International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies

http://TuEngr.com

EFFECTS OF TEMPERATURE AND AMMONIA ON CORAL HEALTH STATUS: A CASE STUDY OF DISC CORAL (*Turbinaria peltata*)

Bussapakorn Udomsap [a], Petchporn Chawakitchareon [a*], Sompop Rungsupa [b]

[a] *Department of Environmental Engineering, Chulalongkorn University, Bangkok 10330, THAILAND*
[b] *Aquatic Resources Research Institute, Chulalongkorn University, Bangkok 10330, THAILAND*

ARTICLE INFO

Article history:
Received 30 January 2018
Received in revised form 15 March 2018
Accepted 19 March 2018
Available online 20 March 2018

Keywords:
Seawater temperature; Ammonia concentration; Coral health status; Active polyps; Lethal concentration.

ABSTRACT

This research focused on the effects of temperature and ammonia on the coral health status of disc coral (*Turbinaria peltata*) by using acute toxicity testing (50% Lethal Concentration: LC_{50}). The acute effects of temperature and ammonia on disc coral were monitored at 24 and 48 hrs. The experiments were carried out in triplicate at temperatures of 30ºC and 33ºC. The concentrations of ammonia were varied at 0, 0.05, 0.07 and 0.1 mgN/L, respectively. The active polyp percentages of disc coral was analyzed with comparison to the health status percentages. According to the findings at 30ºC and at 24 and 48 hrs, and at 33ºC and 24 hrs, the acute toxicity of coral bleaching (LC_{50}) could not be investigated. This is because the coral health status was insufficiently low due to decline, or the mortality percentages were not below 50 percent. On the other hand, at 33ºC and 48 hrs, the acute toxicity of coral bleaching (LC_{50}) could be evaluated. The experimental results strongly indicate that the mortality percentages exceeded 50 percent. These findings were confirmed by Zooxanthellae density in seawater equaling 109.4 cell/ml. Therefore, the LC_{50} at 48 hrs in this study was equal 0.075 mg N/L.

1. Introduction

Corals are marine invertebrates. Classified in the phylum Cnidaria, the structural sequence of limestone results in shapes such as tabulate, massive and branching. Corals acquire food through suspension and autotrophic feeding by Zooxanthellae algea with which the corals share what is called a mutualistic symbiosis. Algae accelerate the formation process of limestone and coral colors. In the other ways, corals provide a residence for algae. Corals grow well at water temperatures between 25-30˚C (Chankong, 2014). When the marine environment changes or conditions become unsuitable, such as when seawater temperatures rise (above 30˚C) or salinity has

*Corresponding author (P.Chawakitchareon). Tel: +66-2-2186674 E-mail: petchporn.c@chula.ac.th.

dropped (Rungsupa et al., 2016 and 2018), algae will produce substances that are toxic to coral tissues. Corals become stressed and drive algae out from the coral tissues. This will result in a loss of coral pigment volume (Fitt et al., 2000). Apart from the effects of temperature and salinity changes on coral, other factors contribute to coral health degradation. Ammonia is one of the factors potentially affecting stress and causing toxicity for corals (Hansuebsai et al., 2018). Current increases in seawater temperature are the most important factors involved in coral health degradation. Moreover, is has been reported that the coral areas in Sichang Island, Chonburi Province, Thailand, have been affected by high ammonia concentrations exceeding set standards (more than 0.07 mgN/L) because the marine ecosystems of Sichang Island have been contaminated by wastewater from municipal activities, cargo ships, human activities, and etc. that increase ammonia concentrations in the area (Regional Environment Office, 2016 and Coverdale et al., 2013).

In addition, current information about the safety effects of ammonia for invertebrates remains limited. Therefore, careful application of the same criteria as that used for fish is currently recommended (Lawson, 1995). Thus, the present study is focused on the effects of temperature and ammonia on the health status of disc coral *(Turbinaria peltata)* by coral health evaluation with the coral health chart that is a standardized, inexpensive, flexible color reference card anyone can use for rapid, broad area assessment of changing coral conditions (Siebeck et al., 2006). The acute toxicity of ammonia concentrations resulting in bleached coral at more than 50% (50% Lethal Concentration: LC_{50}) was calculated by Probit analysis and photographic assessment was used for analysis of the active polyp percentages of *Turbinaria peltata* compared with health status percentages.

2. Methodology

2.1 Acute Effects of Temperature and Ammonia

The experiments were conducted at Sichang Island, Thailand. Acclimated disc coral *(Turbinaria peltata)* was kept in a filtered seawater pond with continuous water flowing for 7 days before starting the experiments which were carried out in triplicate. The selected coral sizes were between 3-4 cm. Coral health was compared with the coral health chart at Levels 5-6; no bleaching was found on the pieces of coral (Figure 1.). The corals were placed in glass cases with filtered seawater that was aerated with temperatures set at 30˚C and 33˚C with a glass heating rod (Figure 2.). Salinity and pH were controlled to remain constant (30 ppt, pH 8). Ammonia concentrations were varied at 0, 0.05, 0.07 and 0.1 mgN/L, respectively.

Zooxanthellae density (Lenore et al., 2012), salinity, pH, temperature and coral health status were measured and recorded at 0, 12, 24 and 48 hrs before changing to regular seawater without adding ammonia. Coral health status was recorded at 24 and 48 hrs with the aim of monitoring coral health recovery which was lower than Level 3 or corals with bleaching on both pieces and parts.

Figure 1: *Turbinaria peltata*

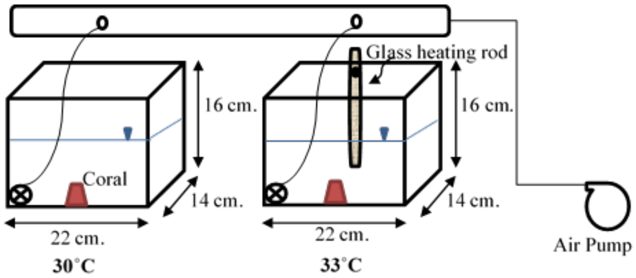

Figure 2: Experiment kit example

2.2 Coral Health Status Evaluation

Coral health status was evaluated by the using the coral health chart (Figure 3.). The colors determination was divided into 4 groups and classified into 6 levels (Siebeck et al., 2006) In which Level 6 is representative of coral in good health (best health) and Level 1 is representative of declining coral health (worst health). After evaluating the coral health status based on colors, the status was then calculated into percentages as shown in Table 1.

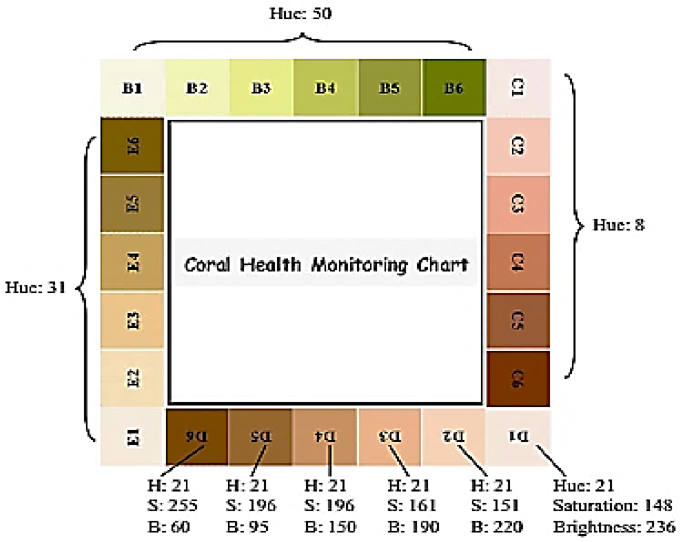

Figure 3: Coral health chart (Siebeck et al., 2006)

*Corresponding author (P.Chawakitchareon). Tel: +66-2-2186674 E-mail: petchporn.c@chula.ac.th.

2.3 Data Analysis

Pictures and amounts of coral with colors lower than Level 3 were recorded. Next, the recorded data was converted into health status categories and mortality percentages in order to calculate the acute toxicity test (LC_{50}) at 48 hrs by using Probit analysis (Finney, 1952). Finally, the correlations between health status percentages and Zooxanthellae density in seawater were determined.

Table 1: Health status and mortality percentages from the coral health chart.

Level	Remark	Health status percentages	Mortality percentages
1	Worst health	16.67	83.33
2	Poor health	33.33	66.67
3	Declining health	50.00	50.00
4	Fair health	66.67	33.33
5	Good health	83.33	16.67
6	Best health	100.00	0.00

2.4 Active Polyp Percentages

The authors found that it was difficult to observe any stress in *Turbinaria peltata*. Therefore, active polyps were recorded by camera, converting true color into 256 gray scales. Fragment areas were selected and counted for active polyps (extend polyps), white spots and non-active polyps and red spots from 256 gray-scale picture (Rungsupa et al., 2018).

Active polyp percentages by calculated = 100*active polyp number/(total polyp counted) (1)

3. Results and Discussion

3.1 Effects of Temperature and Ammonia

In testing at temperatures of 30˚C and 33˚C, the health status and mortality percentages of corals at ammonia concentrations of 0, 0.05, 0.07 and 0.1 mgN/L are shown in Tables 2 and 3. The results indicate that coral health statuses ranged from fair to good at 30˚C and 24 and 48 hrs. (Colors higher than Level 3) and can be calculated at percentages of 74.1-83.3% and 68.5-79.6%, respectively.

At 33˚C and 24 hrs, coral health statuses ranged from poor to good and could be calculated at 54.2-76.7%. Mortality percentages less than 50% would prevent the calculation of ammonia concentrations and their effects on coral bleaching or LC_{50}. At 48 hrs, the coral health statuses ranged from poor to good and could be calculated at 36.7-75.0%.

At 33˚C and 48 hrs with ammonia concentrations of 0.07 and 0.1 mgN/L, the coral health's sensitivity increased the mortality percentages by more than 50% which can be calculated as LC_{50}. The ammonia concentration at 0.1 mgN/L had the highest mortality percentages at 63.3%. When the mortality percentages of the corals exceeded 50% LC_{50} could be calculated by using Probit analysis (Figure 4.). At 33˚C, ammonia concentrations had the effect of coral bleaching or 50-percent coral mortality at 48 hrs which was equal to 0.075 mgN/L. This was related to the Typical

Surface Ocean guideline at less than 0.1 mgN/L (Kallqvist and Svenson, 2013) and LC_{50}, which is close to Marine Water Quality Standards for coral reef conservation that are determined not to exceed 0.07 mgN/L. Moreover, the above findings are related to the Nozawa, (2012) study which found temperatures exceeding 30˚C to have the effects of decreasing the Zooxanthellae density in the coral tissue and increasing seawater temperatures by approximately 1-2˚C, which will result in coral bleaching (Nozawa, 2012 and Buchheim, 2016). In 2010, Thailand reported that the seawater temperature surrounding Phuket Island rise from normal temperatures from 29˚C to 33.5˚C for thirty consecutive days. Consequently, bleaching was observed in all of the corals in the area (Department of Marine and Coastal Resources, 2013).

Table 2: Health status and mortality percentages at 30˚C.

30˚C	Healthy Status (%)				Mortality (%)
Ammonia (mgN/L)	0 hr.	12 hr.	24 hr.	48 hr.	48 hr.
0	100.00	85.19	79.63	79.63	20.37
0.05	100.00	85.42	83.33	77.08	22.92
0.07	100.00	81.48	79.63	75.93	24.07
0.1	100.00	83.33	74.07	68.52	31.48

Table 3: Health status and mortality percentages at 33˚C.

33˚C	Healthy Status (%)				Mortality (%)
Ammonia (mgN/L)	0 hr.	12 hr.	24 hr.	48 hr.	48 hr.
0	100.00	80.00	66.67	63.33	36.67
0.05	100.00	91.67	76.67	75.00	25.00
0.07	100.00	83.33	54.17	47.62	52.38
0.1	100.00	80.00	60.00	36.67	63.33

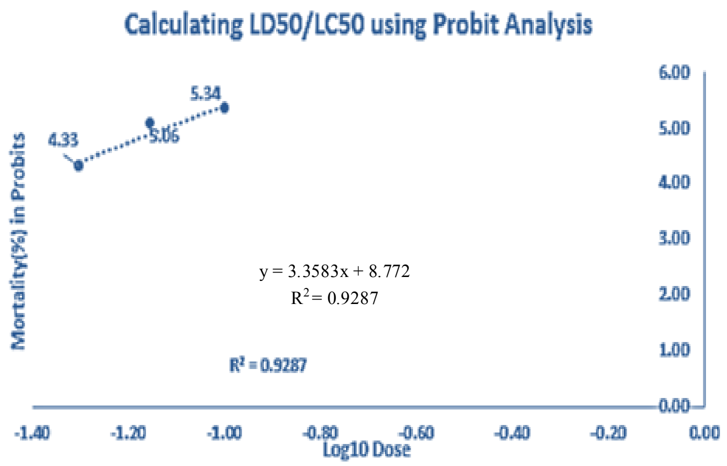

Figure 4: Calculating LC_{50} using Probit analysis at 33˚C.

3.2 Effects of Temperature and Ammonia

According to the measurement of the Zooxanthellae density in seawater at 30˚C and 24 hrs with ammonia concentrations of 0, 0.05 and 0.07 mgN/L, the Zooxanthellae density was between

*Corresponding author (P.Chawakitchareon). Tel: +66-2-2186674 E-mail: petchporn.c@chula.ac.th.

12.7-15.0 cells/ml, and the ammonia concentration was 0.1 mgN/L. The Zooxanthellae density was equal to 26.6 cells/ml. At 33˚C, the Zooxanthellae density at all ammonia concentrations was higher than the Zooxanthellae density at 30˚C. At the ammonia concentration of 0.1 mgN/L, the highest Zooxanthellae density was equal to 45 cells/ml. When compared with the ammonia concentrations at 24 hrs, the temperature was 30˚C at 48 hrs and the Zooxanthellae density increased, except when the ammonia concentration was 0 mgN/L whereby the Zooxanthellae density would decrease. With the passing of time, corals can adjust to new conditions and stop processing mucus secretions. At 33˚C and an ammonia concentration of 0.1 mgN/L, the Zooxanthellae density was equal to 109.4 cells/ml which was correlated with a decline in coral health status (Figure 5.).

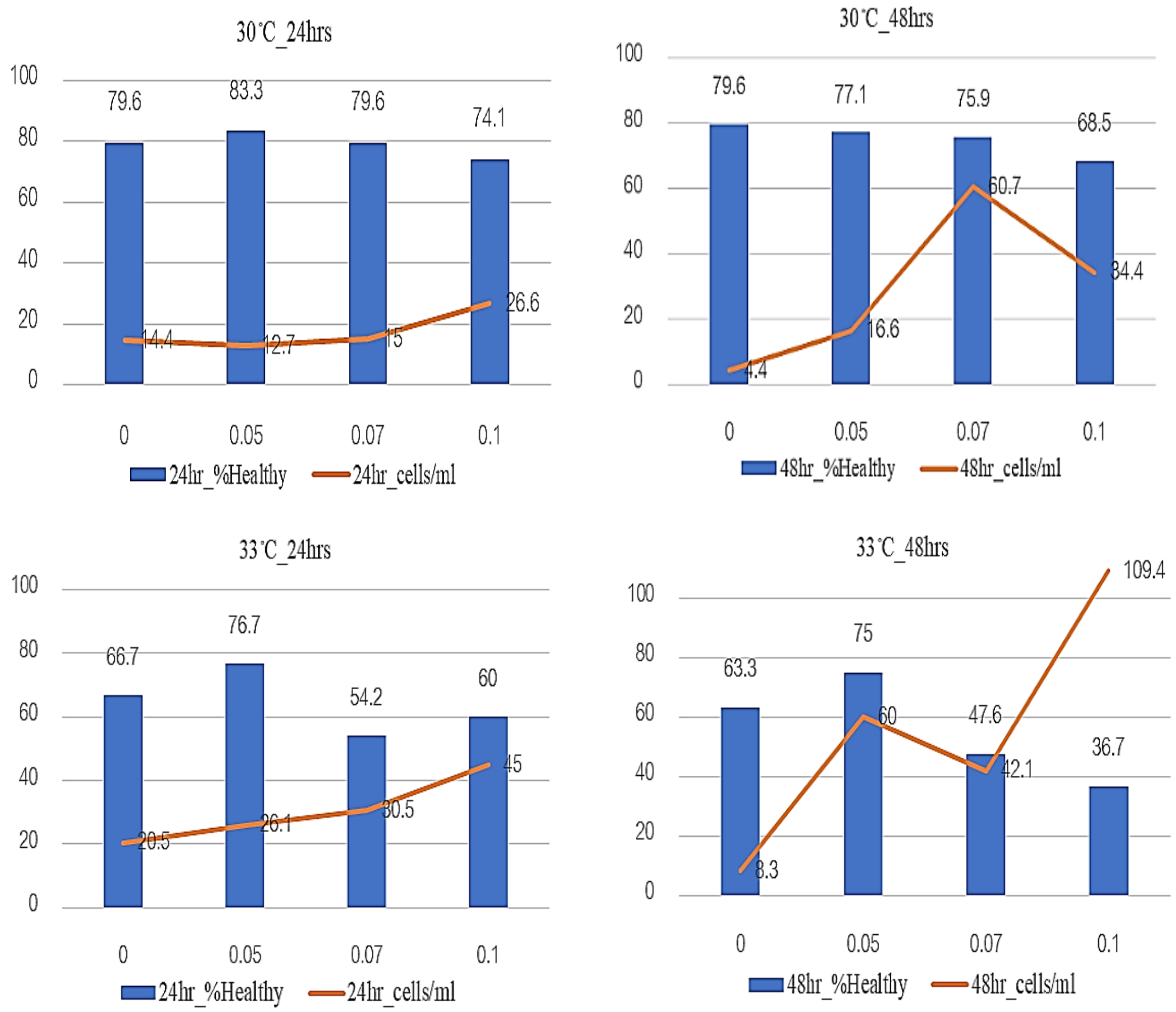

Figure 5: Graph shows the correlations between health status (%) and Zooxanthellae density at a) 30˚C_24 hrs. b) 30˚C_48 hrs. c) 33˚C_24 hrs. d) 30˚C_24 hrs.

According to the observations, corals under stressful conditions would active a defense mechanism caused by unstable conditions by using mucus secretions (Kellog, 2004). If the temperature and ammonia concentrations rise, the corals would accelerate the mucus secretion process (Figure 6. a)), which makes the colors of corals fade as a result of the mucus secretions that make coral tissues slip-out. The result is a decreasing the amount of Zooxanthellae in coral tissues (Kerswell and Jones, 2003) (Figure 6. b)). Thus, there is a correlation with Zooxanthellae density measurement in experimental seawater. At 33 ̊C and 48 hrs with an ammonia concentration of 0.1 mgN/L, coral health status declines. The Zooxanthellae density is measured, the value of density is 109.4 cell/ml, which indicates that corals have the greatest Zooxanthellae slip-out process. Therefore, ammonia is another factor causes coral bleaching or coral health decline, which is a find that concurs with the findings of Baohua et al. (2004), in a study showing that, at a temperature of 32 ̊C, corals have the highest slip-out rate after 18 hrs and ammonia results in more coral bleaching than nitrate (Baohua et al., 2004). Moreover, whether coral bleaching can recover in full or in part depends on the cause of the bleaching, including the severity of stress causing damage inside corals (Arthur et al., 2006). In this experiment, however, the corals were unable to recover from bleaching.

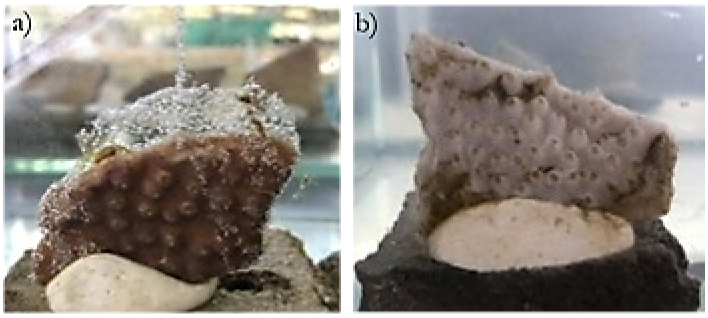

Figure 6: a) Mucus secretion process b) Coral tissue slip-out (at 33 ̊C and 48 hrs with an ammonia concentration of 0.1 mgN/L)

3.3 Active Polyp Percentages

For *Turbinaria peltata*, a disc coral, the other photographic assessment can be used for analysis of stress values (Winter et al., 2009). In this study, active polyp percentages (Table 4) (Figure 6) were compared to health status percentages (Table 5). Determining the active polyp percentages involved counting the polyps which was not exceeding the extend number or fixed area.

Table 4: Active polyp percentages for *Turbinaria peltata*.

33 ̊C	Active polyps			Non active polyps			Active polyp percentages		
Ammonia (mgN/L)	12 hr.	24 hr.	48 hr.	12 hr.	24 hr.	48 hr.	12 hr.	24 hr.	48 hr.
0	57	23	16	1	35	56	98.94	39.70	21.52
0.05	60	11	7	7	55	65	90.11	17.65	8.83
0.07	11	7	1	30	30	40	26.67	16.29	1.45
0.1	12	4	0	48	56	60	21.36	5.97	0.51

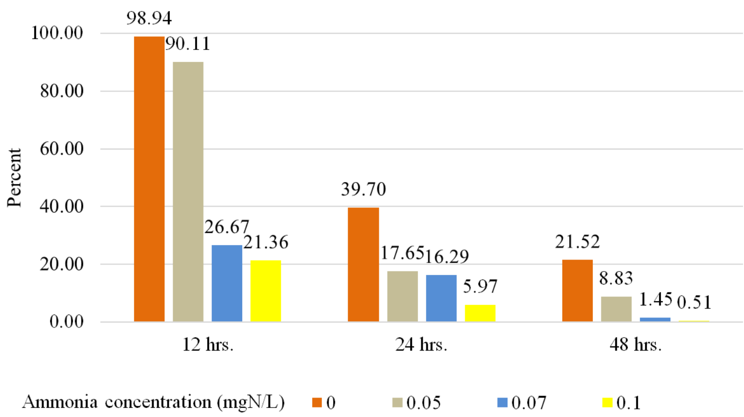

Figure 6: Active polyp percentages for *Turbinaria peltata*.

*Corresponding author (P.Chawakitchareon). Tel: +66-2-2186674 E-mail: petchporn.c@chula.ac.th.

Table 5: Comparison of health status and active polyp percentages

33˚C	12 hr.		24 hr.		48 hr.	
Ammonia (mgN/L)	Health status (%)	Active polyps (%)	Health status (%)	Active polyps (%)	Health status (%)	Active polyps (%)
0	59.26	49.61	48.89	43.06	48.15	38.33
0.05	87.03	47.08	79.63	39.83	31.48	35.07
0.07	59.26	20.95	53.70	1.88	27.78	1.88
0.1	83.33	8.28	51.85	1.75	25.93	1.75

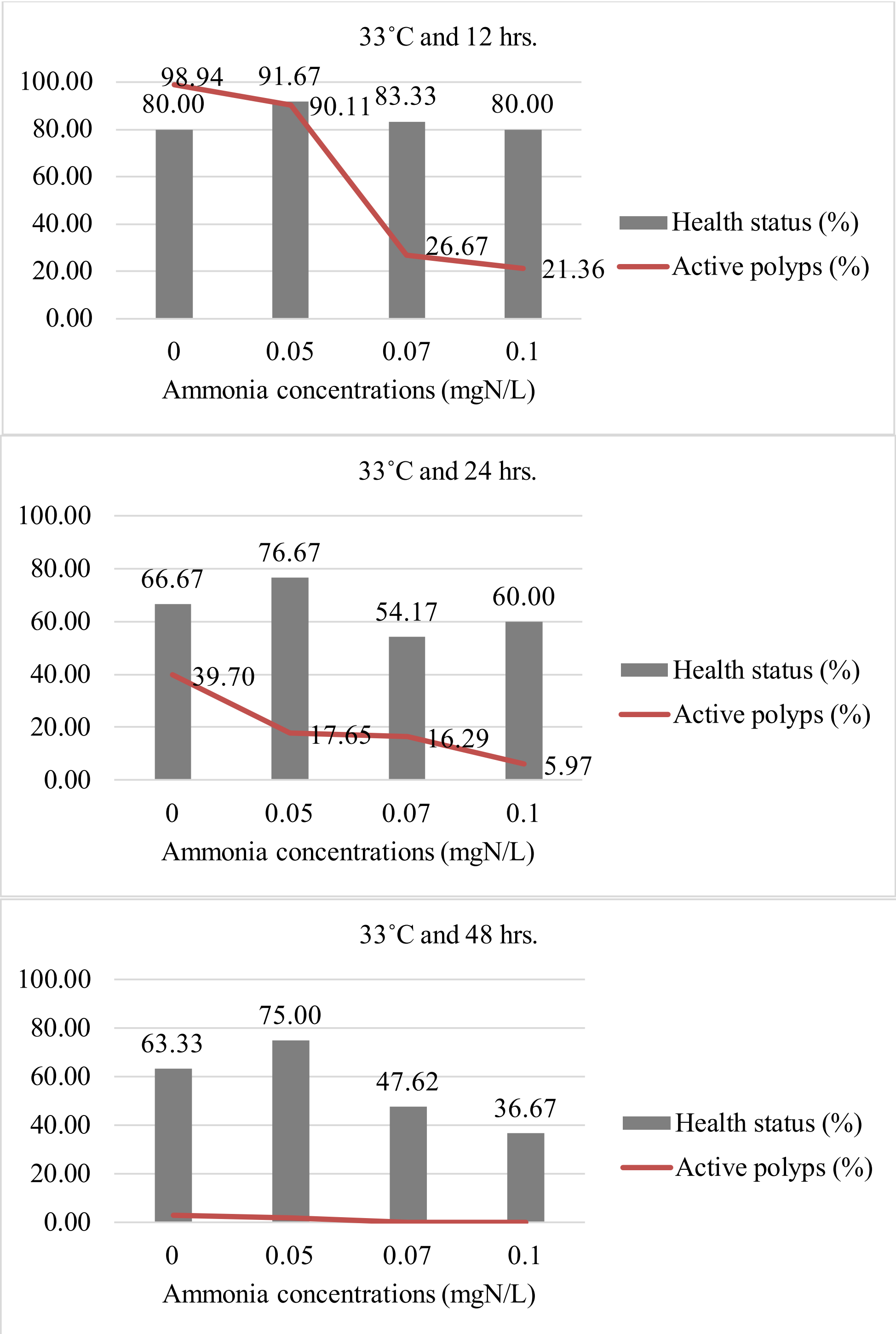

Figure 7: The correlation between health status and active polyp percentages at 33˚C and 12 hrs, 24 hrs, and 48 hrs for *Turbinaria peltata*.

According to Table 5 and Figure 7, health status percentages is correlated with the active polyp percentages. If health statuses are low, the active polyps will be high (Figure 7.). Otherwise, the active polyps will be low when corals have their defense mechanism triggered by unstable conditions (high ammonia concentration). Therefore, these results are correlated with Rungsupa et al. (2018) other studies finding that corals in low salinity with have active polyp percentages at less than or equal to 0.

4. Conclusion

In this study, the results of the temperature and ammonia effects on the coral health status of disc coral (*Turbinaria peltata*) by acute toxicity testing calculated (50% Lethal Concentration: LC_{50}) with Probit analysis at temperatures of 30˚C and 33˚C and time durations of 24 hrs and 48 hrs together with ammonia concentrations of 0, 0.05, 0.07, and 0.1 mgN/L indicated temperatures of 30˚C and 33˚C with ammonia concentrations can increase the bleaching or decline of coral health status and the severity will be determined by follow-up on ammonia concentrations. Moreover, at 33˚C and 48 hrs with an ammonia concentration of 0.1 mgN/L, the highest mortality percentages of coral will occur at 63.3%, which is related to the Zooxanthellae density in seawater equal to 109.4 cell/ml. Thus, it is indicated that corals have to secret mucus to drive algae out from coral tissues and LC_{50} at 48 hrs can be calculated at 33˚C, because corals have to bleach or decline in coral health by more than 50%. After calculations, LC_{50} at 48 hrs was equal to 0.075 mgN/L, but bleaching exceeding 50% could not be found on corals under other conditions in which LC_{50} could not be calculated.

Measuring active polyp percentages is a useful method for monitoring the health status of coral under any stressful conditions. High temperatures, high ammonia concentrations, low salinity and community waste are highly threatening stress factors for shallow water coral. This study reported a correlation between health condition percentages and active polyp percentages. *Turbinaria peltata*, a disc coral, was successfully analyzed for stress value by using active polyp percentages.

5. Acknowledgements

This research was supported by the 90th Anniversary of Chulalongkorn University, Rachadapisek Sompote Fund academic year 2017. The authors would like to thank the staff of Sichang Marine Science Research Station, Chonburi, Thailand, for their assistance and support in the experiments. The authors also would like to express their appreciation for the equipments support from Global Environmental System Leaders Program, Keio University, Japan.

6. References

Arthur, R., Done, T. J., Marsh, H., and Harriott, V. (2006). Local processes strongly influence post-bleaching benthic recovery in the Lakshadweep Island. *Coral Reefs* 25: 427-440.

*Corresponding author (P.Chawakitchareon). Tel: +66-2-2186674 E-mail: petchporn.c@chula.ac.th.

Baohua, Z., Guangce, W., Bo, H., and Tseng, C. K. (2004). Effects of temperature, hypoxia, ammonia and nitrate on the bleaching among three coral species. *Chinese Science Bulletin* 49 (18): 1923-1928.

Buchheim, J. (2016). Coral Reef Bleaching. Odyssey Expeditions-Marine Biology Learning Center Publications. Retrieved from http:// www.marinebiology.org/coralbleaching.htm.

Chankong, A. (2014). Biodiversity of inververtebrate in Thailand sea: Corals. *Conference 2014 International Day for Biodiversity: Island Biodiversity*, June 22-24 2014: Walailak University, Nakhonsithammarat, Thailand.

Coverdale, T. C., Herrmann, N. C. Altieri A. H., and Bertness, M. D. (2013). Latent impacts: the role of historical human activitiy in coastal habitat loss. *Frontiers in Ecology and Environment*, January 2013: 69-74.

Department of Marine and Coastal Resources. (2013). Coral bleaching. Retrieved from http://marinegiscenter.dmcr.go.th/km/coral-blenching/#.WS55qmiGPIV

Finney, D. J. (1952). Probit Analysis (2^{nd} Ed). *Journal of the Institute of Actuaries* 78 (3): 388-390.

Fitt, W. K., McFarland, F. K., Warner, M. E., and Chilcoat. G. C. (2000). Seasonal patterns of tossue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *American Society of Limnology and Oceanography* 45: 667-685.

Hansuebsai, A., Rungsupa, S., Kiyoki, Y., Sasaki, S., and Chawakitchareon, P. (2018). Study the effect of Ammonia by Image Analysis on Healthiness Detection for Coral Quality of Life. *Information Modelling and Knowledge Bases XXIX*, IOS Press, 2018, Volume 301: 343-353.

Kallqvist, T., and Svenson, A. (2013). Assessment of ammonia toxicity in tests with the microalga, Nephroselmis pyriformis, Chlorophyta. Norwegian Institute for Water Research, Kjelsas, Oslo, Norway. *Water Research*. 37(3): 477-484.

Kellogg, C. (2004). Coral Mucus Goes Mainstream-New Discoveries in Microbial Communities. *Journal Marine Ecology Progress Series* 273: 81-88.

Kerswell, P. A., and Jones, J. R. (2003). Effects of hypo-osmosis on the coral Stylophora pistillata: nature and cause of low salinity. *Marine Ecology Progress Series* 253: 145-154.

Lawson, T. B. (1995). Fundamentals of aquaculture engineering. Chapman & Hall, New York. 355 pp.

Lenore, S. C., Arnold, E. G., and Andrew, D. E. (2012). Standard Methods for the Examination of Water and Wastewater 22^{nd} Edition, 10200-Plankton: 1992-2019.

Nozawa, Y. (2012). Annual variation in the timing of coral spawning in a high-latitude environment: influence of temperature. *The Biological Bulletin* 222 (3): 192-202.

Regional Environment Office. (2016). Reports from the environmental quality situation in Eastern of Thailand at 2015. Regional Environment Office 13 (Chonburi). 106 p.

Rungsupa, S., Chawakitchareon, P., Hansuebsai, A., Sasaki, S., and Kiyoki, Y. (2018). Photographic Assessment of Coral Stress: Effect of Low Salinity to *Acropora sp .Goniopora sp .and Pavona sp.* at Sichang Island, Thailand. *Information Modelling and Knowledge Bases XXIX*, IOS Press, 2018, Volume 301: 137-148.

Rungsupa, S., Sesulihatien, W. T., Hansuebai, A., Chawakitchareon, P., Sasaki, S., and Kiyoki, Y. (2016). The Early Step of Healthiness Detection for Coral Quality of Life at Sichang Island, Thailand. *The proceedings of 5th International Conference on Environmental Engineering, Science and Management, Environmental Engineering Association of Thailand*, The Twin Towers Hotel, Bangkok, Thailand, May 11-13, 2016. (11R5-11).

Siebeck, U. E., Marshall, N. J., Klü, A., and Hoegh-Guldberg, O. (2006). Monitoring coral bleaching using a colour reference card. *Coral Reefs* 25 (3): 453-460.

Winters, G., Holzman, R., Blekman, A., Beer, S., and Loya, Y. (2009). Photographic assessment of coral chlorophyll contents: Implications for ecophysiological studies and coral monitoring. *Journal of Experimental Marine Biology and Ecology*: 25-35.

Bussapakorn Udomsap is a graduate student in Department of Environmental Engineering, Chulalongkorn University, Thailand. She earned her bachelor degree in Environmental Science from Mahidol University, Thailand. Her research encompassed coral health related to environmental changes.

Dr.Petchporn Chawakitchareon is an Associate Professor in Department of Environmental Engineering at Chulalongkorn University. She received her B.Sc. (Med.Tech) and M.Sc.(Biochemistry) from Mahidol University. She obtained her PhD in Environmental Engineering from ENTPE-LyonI, France. Dr.Petchporn current interests involve utilization of industrial waste for environmental engineering applications and environmental data mining applications.

Dr.Sompop Rungsupa is a researcher at Aquatic Resources Research Institute, Chulalongkorn University, Bangkok, Thailand. He got his B.Sc. (Marine Science), M.Sc. and Ph.D. (Environmental Science) from Chulalongkorn University. His current research is related to bio-aquatic plants and animals. Dr.Sompop also focuses on the impact of water quality related to circulation system in Thai Abalone (Haliotis asinina).

*Corresponding author (P.Chawakitchareon). Tel: +66-2-2186674 E-mail: petchporn.c@chula.ac.th.

Volume 9 No.1 ISSN 2228-9860 eISSN 1906-9642. http://TUENGR.COM/V09/031.pdf. https://doi.org/10.14456/ITJEMAST.2018.6