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9A Coral bleaching and climate change

Bleaching of the fire corals *Millepora*

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Abstract. The physiology of bleached and unbleached hydrocorals *Millepora* spp. was investigated in Bermuda, Jamaica and the Florida Keys. The fluorescence parameters measured for *M. complantata* decreased by about 50% and the density of *Symbiodinium* by 35% over 2-4 d at 32°C compared to 26°C, indicating that *Symbiodinium* were damaged by the rise in temperature, and subsequently released from the hydrocorals. Bleached and unbleached *M. alcicornis* and *M. complanata* showed normal fluorescence values with a mitotic index of about %15 in *M. complanata*, suggesting that bleached colonies were recovering and growing quickly. The concentration of chlorophyll-*a* within *Symbiodinium* cells did not significantly differ over short-term heat-stress experiments. Loss of *Symbiodinium* from the hosts resulted in decreased photosynthesis and an increase in host respiration rate that correlated with temperature, such that the respiration rate at 32.5-35°C was over 6 times that at 25°C for *M. alcicornis*. Such an increase in metabolic cost associated with a decrease in symbiont density is similar to what happens to scleractinian corals exposed to high seawater temperatures during coral bleaching events and threatens the survival of both hydrocorals and scleractinian reef corals.

Keywords: Hydrocorals, *Millepora*, bleaching

Introduction

There is relatively little information about the hydocoral *Millepora* spp. in the literature on coral reefs (Lewis 1989). Much of the scientific interest in the "fire corals" has centered on the toxicology associated with their nematocysts and effects on humans (i.e. Iguchi et al. 2008). Ecologically, hydrocorals contribute to the structure of reefs and provide protection for mobile species within their branches.

Individual *Millepora* colonies are fast growing (Loya 1976), the white tips of their branches indicating few symbiotic algae (*Symbiodinium* spp.) in that region. In the Caribbean, *Millepora* spp. are found with *Symbiodinium* ITS-types A4a, A3 or a closely related group of types B1, B31a, B32, B37, or B23 of *Symbiodinium* (Finey et al. 2010). High metabolism, including high growth rates, is thought to be facilitated by *Symbiodinium* productivity and the host's ability to feed heterotrophically (Goreau et al.1979, Schonwald et 1997).

Bleaching, or the expulsion of symbiotic dinoflagellates during high-temperature stress, has compromised populations of hydrocorals during the past 30 years (Marshall and Baird 2000, Loya et al. 2001). *Millepora complanata* and *M. alcicornis* were among the first species to exhibit bleaching in the greater Caribbean during the 1973, 1982-3, 1987-88 El Niños (Jaap 1979, 1985; Williams and Bunkley-Williams 1988). Some colonies appeared not to bleach (turn white) during the 1995 El Niño (Fig. 1), whereas all of the remaining colonies appeared to bleach during the 1997-1998 El Niño. Hydrocoral bleaching susceptibility is thought to result partially from their high-light habitat (Banaszak et al 2003) and thin host-tissue content (Loya et al. 2001).

Millepora spp. typically have porous skeletons that make it impossible to remove all of the tissue using common methods applied to scleractinian coral (e.g. water-pik or air brush; Edmunds 1999). In addition, *Symbiodinium* can extend throughout the gastrodermal tissue in hydrocoals, creating internal micro-niches for the symbionts from shaded to very bright light.

While *Millepora spp.* are commonly found on sub-tropical and tropical reefs world-wide, research presented here took place in the Caribbean Sea and surrounding areas. Here I pool data collected between 1991-2012 from Jamaica, Bermuda and the Florida Keys documenting bleaching of *M. alcicornis* and *M. complanata* during experimental heat stress. I conclude that the bleaching patterns in *Millepora* spp. are very similar to those in scleractinian reef corals.

Material and methods

Pieces of *M. alcicornis* and *M. complanata* colonies (4-10 cm^2) were collected from shallow reefs (<3m) off Jamaica, Bermuda, and the Florida Keys between 1991-2012 for laboratory experiments and maintained in fresh seawater before and during experiments. All of the procedures involved short-term (days) exposures of hydrocorals to temperatures 2-7°C higher than ambient seawater temperatures. Temperatures were maintained using water baths, and irradiance was provided by a slide projector at approximately 1000 µmol photons m^{-2} s^{-1}.

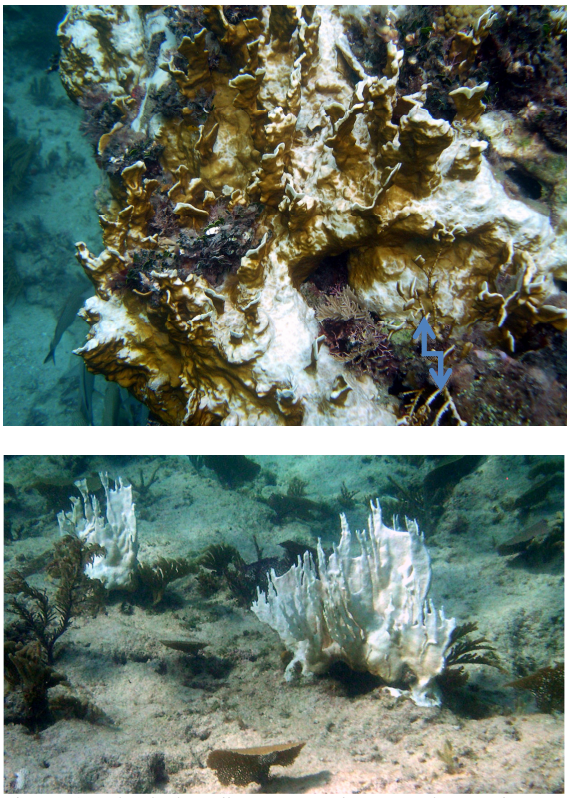

Figure 1. Bleached *Millepora complanata* and *M. alcicornis* (arrows). Photos taken in Puerto Morelos, Mexico by Dustin Kemp.

Photochemical performance of *Symbiodinium* was monitored using saturation pulses from a pulse-amplitude modulated fluorometer (Diving PAM, Walz). Both effective quantum yield ($\Delta F/F_m'$, in the light) and maximum photosynthetic efficiency (F_v/F_m, in the dark) of photosystem II (PSII) of *Symbiodinium* were measured *in situ*. Hydrocoral tissue was removed from both sides of skeleton using a waterpik (Johannes, Weibe 1970). Tissue was homogenized and *Symbiodinium* densities were quantified using a haemocytometer (≥ 6 fields) from replicate samples before and after exposure to high temperature. Mitotic indices were determined from the percent of 1000 symbionts in the doublet stage from *M. complanata* collected approximately every 4h. Symbionts expelled from *M. alcicornis* maintained in 25 and 30°C seawater were collected on days 1,2,3,5, and 6 on glass microfiber filters which were homogenized in 100% acetone and chl *a* measured using a spectrophotometer. To estimate how many *Symbiodinium* were left after waterpiking, the skeleton was pulverized for some hydrocorals, extracting chlorophyll to determine the number of symbionts and a standard curve of chlorophyll-*a* per algal cell.

Surface areas of processed hydrocorals were determined by the aluminum foil to surface area formula (Marsh 1970). Symbiodinium chlorophyll-*a* was extracted in 90% acetone and then calculated from formulas in Jeffery and Humphrey (1975) to determine chlorophyll-*a* cell^{-1}. Photosynthesis and respiration rates were determined from oxygen measurements (YSI Model 5300 Biological Oxygen Meter) in the light or dark in a known volume of 0.22 Millipore-filtered seawater surrounding pieces of *M. alcicornis*. Q_{10} temperature coefficients (rate of respiration 10°C higher: control temperature) were calculated for *M. alcicornis*.

Data was analyzed by comparison of means (s.d.), linear regression or by ANOVA, with significant differences determined at the level of $p<0.05$.

Results

Fluorescence of symbionts

Potential photoinhibition and photodamage resulting in reduced photosynthesis was determined from fluorescence readings on the surface of the hydrocoral. *M. complanata* exposed to 32°C had significantly lower fluorescence ratios than those exposed to an ambient 26°C [$p < 0.05$, with and without the photosynthetic inhibitor 3-(3,4-dichlorophenyl)- ,1-dimethylurea, DCMU, Fig. 2]. The effective quantum yield $\Delta F/F_m'$ decreased faster at 32°C compared to the relatively stable $\Delta F/F_m'$ at 26°C (Fig. 3). Dark acclimated F_v/F_m (maximum quantum yield of PSII) of bleached and unbleached *M. complanata* was 0.60±0.02 (n=3) and for unbleached *M. alcicornis* 0.605±0.02 (n=3), taken in February 1996 in Jamaica when seawater temperatures were at the lowest of the season (ca. 26°C) and the hydrocorals were recovering.

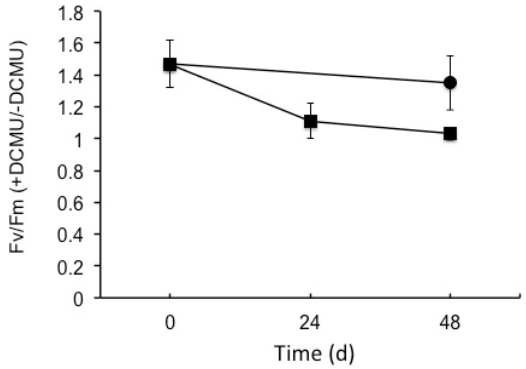

Figure 2. Ratio of fluorescence of the hydrocoral *Millepora complanata* from the Florida Keys; the maximum photosynthetic efficiency (F_v/F_m) with and without the photosynthetic inhibitor DCMU (3-(3,4-dichlorophenyl)- ,1-dimethylurea) maintained at 26°C (circles) and 32°C (squares) over time (d), ± s.d., n=5

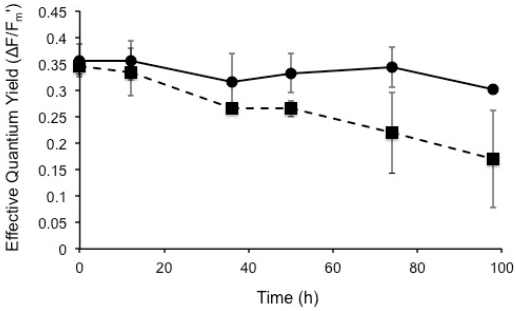

Figure 3. Fluorescence (effective quantum yield = $\Delta F/F_m'$) of the hydrocoral *Millepora complanata* from Jamaica maintained in 26ºC (circles) and 32ºC (squares) over time (hours). Error bars = s.d., n=6

Densities of symbionts

Millepora alcicornis lost approximately one-third of their *Symbiodinium* when maintained at 32°C for 2 days, compared to a 26°C control (Fig. 4). *M. complanata* lost

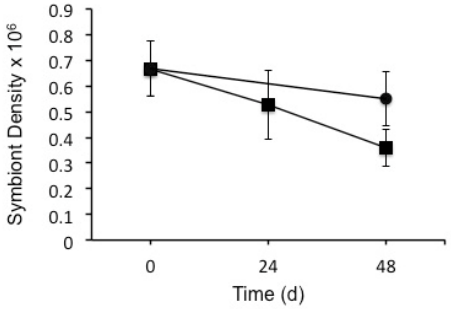

Figure 4. Density of symbiotic algae (*Symbiodinium* sp.) in the hydrocoral *Millepora alcicornis* from Bermuda vs. time in h at seawater temperatures of 26ºC (circles) and 32ºC (squares). Error bars = s.d., n=6

approximately half of their symbionts over a 4 day period at 32°C (0.83±0.12 to 0.39±0.14 x 10^6 cm^{-2}, n=6) compared to 26°C (0.83±0.12 to 0.62±0.14 x 10^6 cm^{-2}, n=6). Symbionts were released from *M. alcicornis* at 1.51±0.63% per day at 30°C and 0.99±0.45% per day at 25°C (ANOVA; Tukey post hoc analysis; $p<0.01$, n=4). These findings are probably an underestimate, assuming that chlorophyll-*a* per symbiont remains constant (Fig. 5).

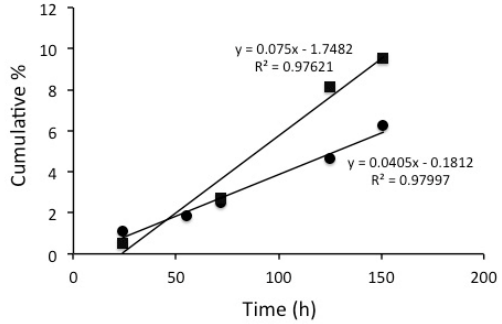

Figure 5. The cumulative percent of symbiotic algae (*Symbiodinium* sp.) released by the hydrocoral *Millepora alcicornis* from Bermuda maintained at 25ºC (circles) and 32ºC (squares) over time (h). Lines are significantly different ($p<0.05$, linear regression).

Chlorophyll-*a* per symbiont cell in *M. complanata* was not significantly different ($P>0.05$) at 0,1 and 2 days of exposure to 32°C seawater (Fig. 6). When *M.*

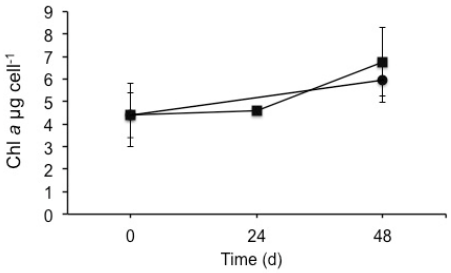

Figure 6. Chlorophyll-*a* per cell of *Symbiodinium* sp. from the hydocoral *Millepora complanata* from Jamaica maintained in 26ºC (circles) and 32ºC (squares) over time (days). Error bars = s.d., n=6

complanata was pulverized after waterpiking, only 0.43% of the symbionts remained in the skeleton (0.0022±00.20 x 10^6 cells out of 5.45±0.81 x 10^6 total symbionts, n=6).

Symbiodinium in *Millepora*

The genetic identity of *Symbiodinium* was not determined for this study, as many of the experiments were conducted between 1987 and 1996 when methods for *Symbiodinium* identification were not available. However, the relatively large diameter of *Symbiodinium* sp. found in *M. complanata* in Jamaica (12.9±1.4 µm, n=20, range 11.3-16.3 µm) is more like Clade A3 or A4a (LaJuenesse 2001). The mitotic index of *Symbiodinium* in *M. complanata* from Jamaica peaked in the morning at a relatively high 15.5±2.6% (s.d., n=4) compared to reef corals (Fig. 7, Fitt et al. 1993).

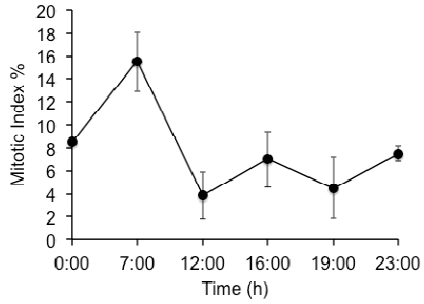

Figure 7. Mitotic index (MI±s.d.) over a diurnal period of *Symbiodinium* sp. from the hydrocoral *Millepora complanata* in Jamaica at ca. 26ºC seawater temperature. Error bars = s.d., n=4-5

Photosynthesis and respiration

Gross photosynthesis (P^G=net oxygen production + oxygen consumption by respiration, data not shown) and respiration rates (R, Fig. 8) of *M. alcicornis* were determined and plotted against temperatures between 25° and 30°C. P^G:R decreased significantly (Fig. 9,

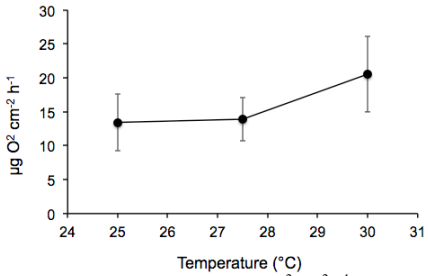

Figure 8. Respiriation rates (ug $O^2cm^{-2}h^{-1}$) vs. temperature (°C) for the hydrocoral *Millepora alcicornnis* from Bermuda (± s.d., n=6). The respiration rate is significantly greater at 30 than 27.5 or 25°C.

p<0.05, ANOVA), and R increased significantly (Fig. 8; p<0.05, ANOVA) with temperature. The calculated Q_{10} of *M. alcicornis* respiration rates

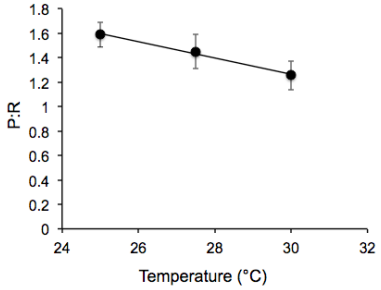

Figure 9. The photosynthesis:respiration ratio (P:R) of the hydrocoral *Millepora alcicornis* (± s.d., n=6) vs. temperature (°C). Experiments conducted in Bermuda.

ranged between 1.35-1.90 between 25.0-32.5°C, then increased to over 6 between 32.5-35.0°C, whereas the symbionts maintained a Q_{10} of about 2 between 26.0-30.0°C before increasing to greater than 4 at 30.0-32.5°C (Fig. 10).

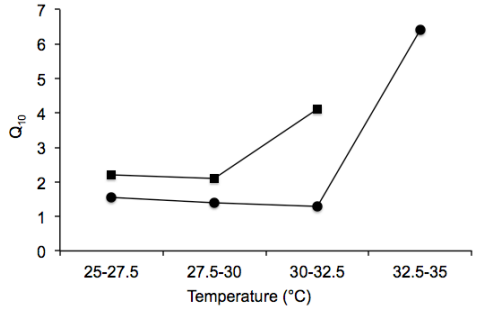

Figure 10. The Q_{10} of the hydrocoral *Millepora alcicornis* (circles) and their *Symbiodinium* (squares) vs. temperature range (°C). Experiments conducted in Bermuda.

Discussion

The heat-stress experiments on *Millepora* spp. demonstrate that these hydrocorals exhibit a bleaching response that is similar to reef corals. Fluorescence ratios (+DCMU/-DCMU) show that photosynthesis was dramatically reduced at 32°C (Fig. 2), with the effective quantum yield ($\Delta F/F_m'$) declining to levels indicating severe photoinhibition probably as a result of photodamage (Fig. 3; Fitt et al. 2001). The maximum quantum yield (F_v/F_m) of *M. complanata* and *M. alcicornis* during the winter (non-bleaching conditions) showed that the symbiotic algae were not stressed and were probably recovering from the past summer's high temperatures. The stress signified by the fluorescence ratios of *Millepora* spp. maintained at 32°C manifests itself in the decline of symbiont density (Fig. 4) and an increase in symbiotic dinoflagellates released over time (Fig. 5). This is a similar pattern to that seen in scleractinian corals experiencing the same level of heat stress (i.e. Warner et al. 1999).

Millepora complanata do not show reductions of chlorophyll-*a* per zooxanthella in short-term laboratory experiments (Fig. 5). This is also similar to some corals exposed to bleaching temperatures in short-term bleaching experiments. These short-term experiments are probably not long enough to see the reductions in chlorophyll-*a* that have been observed in long-term laboratory experiments (i.e. Glynn and D'Croz 1990) or in the time-course of bleaching of pigments from cells that is often seen in corals in nature (i.e. Porter et al. 1989).

Most thermally stressed *M. complanata* take more than 14.5 weeks to recover their normal complement of zooxanthellae after a bleaching event, if they recover at all (Goreau 1964, Jaap 1979, Glynn and De Weerdt 1991). A high mitotic index is characteristic of recovery of symbionts in corals after bleaching (Fitt et al. 1993). The mitotic index of *Symbiodinium* was high (ca. 15%, Fig. 7), which is typical of symbionts from non-stressed *Millepora* sp. (Schonwald et al. 1997, Banaszak et al. 2003) and other hydroids (Fitt 2000). The high growth rate of the symbionts is correlated with the high growth rates of the hydroid (Fitt 2000), probably in addition to growth during recovery.

The density of symbionts in hydrocorals was lower at 32°C than it was at 26°C (Fig. 4), which is significant since it indicates a general decline in photosynthate available for transfer to the host. In other words, the nutritional benefit of *Symbiodinium* to the host decreases during bleaching (Fig. 9). Respiration rates of *M. alcicornis* were significantly greater (p<0.05) at 30.0°C than a 25.0 or 27.5°C (Fig. 8). Increasing metabolism is a common response of invertebrates to increased temperatures (Schmidt-Nielen 1996). A Q_{10} of 2.0 indicates that the respiration rate doubles over a 10°C range. A Q_{10} above 3.0 in Figure 10 indicates physiological stress (Dodds 2007), suggesting a higher metabolic rate at the higher temperatures, requiring an increase in the energy budget. If the symbiotic algae are unable to

supply the required energy to the host, the hydocoral may die (Glynn and De Weerdt 1991), a scenario that has been proposed for scleractinian corals (Thornhill et al. 2011). High Q_{10} is thought to occur just before death in cnidarians exposed to high temperatures. The Q_{10} of zooxanthellae increases substantially between 30.0-32.5°C, while the intact animals experience similar Q_{10} values at slightly warmer temperatures of 32.5-35.0°C (Fig. 10). This indicates that *Symbiodinium* in *Millepora* spp. have a lower tolerance to high temperature stress than the hydocoral host, similar to that seen in reef corals.

In conclusion, the results indicate that *Millepora* hydrocorals and their symbionts respond to high temperature stress in a pattern similar to that seen in scleractinian corals. The survival of this unique group of hydrocorals is threatened by increased sea surface temperatures associated with the frequency of episodes of El Niño.

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