 - 700 nm (PAR), respectively). The hypothesis was tested by subjecting 3 Pyrex® test tubes of zooxanthellae culture to each of the following regimes: full spectrum radiation provided by the fluorescent bulbs, fluorescent radiation lacking UV-B (300 - 320 nm), and fluorescent radiation lacking UV-B and UV-A (320 - 400 nm). Pyrex® was chosen for two reasons: 1) because of its low reduction (13%) of biologically effective radiation (Smith & Baker, 1980), and 2) its common use in microalgae culture. The first tray was fitted with chloroflurocarbon (Aclar®), which transmits wavelengths greater than 280 nm. The second tray was fitted with a cover of polyester film (DuPont Mylar®) that blocked out wavelengths below 320 nm but transmitted most of the longer wavelengthradiation. The third tray was fitted with a cover of UV0 (polycarbonate), which was opaque to both UV-B and UV-A radiation. Culture tubes were laid at a 45° angle; this configuration allowed cultures to receive unshaded irradiance throughout the 12 hour light period. Cell counts were conducted using a hemocytometer and light microscopy every 3 days over the 15 day experimental period. Eight chamber counts per test tubewere taken to provide estimated population densities of zooxanthellae. Data from two runs were tested using ANOVA; no significant difference was found between the three treatments. These results suggest that zooxanthellae raised *in vitro* are not affected by fluorescent-produced UV.

INTRODUCTION

Interest in the ecological importance of solar ultraviolet (UV) radiation has been stimulated by concern over the possible disruption of the earth's protective ozone layer by anthropogenic atmospheric pollution. UV radiation measurements taken by Jerlov (1950) showed that clear, oceanic waters, such as those found over many coral reefs, transmit considerable amounts of UV radiation, yet the previously held view that UV is without ecological importance in all types of waters remained predominant for a considerable period. However, in the last 15 years, numerous observations have given extensive support to Jerlov's view of UV's importance in aquatic environments (see Calkins, 1982 for review). In particular, UV radiation at and above ambient levels has been found to be harmful to many forms of microalgae (McMinn *et al.*, 1994; Cullen et al., 1992; Cullen & Lesser, 1991). Effects of serious UV radiation damage range from reductions in carbon assimilation (Hazzard, 1990) to "bleaching" of chlorophyll and reduction of cell motility (Gerber & Haeder, 1993).

Hermatypic, or reef-building, corals are predominantly found in the euphotic zone of warm, tropical oceans and seas (Falkowski *et al.*, 1990). Their success is partly due to their symbiotic relationship with zooxanthellae (*Symbiodinium* sp.), single-celled dinoflagellate algae that live within the endodermal tissue of the coral animal. These algae require light for photosynthesis, and provide the animal partner with photosynthetic products that contribute to nutrition (Muscatine & Porter, 1977) and aid in the construction of the coral's calcium carbonate skeleton (Goreau, 1961; Pearse & Muscatine, 1971). Zooxanthellae have been isolated from symbiotic partnerships and cultured *in vitro* successfully (Jokiel & York, 1982; Lesser & Shick, 1989). Jokiel and York (1984) demonstrated that *in vitro* cultures of zooxanthellae grown under natural irradiance unshielded from UV were severely impaired in ways similar to other microalgae.

Typical *in vitro* culturing techniques employ the use of fluorescent lighting, which produces both UV-A and UV-B. The experiment described here was designed to test the hypothesis that fluorescent lighting may impair the growth of laboratory cultures of zooxanthellae. If the UV produced by fluorescent lighting is eliminated by using one, or a combination, of filters, it is hypothesized that zooxanthellae division rates will increase.

MATERIALS AND METHODS

Culture of zooxanthellae

Zooxanthellae (*Symbiodinium* sp.) from the sea anemone *Aiptasia pulchella* was obtained from the Coconut Island zooxanthellae collection. All cultures were maintained in Pyrex® test tubes (25 x 150 mm diameter). Pyrex® was chosen for two reasons: 1) because of its low reduction (13%) of biologically effective radiation (Smith & Baker, 1980), and 2) its common use in microalgae culture. The culture tubes were washed with 10% Liqui-Nox®, rinsed with tap water, rinsed with 10% HCl, rinsed with distilled water, stoppered with cotton and autoclaved. One liter of 0.45 micron filtered seawater was filter-sterilized using 0.22 micron Millipore® filter paper and enriched to make "f/2" medium. An initial cell count was made on the Coconut Island master culture using a hemocytometer. A dilution was made from this master culture, and aliquots of forty milliliters, containing a known zooxanthellae density, were placed in the sterile Pyrex® and stoppered with sterile cotton. The cotton stoppers were capped with Parafilm® before the tubes were placed under their respective irradiance regimes.

Lighting

The lighting system used consisted of four 30 watt Westinghouse "Cool White" fluorescent bulbs on a 12:12 hour light:dark photoperiod, which is typically used for *in vitro* microalgae culture. This system provided an total irradiance of 18.5 W $m^{-2}s^{-1}$ (0.047 W $m^{-2}s^{-1}$ at 300 - 320 nm (UV-B), 0.498 W $m^{-2}s^{-1}$ at 320 - 400 nm (UV-A) and 18.0 W $m^{-2}s^{-1}$ at 400 - 700 nm (PAR)), respectively, when measured using a Li-Cor LI-1800UW Underwater Spectroradiometer at Coconut Island, Kane'ohe, Hawai'i on June 16, 1994. Nine test tubes of zooxanthellae culture were split among three treatments. These treatments were: full spectrum radiation provided by the fluorescent bulbs, fluorescent radiation lacking UV-B (300-320 nm), and fluorescent radiation lacking UV-B and UV-A (320-400 nm). The first tray was fitted with chloroflurocarbon (Aclar®), which transmits wavelengths greater than 280 nm. The second tray was fitted with a cover of polyester film (DuPont Mylar®) that blocks out wavelengths below 320 nm but transmits most of the longer wavelength radiation. The third tray was fitted with a cover of UVO (polycarbonate), which is opaque to both UV-B and UV-A radiation. Culture tubes were laid at a 45° angle; this configuration allowed cultures to receive unshaded irradiance throughout the 12 hour photoperiod.

Zooxanthellae cell counts

Cell counts were conducted using a hemocytometer and light microscopy approximately every three days over the 15 day experimental period. Eight chamber counts per test tube were taken to provide estimated population densities. Test tubes were agitated each time a sample drop was extracted to ensure homogeneous mixing of each culture. Cell count data was subjected to ANOVA to determine if there was a difference between cultures of each treatment for each sample day.

RESULTS AND DISCUSSION

Two runs were conducted to test the hypothesis that fluorescent lighting UV may impair *in vitro* cultures of zooxanthellae. Cultures tended to concentrate in areas nearest the irradiance source when left undisturbed. Cells in both motile and non-motile phases were observed during cell counts. Initial cell density per ml for each run was 7000 and 8000, respectively. Over the course of the experiment, cell density increased exponentially for the first twelve days of the experiment before leveling off between Day 12 and Day 15 (Figs. 1 and 2). This leveling off of density may have been a result of cultures reaching senescence. ANOVA was used to determine if the differences between treatments were significant. Differences were found to be not significant.

Cultures grown under the full UV treatment showed slightly higher densities for a majority of the experiment. Jokiel and York (1984) also observed this phenomenon when *Symbiodinium microadriaticum*, *Phaeodactylum tricornutum* and *Tetraselmis* sp. cultures where grown under conditions of light limitation (6% intensity of natural sunlight). One explanation for this higher density is that some species of algae use UV radiation photosynthetically under light-limited growth conditions (Jokiel & York, 1984). Halldal (1968) demonstrated that the action spectrum for photosynthesis in *S. microadriaticum* shows oxygen production in the UV-A range.

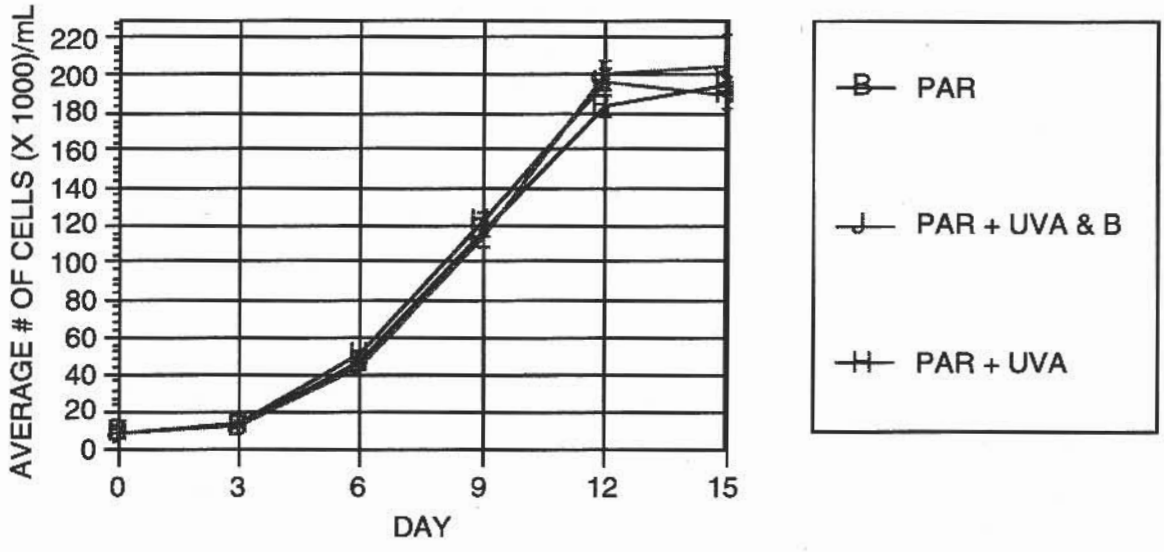

Figure 1. First Run. Zooxanthellae densities when grown under different treatments of PAR, PAR + UV-A, and PAR + UV-A + UV-B.

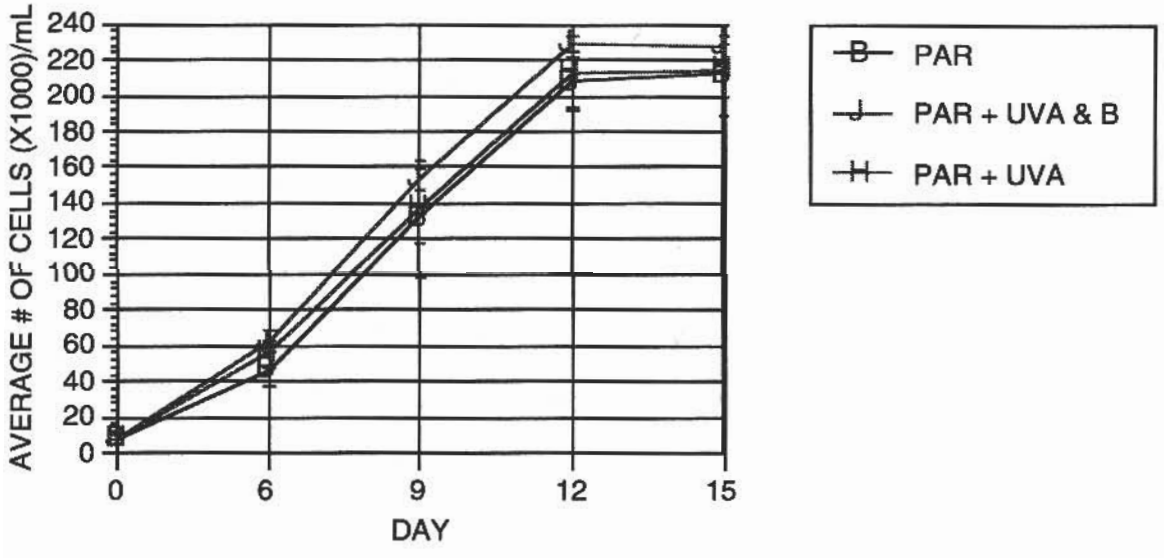

Figure 2:. Second Run. Zooxanthellae densities when grown under different treatments of PAR, PAR + UV-A, and PAR + UV-A + UV-B.

These results, although preliminary, suggest that our stock cultures are not being adversely affected by the UV radiation produced by flourescent lamps. However, the cultures used in this experiment were isolated many years ago and given the rapid division rate of these organisms, untold generations have occurred under these conditions. Therefore, selection for resistant forms might have occurred in these cultures. This could result in the selection of cultures that are UV-tolerant and non-representative of natural populations. The next logical experiment would be to compare response of freshly isolated zooxanthellae of this type with the laboratory forms that have been grown in culture under fluorescent lights since they were isolated from their host over 15 years ago. On the other hand, Jokiel and York (1982, 1984) used the same strain in their work and found the cultured cells to be sensitive to solar UV-A and UV-B. More work dealing with subtleties other than growth needs to be conducted, including analyzes of pigment composition and concentration, to ensure that cultured zooxanthellae are an adequate representation of natural populations.

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