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Responses of coral hosts and their algal symbionts to thermal heterogeneity

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Abstract The effects of high temperature on tropical corals have been studied extensively, but little is known of their response to high frequency thermal fluctuations that are common in many reef habitats. To better understand how oscillatory temperatures affect corals, *Pocillopora meandrina* and *Porites rus* from the lagoon of Moorea were used to test the effects of fluctuating temperatures on coral physiology. Corals were incubated at a stable temperature (28 ºC), or diurnally fluctuating temperatures (26 – 32 ºC) that simulate the conditions in the lagoon. Their response was assessed through dark-adapted maximum quantum yield (F_V/F_M), *Symbiodinium* density, and holobiont respiration. Following incubations, *Symbiodinium* density and F_V/F_M were depressed 25 - 42%, and 20 - 23%, respectively, for both species under fluctuating compared to the stable temperature treatment. Additionally, respiration was 55-63% lower in the fluctuating compared to the stable conditions, although this effect was only found in one of two replicate experiments. These results demonstrate that corals can be strongly affected by diurnal temperature fluctuations in lagoon habitats.

Key Words: temperature, fluctuation, physiology, *Pocillopora meandrina*, *Porites rus*

Introduction

To date, most research on the response of scleractinian corals to thermal stress has considered steady exposures to high temperatures, even though there is evidence that many reefs experience oscillatory thermal regimes with diurnal ranges as great as 9 ºC (Lee et al. 1999, Leichter et al. 2006, Craig et al. 2001). Indirect evidence suggests that such variability can have important effects, for instance, as shown by the association between the variance in temperature and the severity of coral bleaching (McClanahan et al. 2007). The lack of attention to the effects of fluctuating temperatures on corals has important implications, as most efforts to predict the ecological consequences of coral bleaching, as well as the limits of thermal tolerance for corals (e.g., Hoegh-Guldberg 1999), are based on a tacit assumption that temperature is constant on a scale of days to weeks.

In order to understand the full range of responses of corals to thermal stress, studies are required that evaluate the impacts of fluctuating temperature, in addition to the more frequently studied effects of constant temperature regimes. The purpose of this study was to examine the effects of thermal fluctuations that are ecologically relevant for the lagoon of Moorea (i.e., a 6 ºC change within 24 h) on coral physiology. Specifically, we tested the hypothesis that rapid fluctuations in temperature have strong effects on the physiology of the cnidarian host and the *Symbiodinium* symbionts of two species of coral.

Material and Methods

To characterize the thermal regime in the lagoon of Moorea, seawater temperature at ≈3 m depth was measured using loggers (0.02 ºC resolution and 0.0017 Hz sampling frequency) between 2005 and 2006 (Fig. 1a). These temperature records revealed diurnal fluctuation with daytime heating and nighttime cooling (Fig. 1b). To test the hypothesis that such fluctuations affect coral physiology, two consecutive experiments were completed using common lagoon corals -- *Pocillopora meandrina* and *Porites rus* -- in April (trial 1) and May (trial 2) of 2006. Manipulations took place near the end of the Austral summer, which corresponds to a period of higher seawater temperature (Fig. 1a). Corals were collected from 2-3 m depth, and prepared as nubbins. The nubbins were allowed to recover from the collection process for 18-24 h in flowing seawater, and then were placed in the treatment tanks 3 d before the experiments began to allow the corals to acclimate to the ambient conditions at 28 ºC.

Experimental manipulations were used to create a comparison of diurnal thermal fluctuation, and a steady temperature (≈28.6 ºC, which served as the control and simulated mean ambient lagoon conditions during April and May 2006). The fluctuating treatment repeatedly exposed corals to

≈26ºC and ≈32ºC by transferring the nubbins daily from a tank at ≈26 ºC to a tank at ≈32 ºC at 07:00, and back to the ≈26 ºC tank at 19:00, which mimicked extreme diurnal temperature fluctuations in the lagoon. A procedural control was created by moving additional nubbins in and out of a tank held at the ambient temperature (≈28.6 ºC). Nubbins remained submerged in a beaker of seawater during all transfers, and care was taken not to touch the tissue.

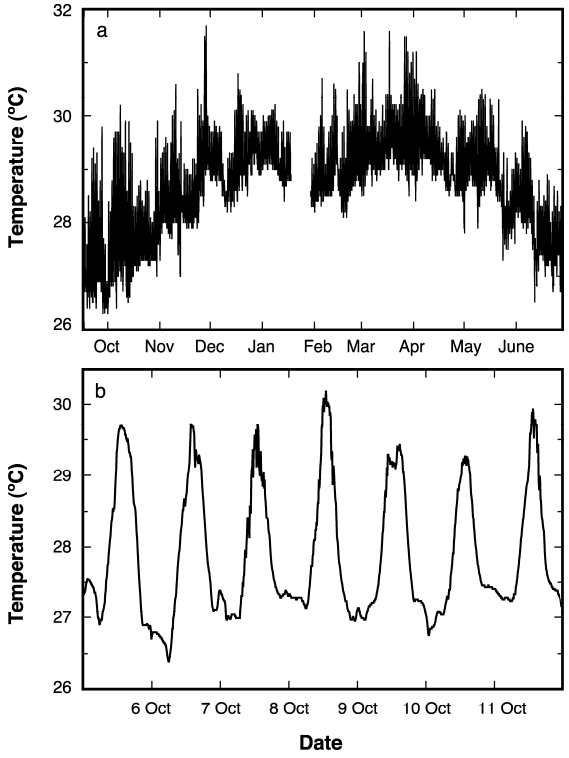

Figure 1. Representative record of seawater temperature in the lagoon (≈3 m depth, 10 min sampling interval) of Moorea from September 2005 – June 2006. (a) The 10-month record shows seasonal variation as well as high frequency changes occurring on a scale of hours-days; the ticks on the abscissa correspond to the first day of each month. (b) Seawater temperature from a 7 day period of strong diurnal fluctuation (~3.5 ºC) during October 2005. The temperature maxima correspond to midday solar warming, and the minima occur between 19:30 – 07:30; the ticks on the abscissa correspond to 0:00 hours.

The manipulations took place in an outdoor microcosm that housed 135 L tanks, each with a separate chiller, heater, and pump, and filled with seawater that was refreshed daily (~20% d^{-1}). To simulate light levels at the collection depth, the tanks were shaded to ≈1,000 µmol photons m^{-2} s^{-1} at noon. Light levels were recorded daily inside each tank, and the temperature was measured every 10 min using loggers (HOBO® and StowAway®, accuracy ±0.2 ºC). Each experiment began at 12:00 on day 1 and ended at 19:00 on day 8, and measurements began immediately following.

Response Variables- Samples sizes differed among the three variables, and also by treatment (Fig. 2). To examine the effect of the treatments, the response of the *Symbiodinium* was measured as maximum dark-adapted quantum yield of PSII (F_V/F_M) and their population density. The performance of the holobiont was measured as dark respiration of the intact coral, which reflects the combined effects of the cnidarian host and the algal symbiont.

F_V/F_M was recorded using a Diving-PAM (Walz, GmbH), and all measurements were made on the final night of the experiment, following 2.5 h of darkness. To determine F_V/F_M, each coral was removed from its treatment tank in a darkened beaker filled with seawater (500 mL), and a single measurement was made using the 5.5 mm fiber-optic probe of the Diving-PAM. A single measurement was used per nubbin to reduce the effect of the measuring light on subsequent F_V/F_M measurements (i.e. subsequent measurements result in significantly increased F_M, $t=2.67$, $df=166$, $p<0.05$). To minimize variation in F_V/F_M due to location of the probe, measurements were taken ~2 cm below the branch tips.

To quantify the *Symbiodinium*, the skeleton was removed from the fixed tissue (5% formalin) by decalcification (10% HCl). The tissue layer resulting from decalcification was homogenized using an ultrasonic dismembrator (Fisher 15-338-550) fitted with a 3.2 mm diameter probe (Fisher 15-33867). The homogenate was suspended in freshwater, and six replicate counts of *Symbiodinium* were completed using a hemocytometer. An aliquot of the slurry was dried at 60 ºC to determine biomass, and the *Symbiodinium* population density was expressed in units of cells mg^{-1}.

Dark respiration was measured as O_2 consumption within a PVC chamber (0.89 L), which was attached to a submersible pump in a closed circuit. As estimated by photographing hydrated brine shrimp eggs, flow speed inside the chamber was 23.1 ± 1.0 cm s^{-1} (mean ± SE, n=15), which approximated the mean flow speeds in the lagoon during the experimental period (R. Carpenter, pers. comm.). A single coral nubbin was placed in the seawater-filled darkened chamber, and the sealed system immersed in a 135 L tank to regulate temperature. The reduction in O_2 concentration due to respiration was measured at ambient temperature (28.2 ± 0.1 ºC [n = 24] in trial 1 and 27.8 ± 0.1 ºC [n = 26] in trial 2) over 25 min using a fiber optic probe (FOXY-R, Ocean Optics). All measurements were completed between 80 and 100% O_2 saturation to avoid O_2-dependent effects on respiration, and the rates of O_2 consumption were corrected for control values obtained by measuring O_2 consumption in a seawater-filled chamber, with no coral. Respiration rates were normalized to surface

area of the coral (obtained by aluminum foil) and reported in units of μmol O_2 cm^{-2} min^{-1}.

Statistical Analysis- The experiment was designed to be analyzed with a mixed-model nested ANOVA in which repeated experiments were used to strengthen the interpretation of treatment effects, but this design was precluded by a trial effect driven by weather-related differences in light and temperature. Due to significant differences in light regime and temperature between trials (data not shown), data were analyzed separately by trial using two-way Model I ANOVAs in which treatment and species were fixed effects, and F_V/F_M, *Symbiodinium* density, and dark respiration were dependent variables. The random placement of nubbins in each tank every day removed potential biases attributed to position effects. Data were assessed for normality and homoscedasticity using a graphical analysis of residuals, and were transformed where necessary (log transformations for respiration).

Results

Light levels within the tanks were significantly higher in trial 2 compared to trial 1 (data not shown), and corals were exposed to a slightly greater diurnal range of temperatures (i.e., a lower minimum and higher maximum) in trial 1 (5.9 ºC) than in trial 2 (5.1 ºC).

Maximum Quantum Yield (F_V/F_M)- For *Pocillopora meandrina, F_V/F_M* over both trials ranged from 0.67-0.76 in the control treatments, and 0.50-0.69 in fluctuating temperature treatments. For *Porites rus*, F_V/F_M in both trials ranged from 0.62-0.71 and 0.51-0.67 in control and fluctuating temperature treatments, respectively. F_V/F_M was significantly affected by treatment ($F_{1,55}$ =162.79, $p < 0.001$) and species ($F_{1,55}$ = 1.178, $p < 0.01$) in trial 1, but there was no interactions of the main effects ($p > 0.05$). A similar outcome of lesser magnitude was observed in trial 2, with F_V/F_M affected significantly by treatment ($F_{1,54}$ = 20.20, $p < 0.001$) and species ($F_{1,54}$ = 39.10, $p < 0.001$), but not the interaction between the two ($p > 0.05$) (Fig. 2a and 2b). These decreases of F_V/F_M corresponded in trial 1 to a reduction in the fluctuating treatment of 23% for *P. meandrina*, and 20% *P. rus*, when compared to the control treatment. The same pattern was present in trial 2, but the reductions of F_V/F_M in the fluctuating treatment were smaller (4% and 3% for *P. meandrina* and *P. rus*, respectively).

Symbiodinium Density- At the conclusion of the two experiments, most of the corals in both of the treatments were slightly pale in color (relative to freshly collected corals). The *Symbiodinium* density changed significantly for both species after exposure to the fluctuating temperatures in trial 1 ($F_{1,26}$ = 5.64, $p < 0.03$), but not in trial 2 ($p > 0.05$) (Fig. 2c and 2d). Mean *Symbiodinium* densities were reduced ~42% in *Porites rus* and ~25% in *Pocillopora meandrina* in the fluctuating treatments of trial 1. Neither the species, nor the interaction of species and treatment, were significant for either trial ($p > 0.05$).

Respiration- Mean dark respiration ranged from 0.017 – 0.046 μmol O_2 cm^{-2} min^{-1} for *Pocillopora meandrina,* and from 0.012 – 0.032 μmol O_2 cm^{-2} min^{-1} for *Porites rus* (Fig. 2e and 2f). Respiration rate was reduced significantly in the fluctuating treatment in trial 1 ($F_{1,9}$ = 13.751, $p < 0.01$) for both *P. meandrina* and *P. rus*, and differed significantly between species ($F_{1,9}$ = 4.955, $p = 0.05$). There was no significant treatment x species interaction for trial 1, or for any factor or interaction in trial 2. The reduction in respiration due to the fluctuating treatment in trial 1 corresponded to a 63% and 55% decrease for *P. meandrina* and *P. rus*, respectively, when compared to the control treatment. In trial 1, the respiration of *P. rus* was ~32% lower than *P. meandrina* at ambient temperature, and ~43% lower in the fluctuating treatment, but in trial 2, the respiration rates were statistically identical in the two corals.

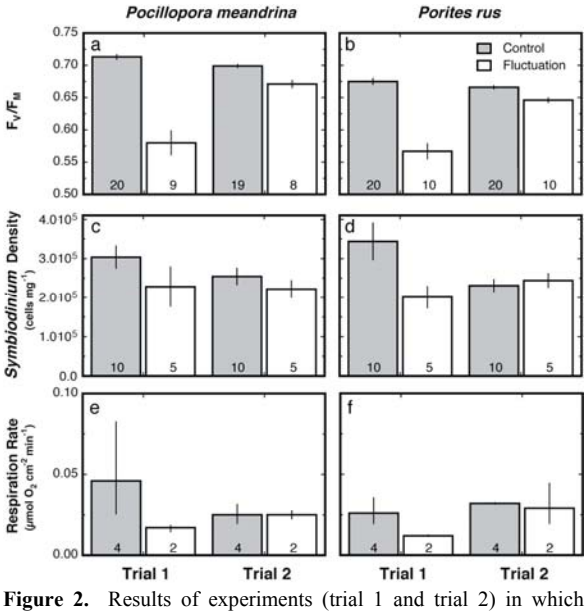

Figure 2. Results of experiments (trial 1 and trial 2) in which corals were exposed to a steady temperature of 28 ºC (control) and a fluctuating treatment consisting of 12 h exposures to ~26 and 32 ºC; (a,b) maximum dark-adapted quantum yield of PSII (F_V/F_M), (c,d) *Symbiodinium* density, and (e,f) dark respiration. Results are displayed as mean ± SE for F_V/F_M and *Symbiodinium* density, and as mean ± SD for back-transformed values for respiration; sample sizes shown within each bar.

Discussion

On many tropical reefs, corals can experience rapid changes in water temperature of a sizable magnitude within minutes to seconds (Leichter et al. 2006, Craig et al. 2001). This study presents some of the first evidence that such temperature oscillations have significant effects on corals, with the direction of response for the measured variables suggesting that the outcome is detrimental to the coral. *Symbiodinium* population density and their function (F_V/F_M), as well as the respiration of the holobiont, all were lower as a result of exposure to fluctuating temperatures, compared with corals exposed to steady temperatures. If corals respond differently not only to a consistent exposure to high temperatures, but also to the thermal oscillations, quantification of their response to fluctuations in temperature will be critical to accurately predict the response of coral communities to the changes in seawater temperature that are forecast for the next century (IPCC 2007).

In the present study, exposure of corals to fluctuating temperatures resulted in a lower efficiency of photophysiology of the algal symbionts, relative to those maintained under control conditions. The magnitude of the decrease of F_V/F_M in *Pocillopora meandrina* and *Porites rus* is likely indicative of chronic irreversible photodamage to PSII of the algal symbionts (Brown et al. 2000, Fitt et al. 2001), and the evidence for this conclusion is twofold. First, there was no sign of the depressed F_V/F_M values returning to the initial levels throughout the experiment (data not shown), as would be expected with reversible photodamage (Fitt et al. 2001). Second, the loss of photosynthetic capacity (F_V/F_M) is juxtaposed with the loss of *Symbiodinium* from the host tissue (Fitt et al. 2001), which is usually interpreted as the terminal phase of coral bleaching, and occurs in association with damage to the symbiont through oxidative stress (Lesser 1997, Fitt et al. 2001). Unfortunately, one limitation of the current study was an inability to compare the effects of the fluctuating temperature treatment and a steady exposure to each of the extreme temperatures, which is necessary to determine whether oscillatory temperatures elicit a response that is the sum of the effects of the component temperatures, or a response of a different nature.

Fluctuating temperatures reduced the F_V/F_M of *Pocillopora damicornis* and *Porites rus* by 19% and 16%, respectively. This reduction is comparable to the decrease in F_V/F_M of *Acropora palifera* and *Pocillopora damicornis* (~8 - 25%) during a natural thermal bleaching event at Heron Island in March 1998, which followed a 2-3 d period over which the seawater temperature was unusually variable (~26-34 ºC within 24h) (Jones et al. 2000). The reductions in *Symbiodinium* density as a result of fluctuating temperatures in the present study (~25 - 42%) fall within the range of losses reported during natural bleaching events (Brown et al. 1995, Jones et al. 2000, Edmunds et al. 2003). Along with clear treatment effects, there was also a significant difference between F_V/F_M for the two species. Determining why these species differ was beyond the scope of this study -- although it is intriguing to speculate that they harbor different taxa of *Symbiodinium* (Rowan 2004) -- but importantly, the absence of a treatment x species interaction for F_V/F_M indicates that *P. rus* and *Pocillopora meandrina* responded to the treatments in similar ways.

In the present study, the fluctuating temperature treatment resulted in decreased respiration compared to the control conditions for both species, at least in one of the two trials. While within the range of values reported for congeners (Edmunds and Davies 1986, Rex et al. 1995), respiration rates characteristically increase with rising temperature. A likely explanation for the lower respiration rate in the fluctuating treatment in the first trial is that it reflected the consequences of a decrease in quantity of photosynthetically fixed carbon translocated to the animal, which is thought to stimulate the metabolism of the host (Szmant and Gassman 1990), either due to a decrease in *Symbiodinium* function (as assessed by F_V/F_M), or a reduction in their population density (Szmant and Gassman 1990, Castillo and Helmuth 2005). In this situation, lower rates of respiration are likely to reflect a negative effect of temperature.

A second explanation for the decline in respiration rates in the first trial, is that it reflects a beneficial response to fluctuating temperatures (Barshis et al. 2008), and therefore could be described as acclimatization. In this situation, lower rates of respiration are arguably a positive response to temperature. However, it is challenging to interpret the implications of changes in metabolic rates, particularly in a mutualistic symbiosis where the holobiont respiration reflects the sum of the parts. Clearly, a more detailed analysis of the causes of the change in respiration rates as a result of fluctuating temperatures in the present study (as well as why it was absent in the second trial) are necessary.

In the present study, the differences between trials in the response of both corals to fluctuating temperature were unexpected given the considerable effort expended to replicate the treatment conditions. A reason for the differences in response to the fluctuating treatment between trials may be temporal variation in environmental history of the experimental corals (i.e., differences in light and temperature prior to collection). While corals were collected from the same location and depth for both trials, the additional

time (11 d) spent at local environmental conditions by the corals used in trial 2 may have had an effect on their ability to respond to extreme fluctuations. As noted previously the second trial was completed following the peak temperatures and therefore it is possible the differences in response in trial 2 may be a result of lower stress on the corals, due to the associated decrease in temperature in the field.

A greater magnitude (~16% greater) of diurnal temperature variation occurred in trial 1 compared to trial 2, and most interestingly was associated with a stronger response to the fluctuations in host and symbiont, at significantly lower light levels. This finding suggests that thermal fluctuation may act in a threshold fashion with regards to the magnitude of the fluctuations. Above this threshold, the effects of large temperature fluctuation at reduced light levels are more severe than the effects below this threshold, where small temperature fluctuation may confer protection against the effects of higher light levels, which are generally thought to accentuate thermal bleaching (Brown 1997, Hoegh-Guldberg 1999).

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