

Experimental effect of temperature and sedimentation on bleaching of the two Red Sea corals *Stylophora pistillata* and *Acropora humilis*

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Abstract. Ammar MSA, Obuid-Allah AH, Al-Hammady MAM. 2013. Experimental effect of temperature and sedimentation on bleaching of the two Red Sea corals *Stylophora pistillata* and *Acropora humilis*. *Nusantara Bioscience* 5: 75-85. At 26°C (the control sample), the loss of zooxanthellae by each of the two studied corals *Stylophora pistillata* and *Acropora humilis* was very low. Cell viability of the two studied corals was similar at 26 and 29°C, but depicted a sharp decline of zooxanthellae lost at 31°C through time. As the temperature increased to 35°C, the loss of zooxanthellae from each host increased both with time and temperature elevation. The coral *A. humilis* had a higher decrease in its zooxanthellae densities than *S. pistillata* at the same treatment. Bleaching temperature threshold was 33°C or less for the two species *S. pistillata* and *A. humilis* where 51% of their zooxanthellae were lost after 24 h of exposure. In samples exposed to sediment concentration of 0.1 mg/cm²/L, zooxanthellae densities of *A. humilis* and *S. pistillata* did not show any decrease after 1 day. However, after 1 day of exposure to 0.5 mg/cm²/L, zooxanthellae densities were significantly different from those of the controls. Increases in sediment concentration to 1 mg/cm²/L caused a decrease in zooxanthellae densities that vary greatly over time. Measurements of zooxanthellae densities of *A. humilis* and *S. pistillata* at this stage revealed a highly significant difference between exposed and control sample. At 1 g/cm²/L, the number of zooxanthellae lost from *A. humilis* was higher than those lost from *S. pistillata* at same time. It is suggested that the normal sedimentation rate for *A. humilis* and *S. pistillata* to be in an order of 1 mg/cm²/L or less.

Key words: *Acropora humilis*, bleaching, Red Sea corals, sedimentation, *Stylophora pistillata*, temperature

Abstrak. Ammar MSA, Obuid-Allah AH, Al-Hammady MAM. 2013. Pengaruh perlakuan suhu dan sedimentasi terhadap pemutihan dua jenis karang dari Laut Merah *Stylophora pistillata* dan *Acropora humilis*. *Nusantara Bioscience* 5: 75-85. Pada suhu 26°C (kontrol), hilangnya zooxanthellae dari dua jenis karang yang diteliti *Stylophora pistillata* dan *Acropora humilis* sangat rendah. Viabilitas sel kedua jenis karang tersebut serupa pada suhu 26 dan 29°C, tetapi pada suhu 31°C terjadi penurunan tajam zooxanthellae sejalan dengan bertambahnya waktu. Pada saat suhu meningkat menjadi 35°C, hilangnya zooxanthellae dari masing-masing inang meningkat sejalan dengan bertambahnya waktu maupun suhu. Karang *A. humilis* mengalami penurunan kepadatan zooxanthellae yang lebih tinggi daripada *S. pistillata* pada perlakuan yang sama. Batas suhu pemutihan karang adalah 33°C atau kurang untuk kedua jenis karang, *S. pistillata* dan *A. Humilis*, dimana 51% dari zooxanthellae-nya hilang setelah 24 jam paparan. Pada sampel yang terpapar sedimen dengan konsentrasi 0,1 mg/cm²/L, kepadatan zooxanthellae *A. humilis* dan *S. pistillata* tidak menunjukkan penurunan apapun setelah 1 hari. Namun, setelah 1 hari paparan sedimen 0,5 mg/cm²/L, kepadatan zooxanthellae secara signifikan berbeda dari kontrol. Peningkatan konsentrasi sedimen 1 mg/cm²/L menyebabkan penurunan kepadatan zooxanthellae yang sangat bervariasi dari waktu ke waktu. Pada tahap ini, pengukuran kepadatan zooxanthellae *A. humilis* dan *S. pistillata* menunjukkan perbedaan yang sangat signifikan antara yang terpapar dan kontrol. Pada konsentrasi sedimen 1 g/cm²/L, jumlah zooxanthellae yang hilang dari *A. humilis* lebih tinggi daripada yang hilang dari *S. pistillata* pada waktu yang sama. Hal ini menunjukkan bahwa, tingkat sedimentasi normal untuk *A. humilis* dan *S. pistillata* berada pada kisaran 1 mg/cm²/L atau kurang.

Kata kunci: *Acropora humilis*, pemutihan, karang Laut Merah, sedimentasi, *Stylophora pistillata*, suhu

INTRODUCTION

Bleaching (loss of pigmentation by corals) is a widespread phenomenon in coral reef ecosystems. Despite this, the underlying of some forms of bleaching are poorly understood. This study explores the conditions that induced bleaching in two zooxanthellae, reef coral species *Acropora humilis* and *Stylophora pistillata* collected from the middle-reef in front of National Institute of Oceanography and Fisheries (NIOF), Hurghada Branch.

Environmental extremes, such as high temperature or irradiance, damage the symbionts' photosynthetic machinery, resulting in the overproduction of oxygen radicals. This leads to eventual cellular damage in the symbionts and/or their hosts and can lead to the expulsion of symbionts and the eventual break down of the symbiosis (Lesser 2006). The loss of zooxanthellae (and/or a reduction in their pigment concentrations) as a result of this process is referred to as "bleaching" (Brown et al. 1999; Fitt et al. 2000).

An important factor affecting coral communities is the widespread, periodic bleaching of coral colonies (Celliers and Schleyer 2002). Coral bleaching results in the breakdown of a mutualistic symbiosis that is essential for the survival of corals, since the polyp receives a substantial part of its energy from the zooxanthellae (Muller-Parker and D'Elia 1997), and any disruption of this relationship will affect photosynthetic potential, coral growth, reproductive output and may eventually kill the coral (Richmond 1997).

Because of the intensity of recent bleaching events and associated mortalities, bleaching is considered by most reef scientists to be a serious and relatively new challenge to the health of world's coral reef (Celliers and Schleyer 2002; Nesa and Hidaka 2009; Obura 2009; Miller et al. 2011). The widespread bleaching of corals on individual reefs has largely been correlated with elevated sea temperature (Celliers and Schleyer 2002; Mc Clanahan et al. 2005).

Seasonal cycles in the quantum yields of chlorophyll fluorescence of corals have also been observed (Warner et al. 2002), revealing seasonal acclimatization in solar irradiance and seawater temperature. Moreover, Nesa and Hidaka (2009) detected a negative correlation between survival time and the zooxanthellae density of tissue balls at 31°C in both *Fungia* sp. and *P. divaricata*. Elevated temperature was found to significantly reduce the amount of zooxanthellae in primary polyps (Anlauf et al. 2010). Exposure to elevated temperatures reduces the photosynthetic rate of zooxanthellae and predisposes their photosynthetic apparatus to further damage (Bhagooli and Hidaka 2004).

Reef-building corals and their zooxanthellae had shown two broad ways that may be able to cope with elevated temperature (Clark 1983). Firstly, by micro-adaptive combinations of symbiotic algae (Rowan et al. 1997), secondly by biochemical defense mechanisms, such as the induction of heat shock proteins (Sharp et al. 1997; Ammar and Mueller 2001). Despite their ability to acclimatize to their thermal limits, reef-building corals do not appear to have acclimatized to the rapid increase in sea temperature over the past 20 years.

Sedimentation is among the factors that lead to escape of symbiotic zooxanthellae from the host coral (Dubinsky and Stambler 1996). Fabricius (2005) regarded sedimentation as an increasing threat to coral reefs. The impacts associated with sedimentation and sediment burial include reduced photosynthesis and increased respiration (Philipp and Fabricius 2003; Weber et al. 2006), tissue mortality (Lirman and Manzello 2009), reduced growth (Lirman and Manzello 2009), and reduced fertilization, larval survivorship, and recruitment (Babcock and Smith 2000).

The degree of coral mortality and bleaching depends on the amount of sediment in the coral communities following a tropical storm in the tropical Atlantic (Nowlis et al. 1997). Burial of corals by sediments for 20 hours resulted in increased discoloration of coral tissue, after 68 hours of burial, up to 98% of the tissue bleached in the first days, about 50% of this tissue disappeared subsequently and bare coral skeleton became exposed or were covered with algae (Wesseling et al. 1999). Cruz-Pinio et al. (2003) found that high sedimentation rates, low light availability and

anthropogenic influence lead to cellular damage and deteriorated coral skeletal density.

Turbidity reduces light levels, photosynthetic potential and possibly coral growth rates (Yentsch et al. 2002; Anthony and Hoegh-Guldberg 2003) while elevated net sedimentation rates increase abrasion and smothering (Fabricius 2005), inhibiting coral growth and reef accretion. In contrast, Palmer et al. (2010) found that nearshore environments directly influenced by fluvial sediments and dominated by terrigenous sedimentation are generally considered marginal for coral reef growth. The energetic costs of sediment clearing can be considerable (Riegl and Branch 1995), and the inability to clear sediments exposes corals to further stress as anoxic conditions under sediments can cause tissue bleaching and subsequent mortality (Weber et al. 2006).

For the purposes of this study, the release of algal symbionts at various temperatures and sediment concentrations ("bleaching response") was studied on the two reef corals *S. pistillata* and *A. humilis* using the protocols of Hoegh-Guldberg and Smith (1989) and Anlauf et al. (2010). The focus here on increasing seawater temperature reflects the choice of an environmental factor that is an integral component of global climate change effects and is tractable to experimental manipulation. Moreover, both temperature and sedimentation have a well-established effect on coral bleaching.

MATERIALS AND METHODS

Two experiments were carried out in the NIOF laboratories using the hermatypic coral *S. pistillata* and *A. humilis* in which corals were exposed to elevated water temperatures and different concentrations of sediments. Corals were collected from the middle reef in front of the National Institute of Oceanography and Fisheries (NIOF), Hurghada Branch, Egypt.

Experiments were conducted in open-topped glass aquaria, under controlled conditions. Seawater was aerated using air pumps and heated and recirculated using submersible pumps and aquarium heaters. Refrigerated coolers and refrigerated water-baths were used to control the temperatures to be within $\pm 0.3^\circ\text{C}$ of the desired level. Temperature readings were made every 5-15 min between 8.00 a.m. and 6.00 p.m. During the night, the readings were made only every 2 hours since the temperature was more stable. The temperatures were increased and decreased following the diurnal variation in water temperature in the field by approximately 6°C . Temperature in the field was between 29°C and 30°C at midday (at 3 m depth).

Small colonies of the two studied species were collected from individual colonies located at 3 m depth from the Middle reef, which is located 200 m offshore between the northern reef and the crescent reef, directly in front of NIOF, Hurghada, Red Sea. Samples were then transferred directly into glass aquaria supplied with air pumps for aeration.

Two corals for studying the effect of temperature were incubated at each temperature test (24, 29, 31, 33 and

35°C). Control samples were placed at room temperature (26°C). An air stone was placed in each aquarium for water circulation and the sample was put in a good light condition during the day for photosynthesis. Three colonies from each species were then taken after 6, 12 and 24 hours and their tissues were removed using seawater jet (Water Pik Teledyne) for counting the zooxanthellae and measuring the chlorophyll concentration. Zooxanthellae were counted using count Rafter cell and the chlorophyll concentration was measured by spectrophotometer method of Jeffrey and Humphrey (1975).

Branches for studying the effect of sediments were put in glass aquaria, exposed to 0.1, 0.5, 1 mg/cm²/L and 1g/cm²/L different concentrations of sediments, an air stone was placed in each aquarium for aeration in good lighting conditions, then three branches of each colony were taken after 1, 5 and 10 days for counting zooxanthellae and measuring chlorophyll concentration.

Biomass measurements

For zooxanthellae density, tissues were stripped from the skeletons (Plate 15) with a jet of recirculated 0.45 µm membrane filtered seawater using a water pikTM (Johannes and Wiebe 1970). The slurry produced from the tissue-stripping process was homogenated in a blender for 30s and the volume of homogenate was recorded. The number of zooxanthellae in 10 ml aliquots of homogenate was measured in triplicate by light microscope (X 400) using Count Rafter Cell. The total number of zooxanthellae per coral was measured after correcting the volume of homogenate. Zooxanthellae density was calculated as a number per unit surface area.

Zooxanthellae number / cm² = counted cells / cell surface area x cell depth x dilution

For chlorophyll analysis, 10-20 ml subsamples of the homogenate were filtered through Whatman GF/C 0.45 filters, which were then homogenized for 30s using a tissue homogenizer. Chlorophyll was extracted twice with 10 ml of acetone 90% for 24 h in darkness at -4 C°. Extracts were centrifuged at 6000 g^{rev/min} for 20 minutes to remove filter fiber from suspension and the supernatants read on a spectrophotometer at 630, 645, 665 and 750 wavelengths. Chlorophyll concentrations were calculated according to the equation of Jeffery and Humphrey (1975), as follow:

$$\text{Ch.a (mg m}^3\text{)} = 11.85D_{663-665} - 1.54 D_{647} - 0.08D_{630} \text{) } v^{-1} V^{-1}$$

D = absorbance at wavelength incubated by subscript, after correction by the cell to cell bank and subtraction of the cell-to-cell blank corrected absorbance at 750 nm

V = volume of acetone (ml)

I = cell (cuvette) length (cm)

V = volume of filtered water (L)

Spectrophotometric reading

When possible, the absorption spectra in the range of 630-750 nm were collected. Our spectrophotometer SPECORD M40 was connected with pc computer. Some of

the earlier data were registered on the paper. The length of cuvettes was chosen according to chlorophyll range (usually L 2cm). Surface area of the bare skeletons remaining after removal of tissue was measured independently using the paraffin wax technique (Stambler et al. 1991), by immersing the skeleton bar in hot wax; the mass of wax added to the skeleton bare was determined by weighing the skeleton bare before and after immersion. A relationship between change in mass and surface area was obtained by immersing a known surface area cubes in the wax.

RESULTS AND DISCUSSION

Experimental effect of elevated temperature on the population density of zooxanthellae of *A. humilis*

The changes observed in zooxanthellae densities lost from *A. humilis* indicate clearly increasing susceptibility to both elevated temperature and prolonged exposure. The control samples maintained at 26°C exhibited no variation in symbiont density at both the beginning and the end of the experiment. In samples exposed to 24°C, zooxanthellae densities were highly significantly different from those at the control sample (P < 0.01, HSD = 0.324), where zooxanthellae densities were 0.78±0.015x10⁶ cells/cm² after 6 hours, 0.77±0.014x10⁶ cells/cm² after 12 hours and 0.64±0.015x10⁶ cells/cm² after 24 hours. *A. humilis* at 29°C had lower counts of symbiotic algae (0.71±0.023 x10⁶ cells/cm² after 6 hours, 0.6±0.015x10⁶ cells/cm² after 12 hours and 0.55±0.015x10⁶ cells/cm² after 24 hours) within host tissue compared to control sample. Moreover, zooxanthellae densities of *A. humilis* were significantly different from those at the control sample (P < 0.01, HSD = 0.18). Increases in temperature to 31°C caused a decrease in zooxanthellae densities and vary greatly over time. Measurements of zooxanthellae densities at this stage revealed a highly significant difference between exposed and control sample (ANOVA, P < 0.01, HSD = 0.296). Where, zooxanthellae densities were 0.62±0.015 x10⁶ cells/cm², 0.5±0.015x10⁶ cells/cm², 0.43±0.015 x10⁶ cells/cm² after 6 hours, 12 hours and 24 hours respectively. At 33°C, the number of zooxanthellae lost from *A. humilis* was decreased to 38% (content after loss= 0.5±0.015x10⁶ cells/cm²) after 6 hours, 48% (content after loss= 0.42±0.015x10⁶ cells/cm²) after 12 hours and to 51% (content after loss= 0.4±0.015x10⁶ cells/cm²) after 24 hours compared to control sample. This indicates that the physiological state of zooxanthellae is clearly influenced by elevated temperatures and the duration of heat exposure. In samples exposed to 35°C, the density of zooxanthellae was (0.41±0.015x10⁶ cells/cm²). This represents a 50% decrease compared to controls after 6 hours. Samples exposed to 35°C and analyzed after 12 hours, showed a zooxanthellae density of (0.3±0.015x10⁶ cells/cm²) (63% decrease) compared to (0.81±0.0125x10⁶ cells/cm²) for controls. While after 24 hours the loss of zooxanthellae was about 75% (content after loss= 0.2±0.015x10⁶ cells/cm²) compared to the control sample (ANOVA, P < 0.01, HSD= 0.503).

Turkey's Studentized Rang Statistical Analysis (HSD) was applied to detect the distinct variance between means of zooxanthellae at a different time of exposure (Table 1). It revealed that the mean value of zooxanthellae densities after 6 hours of exposure was significantly different from those after 12 hours. However, the difference was highly significant between zooxanthellae densities after 12 hours of exposure and 24 hours. This was driven from the data that zooxanthellae densities after 6 hours exposure (0.638×10^6 cells/cm²) were higher than those after 12 hours (0.616×10^6 cell/cm²), which in turn were higher than those after 24 hours (0.503×10^6 cells/cm²).

Table 1. Turkey's studentized rang statistical analysis (HSD) for the experimental effect of changes in temperature (°C) on zooxanthellae density (10^6 cells/cm²) of *A. humilis* by using the sampling frequency as dependent variables.

	After 6 h. (0.638)	After 12 h. (0.766)	After 24 h. (0.503)
After 6 h. (0.638)			
After 12 h. (0.766)	0.128 (Sig)		
After 24 h. (0.503)	0.135 (Sig)	0.263 (H. Sig)	

Note: Number in parentheses = Zooxanthellae density (10^6 cells/cm²). Minimum significant difference 0.0102. H. = Highly significant differences. Sig. = Significant difference

Experimental effect of elevated temperature on chlorophyll contents of *A. humilis*

The amount of chlorophyll per zooxanthellae was inversely related to increased temperature. All chlorophyll concentrations showed a significant decrease with increased temperature ($P < 0.01$ for all cases). Control samples (26°C) had higher content of chlorophyll (2.1 ± 0.11 , 2.1 ± 0.07 and 1.99 ± 0.04 µg/cm² after 6, 12 and 24 hours, respectively) within host tissue compared to test samples. In samples exposed to 24°C, the content of chlorophyll was (2 ± 0.09 , 1.97 ± 0.04 and 1.91 ± 0.06 µg/cm² after 6, 12 and 24 hours respectively). Although the difference was statistically significant (ANOVA, $P < 0.01$, HSD = 0.108). Colonies immediately sampled after 6 hours exposure to 29°C, showed a slight decrease in chlorophyll contents (1.98 ± 0.0106 µg/cm²). While, chlorophyll contents in colonies collected after 12 hours were 1.9 ± 0.04 µg/cm², and after 24 hours were 1.87 ± 0.106 µg/cm². Colonies exposed to 29°C showed a significant difference relative to the control samples (ANOVA, $P < 0.01$, HSD = 0.15).

Measurements of chlorophyll contents at 31°C of exposure revealed a significant difference between exposed and control sample (ANOVA, $P < 0.01$, HSD = 0.42). However, chlorophyll contents were 1.82 ± 0.0104 µg/cm², 1.6 ± 0.08 µg/cm², 1.5 ± 0.02 µg/cm² after 6 hours, 12 hours and 24 hours respectively. An increase in temperature to 33°C caused an increase in the amount of chlorophyll lost from *A. humilis*. Chlorophyll content decreased from 2.1 ± 0.11 µg/cm² in controls to 1.43 ± 0.039 µg/cm² in exposed samples after 6 hours ($P < 0.01$, HSD = 0.73). An analyzed *A. humilis*, after 12 hours of exposure to 33°C, showed a chlorophyll content of 1.37 ± 0.03 µg/cm²

compared to 2.1 ± 0.07 µg/cm² in controls. While after 24 hours it was 1.21 ± 0.08 µg/cm². Exposure of *A. humilis* to 35°C reduced the content of chlorophyll sharply compared to the control sample. However, the difference was statistically highly significant ($P < 0.0001$, HSD = 1.166). About 48% of chlorophyll content was lost from *A. humilis* after 6 hours when incubated at 35°C (1.1 ± 0.13 µg/cm²), after 12 hours about 57% of chlorophyll content (0.9 ± 0.03 µg/cm²) was lost. While after 24 hours the loss of chlorophyll content was about 65% (0.7 ± 0.019 µg/cm²) relative to the control sample.

Turkey's Studentized Rang Statistical Analysis (HSD) was applied to detect the distinct variance between means of chlorophyll contents at different times of exposure (Table 2). It was revealed that, the mean value of chlorophyll contents after 6 hours of exposure was significantly different with those after 12 hours and 24 hours. Also the difference was significant between chlorophyll contents after 12 hours of exposure and 24 hours where the contents of chlorophyll after 6 hours exposure (1.74 µg/cm²) were higher than those after 12 hours (1.65 µg/cm²), which in turn were higher than those after 24 hours (1.53 µg/cm²).

In general, the physiological state of *Symbiodinium* is clearly influenced by elevated temperatures and duration of heat exposure.

Table 2. Turkey's studentized rang statistical analysis (HSD) for the experimental effect of changes in temperature (°C) on chlorophyll concentration (µg/cm²) of *A. humilis* by using the sampling frequency as dependent variables.

	After 6 h. (1.74)	After 12 h. (1.65)	After 24 h. (1.53)
After 6 h. (1.74)			
After 12 h. (1.65)	0.087 (Sig)		
After 24 h. (1.53)	0.206 (H. Sig)	0.11 (Sig)	

Note: Number in parentheses = Chlorophyll concentration (µg/cm²). Minimum significant difference 0.043 .H. = Highly significant differences. Sig. = Significant difference

Experimental effect of elevated temperature on the population density of zooxanthellae of *S. pistillata*

The number of zooxanthellae showed a significant decrease with increasing temperature (ANOVA, $P < 0.01$) and exposure time ($P < 0.01$), additionally there was interaction between temperatures and exposure time ($P < 0.01$). To detect the different effect of changes in temperatures on zooxanthellae densities after different exposure times, Turkey's Studentized Rang Statistical Analysis (HSD) was applied. It was shown that the mean value of zooxanthellae densities exposed to 24°C was significantly different from those in controls (HSD = 0.083). However, the control samples (26°C) had higher densities of zooxanthellae (0.83 ± 0.015 after 6 and 12 hours and $0.82 \pm 0.018 \times 10^6$ cells/cm² after 24 hours) within host tissue compared to test samples. In samples exposed to 24°C, the densities of zooxanthellae were (0.81 ± 0.012 ,

0.73±0.015 and 0.69±0.018x10⁶ cells/cm² after 6, 12 and 24 hours, respectively).

Zooxanthellae densities of *S. pistillata* at 29°C were also significantly different from those of the control sample (P < 0.01, HSD = 0.118). *Stylophora pistillata* had lower counts of symbiotic algae cells (0.76±0.015x10⁶ cells/cm² after 6 hours, 0.75±0.015 x10⁶ cells/cm² after 12 hours and 0.62±0.018x10⁶ cells/cm² after 24 hours) within host tissue compared to control sample. While, the difference between zooxanthellae densities at 31°C and those in control samples were highly significant (P < 0.01, HSD = 0.273), zooxanthellae densities at this stage were 0.66±0.015x10⁶ cells/cm², 0.53±0.015x10⁶ cells/cm², 0.47±0.015x10⁶ cells/cm² after 6 hours, 12 hours and 24 hours respectively.

Measurements of zooxanthellae densities at 33°C revealed a highly significant difference between the exposed and the control sample (ANOVA, P < 0.01, HSD = 0.373). Increases in temperature to 33°C caused a decrease in zooxanthellae densities and vary greatly over time. Zooxanthellae densities at this stage (33°C) were 0.51±0.015x10⁶ cells/cm², 0.44±0.015x10⁶ cells/cm², 0.41±0.015x10⁶ cells/cm² after 6 hours, 12 hours and 24 hours respectively. In samples exposed to 35°C, the density of zooxanthellae was (0.43±0.015x10⁶ cells/cm²). This represents a 48% decrease compared to the control sample after 6 hours. Exposure to 35°C for 12 hours, showed a zooxanthellae density of (0.35±0.015x10⁶ cells/cm²) compared to (0.83±0.015x10⁶ cells/cm²) in controls recording a decrease of 58%. While after 24 hours the loss of zooxanthellae was about 67% (content after loss= 0.27±0.015x10⁶ cells/cm²) relative to the control sample (ANOVA, P < 0.01, HSD= 0.476).

Turkey's Studentized Rang Statistical Analysis (HSD) was applied to detect the distinct variance between means of zooxanthellae at the time of exposure (Table 3). It was detected that the mean value of zooxanthellae densities after 6 hours of exposure was significantly different from those after 12 hours. However, the difference was highly significant between zooxanthellae densities after 12 hours and 24 hours of exposure. This revealed that zooxanthellae densities after 6-hour exposure (0.67x10⁶ cells/cm²) were higher than those after 12 hours (0.605x10⁶ cells/cm²) while zooxanthellae densities after 12 hours were higher than those after 24 hours (0.546x10⁶ cells/cm²).

Table 3. Turkey's studentized rang statistical analysis (HSD) for the experimental effect of changes in temperature (°C) on zooxanthellae density (10⁶ cells/cm²) of *S. pistillata* by using the sampling frequency as dependent variables.

	After 6 h. (0.666)	After 12 h. (0.605)	After 24 h. (0.546)
After 6 h. (0.666)			
After 12 h. (0.605)	0.061 (Sig)		
After 24 h. (0.546)	0.12 (Sig)	0.342 (H. Sig)	

Note: Number in parentheses = Zooxanthellae density (10⁶ cells/cm²). Minimum significant difference 0.01; H. = Highly significant differences. Sig. = Significant difference.

Experimental effect of elevated temperature on chlorophyll contents of *S. pistillata*

The amount of chlorophyll concentration showed a significant decrease with increasing temperature (ANOVA, P < 0.01) and prolonged exposure (P < 0.01). Additionally, there was interaction between the treatments. Control samples (26°C) had chlorophyll contents of (2.2±0.11, 2.1±0.088 and 2±0.015 µg/cm² after 6, 12 and 24 hours, respectively). The contents of chlorophyll in samples exposed to 24°C were (1.98±0.082, 1.94±0.051 and 1.9±0.018 µg/cm² after 6, 12 and 24 hours, respectively). Also, the difference between these samples was statistically significant (ANOVA, P < 0.01, HSD = 0.163). Six hours of exposure to 29°C showed a slight decrease in chlorophyll contents (1.84±0.056 µg/cm²). While, chlorophyll content in colonies exposed for 12 hours was 1.83±0.046 µg/cm², while it was 1.8±0.015 µg/cm² after 24 hours. Colonies exposed to 29°C showed a significant difference from the control samples (ANOVA, P < 0.01, HSD = 0.28).

Measurements of chlorophyll content at 31°C of exposure revealed also a significant difference between exposed and control sample (ANOVA, P < 0.0001 and HSD = 0.43). Also, chlorophyll contents were 1.7±0.016 µg/cm², 1.67±0.045 µg/cm², 1.62±0.018 µg/cm² after 6 hours, 12 hours and 24 hours respectively. An increase in temperature to 33°C caused an increase in chlorophyll content loss from *S. pistillata*. Chlorophyll content decreased from 2.2±0.11 µg/cm² in controls to 1.5±0.028 in samples exposed for 6 hours (P < 0.01, HSD = 0.73).

An analyzed *S. pistillata* after 12 hours of exposure to 33°C showed a chlorophyll content of 1.1±0.035 µg/cm² compared to 2.1±0.088 µg/cm² in controls. While after 24 hours it was 0.9±0.015 µg/cm² compared to 2±0.015 for the controls. Exposure of *S. pistillata* to 35°C sharply reduced the content of chlorophyll relative to the control sample. In addition, the difference was statistically highly significant between both samples (P < 0.0001, HSD = 1.36). About 63% of chlorophyll content was lost from *S. pistillata* after 6 hours when incubated at 35°C (content after loss= 0.81±0.023 µg/cm²), while it was 65% after 12 hours (content after loss= 0.74±0.007 µg/cm²). While after 24 hours, the loss of chlorophyll content was about 66% (content after loss= 0.68±0.015 µg/cm²) relative to the control sample.

Table 4. Turkey's studentized rang statistical analysis (HSD) for the experimental effect of changes in temperature (°C) on chlorophyll concentration (µg/cm²) of *S. pistillata* by using the sampling frequency as dependent variables.

	After 6 h. (1.67)	After 12 h. (1.56)	After 24 h. (1.48)
After 6 h. (1.67)			
After 12 h. (1.56)	0.11 (Sig)		
After 24 h. (1.48)	0.19 (Sig)	0.08 (Sig)	

Note: Number in parentheses = Chlorophyll concentration (µg/cm²). Minimum significant difference 0.0304. Sig. = Significant difference

Turkey's Studentized Rang Statistical Analysis (HSD) was applied to detect the distinct variance between means of chlorophyll contents at different times of exposure (Table 4). It was revealed that the mean value of chlorophyll contents after 6 hours of exposure was significantly different from those after 12 hours (ANOVA, $P < 0.0001$, HSD = 0.11) and 24 hours. Also the difference was significant between chlorophyll contents after 12 hours of exposure and 24 hours, this revealed the recorded data that the contents of chlorophyll after 6 hours of exposure ($1.67 \mu\text{g}/\text{cm}^2$) were higher than those after 12 hours ($1.56 \mu\text{g}/\text{cm}^2$), and chlorophyll contents after 12 hours were higher than those after 24 hours ($1.48 \mu\text{g}/\text{cm}^2$).

Experimental effect of increased sediment concentrations on the population density of zooxanthellae density of *A. humilis*

The changes observed in zooxanthellae densities lost from *A. humilis* indicated an increased susceptibility to both increased sediment concentrations and prolonged exposure. The control samples exhibited slight variations in algal density at the beginning and the end of the experiment. In samples exposed to $0.1 \text{ mg}/\text{cm}^2/\text{L}$, zooxanthellae densities did not show any decrease after 1 day ($0.8 \pm 0.025 \times 10^6$ cells/ cm^2 in controls and exposed samples). However, after 5 days of exposure to $0.1 \text{ mg}/\text{cm}^2/\text{L}$, zooxanthellae densities decreased from $0.79 \pm 0.015 \times 10^6$ cells/ cm^2 (controls) to $0.76 \pm 0.007 \times 10^6$ cells/ cm^2 , while after 10 days zooxanthellae densities were $0.73 \pm 0.014 \times 10^6$ cells/ cm^2 compared to $0.77 \pm 0.01 \times 10^6$ cells/ cm^2 for the controls. The mean of zooxanthellae densities in samples exposed to $0.1 \text{ mg}^2/\text{cm}/\text{L}$ were significantly different from those of the controls ($P < 0.01$, HSD = 0.023). *Acropora humilis* at $0.5 \text{ mg}/\text{cm}^2/\text{L}$ had lower counts of symbiotic alga cells ($0.75 \pm 0.015 \times 10^6$ cells/ cm^2 after 1 day, $0.7 \pm 0.015 \times 10^6$ cells/ cm^2 after 5 days and $0.65 \pm 0.01 \times 10^6$ cells/ cm^2 after 24 hours) within host tissue compared to the control sample. Moreover, zooxanthellae densities were significantly different from those of the control sample ($P < 0.01$, HSD = 0.086).

Increases in sediment concentration to $1 \text{ mg}^2/\text{cm}/\text{L}$ caused a decrease in zooxanthellae densities and vary greatly over time. Measurements of zooxanthellae densities at this stage revealed a highly significant difference between exposed and control sample (ANOVA, $P < 0.0001$ and HSD = 0.173). Where, zooxanthellae densities were $0.72 \pm 0.015 \times 10^6$ cells/ cm^2 , $0.68 \pm 0.015 \times 10^6$ cells/ cm^2 , $0.44 \pm 0.01 \times 10^6$ cells/ cm^2 after 1 day, 5 days and 10 days, respectively. At $1 \text{ g}/\text{cm}^2/\text{L}$, the number of zooxanthellae lost from *A. humilis* was decreased to 11% (content after loss = $0.71 \pm 0.012 \times 10^6$ cells/ cm^2) compared to control sample after 1 day, 24% (content after loss = $0.6 \pm 0.015 \times 10^6$ cells/ cm^2) after 5 days and to 60% (content after loss = $0.31 \pm 0.012 \times 10^6$ cells/ cm^2) after 10 days, indicating that the physiological state of zooxanthellae is clearly influenced by elevated sedimentation rate and the duration of exposure. In addition, the differences in zooxanthellae densities between samples exposed to $1 \text{ g}/\text{cm}^2/\text{L}$ and controls were highly significant (ANOVA, $P < 0.01$, HSD = 0.246).

Turkey's Studentized Rang Statistical Analysis (HSD) was applied to detect the distinct variance between means of zooxanthellae densities after different times of exposure (Table 5). It was revealed that, the mean value of zooxanthellae densities after 1 day of exposure was significantly different from those after 5 days. However, zooxanthellae densities after 10 days of exposure were highly significantly different from the sample of 1 day of exposure and 5 days. This revealed the result that zooxanthellae densities after 1 day exposure (0.755×10^6 cells/ cm^2) were higher than those after 5 days (0.706×10^6 cells/ cm^2), also those after 5 days were higher than those after 10 days (0.58×10^6 cells/ cm^2).

Table 5. Turkey's studentized rang statistical analysis (HSD) for the experimental effect of changes in sedimentation on zooxanthellae density (10^6 cells/ cm^2) of *A. humilis* by using the sampling frequency as dependent variables.

	After 1 day (0.755)	After 5 days (0.706)	After 10 days (0.58)
After 1 day (0.755)			
After 5 days (0.706)	0.049 (Sig)		
After 10 days (0.58)	0.175 (H. Sig)	0.126 (H. Sig)	

Note: Number in parentheses = Zooxanthellae density (10^6 cells/ cm^2). Minimum significant difference 0.01. H. = highly significant differences. Sig. = Significant difference

Experimental effect of increased sediment concentrations on chlorophyll contents of *A. humilis*

Chlorophyll contents within the host tissue showed a significant decrease with increased sedimentation rate ($P < 0.01$ for all cases). Control samples had higher content of chlorophyll (1.99 ± 0.15 , 1.73 ± 0.015 and $1.31 \pm 0.018 \mu\text{g}/\text{cm}^2$ after 1, 5 and 10 days, respectively) compared to the test samples. In samples exposed to $0.1 \text{ mg}/\text{cm}^2/\text{L}$ the content of chlorophyll was (1.99 ± 0.02 , 1.71 ± 0.015 and $1.27 \pm 0.01 \mu\text{g}/\text{cm}^2$ after 1, 5 and 10 days, respectively). In addition, the difference between 1, 5 and 10 days samples was statistically significant (ANOVA, $P < 0.01$, HSD = 0.02). Colonies immediately sampled after 1 day exposure to $0.5 \text{ mg}/\text{cm}^2/\text{L}$ showed a slight decrease in chlorophyll contents ($1.91 \pm 0.01 \mu\text{g}/\text{cm}^2$). While chlorophyll contents were $1.66 \pm 0.015 \mu\text{g}/\text{cm}^2$ in colonies collected after 5 days and $1.1 \pm 0.05 \mu\text{g}/\text{cm}^2$ after 10 days. Colonies exposed to $0.5 \text{ mg}/\text{cm}^2/\text{L}$ showed a significant difference in chlorophyll contents compared to the control samples (ANOVA, $P < 0.001$, HSD = 0.121).

Chlorophyll contents of samples exposed to $1 \text{ mg}/\text{cm}^2/\text{L}$ was highly significantly different from the control sample (ANOVA, $P < 0.01$, HSD = 0.242). Chlorophyll contents were $1.84 \pm 0.015 \mu\text{g}/\text{cm}^2$, $1.58 \pm 0.015 \mu\text{g}/\text{cm}^2$ and $0.89 \pm 0.01 \mu\text{g}/\text{cm}^2$ after 1 day, 5 days and 10 days respectively. Exposure of *A. humilis* to $1 \text{ g}/\text{cm}^2/\text{L}$ sharply reduced the content of chlorophyll in relative to the control sample, giving a high significance of difference between both treated and control sample ($P < 0.01$, HSD = 0.374). About 10% of chlorophyll content was lost from *A. humilis* after 1 day when exposed to $1 \text{ g}/\text{cm}^2/\text{L}$ (content after loss =

1.79±0.02 µg/cm²), after 5 days for about 18% of chlorophyll content (content after loss = 1.42±0.012 µg/cm²) was lost. While after 10 days the loss of chlorophyll content was about 47% (content after loss = 0.7±0.019 µg/cm²) relative to the control sample.

Turkey's Studentized Rang Statistical Analysis (HSD) was applied to detect the distinct variance between means of chlorophyll contents at different times of exposure (Table 6). It was revealed that the mean value of chlorophyll contents after 1 day of exposure to different concentrations of sediments was significantly different from those after 5 days. However, the difference was highly significant between chlorophyll contents of 10 days samples and controls. In addition, the difference was highly significant between chlorophyll contents of 10 days and 5 days of exposure. Contents of chlorophyll after 1 day of exposure (1.904 µg/cm²) were higher than those after 5 days (1.62 µg/cm²), which in turn were higher than those after 10 days (1.056 µg/cm²).

Table 6. Turkey's studentized rang statistical analysis (HSD) for the experimental effect of changes in sedimentation rates (mg/cm²/L) on chlorophyll concentration (µg/cm²) of *A. humilis* by using the sampling frequency as dependent variables.

	After 1 day (1.904)	After 5 days (1.62)	After 10 days (1.056)
After 1 day (1.904)			
After 5 days (1.62)	0.284 (Sig)		
After 10 days (1.056)	0.848 (H. Sig)	0.564 (H. Sig)	

Note: Number in parentheses = Chlorophyll concentration (µg/cm²). Minimum significant difference 0.015. H. = highly significant differences. Sig. = Significant difference

Experimental effect of increased sediment concentrations on the population densities of zooxanthellae of *S. pistillata*

The number of zooxanthellae showed a significant decrease with increasing sediment concentrations (ANOVA, $P < 0.01$) and exposure time ($P < 0.01$). Additionally, there was an interaction between temperature and exposure time ($P < 0.01$). To detect the distinct different effects of changes in sediment concentrations on zooxanthellae densities after different exposure times, Turkey's Studentized Rang Statistical Analysis (HSD) was applied. It was shown that the mean value of zooxanthellae densities exposed to 0.1 mg/cm²/L was significantly different from those in the controls (HSD = 0.02). However, control samples had higher densities of zooxanthellae (0.81±0.015, 0.8±0.015 and 0.78±0.015x10⁶ cells/cm² after 1, 5 and 10 days respectively) compared to test samples. While, in samples exposed to 0.1 mg/cm²/L, the densities of zooxanthellae were (0.8±0.015 after 1 day, 0.78±0.007 after 5 days and 0.75±0.01x10⁶ cells/cm² after 10 days, respectively). Zooxanthellae densities of *S. pistillata* exposed to 0.5 mg/cm²/L had also a significant difference from those of the control sample ($P < 0.01$, HSD = 0.073). Recorded data revealed that, *S. pistillata* had lower counts of symbiotic algae cells (0.77±0.015x10⁶ cells/cm², 0.72±0.015x10⁶ cells/cm² and 0.68±0.01x10⁶

cells/cm² after 1, 5 and 10 days respectively) within host tissue compared to control sample. The difference was highly significant between zooxanthellae densities at 1 mg/cm²/L and those in control samples ($P < 0.01$, HSD = 0.156). At this stage zooxanthellae densities were 0.74±0.015x10⁶ cells/cm², 0.69±0.018x10⁶ cells/cm², and 0.49±0.01x10⁶ cells/cm² after 1 day, 5 days and 10 days respectively. An increase in sediment loading to 1 g/cm²/L caused a decrease in zooxanthellae densities which vary greatly over time.

Measurements of zooxanthellae densities after exposure to 1 g/cm²/L revealed a high significant difference between exposed and control samples (ANOVA, $P < 0.01$, HSD = 0.226). Zooxanthellae densities at this stage were 0.71±0.014x10⁶ cells/cm² after 1 day, 0.63±0.015 x10⁶ cells/cm² after 5 days and 0.37±0.015x10⁶ cells/cm² after 10 days. This represents a 13.4% decrease in zooxanthellae density after 1 day and 31% after 5 days compared to controls. While after 24 hours the loss of zooxanthellae was about 53.6% relative to the control sample.

Turkey's Studentized Rang Statistical Analysis (HSD) was applied to detect the distinct variance between means of zooxanthellae at the time of exposure (Table 7). It was revealed that the mean value of zooxanthellae densities after 5 days of exposure was significantly different from those after 1 day. However, the difference was highly significant between zooxanthellae densities after 10 days of exposure and those after 1 and 5 days. However, zooxanthellae densities after 1 day of exposure were 0.766 x10⁶ cells/cm², being higher than those after 5 days (0.724 x10⁶ cells/cm²). However, which in turn were higher than those after 10 days (0.614x10⁶ cells/cm²).

Table 7. Turkey's studentized rang statistical analysis (HSD) for the experimental effect of changes in sedimentation rates (mg/cm²/L) on zooxanthellae density (10⁶ cells/cm²) of *S. pistillata* by using the sampling frequency as dependent variables.

	After 1 day (0.766)	After 5 days (0.724)	After 10 days (0.614)
After 1 day (0.766)			
After 5 days (0.724)	0.042 (Sig)		
After 10 days (0.614)	0.152 (H. Sig)	0.11 (H. Sig)	

Note: Number in parentheses = Zooxanthellae density (10⁶ cells/cm²). Minimum significant difference 0.0096. H. = highly significant differences. Sig. = Significant difference.

Experimental effect of increased sediment concentrations on chlorophyll contents of *S. pistillata*

The amount of chlorophyll showed a significant decrease with increasing sediment concentration (ANOVA, $P < 0.01$) and prolonged exposure ($P < 0.01$). Additionally, there was interaction between the treatments. Control samples had chlorophyll contents 2±0.015, 1.84±0.018 and 1.34±0.015 µg/cm² after 1, 5 and 10 days respectively. The contents of chlorophyll in samples exposed to 0.1 mg/cm²/L was (1.98±0.015 µg/cm² after 1 day, 1.74±0.013 µg/cm² after 5 days and 1.32±0.013 µg/cm² after 10 days). The difference between exposed and controls was

statistically significant (ANOVA, $P < 0.01$, HSD = 0.044). One day exposure to 0.5 mg/cm²/L showed a slight decrease in chlorophyll contents (1.93±0.01 µg/cm²). However, chlorophyll contents in colonies analyzed after 5 and 10 days were 1.69±0.013 µg/cm² and 1.21±0.013 µg/cm² respectively. Colonies exposed to 0.5 mg/cm²/L showed a significant difference relative to the control samples (ANOVA, $P < 0.0001$, HSD = 0.114). While, measurements of chlorophyll content in samples exposed to 1 mg/cm²/L expressed a high significant difference between exposed and control sample (ANOVA, $P < 0.01$, HSD = 0.242). Where is chlorophyll contents were 1.87±0.013 µg/cm², 1.61±0.013 µg/cm², 0.97±0.013 µg/cm² after 1 day, 5 days and 10 days respectively.

An increase in sediment concentration to 1 g/cm²/L caused a sharp loss in chlorophyll content from *S. pistillata*. Chlorophyll content decreased from 2±0.15 µg/cm² in controls to 1.83±0.016 µg/cm² in exposed samples after 1 day (8.5% decrease). An analyzed *A. humilis* after 5 days of exposure to 1 g/cm²/L, showed a chlorophyll content of 1.47±0.007 µg/cm² compared to 1.84±0.088 µg/cm² in controls (20% decreased). After 10 days it was 0.81±0.016 µg/cm² compared to 1.34±0.013 µg/cm² in controls (40% decrease). The difference between 1 g/cm²/L exposure and controls was statistically highly significant ($P < 0.01$, HSD = 0.354).

Turkey's Studentized Rang Statistical Analysis (HSD) was applied to detect the distinct variance between means of chlorophyll contents at different times of exposure. It was revealed that the mean value of chlorophyll contents after 1 day of exposure was significantly different from those after 5 days but it was highly significantly different from those after 10 days. In addition, the difference between chlorophyll contents after 5 and 10 days of exposure was highly significant. However, the measured chlorophyll content after 1 day of exposure (1.92 µg/cm²) was higher than those after 5 days (1.66 µg/cm²) which in turn was higher than those after 10 days (1.129 µg/cm²).

Table 8. Turkey's studentized rang statistical analysis (HSD) for the experimental effect of changes in sedimentation rates (mg/cm²/L) on chlorophyll concentration (µg/cm²) of *S. pistillata* by using the sampling frequency as dependent variables.

	After 1 day (1.921)	After 5 days (1.669)	After 10 days (1.129)
After 1 day (1.921)			
After 5 days (1.669)	0.252 (Sig)		
After 10 days (1.129)	0.792 (H. Sig)	0.54 (H. Sig)	

Note: Number in parentheses = Chlorophyll concentration (µg/cm²). Minimum significant difference 0.01. H. = highly significant differences. Sig. = Significant difference.

Experimental effect of changes in temperature on bleaching

The changes observed in zooxanthellae lost from the two corals *A. humilis* and *S. pistillata* clearly indicate increased susceptibility to both elevated temperature and prolonged exposure. At 26°C (the control sample), the loss

of zooxanthellae by each of these corals was very low. Cell viability of these corals was similar at 26 and 29°C, but depicted a sharp decline of zooxanthellae lost from these corals at 31°C through time. This result confirms the result of Strychar et al. (2004) that zooxanthellae lost from *Acropora hyacinthus*, *Favites complanata*, and *Porites solida* at 32°C was greater than that at 28°C. However, Berkelmans and Willis (1999) found a temperature increase of 2-4°C is to have caused coral bleaching within days while a temperature increase of 1-2°C caused bleaching within weeks. Moreover, Nesa and Hidaka (2009) detected a negative correlation between survival time and the zooxanthellae density of tissue balls at 31°C in both *Fungia* sp. and *Porites divaricata*. This relationship was clearly observed in the Caribbean basin during the 1980s and 1990s, when annual coral bleaching increased logarithmically with sea surface temperature anomalies (McWilliams et al. 2005). A 0.1°C rise in regional sea surface temperature resulted in a 35% increase in the number of areas that reported bleaching, and mass bleaching events occurred at regional sea surface temperature anomalies of 0.2°C and above (Baker et al. 2008).

As the temperature increased to 35°C in the present experiment, the loss of zooxanthellae from each host increased both with time and temperature elevation. This result agrees with the finding of Riegl (2002) that bleaching mortalities were reported in Abu Dhabi at 1996 when temperatures remained above 35°C for 3 weeks. Elevated temperature was found to significantly reduce the amount of zooxanthellae in primary polyps (Anlauf et al. 2010).

The result reported in the present experiment indicated that *A. humilis* had a higher decrease in its zooxanthellae densities than *S. pistillata* at the same treatment; where 50% of zooxanthellae in the host tissue of *A. humilis* were lost after 6 hours while *S. pistillata* lost 48% of zooxanthellae after equal time of exposure. Differences in the response of these species of coral to thermal stress may result from difference in hospice irradiances driven by the combination of skeletal architecture and light scattering properties (Enríquez et al. 2005). An additional source of variation in the response to thermal stress in these corals may originate from differences in tissue thickness that is associated with difference in the initial protein content (Warner et al. 2002). Fitt et al. (2009) found physiological and biochemical differences of both symbiont and host origin in the response to high-temperature stress of *Porites cylindrica* and *S. pistillata*. Ferrier-Pagès et al. (2010) studied the changes in feeding rates of three scleractinian coral species between normal and short-term stress conditions, and assessed the effect of feeding on the photosynthetic capacity of corals exposed to thermal stress. He found that *S. pistillata* significantly decreased its feeding rates at 31°C, while rates of *Turbinaria reniformis* and *Galaxea fascicularis* were increased between 26 and 31°C. Exposure to elevated temperatures reduces the photosynthetic rate of zooxanthellae and predisposes their photosynthetic apparatus to further damage (Jones et al. 1998; Bhagooli and Hidaka 2004).

In the present experiment, the bleaching temperature threshold was 33°C or less for the two species *S. pistillata*

and *A. humilis* where 51% of their zooxanthellae were lost after 24 h of exposure. This result confirms that of Leletkin (2002) that water temperature of 32°C and above inevitably caused coral bleaching. However, the thermal bleaching threshold for primary polyps might be below that reported (30–31°C) from most adult coral species in the eastern Pacific (D'Croz et al. 2001; Hueerkamp et al. 2001). Bleaching temperature thresholds vary locally, and conditions that result in coral mortality in some regions can have no effect on corals in others. For example, while 30.5°C and 30.8°C represent bleaching thresholds for at least some regions of the Caribbean and Great Barrier Reef, respectively (Berkelmans et al. 2004; Manzello et al. 2007), temperatures as high as 35.5°C do not affect corals in the Arabian Gulf or in the Samoan Manu'a Islands (Craig et al. 2001; Riegl 2002; Birkeland et al. 2008). Indeed, individual corals have been reported surviving in Abu Dhabi at temperatures up to 40°C (Kinsman 1964), although mortality did occur in this region in 1996 when temperatures remained above 35°C for 3 weeks (Riegl 2002).

Experimental effect of changes in sedimentation rate on bleaching

In samples exposed to 0.1 mg/cm²/L, zooxanthellae densities of *A. humilis* and *S. pistillata* did not show any decrease after 1 day. However, after 1 day of exposure to 0.5 mg/cm²/L, zooxanthellae densities were significantly different from those of the controls. This result agrees with the laboratory finding of Peters and Pilson (1985) who examined the effect of heavy sedimentation rate on corals using both symbiotic and asymbiotic colonies of *Astrangia danae* at a rate of 200 mg cm⁻² day⁻¹ for 4 weeks. He found no difference from controls while slight adverse effects relative to controls were noted after increasing the sand applications to three times per day. The effects of varying rates of sedimentation (0.5 to 325 mg cm⁻² d⁻¹) on settlement rates of *Acropora millepora* larvae were examined experimentally, in aquaria. Higher sedimentation rates reduced the number of larvae settling on upper surfaces, but total numbers of settled larvae were not significantly affected by sedimentary regime (Babcock and Davies 1990). Increases in sediment concentration to 1 mg/cm²/L caused a decrease in zooxanthellae densities and vary greatly over time. Measurements of zooxanthellae densities of *A. humilis* and *S. pistillata* at this stage revealed a highly significant difference between exposed and control sample. However, Sofonia and Anthony (2008) found no effect of sediment loads greater than 110 mg cm⁻² on any of the physiological variables of *Turbinaria mesenterina*, that tolerant to sediment loads an order of magnitude higher than most severe sediment conditions in situ. Likely mechanisms for such tolerance are that: (1) colonies covered in sediment in low-flow were able to clear themselves rapidly (within 4–5 h) and (2) sediment provides a source of food. These results suggest that intensified sediment regimes on coastal reefs may shift coral communities towards dominance by a few well-adapted species (Weber et al. 2006; Palmer et al. 2010).

Cruz-Pinion et al. (2003) found that high sedimentation rates, low light availability and anthropogenic influence

lead to cellular damage and deteriorated coral skeletal density. At 1 g/cm²/L, the number of zooxanthellae lost from *A. humilis* was higher than that was lost from *S. pistillata* at same time. This result agrees with the finding of Fabricius et al. (2007). Who reported a contrast between species susceptibility at 39.6 mg cm⁻² day⁻¹ sedimentation rate in Ngardmau, Palau, Micronesia, that small polyp corals such as *Porites rus* suffered greatest mortality while damage in *Galaxea fascicularis* was less severe as sediments were shifted and removed by the large polyps. However, *Acropora* spp. appeared partially bleached although little time sediment remained on branches. With reference to the present experiment, we can suggest that the normal sedimentation rate for *A. humilis* and *S. pistillata* to be in an order of 1 mg/cm²/L or less. Chronic rates and concentrations above these values are high. This result is conflicted with the finding of Rogers (1990), who concluded that the mean sediment concentration was < 10 mg/L at reefs subject to stresses from human activities. Sedimentation is regarded as an increasing threat to coral reefs (reviewed by Fabricius 2005). The impacts associated with sedimentation and sediment burial include reduced photosynthesis and increased respiration (Philipp and Fabricius 2003; Weber et al. 2006), tissue mortality, reduced growth (Rice and Hunter 1992; Lirman and Manzello 2009), and reduced fertilization, larval survivorship, and recruitment (Babcock and Smith 2000). Turbidity reduces light levels, photosynthetic potential and possibly coral growth rates (Yentsch et al. 2002; Anthony and Hoegh-Guldberg 2003); however, elevated net sedimentation rates increases abrasion and smothering (Rogers 1990; Fabricius 2005). On the other hand, Palmer et al. (2010) found that nearshore environments directly influenced by fluvial sediments and dominated by terrigenoclastic sedimentation are generally considered marginal for coral reef growth. The same author did not mention if there is some way of washing or cleaning of these sediments. The energetic costs of sediment clearing can be considerable (Riegl and Branch 1995), and the inability to clear sediments will expose corals to further stress because anoxic conditions due to sediments can cause tissue bleaching and subsequent mortality (Weber et al. 2006).

Increased temperature and sedimentation rate are both known to cause physiological stress in corals (Weber et al. 2006; Lirman and Manzello 2009; Obura 2009). Corals are not homoiothermic (Hoegh-Guldberg and Smith 1989) and short-term temperature and sediment stress can cause changes in basal metabolism, such as respiration and zooxanthellae photosynthesis (Nyström et al. 2001; Philipp and Fabricius 2003; Weber et al. 2006).

Some key questions on the present work and their answers

Q: How does the fact that “the temperature treatments were just 6, 12 or 24 hours long” relate to the aims and how does this design advance our understanding of temperature change as a result of climate change effects? **A:** These experimental choices should not be construed to mean that the response of reef corals to global climate change can be fully understood without addressing other facets of global climate change and through a comprehensive analysis of

the coral holobiont. This study addresses mainly the early effects of heat stress on symbiotic dinoflagellates (zooxanthellae) within the tissues of a common reef-coral.

Q: Why were the experiments conducted under constant temperature conditions and how does this design advance our understanding of temperature change as a result of climate change effects? **A:** The experiments were conducted under constant temperature conditions as they were conducted in an aquarium, and to maintain a stable reef tank ecosystem, a constant temperature is required. Also, as the reef tank is a closed system and most types of coral can thrive a few degrees above or below the ideal level in such a closed system, the study examined the early effects of heat stress on symbiotic dinoflagellates (zooxanthellae) within the tissues of a common reef-coral.

Q: How are results from a constant temperature experiment reconciled with the normal cyclic conditions experienced by the corals in their natural habitat? **A:** The temperatures in the present experiment were increased and decreased following the diurnal variation in water temperature in the field by approximately 6 °C. Temperature in the field was between 29 °C and 30 °C at midday (at 3 m depth). Therefore, corals for studying the effect of temperature were incubated at each temperature test (24, 29, 31, 33 and 35 °C). Control samples were placed at room temperature (26°C). Since coral bleaching occurs when the thermal tolerance of corals and their photosynthetic symbionts (zooxanthellae) is exceeded, corals in the present experiment, beside being subjected to the normal cyclic temperature, they were subjected to some few degrees below and above the normal cyclic temperature.

Q: How quickly was ambient temperature changed during experimentation and how does this affect interpretation of the outcome? **A:** Bleaching can be induced by short-term exposure (i.e., 1 day) at temperature elevations of 3°C to 4°C above normal summer ambient or by long-term exposure (i.e., several weeks) at elevations of 1°C to 2°C. Temperature elevations above summer ambient, but still below the bleaching threshold (as in the present work), could impair growth and reproduction by the effect of increased zooxanthellae expulsion. In 1998 Red Sea corals were perilously close to their bleaching threshold during the summer months, and localized bleaching did occur. In some cases, local warming of surface water on shallow reef flats exceeded this threshold temperature and caused localized coral bleaching.

Q: What is briefly the design of the sedimentation experiments and how do they allow the conclusions that are postulated. **A:** The present experiment examined the effect of different sedimentation rates on corals. For detection of the short-term sediment, branches for studying the effect of sediments were put in glass aquaria, exposed to 0.1, 0.5, 1 mg/cm²/L and 1g/cm²/L different concentrations of sediments. The present experiment postulated that the normal, safe sedimentation rate for *A. humilis* and *S. pistillata* to be in order of 1mg/cm²/L or less.

Q: Exactly how do the outcomes of this work advance our understanding? **A:** Threshold temperatures, as well as normal safe sedimentation rate determined in the present work, can be applied directly to reef aquaria where they are

closed systems exactly like those used in our experiments. These experimental choices should not be construed to mean that the response of reef corals to global climate change or to natural or human-made sedimentation in the sea can be fully understood without a comprehensive analysis of the coral holobiont and the surrounding environmental conditions.

CONCLUSIONS

The result reported in the present experiment indicated that *Acropora humilis* had a higher decrease in its zooxanthellae densities than *Stylophora pistillata* at the same treatment after equal time of exposure to temperature. In the present experiment, the bleaching temperature threshold was 33°C or less for the two species *S. pistillata* and *A. humilis* where 51% of their zooxanthellae were lost after 24 h of exposure. On exposure to sedimentation, number of zooxanthellae lost from *A. humilis* was higher than those lost from *S. pistillata* at same time. The normal sedimentation rate for *A. humilis* and *S. pistillata* was found to be in an order of 1 mg/cm²/L or less.

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