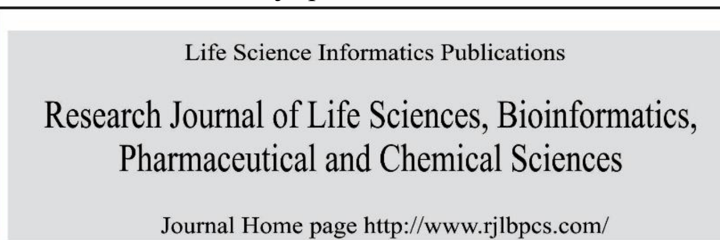

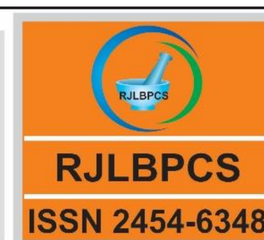

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DETECTION OF CORAL BLEACHING USING LASER INDUCED FLUORESCENCE SPECTRAL SIGNATURES

P.V. Anu Baburaj*[1], Narayanan Subhash[1], M.K Anil [2], Rani Mary George[2], Nita Sukumar[1], K.V Baiju [3]

1. National Centre for Earth Science Studies, Trivandrum, India.
2. Central Marine Fisheries Research Institute, Trivandrum, India.
3. Govt. Women's College, Trivandrum, India.

ABSTRACT: Corals are highly sensitive to elevation in temperature, which ultimately results in coral bleaching. This study focuses on detection of coral bleaching by investigating the variations in coral fluorescence signatures, in particular the fluorescence intensity ratio F680/F735 of the chlorophyll fluorescence emission, in response to elevated temperature in the coral species *Goniopora stokesi.* The corals were grown under controlled conditions and exposed to elevated temperatures of 30, 31, and 32°C. Laser-induced fluorescence (LIF) spectral signatures of corals were recorded during each treatment using a portable fiber-optic fluorosensor. All the corals studied showed a prominent peak around 495 nm from the host fluorescent proteins and two peaks at 680 and 735 nm from the chlorophyll in Zooxanthellae. The average fluorescence intensity ratio values at different temperature points were tested using ANOVA and the F value was found to be statistically significant ($P<0.0005$) indicating that the average fluorescence intensity ratio values at each temperature differ significantly. A simple regression model was constructed to understand the effect of temperature on coral fluorescence ratio and the constructed model appears to be ideal ($F=206.67$, $P<0.001$) to explain the variation in F680/F720 ratio due to elevated temperature. The study indicated that a 1°C rise in temperature will significantly reduce the F680/F720 ratio by 28%. Thus, the LIF technique has the potential to be used to detect declining coral health prior to the appearance of visible signs of bleaching and the F680/F735 intensity ratio could be used as a non invasive means to detect coral bleaching.

KEYWORDS: Coral bleaching, Coral fluorescence, Temperature stress, Laser-induced fluorescence.

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Corresponding Author: Dr. P.V. Anu Baburaj* Ph.D.
National Centre for Earth Science Studies, Trivandrum, India.
Email Address: annuak@gmail.com

1. INTRODUCTION

Coral reefs are one of the most productive ecosystems of the world. However, coral reefs worldwide are under rapid decline due to both natural and anthropogenic stressors [1], [2]. Coral bleaching occurs when environmental changes disrupt the coral algal symbiosis leading to the expulsion of zooxanthellae from the coral host and reduction in photosynthetic pigments within the symbionts [3], [4], [5], [6] that leads to corals losing their colour. Elevated sea surface temperature is the major cause for the many devastating bleaching events that have occurred since 1980s which is believed to be associated with global warming due to anthropomorphic release of carbon dioxide and other gases [7], [8],[9], [10]. Laser-induced fluorescence (LIF) signatures of corals are used to understand the effect of stress elements, such as sea surface temperature, salinity, and marine pollution on fluorescent pigments and serve as indicators of coral health. Fast Reception Rate (FRR) fluorometers and underwater Pulse Amplitude Modulated (PAM) fluorometers were developed and used in situ to evaluate changes in photosynthetic parameters [11], [12]. Hardy et al. [13] measured the in vivo LIF in different species of Caribbean corals. These studies were primarily concerned with the chlorophyll fluorescence emission at 685 and 740 nm with excitation at 532 nm or 337 nm. Myers et al [14] used fluorescence imaging to investigate micro distribution of chlorophyll pigments in corals. Zawada and Jaffe [15] used a low-light level underwater multispectral imaging system to study changes in the fluorescence of Caribbean coral *Montastraea faveolata* during heat induced bleaching. They developed a normalized difference ratio between green and orange pigments to facilitate comparison with chlorophyll fluorescence as a bleaching indicator. Rodrigues et al [11] used chlorophyll fluorescence to monitor long term bleaching and recovery of *Porites compressa* and *Montipora capitata* using a PAM fluorometer. Changes in minimum (F_0), maximum (F_m) and variable (F_v) fluorescence throughout bleaching and recovery indicated periods of initial photoprotection followed by photodamage in both species, with *P. compressa* requiring less time for photosystem II repair than *M. capitata* indicating that *P. compressa* is more resilient to bleaching stress. Manzello et al. [12 remotely monitored chlorophyll fluorescence of two reef-forming corals during the 2005 bleaching event and found that colonies exhibited photosystem II inactivation coincident with thermal stress. These studies could determine the extent of coral bleaching but could not provide any early warning signs until the bleaching process has been initiated and the damage is already been done. Therefore, being able to detect declining coral health prior to the appearance of visible signs of bleaching would be advantageous. Here, we use a portable fiber-optic

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fluorosensor to record changes in fluorescent spectral signatures in two species of corals in situ, subjected to elevated temperature in a controlled marine environment. The corresponding changes in F680/F735 fluorescent intensity ratios at different temperatures were calculated and compared to understand the early symptoms of temperature rise. A simple regression model was constructed in order to find out the effect of elevated temperature on the average fluorescence intensity ratio values at each treatment and the results are presented.

2. MATERIALS AND METHODS

A. Sample collection and coral growth

Samples of *Goniopora stokesi* were collected from the Islands of Vaan and Koswari in the Gulf of Mannar from a depth of 2-3 m respectively. The collected corals were placed separately in plastic containers, sealed at depth and brought to the surface. The samples were then transported to the laboratory at Central Marine Fisheries Research Institute (CMFRI) and placed in large temperature controlled glass tanks of dimension 3x2x2 feet containing filtered seawater. There were a set of four tanks, comprising of two replicate tanks and two treatment tanks, for each temperature treatment. The experiments were carried out under permission from the Central Marine Fisheries Research Institute (CMFRI) and National Centre for Earth Science Studies. The control temperature was set at 29°C in accordance with the mean sea surface temperature (SST) at the collection site. All the corals placed inside the tanks were allowed to acclimatize to 29°C for 2 weeks. The corals exhibited no signs of bleaching or diseased condition during this period. The temperature in all the four the tanks were controlled using an immersible heater and the temperature was monitored and controlled with a digital thermostat. Lighting was provided for all the samples using T5 lamps (5000 lm) for 12 h per day. After acclimation for 2 weeks at 29°C the corals were subjected to temperature stress by gradually raising the temperature of the sea water bath in 2 tanks to 30, 31 and 32°C and while the control group of corals in the other 2 tanks were maintained at 29°C. On increasing the set temperature by 1°C, an acclimatization time of four days was given for the photosynthetic functions of corals to stabilize. Fluorescence spectra of the corals were recorded for each treatment afterwards.

B. LIF measurement setup

The schematic of the portable fiber-optic fluorosensor used in the study to record the LIF spectra of corals is shown in Fig.1. A diode laser (M/s CNI, PRC) emitting at 404 nm with an output power of 50 mW (CW) was used for excitation of fluorescence and a miniature fiber-optic spectrometer (Ocean Optics, USA, Model: USB2000FL) connected to the USB port of a laptop computer for recording the emission from corals in visible range with a spectral resolution of 8 nm. The 3-m long fiber-optic probe used for sampling has a central fiber (400 μm dia.) to deliver the excitation beam and six surrounding fibers (each of 400 μm dia.) to collect the LIF emission. The back-scattered laser light was removed from the fluorescence signal using a long-pass filter (Schott GG420)

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mounted in an inline filter holder. The probe tip was positioned at a distance of 3 mm from the coral head with the help of a PVC sleeve inserted over the cylindrical SS ferrule casing. This geometry facilitated optimal overlap between excitation light and collection area and also prevented the ambient light from entering the detection system.

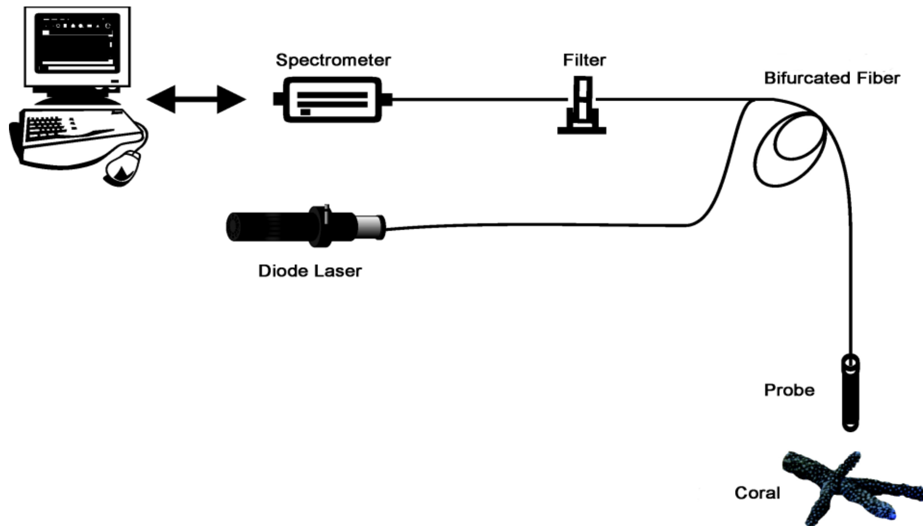

Fig.1. Schematic of the portable fiber-optic fluorosensor

C. Data acquisition and analysis

The probe was placed with the sleeve lightly touching the coral surface and the LIF spectra was recorded using the OOI Base32 software (Ocean Optics, Inc., USA) configured to record the spectra averaged for 40 scans with a boxcar width of 10 nm and an integration time of 100 ms. Eight sets of measurements were taken from different parts of each coral and the averaged spectral data was used for further analysis. In order to determine the spectral regions of maximum variation during heat induced bleaching, the spectra were normalized to the peak intensity of fluorescent protein emission around 495 nm.

D. Statistical Analysis

The F680/F720 fluorescence intensity ratio values at different temperatures (29, 30, 31and 32°C) were calculated. Initially, the average fluorescence intensity ratio values at different temperatures were tested for significance using one way analysis of variance (ANOVA) followed by Scheffe's Post Hoc comparisons for pair-wise significance. The effect of elevated temperature on F680/F720 fluorescence intensity was studied by constructing a simple linear regression model by taking average F680/F720 fluorescence intensity ratio values as the dependent variable and temperature as the independent variable. A p-value less than 0.05 is considered to be statistically significant.

The constructed model is of the form

$FV=\alpha+\beta\times \text{Temperature}$...... (1)

where α is the intercept and β is slope of the regression line.

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3. RESULTS AND DISCUSSION

After acclimation of *Goniopora stokesi samples* for 2 weeks at 29°C, the seawater temperature of the treatment tank was raised to 30°C. No visible signs of bleaching were observed and the corals remained healthy at 30°C throughout the treatment period. However, as the temperature was raised to 31°C small areas on the corals began to show signs of bleaching and bleaching progressed as the temperature was further raised to 32°C. We noticed that at 32°C the corals were almost fully bleached. Photographs showing the effect of elevated temperature on corals of *Goniopora stokesi* are given in fig. 2.

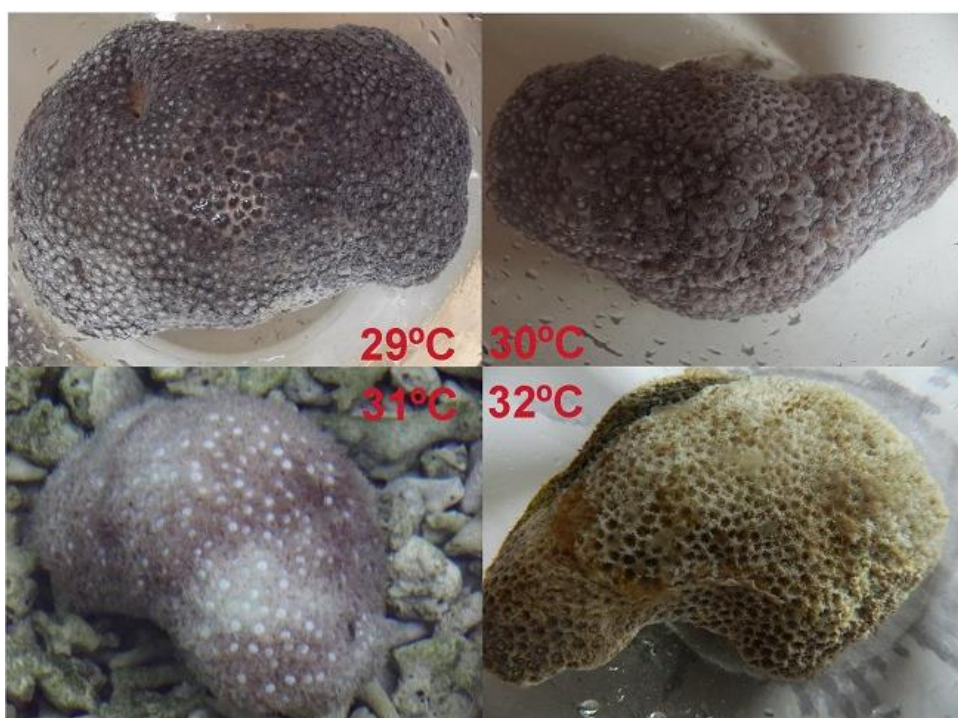

Fig. 2. Images of *Goniopora stokesi* at different temperature treatments

A. Spectral features

The LIF spectra of *Goniopora stokesi* consists of an emission band at 495 nm from the fluorescent proteins and two chlorophyll emission peaks at 680 and 735 nm. The averaged LIF spectra, normalised to the maximum intensity at 495 nm, is shown in fig. 3. As compared to the 495 nm peak, the chlorophyll fluorescence emission shows a decreasing trend in intensity with temperature rise. Although the chlorophyll fluorescence intensity at 30 and 31°C do not vary much, the decreasing trend associated with elevated temperature and bleaching events can be clearly evidenced in the LIF spectra recorded at 32°C. The fluorescence intensity ratios F680/F735 calculated from the raw spectral data at different temperature settings show a decreasing trend with increase in temperature and are given in Table 1 for the species studied.

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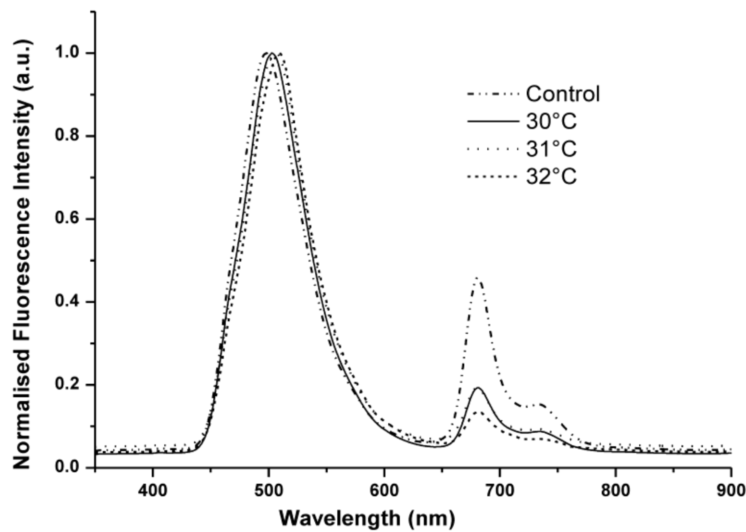

Fig.3. Averaged fluorescent spectra of corals at each treatment normalised to the maximum intensity at 495 nm

Table 1: Fluorescence intensity ratio F680/F735 of coral samples calculated from the raw spectral data

Sample No.	Control 29°C	Treatment 1 30°C	Treatment 2 31°C	Treatment 3 32°C
1	2.63	2.17	1.94	1.66
2	2.50	2.08	1.91	1.52
3	2.43	2.09	1.95	1.79
4	2.63	2.01	1.97	1.80
5	2.51	2.04	1.89	1.64
6	2.65	2.29	1.96	1.73

B. Temperature variance of fluorescence intensity ratio

The average fluorescence intensity ratio values at different temperature points were tested using ANOVA and the details are shown in Table 2. From Table 2, it can be seen that the F value is statistically significant ($P<0.0005$) indicating that the average fluorescence intensity ratio values at each temperature differ significantly. Scheffe's test also showed significant difference between every pair of temperatures ($P<0.01$). At the average F680/F720 was 2.56±0.04, 2.11±0.04, 1.94±0.01 and 1.69±0.04 for 29°C, 30°C, 31°C and 32°C respectively which is plotted in fig. 4. The decrease in the average fluorescence ratio F680/F735 was more pronounced as the temperature was raised from 29 to 30°C albeit the fact that no visible symptoms were seen. The F680/F735 ratio showed a variance of 17.57% between the corals that were maintained at 29°C with respect to those

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at 30°C. As the temperature of the corals were increased to 31°C, the F680/F735 ratio decreased further by 8.05%. Further, a variance of 12.73% was observed in the F680/F735 ratio between the corals maintained at 31 and 32°C.

Table 2: Data and Test of Significance of average fluorescence intensity ratio values at different temperatures

Temperature	AM	SE	F	P
29°C	2.56	0.04	104.70	<0.0005***
30°C	2.11	0.04		
31°C	1.94	0.01		
32°C	1.69	0.04		

***: significant at 0.001 level

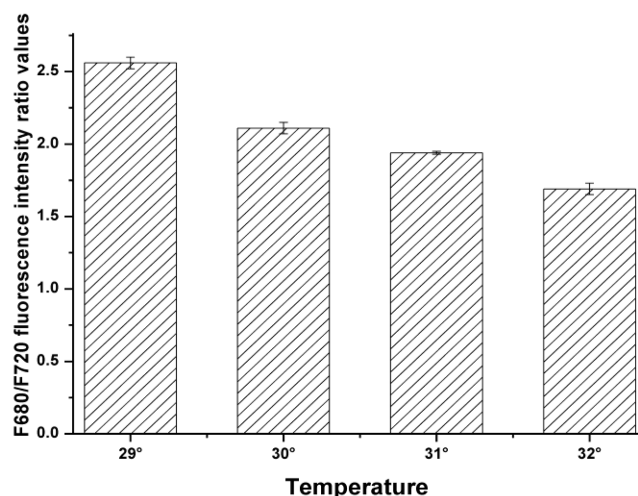

Fig.4. Comparative bar diagram of average F680/F720 fluorescence intensity ratios

C. **Effect of elevated temperature on the average F680/F720 ratio**

The simple regression model was constructed for studying the effect of temperature on F680/F720 fluorescence intensity ratio using eqn. 1.

where α is the intercept and β is slope of the regression line. The values of α and β were estimated from the sample data using the principle of least squares. The significance of the model (eqn. 1) for explaining the effect of temperature on F680/F720 fluorescence intensity ratio was tested using ANOVA and the significance of the estimated values of α and β was tested using t-test. The coefficient of determination (R^2) was used for assessing the explanatory power of the regression model. Data on the test of significance of regression on F680/F720 fluorescence intensity ratio variance with temperature is shown in Table 3.

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Thus the constructed model (1) using the estimated values can be written as

$$F680/F720=10.56-0.28\times \text{Temperature} \quad (2)$$

As can be evidenced from Table 3, the constructed model appears to be sufficient (F=206.67, P<0.001) to explain the variation in F680/F720 ratio due to elevated temperature. Moreover the slope coefficient is negative (-0.28) and is significant using t-test (t=14.37, P<0.001). The R^2 value is 0.903 indicating that more than 90% of the variation in F680/F720 fluorescence intensity ratio is attributed to the temperature change. This shows that slight increase in temperature could significantly reduce the F680/F720 fluorescence intensity ratio; i.e. on an average a 1°C rise in temperature will significantly reduce the F680/F720 ratio by 28%. The scatter diagram and the line of regression of F680/F720 fluorescence intensity ratio on temperature are given in fig. 5.

Table 3: Data and Test of Significance of regression of F680/F720 fluorescence intensity ratio on temperature

Parameter	Values	t	P	F	P
α	10.56	17.88	<0.0005***	206.67	<0.0005***
β	-0.28	14.37	<0.0005***		

***: significant at 0.001 level, R2=0.903

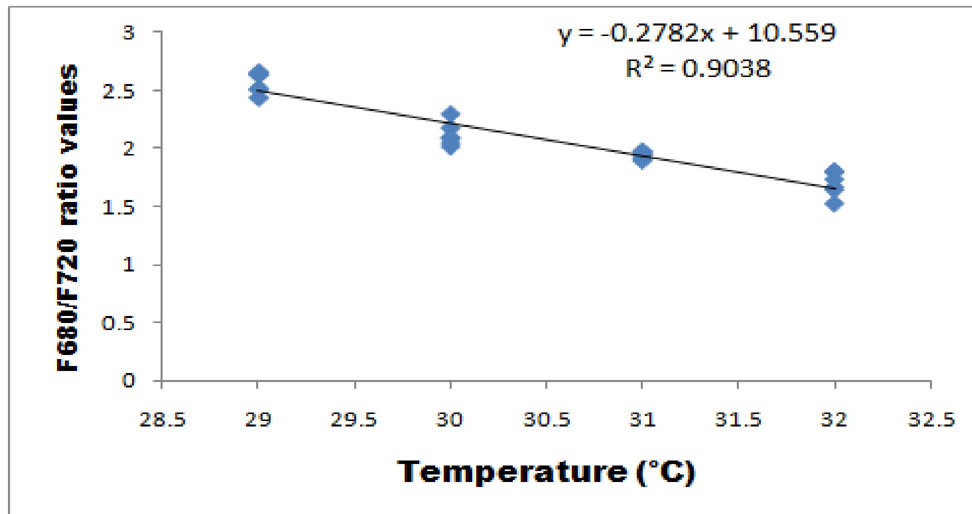

Fig.5 Scatter diagram and the line of regression of F680/F720 fluorescence intensity ratio on temperature

DISCUSSION

The present study has determined the variation in fluorescence spectral signatures and fluorescence intensity ratio with respect to heat induced stress in corals. It was observed that with respect to the fluorescent protein emission at 495 nm, the chlorophyll fluorescence intensity at 680 and 735 nm decreases with increase in temperature. Induced chlorophyll fluorescence has long been used to understand the photosynthetic mechanisms and to assess the physiological conditions in plants and phytoplanktons [16]. Photosynthesis is a sensitive indicator of thermal stress in corals. Iglesias –

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Prieto et al. [17] reported that photosynthesis is impaired at temperature above 30°C and ceases completely at 34-36°C. This suggests that elevated temperature adversely affects the photosynthesis in symbiotic dinofalgellate rather than adversely affecting the animal hosts and relegating the symbiont to a more passive role. Hardy et al. [13] reported that the fluorescence spectra of Caribbean coral *Solenastrea bournoni* have distinct chlorophyll peaks at 685 and 740 nm that gradually decreased in intensity when the temperature was either raised suddenly to a higher value (35-40ºC) and brought to normal room temperature (27ºC) or kept at higher values of 35ºC over 5 day period. Further, their results showed the absence of these two peaks in fully bleached corals. In the present study the intensity of chlorophyll emission decreased gradually as the temperature was increased by 1°C. Intra cellular Calcium concentration is known to play a pivotal role in the subcellular mechanism of coral bleaching induced by elevated temperature. Heat induced bleaching was inhibited when the ambient calcium concentration in the sea water was reduced or depleted [18]. Strychar and Sammarco [19] investigated the effects of heat stress on pigments of Zooxanthellae and observed that all phytopigments decreased linearly as temperature was increased. This indicates that decrease in symbiodinium phytopigments in response to heat stress may be a need to adapt while their host coral may already be adapted. Roth and Deheyn [20] conducted cold and heat treatments on the branching coral *Acropora yongei* and found that fluorescence from both the green fluorescent protein and chlorophyll decreased with declining coral health. However, no specific trend was observed in the fluorescent intensity of fluorescent protein with respect to elevated temperature stress in *G.stokesi.* The present study utilized changes in the fluorescence intensity ratio F680/F735 as an indicator of coral stress. It is observed that the F680/F735 ratio decreases as the corals were subjected to elevated temperature with the changes becoming more pronounced as the temperature was increased from 29 to 30°C. The fluorescence intensity ratios at different temperatures were first compared to understand the variation in fluorescence as the temperature was raised by 1°. The average F680/F735 ratio values were computed and were tested using ANOVA and the F value was found to be statistically significant with $P < 0.0005$. Scheffe's test also showed significant difference between every pair of temperatures ($P<0.01$). As the variation in fluorescence with increase in temperature was obvious, a simple regression model was constructed to find the effect of temperature on the average F680/F735 ratio. The constructed model can be used to identify the variation in average F680/F735 ratio due to elevated temperature. Moreover, a R^2 value of 0.903 indicates that more than 90% of the variation in fluorescence can be attributed to elevated temperature alone. The average F680/F735 ratio showed a variance of 17.57% between the corals maintained at 29°C and those maintained at a constant temperature of 30°C for 4 days. As the corals were subjected to 31 and 32°C, the F680/F735 ratio decreased further by 8.35 and 12.73%, respectively for each degree of temperature rise. Although, the maximum decrease percentage in the F680/F735 ratio (17.57%) was observed during the initial phases of temperature rise (29-30 °C), the
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visible signs of coral bleaching were seen only when the temperature of the corals reached 32°C. This clearly indicates LIF signatures of corals have the potential to detect coral bleaching at an earlier stage, even before the appearance of visible signs of bleaching.

4. CONCLUSION

Corals are highly sensitive to temperature changes and one of the major threats to reef forming corals is the rise in temperature of the marine environment. Coral fluorescence arises from the photosynthetic pigments in the zooxanthellae and is an important parameter for assessing coral health. The fluorescence intensity in general showed a decreasing trend in response to elevated temperature and the chlorophyll fluorescence ratio F680/F735 showed marked variation as the heat induced bleaching progressed. The pronounced decrease in the F680/F735 ratio for a small increase in initial temperature of 1°C shows that this ratio can serve as an indicator of bleaching prior to the appearance of visible signs in corals subjected to elevated temperature stress in coastal waters. Therefore, the fluorescence intensity ratio F680/F735 can serve as a proxy to assess the extent of damage to coral tissues due to elevated temperature stress. Further in situ studies on coral habitats in a marine environment would be required to confirm the effectiveness of the changes noticed in the fluorescence ratio (F680/F735) as an indicator of the early symptoms of coral bleaching and for its possible application in coral health monitoring from a remote sensing platform.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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