# Effects of Temperature and Salinity on Coral Bleaching in Laboratory

Kamonphon Patthanasiri, Thaithaworn Lirdwitayaprasit, Thamasak Yeemin, Ing-on Thongcamdee, and Nantapak Potisarn

Abstract—Coral bleaching occurs when cell density or the concentration of photosynthetic pigments of the endosymbionts, zooxanthellae are decreased. This incident may possibly be caused by some environmental stresses, especially under conditions of elevated temperature, decrease in water salinity, or a combination of these factors. To determine the role of temperature and salinity on zooxanthellae and coral bleaching this study was conducted in aquariums under laboratory conditions on cauliflower coral Pocillopora damicornis. The samples were collected from three sites around Samaesan Island, Chonburi, Thailand. Three sets of experiments were conducted at three levels of temperature: room temperature 27 (control), 30, and 33 °C respectively. At each temperature level, three levels of salinities; 10, 20 and 30 (control) psu were tested as well. Coral bleaching percentage and zooxanthellae density in the water column were observed every 6 hours during the period of 72 hours. The results showed that when coral exposed to the highest temperature (33 °C) under the lowest salinity (10 psu), 50-90% bleaching was found and higher symbiont densities in the water column were detected. These results suggested that the combination of the high temperature and low salinity had synergistic effects on coral bleaching and zooxanthellae.

 $\begin{tabular}{ll} \it Index & \it Terms $--$ Bleaching, & salinity, & temperature, \\ \it zoox anthellae. \end{tabular}$ 

#### I. INTRODUCTION

Bleaching or the paling of coral color occurs when cell densities or/and the concentration of photosynthetic pigments of zooxanthellae in coral are decreased [1], [2] which may result from some environmental stresses such as an increase in temperature, solar radiation, disease, decreased salinity, sedimentation, inorganic nutrients, or a combination of these factors. Widespread coral bleaching has mainly been observed where daily sea surface temperature exceeds the mean summer maximum temperature by only 1-2 °C [3]

In recent decades, coral bleaching has become more severe resulting in a mass mortality of corals. Coral bleaching has increased in frequency, intensity and covered a larger

Manuscript received May 15, 2021; revised September 8, 2021. (The study is a part of the dissertation of the Department of Marine Science, Chulalongkorn University)

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geographic scale. The frequency and severity of bleaching events occurred under thermal stress. Due to the increased temperature, the growth rate, density and photosynthetic efficiency were decreased because of its effect on photosynthesis of zooxanthellae, especially at 32-34 °C. [4]

The cause of coral bleaching is not only from the increase in temperature but also light and salinity intensity as well. For instance, Reference [5], [6] reported the salinity (especially hyposalinity) effect on the rates of photosynthesis of zooxanthellae. Moreover, the combination of high temperature and low salinity treatment may aggravate the effects of coral bleaching. [7], [8]

In Thailand, reported severe bleaching as a result of the increasing sea surface temperature from 29 °C to 30 °C for long periods in 2010 [9] and the years after, the severe flooding during the monsoon season in Thailand in the year 2011 diluted seawater around Si Chang Island to 11 psu which resulted in coral bleaching. [10]

In order to better understand and test the effects of temperature and salinity, both separately and in combination, toward the change of zooxanthellae in the cauliflower coral *Pocillopora damicornis* a laboratory condition was chosen. It was decided the range of temperatures investigated would be from 27 to 33 °C and the range of salinity from 10 to 30 psu. These tested conditions are similar to the natural conditions of the corals around Samaesan Island where the specimens were collected.

# II. METHOD

# A. Research Organism

This study examined the effects of temperature and salinity on cauliflower coral *Pocillopora damicornis*, a common shallow water scleractinian found throughout the Gulf of Thailand and dominant in the study area. The samples were collected from three sites around Samaesan Island, Chonburi province, Thailand, namely: station A Khao Mha Jor (12.5986, 100.9465) station B Pla Muek Island (12.5871, 100.9438) and station C Had Tien diving point (12.5688, 100.9602)

## B. Coral Collection and Culturing

The cauliflower corals *Pocillopora damicornis* were randomly collected at the depth of 1.5-4 m. from the three sites around Samaesan Island. Fragmentation of the coral was carried out and nursed for two months at Khao Mha Jor station. Just before the experiment, all samples were immediately brought to the laboratory at Eastern Gulf Fisheries and Development Center, Rayong.

doi: 10.18178/ijesd.2022.13.1.1367

## C. Experimental Setup

To test the effects of temperature and salinity on coral bleaching, this study carried out three experiments at room temperature 27 (control) 30 and 33 °C respectively. In each experiment at the set temperature, three levels of salinities; 10, 20 and 30 (control) psu were also tested. The nubbins were randomly distributed in three aquariums. Each aquarium is divided into three compartments, (Station A - Khao Mha Jor, B - Pla Muek Island, and C - Had Tien diving point). In each aquarium, seawater within the compartment is aerated, recirculated, and the aquariums were each placed under lights with an intensity of about 4,000 lux on a 12 hour photoperiod without feeding.

At 30 psu salinity one set of aquariums was kept at 27  $^{\circ}$ C as control. The other aquariums were set for testing at 30 and 33  $^{\circ}$ C where the temperature was adjusted at the rate of + 1  $^{\circ}$ C per day until the temperature reached the set level. Once all aquariums reached the set temperature, the experiments were then started.

On the other hand, in the test for salinity effect one aquarium was set aside at 30 psu as control while the other aquariums for testing at 20 and 10 psu their salinity were decreased at the rate of 5 psu for every 6 hours until the set salinities were reached and the experiments started.

# D. Measurement on Degree of Bleaching by Coral Health Chart

Coral bleaching percentage was measured by a coral health chart (provided by the University of Queensland) that recorded changes in coral colour.

- 1) Select the lightest area, avoiding the tips of branching areas
- 2) Hold the colour chart next to the selected area.
- 3) Rotate the chart until you find the closest colour match.
- 4) Record the matching colour code along with coral type on the data sheet.
- 5) Repeat step 1-4 for the darkest area.

# E. Cell Morphology Observation

In all treatments during the experimental period, Cell morphology of zooxanthellae in the water column was also observed in shape, size (diameters), color, organelles and others

# III. RESULTS

#### A. Symbiont Densities in the Water Column

Prior to starting the experiment, symbiont cells in the water column counts range from 0 to 2 cells/ml. During the period of experimentation at 30 psu, zooxanthellae in the water column were not found at the control temperature (27 °C). Very low symbiont densities in the water column were observed at the high temperature levels (30 and 33 °C) with the symbiont densities ranging from 0-2 cells/ml.

At the 20 psu level, the symbiont densities range from 44-772 cells/ml. at both 30 and 33 °C. While the highest symbiont densities were found to be at the lowest salinity level (10 psu) especially during exposure to higher temperature, that of 33 °C, with up to 1870 cells/ml. from

Had Tien diving point station.

Results in this study showed that the symbiont started to leave their hosts once the salinity decreased and the temperature increased with an exception at 20 psu where the symbiont density in the water column at 30 °C was higher than that of 33 °C. The highest symbiont densities in the water column were detected in the lowest salinity (10 psu) under the highest temperature treatments (33 °C). Moreover, those cells in the water column were similar to normal cells in shape and size but they were clearly pale in colour and had less cytoplasmic organelles than the normal cells. [Fig 1 and 2]

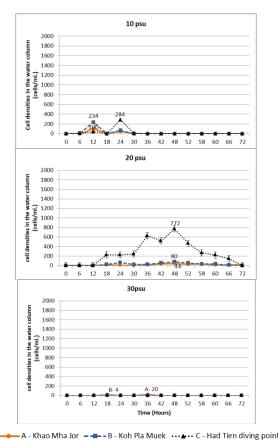
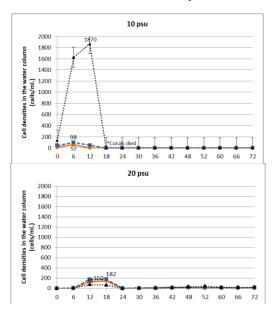


Fig. 1. Symbiont densities in the water column at the temperature levels 30 oC under the 10, 20 and 30 psu.



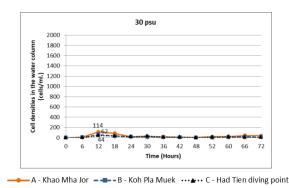


Fig. 2. Symbiont densities in the water column at the temperature levels 33 oC under the 10, 20 and 30 psu.

#### B. Coral Bleaching (Measured by Coral Health Chart)

At the control temperature  $27\,^{\circ}C$ , under the control salinity of 30 psu and 20 psu level the normal coral branches (no bleaching) were found but under the lowest salinity level of 10 psu 50-70% bleaching was found at all stations (Lightest area color: Station A – D1 65%, Station B – D1 50%, and Station C – D1 70%). [Fig 3]

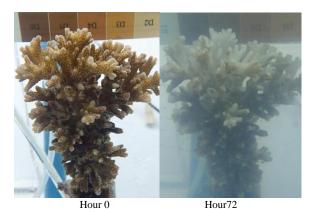
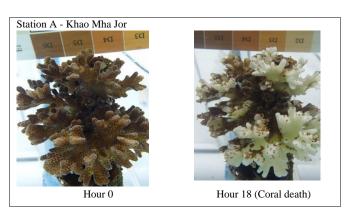


Fig. 3. Coral branches at the control temperature (27 oC) under 10 psu at Hour 0 and Hour 72

At the midrange 30  $^{\circ}$ C, under the control salinity (30 psu) it was found that there were normal coral branches (no bleaching). Under the 20 psu treatment bleaching had occurred after 36 hours. At 72 hours, the area of bleached corals reached 5-50% (Lightest area color: Station A – D2 5%, Station B – D1 10% and Station C – D1 50%).

In the lowest salinity treatment (10 psu), 40-70% of bleaching was found after 18 hours and coral branches from Station A died, corals from Station B died after 24 hours, whereas corals from Station C remained alive, as shown in Fig. 4.



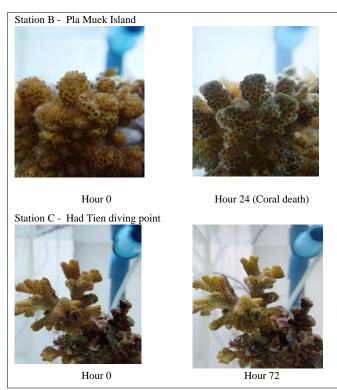


Fig. 4. Coral branches from 3 stations a). Khao Mha Jor, b). Pla Muek Island and c). Had Tien diving point at 30 oC under the lowest salinity (10 psu).

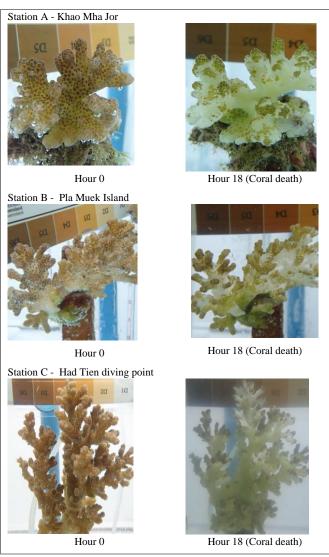


Fig. 5. Coral branches from 3 stations a). Khao Mha Jor, b). Pla Muek Island and c). Had Tien diving point at 33 °C under the lowest salinity (10 psu).

For the coral exposed to the lowest salinity of 10 psu, 50-90% bleaching (Lightest area color: Station A-D1 90%, Station B-D1 70% and Station C-D1 90%) took place before their death on the 18th hour, as shown in Fig. 5.

#### IV. DISCUSSION

The highest number of zooxanthellae released and coral bleaching were detected in the lowest salinity (10 psu) under the highest temperature (33 °C) treatments. This result indicates that the high temperature and low salinity affect zooxanthellae and cause coral bleaching. Under the increased temperature condition, growth rate density photosynthetic efficiency of zooxanthellae were decreased because of the elevated temperature, especially at 32-34 °C where it affects the photosynthesis of zooxanthellae in living corals. [11], [12] However, the tolerance of symbiotic algae to temperature stress may vary depending on host coral species. Moreover, Reference [5] reported the changing in salinity, especially hyposalinity, affected the rates of photosynthesis of zooxanthellae. Beyond that the reduction in salinity in surface waters caused massive mortality of coral reef organisms.

Under the control salinity treatment (30 psu) the stressed coral had changes to the darkest area moving from D6 to D4 but did not bleach the coral to a white skeleton (D1 color) even at the exposure of 33 °C. On the other hand, 50-70% bleaching was found at all stations when under the lowest salinity level of 10 psu, including the corals exposed to the control temperature (27 °C). Since the salinity at every temperature test shows a more intense effect on the number of zooxanthellae released and coral bleaching, the result may differ from the widespread belief that seawater temperature is the main factor inducing coral bleaching. That raised a question of why salinity becomes a stronger inducer than temperature.

In our experiments, the tests have been carried out in aquariums that can contain limited amounts of seawater. In addition, the water in each aquarium was under controlled conditions and was uniform in water quality all around the tested specimens. On the contrary, in nature the cauliflower corals fill the space on the seafloor in both the horizontal and vertical plain. It is necessary to point out the differences between the condition of water mass in the aquarium and in nature. Uniformity of water quality was maintained in the aquariums while in nature uniformity in water quality in both the horizontal and vertical plain is nearly impossible due to the actions of water flow both by wave and intertidal current, variation in light intensity during the daytime, concentration of suspended particulates in water mass, the balance between replenishment rate and bleaching of the coral, and other unknown factors. Since the tests were carried out only for salinity and temperature, emphasis will be made on only these two factors.

In conclusion, in this study we found the highest bleaching percentage when corals exposed to the lowest salinity (10 psu) at every temperature level. Moreover, we found 50-90% bleaching (highest percentage) and the highest symbiont densities in the water column when corals were exposed to the highest temperature level (33 °C) under the lowest salinity

level (10 psu). It suggests that the combination of high temperature and low salinity can affect the coral bleaching and zooxanthellae in living corals. The high temperature and low salinity treatment had synergistic effects on scleractinian coral fragment. [7], [13]

#### CONFLICT OF INTEREST

The authors declare that we have no conflict of interest.

#### **AUTHOR CONTRIBUTIONS**

Kamonphon Patthanasiri, Thaithaworn Lirdwitayaprasit and Thamasak Yeemin carried out the research design and analyzed the data; Kamonphon Patthanasiri, Ing-on thongcamdee and Nantapak Potisarn carried out the field and laboratory study. The authors' contribution is reflected in all the research approved in its final version.

#### ACKNOWLEDGMENT

The authors would like to thank the Royal Thai Marine Corps for the assistance with the sample collecting and The Eastern Gulf Fisheries and Development Center, Rayong for the laboratory. This study was supported by the Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, the graduate school Chulalongkorn University, and the Science Achievement Scholarship of Thailand.

#### REFERENCES

- G. P. Podest á and P. W. Glynn, "Sea surface temperature variability in Panama and Galapagos: Extreme temperature causing coral bleaching," *Journal of Geophysical Research: Oceans*, vol. 102, pp. 15749-15759, July 1997.
- [2] T. C. Jeunesse and R. K. Trench, "Biogeography of two species of Symbiodinium (Freudenthal) inhabiting the intertidal sea anemone Anthopleura elegantissima (Brandt)," The Biological Bulletin, vol. 199, pp. 126-134, Oct 2000.
- [3] R. J. Jones, O. Hoegh-Guldberg, A. W. D. Larkum, and U. Schreiber, "Temperature induced bleaching of corals begins with impairment of the CO<sub>2</sub> fixation mechanism in zooxanthellae," *Plant Cell and Environment*, vol. 21, pp. 1219-1230, March 2002.
- [4] R. Rodolfo-Metalpa, C. Richard, D. Allemand, C. N. Bianchi, C. Morri, and C. Ferrier-Pag ès, "Response of zooxanthellae in symbiosis with the Mediterranean corals Cladocoracaespitosa and Oculinapatagonica to elevated temperatures," *Marine Biology*, vol. 150, pp. 45-55, May 2006.
- [5] C. Ferrier-Pages, J. P. Gattuso, and J. Jaubert, "Effect of small variations in salinity on the rates of photosynthesis and respiration of the zooxanthellae coral Stylophora pistillata," Marine Ecology Progress Series, vol. 181, pp. 309-314, May 1990.
- [6] R. Berkelmans, A. M. Jones, and B. Schaffelke, "Salinity thresholds of *Acropora* spp. on the Great Barrier Reef," Springer-Verlag, vol. 31, pp. 1103-1110, July 2012.
- [7] M. Dias, C. Madeira, N. Jogee, A. Ferreira, R. Gouveia, H.Cabral, M. Diniz, and C. Vinagre, "Oxidative stress on deleratinian coral fragments following exposure to high temperature and low salinity," *Ecological Indicators*, vol. 107, Dec 2019.
- [8] R. A. Kinzie, M. Takayama, S. R. Santos, and M. A. Coffroth, "The adaptive bleaching hypothesis: experimental tests of critical assumptions," *The Biological Bulletin*, vol. 200, pp. 51-58, Nov 2000.
- [9] R. P. Dunne, "The record of sea temperature during the 2010 coral bleaching at Phuket, Thailand-different datasets, different perspectives-unexplained errors in hadisst 1.1.," *Phuket Marine Biological Center Research Bulletin*, vol. 71, pp. 11-18, 2012.
- [10] T. Yeemin, "Climate change impacts and implications. Synthesis of status and knowledge on impacts, recovery and management of coral reefs from coral bleaching phenomenon," 2011.
- [11] G. Yamashita et al., "Acropora recruits harbor "rare" Symbiodinium in the environmental pool," Springer-Verlag, vol. 32, pp. 355-366, Nov 2012.

- [12] K. R. Trench, "The cell biology of plant Animal symbiosis," Ann. Rev. Plant Physiol, vol. 30, pp. 485-531. 1979.
- [13] Z. Zhou, S. Zhao, J. Ni, Y. Su, L. Wang, and Y. Xu, "Effects of environmental factors on C-type lectin recognition to zooxanthellae in the stony coral *Pociilopora damicornis*," *Fish and Shellfish Immunology*, vol. 79, pp. 228-233, Aug 2018.

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