

Title: "The effect of sudden changes in temperature, light and salinity on the density and export of zooxanthellae from the reef corals Stylophora pistillata (Esper, 1797) and Seriatopora hystrix (Dana, 1846).

Ove Hoegh-Guldberg

Department of Biology

U.C.L.A.

CA 90024

U.S.A.

G.Jason Smith

Hopkins Marine Station

Pacific Grove

CA 93950

and

Department of Molecular Genetics and Cell Biology

University of Chicago Chicago IL60637 U.S.A.

ABSTRACT

The bleaching (loss of pigmentation by corals) is a widespread phenomenon in coral reef ecosystems. Despite this, the underlying causes of some forms of bleaching are poorly understood. This study explores the conditions that induce bleaching in two species of reef coral-zooxanthellae associations from Lizard Island (Great Barrier Reef, Australia). Bleached Stylophora pistillata and Seriatopora hystrix collected from the edge of Lizard Island lagoon had the same amount of chlorophyll *a* per zooxanthellae as normal-looking corals yet had reduced population densities of zooxanthellae when compared to normal colonies. In this case, the lack of pigment in the bleached corals was explained by low numbers of zooxanthellae and not by pale zooxanthellae. This is contrary to results obtained by some other workers and suggests that closer inspection of the underlying reasons for the pale color of bleached corals is warranted. In laboratory experiments, sudden exposures to full sunlight induced bleaching of S. pistillata previously grown at 25% sunlight. The pale color of the high sunlight colonies was explained by the low pigment content of the zooxanthellae rather than low densities of zooxanthellae. In addition, the specific expulsion rate (u_x) was not influenced by sudden increases in solar irradiance. Sudden exposures to elevated temperatures ($> 30^\circ\text{C}$) resulted in bleached S. pistillata and S. hystrix. Bleached corals in this case had reduced densities of zooxanthellae despite normal zooxanthella pigment contents. U_x of both corals was very sensitive to temperature. Seven-hour exposures to 30°C and 32°C resulted in specific expulsion rates in excess of 1000 times that of controls. U_x remained high even when corals were returned to control temperatures (27°C). After

high temperature stress, S. pistillata and S. hystrix had high and sustained colony respiratory rates (r_c), reduced colony photosynthetic rates ($P_{c\ net\ max}$) and gross photosynthetic to respiratory ratios ($P_{c\ g\ max}/r_c$). The oxygen metabolism of S. pistillata and S. hystrix remained abnormal for up to four d after a seven-h-exposure to 32°C. Recovery was evident 19 d later. By this time, the mean population density of zooxanthellae and $P_{c\ g\ max}/r_c$ ratios of exposed fragments had increased.

Key words: corals, light, mass bleaching, stress, temperature, zooxanthellae.

INTRODUCTION

Zooxanthellae (endosymbiotic dinoflagellates) are found in the endodermal tissues of reef-building corals. The population density of zooxanthellae per coral surface area in reef-building corals ranges between 0.5 and 5×10^6 cell.cm⁻² (Drew 1972, Porter et al. 1984). Under several extraordinary or "stress" conditions, corals rapidly lose their characteristic brown coloration, a phenomenon referred to by Yonge & Nichols (1931a) as "bleaching". The occurrence of the simultaneous bleaching of reef-building corals in large areas of tropical seas (Glynn 1984, Roberts 1987, 1988) has recently commanded widespread attention from both scientists and policy makers alike.

Few experimental studies have explored the variables that cause corals to bleach. Vaughan (1914) found that corals would bleach if deprived of light for 43 d. Yonge & Nichols (1931a) confirmed this observation and also reported that corals would bleach if starved for periods from 24 to 92 d, depending on the species. Elevated temperature also causes corals to bleach. Yonge & Nichols (1931b) reported that Favia rapidly bleached when maintained for 2 to 4 h in seawater heated to 36° C. Jokiel & Coles (1977), and Coles & Jokiel (1978), showed that a number of coral species bleach when exposed to water temperatures several degrees higher than normal (30-32°C) for longer periods of time.

Several field studies have attempted to correlate changes in environmental variables with coral bleaching. Two of these studies attributed localized coral bleaching to reduced seawater salinities following the input of large amounts of fresh water during tropical storms (Egana & DiSalvo 1982,

Goreau 1964) and others have found correlations between periods of abnormally high sea temperature and widespread coral bleaching (Jokiel & Coles 1974, Jaap 1979, Glynn 1984, Lasker et al. 1984). Recently, the evidence in support of a connection between elevated sea temperatures and bleaching has been further strengthened (Atwood et al. 1988, Causey 1988, Causey et al. 1988).

Some investigators have suggested that elevated levels of ultra-violet radiation have led to the bleaching of corals (Fisk & Done 1985, Harriot 1985, Oliver 1985, and Goenaga et al. 1988). The evidence in this case, however, is circumstantial and is restricted to the observations that (1) periods of low turbidity, calm seas and hence increased solar irradiance, have preceded some bleaching events, and (2) corals tend to first bleach first on their upper surfaces.

The majority of studies assume that the pale color of bleached corals is explained by low numbers of zooxanthellae (Yonge & Nichols 1931a,b, Goreau 1964, Glynn 1984, Lasker et al. 1984, Fisk & Done 1985, Harriot 1985, Oliver 1985, Roberts 1987, 1988). There is, however, little quantitative evidence to substantiate this assumption. Bleaching could also be due to the loss of photosynthetic pigments from zooxanthellae while the number of zooxanthellae remains constant. Light-adapted Stylophora pistillata, for example (Porter et al. 1984) may be up to 10 times paler than the relatively dark shade-adapted forms. Porter et al. (1984) found that it was the concentration of photosynthetic pigments per zooxanthella and not the overall number of zooxanthellae that was reduced. A recent study by Reese et al. (1988) has shown that bleached Montastrea annularis from southeast Florida were pale because of both low zooxanthella pigment concentrations and population densities in the host coral. Accompanying the assumption that bleaching occurs because corals lose zooxanthellae has been the assertion that low densities of zooxanthellae occur because zooxanthellae are expelled from the symbiosis during bleaching. The evidence to support this claim is qualitative and is restricted

to observations of pellets containing zooxanthellae being extruded during bleaching (Yonge & Nichols 1931a,b, Fankboner & Reid 1981). Whether or not zooxanthellae may also undergo digestion or disintegrate in situ has not been answered (Muscatine 1973, Trench 1974, 1979) and it is conceivable that these two "sinks" for zooxanthellae may be significant relative to expulsion. To date, no one has measured the rate at which zooxanthellae are expelled and/or digested by corals during bleaching.

This paper experimentally explores aspects of bleaching for two pocilloporid corals growing near Lizard Island in the northern region of the Great Barrier Reef. Both naturally and experimentally bleached corals were used to answer the following questions: (1) Is color loss in bleached corals collected near Lizard Island due to the loss of zooxanthellae or to the reduced amount of photosynthetic pigment per zooxanthella? (2) Are there differences in the characteristics of bleaching when corals are exposed to elevated light as opposed to elevated temperature or reduced salinity? (3) Does the rate at which zooxanthellae are ejected from corals increase as corals are subjected to conditions that cause bleaching? The last part of this paper examines the recovery of corals following exposure to elevated temperature in an attempt to document the recovery of corals following a short-term temperature shock.

MATERIALS AND METHODS

(A) Collection and Maintenance of corals.

(i) All experiments were performed on the corals Stylophora pistillata and Seriatopora hystrix collected at 5m depth from Lizard Island (14° 14' S, 144° 28' E) during the early part of May 1987. The pieces of bleached and normal colonies were transported back to the laboratory at Lizard Island research station where they were analyzed for a range of biomass characteristics (section D) within three h of collection.

(ii) Colonies of Stylophora pistillata and Seriatopora hystrix measuring 30-60 cm in diameter were collected from 6m depth at the edge of Lizard Island lagoon directly opposite the research station. After collection, the colonies were transferred to tubs of seawater and were transported back to the seawater aquarium at Lizard Island Research Station. Within a few hours of collection, using a pair of wire cutters, tips (3-6 cm long) were clipped off from the colonies and were placed in small racks constructed from microscope slides, monofilament netting and plastic piping (5 cm diameter, Fig. 1a). The racks containing the tips were immersed in tubs of seawater (approximately 12 cm depth) receiving a continuous supply of fresh seawater and were illuminated by sunlight reduced to approximately 25% incidence irradiance by two layers of black plastic mesh (Fig. 1b). New tissue had completely grown over the broken ends of the colony tips by the end of four weeks, at which time the tips (referred to from now on as colonies) were used in the experimental studies.

(B) Experimental treatments.

Two groups of experiments were done.

(i) The effect of high light exposure and continuous darkness.

(a) Colonies were placed in 40 ml of filtered seawater in small containers that were floated in several large flow-through aquaria (27°C - 28°C). One set (n = 8) received full sunlight and another (control, n = 8) received 25% full sunlight. Black plastic sheets were used to shield a third set (n = 8) from all light. Each day, just prior to nightfall, the water in each container was briskly squirted (using a glass pipette) against the sides of the container (to remove any zooxanthellae clinging to the sides of the container) and was replaced with fresh filtered (GF/C Whatman) and aerated seawater (35 ppt). The seawater removed from the containers was filtered and the filters stored at -20°C for later chlorophyll analysis. At the end of the first day, three colonies (randomly selected) were taken for measurement of oxygen flux (section C) and then killed for biomass analysis (section D). The experiment was terminated at the end of 10 days at which time another 3 colonies were taken from each treatment for a similar set of oxygen flux and biomass measurements.

(b) The effect of sudden exposure to sunlight on the rate at which zooxanthellae move out of S. pistillata and S. hystrix was also investigated. Colonies of both species in containers (as described above) were placed in 25% or 100% full sunlight. Each hour for the first 6 h, the seawater in the containers was replaced as described above, the old seawater filtered and the filters stored frozen for chlorophyll fluorescence analysis. Other measurements were made 24 and 30 h after the beginning of the experiment. Thirty h after the beginning of the experiment, the colonies were killed and processed for measurement of biomass parameters (section D).

(ii) The effect of sudden exposure to elevated temperature and reduced salinity on S. pistillata and S. hystrix.

(a) Using submersible water heaters (Exotic Inc., Cat. no. 1257), the temperature in four glass aquaria (70 x 40 x 30 cm) containing fresh seawater (35 ppt) was maintained at 27°C, 30°C, 32°C and

34°C. Observations made every four h verified that the temperature remained constant ($\pm 1^\circ\text{C}$). A set of smaller aquaria (30 x 20 x 20 cm) were filled with seawater diluted to 30 ppt, using double distilled water, and were placed inside each of the larger ones, with the lip of the smaller one above the water line of the larger so that the two volumes did not mix. An airstone was placed in each aquarium for water circulation. Fifteen colonies of both S. pistillata and S. hystrix (still in their racks) were placed in each of the larger (35 ppt) and smaller (30 ppt) aquaria. After seven h, 6 colonies (selected randomly) of each species were transferred from the 32°C (35ppt) treatment to the 27°C (35ppt) treatment. Two of these colonies were used for oxygen flux and biomass measurements immediately. After four d, three colonies from each treatments were used for oxygen flux and biomass measurements. These measurements were also repeated at the end of 23 d for 3 colonies (of each species) transferred from 32°C (35ppt) to 27°C (35ppt) after six h.

(b) To investigate the short-term effect of temperature on the rate at which zooxanthellae leave S. pistillata and S. hystrix, 4 colonies were placed in 40 ml of filtered (GF/C Whatman) seawater inside containers which were floated in one of three aquaria maintained at 27°C, 30 °C and 32°C. Every h, the water in each container was replaced with fresh aerated and filtered seawater, pre-heated to the incubation temperature. The seawater removed from the containers was filtered and the filters stored frozen for later measurement of chlorophyll. At the end of 7 h, the containers containing the colonies were transferred to 27° and seawater sampled after five, 11, 17, 23 and 29 h. The experiment was terminated after the last sample and the colonies processed for biomass determinations.

(C) Closed volume measurement of oxygen flux (photosynthesis and respiration).

Rates of photosynthesis and respiration were measured by monitoring changes in dissolved oxygen within chambers (75 ml) containing single colonies. Each chamber contained a stirbar powered by a

submersible magnetic stirrer ("Variomag" magnetic stirrer, Telesystems Inc.). A dual optic fiber light source (Reichert Scientific Instruments) illuminated the chambers from opposite sides.

Data from each experiment were collected using an Acorn BBC computer. Light was measured using a light dependent resistor (LDR1, Dick Smith), calibrated using a LI-COR quantum sensor (LI 1935A). Temperature was measured using a small copper/constantan thermocouple (and electronics employing an AD595 temperature chip, Analogue Devices) which was water-proofed and set inside the chamber. Oxygen was measured using Clark-type polarographic electrodes constructed according to the method of Mickel *et al.* (1983) and, together with the light and temperature sensors, connected via a multi-gain/multi-channel connector box (Hoegh- Guldberg, in prep.) to the Acorn BBC computer. Light, temperature and oxygen were measured every 30 s with each measurement representing the mean of 150 samples of the voltage output of each sensor. Each sensor was calibrated at the beginning and end of each experiment. The voltage output of the thermocouple circuitry was calibrated to within $\pm 0.1^{\circ}\text{C}$ using a mercury bulb thermometer (Eveready thermometer Co.). The voltage response of the polarographic oxygen electrodes were calibrated using air saturated seawater and a saturated solution of $\text{Na}_2\text{S}_2\text{O}_3$ (zero oxygen content), and the tables of Hitchman (1978), using the appropriate correction for salinity and temperature at a barometric pressure of 760 mm Hg.

Each colony was allowed to adjust to chamber conditions for at least 20 min before sampling began. The chambers were then exposed to different light levels, each exposure lasting 15-20 min. By varying both the intensity setting and position of the lamp, the intensity of the light was varied between 0 and $1500 \text{ } \mu\text{mol (photon).m}^{-2}.\text{s}^{-1}$. Dark conditions were created by covering the chambers with sheets of black plastic. The oxygen flux of each colony at each light intensity was measured at

least twice. Temperature during each run was kept between 27°C and 28°C. If either temperature increased above 28°C or the concentration of oxygen in the chamber decreased below 2 ppm, the experiment was terminated and the chamber flushed with fresh unaerated seawater. In these cases, sampling was discontinued for 20 - 30 min to allow the colonies to adjust to the new chamber conditions. Total changes in oxygen were calculated by multiplying the changes in oxygen concentration by the total volume of the chambers corrected for the volume of the colonies.

Immediately following each experimental trial, the colonies were killed and biomass parameters measured. Rates of change in oxygen concentration were standardized to each of four variables: colony surface area, protein content of the colony, chlorophyll *a* content and number of zooxanthellae found within a colony. The respiratory rate (the rate of consumption of oxygen in darkness, r_c) and the net photosynthetic rate (the net production of oxygen in the light, $P_{c\ net}$) of the intact coral, and the ratio of the gross maximum photosynthetic rate ($P_{c\ g\ max} = r_c + P_{c\ net\ max}$) to the respiratory rate ($P_{c\ g\ max}/r_c$) were calculated. The conventions used for calculating these quantities are as described by Muscatine (1980). The colonies were exposed to a series of light intensities (*I*). Data obtained were fitted to a hyperbolic function ($P_{c\ net} = P_{c\ g\ max} e^{-I/P_{c\ g\ max}} + r_c$) using the Marquardt algorithm (Marquardt 1963).

(D) Biomass measurements

Colonies were blotted on moist tissue paper to remove excess water and weighed using a top-loading balance. The tissue on the colonies was then removed using a dental Water-Pik (Delux model, Teledyne). The volume of the resulting homogenate was measured and mixed thoroughly. First, three 2 ml samples were stored at - 20°C for protein measurement. Second, buffered (5 %) formalin solution (2 ml) was added to another 10 ml subsample of the homogenate which was set

aside for measuring the concentration and mitotic index (ratio of dividing cells to total cells) of zooxanthellae in the homogenate. Finally, three 16 ml subsamples were filtered using GF/C (Whatman) filters, which captured the zooxanthellae on the top surface of the filter paper. The filters were covered with aluminum foil to exclude light and were frozen (-20°C) for measurement of chlorophyll a.

The total protein concentration of the homogenate was determined using the Hartree (1972) modification of the Lowry protein assay. The chlorophyll fluorescence was determined by grinding the frozen filters in 3.22 ml of acetone and centrifuging the resulting solution until clear. The amount of fluorescence due to chlorophyll was measured using a Turner Fluorometer (model 111; excitation filters: Corning C/S2A and C/S 5-60; emission filter: Corning C/S 2-64). Total fluorescence measurements were calibrated to total chlorophyll a content ($r^2 = 0.90$, Jeffrey and Humphrey, 1975) measured for 30 samples. Using this method, chlorophyll fluorescence measurements were converted to concentrations of chlorophyll a.

Zooxanthellae appeared intact following isolation. The concentration of zooxanthellae in the homogenate was determined using a hemacytometer (Bright-Line, American Optical Corp.) and eight separate cell counts. The mitotic index was determined by counting the number of dividing cells in three samples of 500 zooxanthellae. Populations of zooxanthellae in both species of corals show synchronous division (Smith and Hoegh-Guldberg, 1987, Hoegh-Guldberg and Smith 1989) and consequently the specific growth rate of these populations was calculated using the formula for synchronous populations of zooxanthellae as described by Wilkerson et al. (1983).

The surface area of the bare skeletons remaining

after removal of tissue was measured independently using the Varathane/dye method of Hoegh-Guldberg (1988) and the aluminium foil method of Marsh (1970). Total protein, chlorophyll a content and zooxanthellae number were determined by multiplying each sample parameter by the total volume of the homogenate.

(E) Calculation of specific expulsion rates.

The specific expulsion rate was measured using the methodology of Hoegh-Guldberg *et al.* (1987), with the following modifications. The fluorescence due to chlorophyll appearing in the seawater surrounding the colonies was used as a measure of the total number of zooxanthellae moving out of corals. The in situ population sizes were estimated as chlorophyll fluorescence. Population sizes were determined by multiplying the average chlorophyll fluorescence per colony surface area before and after the experiment by the total area of the colony. The amount of fluorescence per surface area before the experiment was determined from the control colonies. The specific expulsion rate was calculated as:

$$U_x = F_{out} / (F_{in\ situ} \times t)$$

where F_{out} = Fluorescence appearing in incubation medium; $F_{in\ situ} = 0.5(F_b + F_a)$ or the average total colony fluorescence before (F_b) and after (F_a) the experiment, and t = period in days over which expulsion was measured.

(F) Statistical analysis.

Experimental data were examined using two factor ANOVA and Student t-tests, after first checking for normality of the data and homoscedasticity of sample variances. Calculations were done using the computer software package STATGRAPHICS (Statistical Graphics Corp.).

RESULTS

(A) The chlorophyll content and population density of zooxanthellae in normal and bleached colonies collected near Lizard Island.

The total chlorophyll content of bleached specimens of S. pistillata and S. hystrix was about a third that of normal looking colonies (27 % and 29 % respectively, Table I). In both species, the low chlorophyll content of bleached colonies can be explained by reduced numbers of zooxanthellae and not by reduced amounts of chlorophyll per zooxanthella in these colonies.

(B) Experimental treatments:

(i) The effect of exposure to either high light or continuous darkness.

After exposure to full sunlight for 8 h, colonies of S. pistillata were visibly paler on their upper surfaces as compared to their lower surfaces. Examination of the chlorophyll content and number of

zooxanthellae in the tissues of the corals at this stage did not reveal any statistically significant differences between exposed and control corals ($p > 0.05$, data not shown). By the end of 10 d the upper surfaces of colonies exposed to full sunlight were white. Despite the pale color, the tentacles of coral polyps could still be seen protruding from these areas. The pale color of colonies exposed to full sunlight resulted from reduced chlorophyll per zooxanthella ($p < 0.05$) and not reduced numbers of zooxanthellae (Fig. 2). Both the amount of chlorophyll fluorescence and the number of zooxanthellae per area decreased when colonies were maintained in the dark ($0.05 > p < 0.10$; Fig. 2). The mean of chlorophyll fluorescence per zooxanthella in corals incubated in the dark for 10 d was also lower (though not statistically different) from that of zooxanthellae obtained from control (25 % full sunlight) treatments ($p > 0.10$).

Close fits of the oxygen flux and irradiance data to a hyperbolic function were obtained (Fig. 3). There was no evidence of photoinhibition up to irradiances of $1500 \text{ umol (photon).m}^{-2}.\text{s}^{-1}$ (approximately equal to full sunlight). The best fit to this model for three colonies of both *S. pistillata* and *S. hystrix* estimated $P_{g \text{ c max}}$ as 33.3 ± 1.15 and $28.1 \pm 1.11 \text{ ug O}_2.\text{cm}^{-2}.\text{h}^{-1}$ respectively. The estimate of the photosynthetic efficiency per surface area (ϵ) for *S. pistillata* and *S. hystrix* respectively was 0.085 ± 0.0059 and $0.119 \pm 0.0137 \text{ ug O}_2.\text{cm}^{-2}.\text{h}^{-1}/\text{umol (photon).m}^{-2}.\text{s}^{-1}$, and the estimate of the colony respiratory rate (r_c) was 10.7 ± 0.61 and $8.9 \pm 0.74 \text{ ug O}_2.\text{cm}^{-2}.\text{h}^{-1}$. The photosynthetic response of *S. pistillata* to light saturated at approximately $800 \text{ umol (photon).m}^{-2}.\text{s}^{-1}$ (I_k c.a. $400 \text{ umol (photon).m}^{-2}.\text{s}^{-1}$, Fig. 3). The photosynthetic rate of zooxanthellae from *S. hystrix* saturated at light intensities of approximately $400 \text{ umol (photon).m}^{-2}.\text{s}^{-1}$ (I_k c.a. $300 \text{ umol (photon).m}^{-2}.\text{s}^{-1}$, Fig. 3). All other measurements were carried out in either total darkness (respiratory rate measurements) or at light intensities of $1500 \text{ umol (photon).m}^{-2}.\text{s}^{-1}$ (light saturated photosynthesis).

Colonies in containers exposed to either full sunlight or continuous darkness for one d, had mean gross photosynthetic rates that were lower than those of control colonies (25 % full sunlight, Fig. 4), although this difference was not statistically significant ($p > 0.05$). After 10 d, the measurements were repeated (Fig. 4). The mean gross photosynthetic rate of zooxanthellae in colonies exposed to full sunlight and continuous darkness was lower ($p < 0.05$) than the mean rate measured for zooxanthellae in control colonies. In all three treatments, the mean respiratory rate of the colonies was higher than that of the colonies controlling for the effect of the containers ($9.0 \pm 0.79 \text{ ug O}_2\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$, $p < 0.05$). The respiratory rates of the colonies maintained for 10 d in the containers also increased as maintenance light level decreased. Mean gross photosynthetic rates were slightly higher at day 10 than at day 1. This difference was most pronounced in the control light level, with a 43 % increase at day 10 as compared to day 1 (Fig. 4).

The specific expulsion rate of zooxanthellae from S. pistillata under normal light conditions (25 % full sunlight) between ranged between $1 - 20 \times 10^{-4} \text{ d}^{-1}$ (Fig. 5a). Exposing the colonies to full sunlight caused a slightly elevated specific expulsion rate at day 1 ($p < 0.05$) and again between days 4 and 5 (Fig. 5a). In a second experiment, the expulsion of zooxanthellae was examined for S. pistillata and S. hystrix exposed to full sunlight for a shorter time period (Fig. 5b). Specific expulsion rates varied between 0.1 and $10.0 \times 10^{-4} \text{ d}^{-1}$, and were similar to the mean specific expulsion rates of colonies exposed to normal light levels. In this case, the specific expulsion rates in full sunlight were indistinguishable from control rates.

(ii) The effect of elevated temperature and reduced salinity.

Corals exposed to normal (27°C) or slightly elevated (30°C) temperatures did not show any visual signs of bleaching after four d. Both species exposed to 32°C , on the other hand, were paler than

controls. Polyps in both species began closing at the end of two h of exposure and inspection using a Leitz microscope revealed zooxanthellae disappearing from regions between the polyps (Fig. 6a). This distribution of zooxanthellae gave the colonies a speckled appearance. Pellets of zooxanthellae were also occasionally observed collecting in the pharyngeal regions of the polyps (Fig. 6b).

All colonies exposed to 34°C died within 8 hours. Mortality was also higher in the 32°C treatment after 4 d (50 % mortality in S. pistillata and 40 % in S. hystrix) as compared to 30°C (10 % in S. pistillata and 20 % in S. hystrix). No colonies died in the 27°C treatment. Reducing the salinity to 30 ppt had no effect on any of the parameters measured in this study (ANOVA, $p > 0.05$). Consequently the data for the two salinities were pooled for each temperature. The number of zooxanthellae per area was lower in S. pistillata and S. hystrix colonies exposed to 32°C for 4 d (Fig. 7a, $p < 0.01$) than in colonies exposed to 27°C. Exposure to 30°C reduced the mean number of zooxanthellae relative to colonies incubated at 27°C, although the difference was not statistically significant ($p > 0.05$). The amount of chlorophyll fluorescence per zooxanthella was not effected by temperature (Fig 7b).

The response of the gross photosynthetic rate to a 4-d- exposure to increased temperature varied. Zooxanthellae in S. pistillata colonies had a higher gross photosynthetic rate (measured at 27°C) when the colonies were incubated at 30°C for 4 d, as opposed to being incubated at either 27°C or 32°C (Fig. 8a). Zooxanthellae in S. hystrix colonies on the other hand, did not show any effect of temperature on their gross photosynthetic rate (Fig. 8a). The respiratory rate of the colonies (measured at 27°C) increased as maintenance temperature was increased. The gross photosynthetic rate of the colonies was compared to the respiratory rate of the colonies via the ratio of the gross photosynthetic rate to respiratory rate ($P_{c\ g\ max}/r_c$) ratio. As a result of the trends in the gross photosynthetic and respiratory rates, $P_{c\ g\ max}/r_c$ decreased with increasing maintenance temperature

(Fig. 8c).

Elevating the temperature to 30 - 32°C increased the rate at which zooxanthellae moved out of, or were expelled from, the corals (Fig. 9). Thirty minutes after the beginning of exposure, specific expulsion rates of colonies kept at both 30°C and 32°C treated colonies were 100 times larger than for colonies kept at 27°C. Three h after the beginning of exposure, the specific expulsion rate increased sharply and by the end of four h was approximately 500 to 1000 times higher than control colonies incubated at 27°C. The specific expulsion rate decreased after colonies incubated at 32°C for 7 h were placed back in 27°C seawater, but remained two orders of magnitude higher than control colonies for up to 25 h after exposure.

Colonies exposed to 32°C for 6 h, and examined 4 d after being placed back at 27°C, had lower numbers of zooxanthellae per surface area when compared to control colonies or colonies sampled immediately after a 4-h-exposure to 32°C seawater (Figs. 10, 11). Signs of recovery were evident 23 d later. By the end of 23 d there was an increase (though not statistically significant; $p > 0.05$) in the number of zooxanthellae per surface area in both species (Figs. 10, 11). After four d, the recovering colonies still showed increased respiratory rates relative to controls (Fig. 10, 11). After 23 d, respiratory rates had decreased to levels typical of untreated control colonies measured at the same time. Gross photosynthetic rates did not vary with time after exposure of the colonies to elevated temperature. $P_{c\ g\ max}/r_c$ ratios in both species decreased immediately after exposure to 32°C and were still lower than controls 4 d later. By the end 23 d, however the $P_{c\ g\ max}/r_c$ ratios were indistinguishable from control $P_{c\ g\ max}/r_c$ ratios.

DISCUSSION

(A) The chlorophyll content and population density of zooxanthellae in bleached *S. pistillata* and *S. hystrix* collected near Lizard Island.

Originally, coral bleaching referred to the the loss of brown pigment by corals (Yonge & Nichols 1931a). More recently bleaching has been taken to be synonymous to the loss of zooxanthellae by corals (Goreau 1964, Fisk & Done 1985; Harriot 1985; Oliver 1985; Roberts 1987, 1988) despite the fact that bleaching (by the original definition) has been reported to occur when zooxanthellae lose photosynthetic pigment (hence bleach) as they photo-adapt to high light conditions (Porter et al. 1984). The population density of zooxanthellae remains constant in this case (Porter et al. 1984). Several authors have recognized this problem and have been careful to retain the original meaning of the term 'bleaching' (Jokiel & Coles 1977; Glynn 1983; Lasker et al. 1984).

Bleached specimens of S. pistillata and S. hystrix collected from near Lizard Island had reduced numbers of zooxanthellae, which had either the same as or greater pigment concentrations than zooxanthellae from normal looking S. pistillata and S. hystrix. In another study, Kleppel et al. (1989; see also Reese et al. 1988) found that zooxanthellae isolated from bleached Montastrea annularis had only 3 - 19 % of the carotenoid and chlorophyll pigment of the zooxanthellae isolated from normal colonies. This further demonstrates that bleached corals do not always have lower than normal population densities of zooxanthellae. The difference between the results of Kleppel et al. (1988) and this study may reflect differences due to species, the factor initiating bleaching or in the amount of recovery that has occurred by the time samples are taken from an affected colony. In both studies, the amount of time between the actual advent of bleaching and sampling is unknown. Bleaching may initially involve a decline in the pigment content and population density of zooxanthellae, and may be followed by a period in which the pigment content of the zooxanthellae recovers despite the population density of zooxanthellae remaining low.

(B) Experimental treatments:

(i) The effect of exposing either high light or continuous darkness.

When S. pistillata colonies were exposed to full sunlight for 10 days, they became visibly bleached on the upper surfaces. This was due to a reduction in the amount of photosynthetic pigments (measured as chlorophyll fluorescence) per zooxanthella and not to a reduction in the number of zooxanthellae per surface area (Table II). This result is in accord with observations collected for

light-adapted S. pistillata from the Red Sea (Porter et al. 1984). Light-adapted S. pistillata is pale because the zooxanthellae have reduced amounts of photosynthetic pigments relative to darker shade-adapted S. pistillata. Both ecotypes have the same number of zooxanthellae per surface area. Coles & Jokiel (1978) also reported that corals from Hawaii bleach when exposed to full sunlight and that the underlying reason is probably due to a reduction in the amount of photosynthetic pigment per zooxanthella.

There is no evidence from our studies that sudden increases in solar irradiance can initiate large increases in the expulsion of zooxanthellae. Not only are decreases in the population density of zooxanthellae minimal but specific expulsion rates are only slightly higher in those colonies exposed to full sunlight. The growth rate of zooxanthellae in S. pistillata growing under light levels 25 % full sunlight at Lizard Island is approximately $0.012 \pm 0.0019 \text{ d}^{-1}$ (Smith and Hoegh-Guldberg, 1987), which is an order of magnitude higher than the average specific expulsion rate measured in any experimental treatment described here. Since the rate at which zooxanthellae are being produced by population growth greatly exceeds the rate at which they are being lost through expulsion, the significance of the slight stimulation of U_x by increased solar irradiance is further diminished.

In S. pistillata placed in the dark for 10 days, zooxanthellae tend to have reduced amounts of photosynthetic pigments and to be less numerous. As with the high light treatments described above, the rate at which zooxanthellae moved out of colonies during the 10 days was not significantly different from the rate at which zooxanthellae moved out of (or were expelled from) colonies maintained under control light levels. Despite low expulsion rates, the number of zooxanthellae in dark treated S. pistillata was lower than that in control (25 % full sunlight) colonies. This suggests

that other sinks for zooxanthellae within this experiment exist. Without knowing the precise effects of darkness on zooxanthellae after expulsion, however, one cannot assume that the zooxanthellae "missing" from the populations in situ (and not found outside the corals) were necessarily not expelled.

The respiration rates of colonies kept in small containers for 10 days were significantly higher than that of colonies not kept in small containers. The reason for this difference is not clear but may relate to such factors as the reduced circulation or oxygen levels (at night), or to increased bacterial colonization of colony surfaces weakened by the stress of being confined. Respiratory rates also increased as incubation light intensity decreased. The respiration rate of corals has been reported to vary with depth; corals from shallower habitats have higher respiration rates than corals from deeper habitats (Wetthey & Porter 1976, Redalje 1976, Davies 1980, Zvalinski et al. 1980, Porter et al. 1984, McCloskey & Muscatine 1984, Gattuso 1985). Cnidarian respiration rates are also influenced by the rate of food intake (Fitt & Pardy 1981; Fitt et al. 1982) and by previous periods of photosynthetic activity (McCloskey & Muscatine 1984, Edmunds & Davies 1988), the prevailing hypothesis being that increased respiration rates are increased due to increased respiratory substrate levels following feeding or photosynthesis. These results contrast with those found in this study, and may be due to the different experimental approaches. McCloskey & Muscatine (1984), for example, collected data on the respiratory rates of corals at night, with corals being exposed to their natural (shade or light) conditions by day. In the present study, however, the respiratory rate at different maintenance light levels was measured before and after the corals were exposed to saturating irradiances for the determination of light saturated photosynthetic rates.

(ii) The effect of high temperature and reduced salinity.

Exposing S. pistillata and S. hystrix to seawater at a reduced salinity of 30 ppt at several temperatures did not effect any biomass or metabolic variable measured in this study. Harriot (1983) did not find any evidence of salinity decreasing below 30 ppt during the 1982 bleaching event around Lizard Island, and in fact found evidence to the contrary; rainfall immediately preceding the occurrence of bleaching was below average (Harriot 1985). Reducing salinity even further (to 23 ppt) caused death, without any reduction in the population density of zooxanthellae within 48 h (Hoegh-Guldberg, unpublished data). It is concluded, therefore, that exposure to reduced salinity for 4 - 10 d does not induce bleaching characteristic of that seen for S. pistillata or S. hystrix around Lizard Island.

Increasing the maintenance temperature of S. pistillata and S. hystrix had a dramatic effect on both biomass and respiration (Table II). Corals heated up to 34°C in an aerated tank died within 8 h. Corals survived exposure to 32° and 30°C water although the mortality increased considerably with increased temperature. The temperature sensitivity of reef corals is well known (Gardiner 1903, Wood-Jones 1910, Mayer 1918, Yonge & Nichols 1931 b , Kinsman 1964, Coles et al. 1976). Excellent data for the existence of geographical variation in temperature tolerance has also been reported by Coles et al. (1976). They found that corals from Enewetak (average water temperature c.a. 28.5°C) could survive a 10 h exposure to 35.6° while most corals from Hawaii (average water temperature c.a. 24.5°C) died when water temperatures were raised to 32.4°C. The upper limit for S. pistillata and S. hystrix at Lizard Island appears to be about 33°C, which is similar to that reported for Hawaiian corals. As water temperatures at Lizard Island vary from 24°C to 29°C (N. Quinn, pers. comm.), the data collected during this study compares well with the data of Coles et al. (1976).

Coral colonies exposed to 32°C had visibly paled by the end of 4 h. Pale color was primarily due to low numbers of zooxanthellae and was not due to reduced pigment per zooxanthella. Soon after exposure to elevated temperatures, zooxanthellae in S. pistillata and S. hystrix began moving from the area between the polyps and began accumulating in the areas immediately adjacent to the pharynx of each polyp, as reported by other researchers for heat-stressed corals (Yonge & Nichols 1931 b, Coles & Jokiel 1978). In our study, the rate at which zooxanthellae were expelled from corals was temperature dependent, and increased dramatically as corals were heated to temperatures of 30° or more. Thirty minutes after the beginning of exposure to seawater at 32°C the specific expulsion rates of S. pistillata and S. hystrix rose to levels 100 times that of colonies incubated at 27°C. After 3 h of exposure, the specific expulsion rate was 500 - 1000 times that measured for colonies incubated at 27°C, and 3 to 10 times higher than the rate of production of new zooxanthellae ($0.012 \pm 0.0019 \text{ d}^{-1}$ and $0.0557 \pm 0.0169 \text{ d}^{-1}$, for S. pistillata and S. hystrix respectively). This observation supports the speculations of a large number of workers (Mayer 1918, Yonge & Nichols 1931 b, Goreau 1964, Jokiel & Coles 1977, Coles & Jokiel 1978, Egana & Disalvo 1982, Roberts 1987, 1988) that the major reason for the paling of corals following exposure to elevated water temperature is the increased expulsion of zooxanthellae that occurs at these higher temperatures.

The respiratory rates of colonies exposed to elevated temperatures were several times those of colonies exposed to 27°C. Light saturated rates of photosynthesis were less consistently affected by maintenance temperature. Light saturated photosynthetic rates were unaffected in S. hystrix and were increased for S. pistillata at 30°C relative to rates at 27° and 32°C. $P_{c \text{ g max}}/r_c$ ratios decreased as temperature increased. This was primarily due to the temperature dependent increase in respiration and a decrease in the number photosynthetically active cells per surface area as the zooxanthellae were expelled. Although this study reflects 'residual' effects of temperature on zooxanthellae biomass and

oxygen metabolism, there is some similarity of these trends with those found by Coles and Jokiel (1977). These authors found a rapid decrease in $P_{c\ g\ max}/r_c$ as the measurement temperature increased, and used this to explain the increased mortality of corals as temperature increases. The residual effects of temperature found in this study may reflect the induction of respiratory acclimation to high temperature. However, it is equally possible that the elevated rates of respiration reflect the increased colonization by bacteria of coral surfaces weakened by temperature stress. More work is required before the meaning of these residual effects of temperature can be understood.

Four d after a 7 h exposure to 32°C, *S. pistillata* and *S. hystrix* still showed elevated respiratory rates and higher than normal specific expulsion rates. Despite this, polyps were completely expanded by the end of the first day of after the removal of the temperature stress. Twenty three d later, however, the mean number of zooxanthellae per surface area had increased and respiratory rates decreased back to control levels. These trends were also reflected in the $P_{c\ g\ max}/r_c$ ratio which had recovered to levels seen prior to the onset of temperature stress.

The question of why corals bleach in nature is still unanswered. However, it is clear from this study that measuring the amount of photosynthetic pigment per zooxanthella and the population density of zooxanthellae in bleached corals can offer new insight on issues ranging from the time course of bleaching events to the similarity or difference of mass bleaching events in nature. The response of *S. pistillata* and *S. hystrix* to elevated temperature matches the speculations of several field studies that have proposed that elevated sea temperatures are responsible for causing the expulsion of zooxanthellae from reef corals (Table II). Contrary to the effect of elevated temperature, reducing the salinity of seawater to 30 ppt or exposing *S. pistillata* and *S. hystrix* to sudden increases in solar

irradiation does not induce the mass expulsion of zooxanthellae. Despite this, exposure to elevated light can result in corals which appear bleached but are not low in numbers of zooxanthellae. There is little evidence, therefore, that reduced salinity or elevated solar irradiance are instrumental in causing the rapid expulsion of zooxanthellae from S. pistillata and S. hystrix, as has been characterized for recent mass bleaching events.

ACKNOWLEDGMENTS

Support for this project was provided by a Lizard Island/Australian Museum Bicentenary Fellowship. We would also like to acknowledge the ready assistance of Dr.s B.L. Kojis and N.J. Quinn, and staff of Lizard Island Research Station. Additional support was provided by NSF #OCE 8510518 to L. Muscatine.

REFERENCES

Atwood, D.K., J.C. Sylvester, J.E. Corredor, J.M. Morell, A.

Mendez, W.J. Nodal, B.E. Huss & C. Foltz, 1988. Sea surface temperature anomalies for the Caribbean, Gulf of Mexico, Florida Reef Track and the Bahamas considered in the light of the 1987 regional coral bleaching event.

Proc. Assoc. Is. Mar. Lab. Carib. Vol. 21, p. 47.

Causey, B.D., 1988. Observations of environmental conditions preceding the coral bleaching event of 1987.

Proc. Assoc. Is. Mar. Lab. Carib. Vol. 21, p. 48.

Causey, B.D., J.C. Halas, J.H. Hudson & W.C. Jaap, 1988. Zooxanthellae expulsions in Florida reefs during 1987.

Proc. Assoc. Is. Mar. Lab. Carib. Vol. 21, p. 51.

Coles, S.L. & P.L. Jokiel, 1977. Effects of temperature on photosynthesis and respiration in hermatypic corals.

Mar. Biol., Vol. 43, pp. 209-216.

Coles, S.L. & P.L. Jokiel, 1978. Synergistic effects of temperature, salinity and light on the hermatypic coral Montipora verrucosa. Mar. Biol., Vol. 49, pp. 187-195.

Coles, S.L., P.L. Jokiel & C.R. Lewis, 1976. Thermal tolerance in tropical versus subtropical Pacific reef corals.

Pacif. Sci., Vol. 30, pp. 159-166.

Davies, P.S. 1980. Respiration in some Atlantic reef corals in relation to vertical distribution and growth form.

Biol. Bull. Woods Hole Mass. Vol. 158, pp. 187-194.

Drew, E.A., 1972. The biology and physiology of algal-invertebrate symbiosis. II. The density of algal cells in a number of hermatypic hard corals and alcyonarians from various depths. J. Exp. Mar. Biol. Ecol., Vol. 9, pp. 71-75.

Edmunds, P.J. & P.S. Davies, 1988. Post-illumination stimulation of respiration rate in the coral Porites porites. Coral Reefs Vol. 7(1), pp. 7-9.

Egana, A.C. & L.H. DiSalvo, 1982. Mass expulsion of zooxanthellae by Easter Island corals. Pacif. Sci., Vol. 36, pp. 61-63.

Fankboner, P.V. & R.G.B. Reid, 1981. Mass expulsion of zooxanthellae by heat-stressed corals: a source of food for giant clams? Experientia, Vol. 37, pp. 251-252.

Fisk, D.A. & T.J. Done, 1985. Taxonomic and bathymetric patterns of bleaching in corals, Myrmidon Reef, QLD. Proc. 5th Int. Coral Reef Symp., Vol. 6, pp. 149-154.

Fitt, W.K. & R.L. Pardy, 1981. Effects of starvation, and light and dark on the energy metabolism of symbiotic and aposymbiotic sea anemones, Anthopleura elegantissima. Mar. Biol., Vol. 61, pp. 199-205.

Fitt, W.K., R.L. Pardy & M.M. Littler, 1982. Photosynthesis, respiration and contribution to community productivity of the symbiotic sea anemone, Anthopleura elegantissima

(Brandt, 1835).

J. Exp. Mar. Biol. Ecol., Vol. 61, pp. 213-232.

Gardiner, J.S., 1903. The fauna and geography of the Maldive and Laccadive Archipelagoes. Vol. 1. At the University Press, Cambridge.

Gattuso, J.-P., 1985. Features of depth effects on Stylophora pistillata, an hermatypic coral in the Gulf of Aqaba (Jordan, Red Sea).

Pro. 5th Int. Coral Reef Symp., Vol. 6, pp. 95-100.

Glynn, P.W. 1984. Widespread coral mortality and the 1982-83 El Nino warming event. Environ. Conserv., Vol. 11, pp. 133-146.

Goenaga, C., V. Vicente & R. Armstrong, 1988. Aposymbiosis in Puerto Rican zooxanthellate cnidarians.

Proc. Assoc. Is. Mar. Lab. Carib. Vol. 21, p. 49.

Goreau, T.F. 1964. Mass expulsion of zooxanthellae from Jamaican reef communities after hurricane Flora.

Science, Vol. 145, pp. 383-386.

Harriot, V.J., 1983. Reproductive ecology and population dynamics in a scleractinian coral community. Ph.D. thesis, James Cook University of North Queensland, Australia.

Harriot, V.J., 1985. Mortality rates of scleractinian corals before and during a mass bleaching event.

Mar. Ecol. Prog. Ser., Vol. 21, pp. 81-88.

Hartree, E.F., 1972. Determination of protein: a modification of the Lowry method that gives linear photometric response. Analyt. Biochem., Vol. 48, pp. 422-427.

Hitchman, M.L., 1978. Measurement of dissolved oxygen. Chemical Analysis. A series of monographs on analytical chemistry and its applications. John Wiley and sons, New York, Vol. 49, pp. 200-210.

Hoegh-Guldberg O., 1988. A method for determining the surface area of corals. Coral Reefs, Vol. , pp. .

Hoegh-Guldberg O. & G.J. Smith, 1989. The effect of symbiont population density and external nutrients on the biomass and oxygen flux of the reef corals Seriatopora hystrix (Dana, 1846) and Stylophora pistillata (Esper, 1797).

Submitted to Mar. Ecol. Prog. Ser.

Hoegh-Guldberg, O., L.R. McCloskey & L. Muscatine, 1987. Expulsion of zooxanthellae by symbiotic cnidarians from the Red Sea. Coral Reefs, Vol. 5, pp. 201-204.

Jaap, W.C. 1979. Observations on zooxanthellae expulsion at Middle Sambo Reef, Florida Keys.

Bull. Mar. Sci., Vol. 29, 414-422.

Jeffrey, S.W. & G.F. Humphrey, 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton.

Biochem. Physiol. Pfl., Vol. 167, pp. 191-194.

Jokiel, P.L. & S.L. Coles, 1974. Effects of heated effluent on hermatypic corals at Kahe Point, Oahu.

Pacif. Sci., Vol. 28, pp. 1-18.

Jokiel, P.L. & S.L. Coles, 1977. Effects of temperature on the mortality and growth of Hawaiian reef corals.

Mar. Biol., Vol. 43, pp. 201-208.

Kleppel, G.S., R.E. Dodge & C.J. Reese, 1988. Changes in pigmentation associated with bleaching in stoney corals.

Submitted to Science.

Kinsman, D.J.J., 1964. Reef-coral tolerance of high temperatures and salinities. Nature (London), Vol. 202, pp. 1280-1282.

Lasker, H.R., E.C. Peters & M.A. Coffroth, 1984. Bleaching of reef coelenterates in the San Blas Islands, Panama.

Coral Reefs, Vol. 3, pp. 183-190.

Marsh, J.A., 1970. Primary productivity of reef-building calcareous red algae. Ecology, Vol. 51, pp. 255-263.

Marquardt, D.W., 1963. An algorithm for least-squares estimation of nonlinear parameters.

J. Soc. ind. appl. Math., Vol. 11, 431-441.

Mayer, A.G., 1918. Ecology of the Murray Island coral reef.

Pap. Dep. Mar. Biol. Carn. Inst. Washington, Vol. XIX, pp. 1-47.

McCloskey, L.R. & L. Muscatine, 1984. Production and respiration in the Red Sea coral Stylophora pistillata as a function of depth. Proc. R. Soc. London Ser. B, Vol. 222, pp. 215-230.

Mickel, T.J., L.B. Quetin & J.J. Childress, 1983. In Polarographic oxygen sensors. Aquatic and physiological applications. ed. E. Gnaiger and H. Forstner. Springer-Verlag, Berlin, Heidelberg and New York.

Muscatine, L., 1973. Nutrition of corals. In Biology and Geology of Coral Reefs, ed. A.O. Jones and R. Endean, Vol. 2(1), pp. 77-115; New York, London, Academic Press.

Muscatine, L., 1980. Productivity of Zooxanthellae. In Primary productivity in the sea. (Falkowski, P.G. ed.) pp. 381-402. New York: Plenum Publishing Corp.

Oliver, J., 1985. Recurrent seasonal bleaching and mortality of corals on the Great Barrier Reef.

Proc. 5th Int. Coral Reef Congress, Vol. 4, 201-206.

Porter, J.W., L. Muscatine, Z. Dubinsky & P.G. Falkowski, 1984. Primary production and photo-adaptation in light- and shade-adapted colonies of the symbiotic coral, Stylophora pistillata.

Proc. R. Soc. London Ser. B, Vol. 222, pp. 161-180.

Redalje, R., 1976. Light adaptation strategies of hermatypic corals. Pacif. Sci., Vol. 30, p 212.

Reese, C.J., G.S. Kleppel & R.E. Dodge, 1988. The physiological implications of bleaching of corals off southeast Florida. Proc. Assoc. Is. Mar. Lab. Carib. Vol. 21, p. 66.

Roberts, L., 1987. Coral bleaching threatens Atlantic reefs. Science, Vol. 238, pp. 1228-1229.

Roberts, L., 1988. Corals remain baffling. Science, Vol. 239, p.14.

Smith, G.J. & O. Hoegh-Guldberg, (1987). Variation in the growth rate of zooxanthellae with coral host colony size is not controlled by changes in the duration of cytokinesis.

E.O.S. Vol. 68, p 1724.

Trench, R.K., 1974. Nutritional potentials in Zoanthus sociatus (Coelenterata, Anthozoa).

Helgolander wiss. Meeresunters., Vol. 26, pp. 174-216.

Trench, R.K., 1979. The cell biology of plant-animal symbiosis. Ann. Rev. Plant Physiol., Vol. 30, pp. 485-532.

Vaughan, T.W., 1914. Reef corals of the Bahamas and of southern Florida. Yb. Carnegie Institute Washington., No. 13, pp. 222-226.

Yonge, C.M. & A.G. Nichols, 1931 a. Studies on the physiology of corals. V. The effect of starvation in light and in darkness on the relationship between corals and zooxanthellae.

Sci. Repts. Gr. Barrier Reef Exped. Vol. 1(7), pp. 177-211.

Yonge, C.M. & A.G. Nichols, 1931 b. Studies on the physiology of corals. IV. The structure, distribution and physiology of the zooxanthellae.

Sci. Repts. Gr. Barrier Reef Exped. Vol.1(6), pp. 135-176.

Wetley, D.S. & J.W. Porter, 1976. Sun and shade differences in productivity of coral reefs.

Nature (London), Vol. 262, pp.281-282.

Wilkerson, F.P., G. Muller-Parker & L. Muscatine, 1983.

Temporal patterns of cell division in natural populations of endosymbiotic algae.

Limnol. Oceanogr., Vol. 28, pp. 1009-1014.

Wood-Jones, F., 1910. Corals and atolls. Lovell Reeve and Co., London.

Zvalinski, V.I., V.A. Letetkin, E.A. Titlyanov & M.G. Shaposhnikova, 1980. Photosynthesis and adaptation of corals to irradiance. Photosynthetica, Vol. 14, pp. 422-430.

FIGURE LEGENDS

Figure 1. Conditions for maintaining coral fragments (colonies): a) Coral colonies attached to small pieces of microscope slides

and secured to racks made of pvc piping. b) Tubs of seawater that housed racks of coral colonies.

Tubs received a continuous supply of fresh seawater and were illuminated by sunlight reduced to 25 % by black plastic mesh.

Figure 2. Fluorescence due to chlorophyll per zooxanthella and per area, and the number of zooxanthellae per area of colonies maintained for 10 days under 3 different light regimes. Bars are full sunlight (A), control (25 % full sunlight, B) and darkness (C). Shown are means (\pm S.E.M., $n = 3$) and statistically significant differences from control treatments ($p < 0.05$) are indicated by asterisk.

Figure 3. Photosynthesis-irradiance responses of Stylophora pistillata and Seriatopora hystrix. Hyperbolic functions [$P_{c \text{ net max}} = P_{g \text{ c max}}(1 - e^{-al/P_{g \text{ c max}}}) + r_c$] were fitted using repeated iterations and the Marquardt (1963) algorithm for non-linear regression. Each curve represents data from three colonies of each species maintained for 4 weeks in the tubs shown in Fig. 1b.

Figure 4. Gross photosynthetic and respiratory rates for Stylophora pistillata maintained for 1 d and 10 d in full sunlight, 25 % full sunlight (control) and darkness. Shown are 95 % confidence intervals ($n = 3$ colonies).

Figure 5. Specific expulsion rates for Stylophora pistillata under different light regimes. a) Specific expulsion rate of S. pistillata maintained for 10 days in full sunlight, 25 % full sunlight (control) and darkness. Shown are $x \pm$ SEM ($n = 4$). b) Specific expulsion rates of S. pistillata and S. hystrix in the period immediately following sudden exposure to full sunlight. Bar indicates darkness due to the fall of night. Shown are $x \pm$ SEM ($n = 4$).

Figure 6. Seriatopora hystrix after exposure to 32°C seawater for 7 h and recovery at 27°C for 6 h. a) surface of coral colony b) bolus of zooxanthellae collecting in pharynx of polyp. Each polyp is approximately 1 mm across.

Figure 7. a) Population density and b) fluorescence per zooxanthella for Stylophora pistillata and Seriatopora hystrix

maintained at three different temperatures for 4 d. Shown are $\bar{x} \pm \text{SEM}$ ($n = 3$).

Figure 8. a) Gross photosynthetic rates of colonies at light saturation ($P_{c\ g\ max}$), b) colony rates of respiratory rates (r_c) and c) $P_{c\ g\ max}/r_c$ ratios for Stylophora pistillata and Seriatopora hystrix maintained at different temperatures for 4 d. Shown are $\bar{x} \pm \text{SEM}$ ($n = 4$).

Figure 9. Specific expulsion rates for Stylophora pistillata and (b) Seriatopora hystrix maintained at 30°C and 32°C. Shown are $\bar{x} \pm \text{SEM}$ ($n = 4$). Control rates (at 27°C) were $1.60 \pm .54\ d^{-1}$ and $3.65 \pm 1.93\ d^{-1}$ for S. pistillata and S. hystrix respectively.

Figure 10. Population density (top panel), and $P_{c\ g\ max}$, r_c and $P_{c\ g\ max}/r_c$ (bottom panel) for colonies of Stylophora pistillata at various times after exposure to seawater at 32°C for 7 h. Shown are $\bar{x} \pm \text{SEM}$ ($n = 3$).

Figure 11. Population density (top panel), and $P_{c\ g\ max}$, r_c and $P_{c\ g\ max}/r_c$ (bottom panel) for colonies of Seriatopora hystrix at various times after exposure to seawater at 32°C for 7 h. Shown are $\bar{x} \pm \text{SEM}$ ($n = 3$).