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Effects of Salinity and Nitrate on Coral Health Levels of *Acropora sp.*

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Abstract

Changes of marine environmental conditions such as low salinity and nutrient enrichment in seawater can be mainly affected on coral health levels or can adversely cause mass coral bleaching. According to these unsuitable conditions, leading to the objective of this study is to study the effect of salinity and nitrate on coral health levels in branching coral (*Acropora sp.*) at the salinity vary in 15, 20, 25, and 30 psu, the concentration of nitrate vary in 20, 60, and 100 µg-N/l with triplicate experiments. The experimental results showed that 30 psu of salinity at 96 hours was unable to calculate LC_{50} (Lethal Concentration) because of insufficiently lower in declining coral health level or lower in motility percentage. However, the results showed that 15 psu of salinity at 96 hours was able to calculate LC_{50} using Probit analysis, LC_{50} 15 psu of salinity at 96 hours was equally to 89.50 µg-N/l Moreover, high nitrate concentrations showed more paling color and stimulating to release mucus in *Acropora sp*.

Keywords : salinity; nitrate; coral health levels; coral bleaching; LC_{50}

Introduction

Corals and coral reefs play an important role both direct and indirect way as providing vital ecosystem services as a mainly source of food and habitation, protecting the shoreline from storms and wave action and conducting economically valuable as tourist attractions, fishing and generating coastal developments for the purpose of tourism that increasingly revenue to government and private sector. In contrast, human activity by expanding in coastal development is the main threat of coral, leading to discharge wastewater into the sea, and overfishing. The status of coral reef in Thailand in 2015, was continually declining in both the Gulf of Thailand and Andaman side. In 2015, the percentage of live coral cover was 28.3% remarked on high damage level and 50.0% in live coral cover remarked on vary high damage level, while the previous status in 2008 was 19.1% and 18% in live coral cover respectively. Moreover, there are various factors, caused declining coral health as low salinity events, inducing salinity stress in coral by losing control processes for homeostasis which leads to the reduction of the zooxanthellae and chlorophyll concentration, to against growth and reproduction, and to severely result in coral bleaching [1]. Others declining coral health's factor as nutrient enrichment, including dominantly nitrate and ammonia from nutrient runoff from human activities on coastal or directly discharge untreated wastewater. Nutrient enrichment can increasingly promote the growth rate of coral reef organisms. The other studies also found that high ammonia or nitrate concentrations, resulting in loss of zooxanthellae in coral from an imbalance between coral and zooxanthellae [2]. Therefore, this study is focused on the effects of salinity and nitrate on the health status of branching coral (*Acropora sp.*) with acute toxicity test. Coral health evaluation conducted coral health chart, used for calculating health status and mortality percentages and conducted photographic assessment, used for analysis of the active polyp percentages of *Acropora sp*. [3]. The acute toxicity of nitrate concentrations resulting in bleached coral at more than 50% (50% Lethal Concentration: LC50) was calculated by Probit analysis, in order to use as baseline information for monitoring nitrate concentration in seawater that adversely effects on coral health which can result in coral bleaching.

Methodology

Acute Effects of Salinity and Nitrate

Branching coral or *Acropora sp.*, colony size 15 centimeter or more, was kept in a filtered seawater pond with continuous water flowing for 7 days for acclimation. Then the selected corals were checked its color by using coral health chart in the level of 6 and were cut into 3-4 centimeters from the tip of coral. Settling 3 pieces of selected coral into a chamber, sizing of 14×22×16 centimeter. Salinity was varied at 15 20 25 and 30 psu by mixing between filtered seawater (30 psu) and filtered water (0 psu). After that, adding potassium nitrate (KNO_3) to get the various concentration of nitrate at 20 60 and 100 µg-N/l respectively as shown in Figure 1. Temperature and pH were constantly controlled at 30°C and pH 8. In monitoring, coral health status was recorded and photographed by using Olympus stylus TG-4 in Macro mode at 0 12 24 48 72 and 96 hours. After that, replacing seawater with regular seawater without adding nitrate. Coral health status was recorded at 24 and 48 hours 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 Experiment kit example

Active Polyp Percentages

Converting the pictures, photographed in macro mode at 0, 12, 24, 48, 72 and 96 hours from true color (RGB color) into grayscale. The extend polyps or active polyps obviously appear white spots in grayscale. Counting the active polyps in fixed coral's areas and calculating active polyp percentages [3] using the equation (1) below. Its percentage can be comparable to coral health status, shown in Table 1.

$$\text{Active polyp percentages} = \frac{\text{Number of active polyp} \times 100}{\text{Total of polyp counted}} \quad (1)$$

Table 1 Active polyp percentages and health status

Active polyp percentages	Remark
> 50	Good health
25-50	Decline health
< 25	Poor health

Coral Health Status Evaluation

Evaluation of coral health status in *Acropora sp.* could apply Coral Health Chart [4] in Figure 2 to estimate its health status. The color of each side was divided into 4 groups and classified into 6 levels in each side [5]. In level 6 is the representative of coral in good health (best health) and level 1 is representative for declining coral health (worst health). After evaluating the coral health status based on colors, these statuses were calculated into percentages as shown in Table 2.

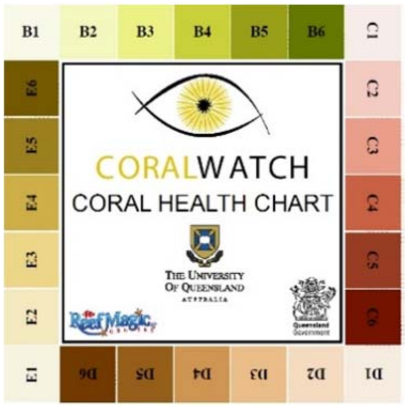

Figure 2 Coral health chart [5]

Data Analysis

Pictures and amounts of color at 0, 12, 24, 48 and 96 hours were used for converting into health status and mortality percentages with Table 2. The corals with health status lower than level 3 or having a mortality percentage higher than 50 would be inducted to calculate the acute toxicity test (LC_{50}) by using Probit analysis [6].

Table 2 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

Results and Discussion

The result in Table 3 found that coral at salinity 30 psu at 24 and 96 hrs showed in health status levels 4 to 5 (72.22-83.33% in health status) as remarked on fair to good health. The lowest mortality percentage was found in nitrate concentration at 20 µg-N/l which had 16.67% in health status. Salinity at 15 psu at 72 hrs, showed in health status level 3 to 4 as remarked on declining to fair health. The lowest health status percentage was found in nitrate concentration at 100 µg-N/l at 72 hrs which had 55.56% in health status or which it was lower than 50% in mortality. Mortality percentages lower than 50 would prevent the calculation of nitrate concentrations and their effects on coral bleaching or LC50. Therefore, at 72 hrs with 44.44% in mortality could not be calculated into LC_{50}.

Table 3 Health status and mortality percentages at salinity 15, 20, 25 and 30 psu.

Salinity 15 psu			Healthy Status (%)				Mortality (%)
Nitrate (µg-N/l)	0 hr.	12 hr.	24 hr.	48 hr.	72 hr.	96 hr.	96 hr.
20	100.00	83.33	83.33	83.33	77.78	66.66	33.33
60	100.00	83.33	83.33	83.33	72.22	61.11	38.89
100	100.00	83.33	83.33	83.33	55.56	44.44	55.56
Salinity 20 psu							
Nitrate (µg-N/l)	0 hr.	12 hr.	24 hr.	48 hr.	72 hr.	96 hr.	96 hr.
20	100.00	83.33	83.33	83.33	83.33	72.22	27.78
60	100.00	83.33	83.33	83.33	72.22	66.67	33.33
100	100.00	83.33	83.33	83.33	66.67	50.00	50.00
Salinity 25 psu							
Nitrate (µg-N/l)	0 hr.	12 hr.	24 hr.	48 hr.	72 hr.	96 hr.	96 hr.
20	100.00	83.33	83.33	83.33	83.33	77.78	22.22
60	100.00	83.33	83.33	83.33	77.78	77.78	22.22
100	100.00	83.33	83.33	83.33	83.33	83.33	16.67
Salinity 30 psu							
Nitrate (µg-N/l)	0 hr.	12 hr.	24 hr.	48 hr.	72 hr.	96 hr.	96 hr.
20	100.00	83.33	83.33	83.33	83.33	83.33	16.67
60	100.00	83.33	83.33	83.33	83.33	77.78	22.22
100	100.00	83.33	83.33	83.33	72.22	72.22	27.78

Whereas, at salinity 15 psu and 96 hrs, the coral showed in health status levels 2 to 3 (44.44-66.66% in health status) as remarked on poor to declining health. The lowest health status percentage was found in nitrate concentration at 100 µg-N/l which had 44.44% in health status or 55.55% in mortality that it was higher than 50% in mortality. Therefore, at 96 hrs with 55.55% in mortality could be calculated into LC_{50} using Probit analysis. Nitrate concentrations that affected on coral bleaching or 50-percent coral mortality at 96 hrs was equal to 89.50 µg-N/l, shown in Figure 3.

In table 3, the results showed the effect of salinity on the coral separately. In constant nitrate concentration, the lowest concentration of nitrate at 20 µg-N/l at salinity 15, 20, 25 and 30 psu at 96 hr had mortality percent as 33.33, 27.78, 22.22 and 16.67 respectively. It can be concluded that mortality in *Acropora sp*. was dominantly caused by lower salinities (15, 20 and 25 psu) than normal salinity (30 psu). Mortality in corals after exposure to low salinity was influenced by tissue sloughed-off [7]. Moreover, the result also showed that the coral at same salinity (15, 20, 25 and 30 psu) had the lowest mortality percentages, showed in nitrate concentration at 20 µg-N/l while highest mortality percentages showed in nitrate concentration at 100 µg-N/l (in salinity 15, 20 and 30 psu). Indicating that stressed in *Acropora sp.* in salinity between 15-30 psu was also caused by higher nitrate concentration (100 µg-N/l). Also, stressed in coral, caused by nitrate has the effects of decreasing the Zooxanthellae density and chlorophyll in the coral tissue [8]. Even if, nitrate can increase Zooxanthellae density and chlorophyll in the coral tissue at first [9, 10]. Different coral species have individual resistance and response for nitrate concentration differently, for example, *Pocillopora damicornis* mainly affected by nitrate while *Porites lobate Dana* can resist temperature and nitrate concentration in 232.4 µg-N/l. However, *Acropora sp.* also responses to ammonia which is an important form of nitrogen in seawater. There was research confirmed that LC_{50} of ammonia concentration at 0.054 mg-N/l can cause coral bleaching or 50-percent coral mortality in 48 hrs. [11].

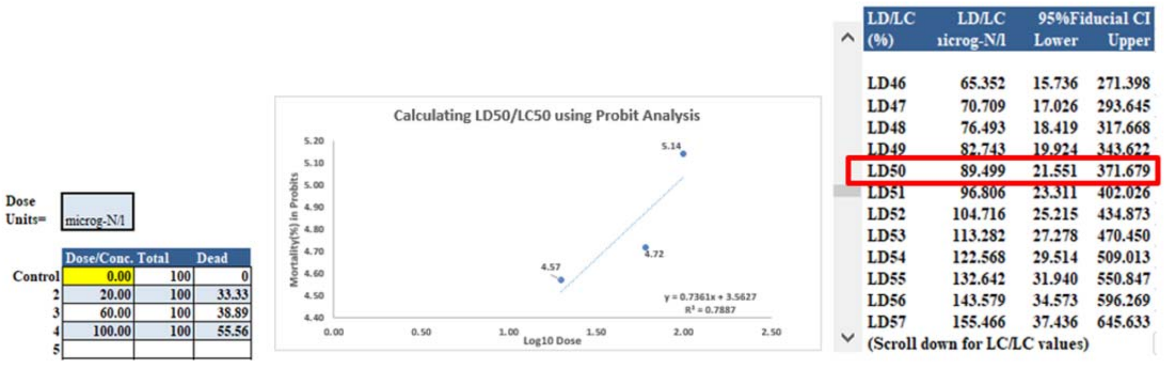

Figure 3 Calculating LC_{50} using Probit analysis at salinity 15 psu.

According to Figure 4 a), the highest active polyp percentages at salinity 30 psu and 0 hr in nitrate concentrations of 20, 60 and 100 µg-N/l were equal to 70 74 and 43% in active polyp. Similarly, at salinity 15 psu and 0 hr, Figure 4 b) had the highest active polyp percentages which were respectively equal to 85 75 and 95% in active polyp.

Next more hour, between 24 and 96 hrs at salinity both 15 and 30 psu found decreasing of active polyp percentages. At salinity 15 psu in Figure 4 b) showed decreasing with rapid in 48 hr, unlike at salinity 30 psu showed decreasing with slightly. Likewise, at salinity 15 psu and 48 hr, the coral started releasing mucus in Figure 5. Also, the lowest active polyp percentages were found in salinity 15 psu and 96 hr, Figure 4 b), including 0, 2 and 0% in active polyp that correlated with the highest mortality percentages in Table 3, ranged between 33.33-55.56% in mortality.

Moreover, in testing at salinity 15 psu with a nitrate concentration of 100 μg-N/l found that *Acropora sp.* released mucus coat themselves at first time in 48 hours, shown in Figure 5 and its mucus increasingly thicker in 72 and 96 hrs respectively. Releasing mucus in coral is affected by living in severe environmental conditions that triggered defence mechanism of coral to against exposing severe environment.

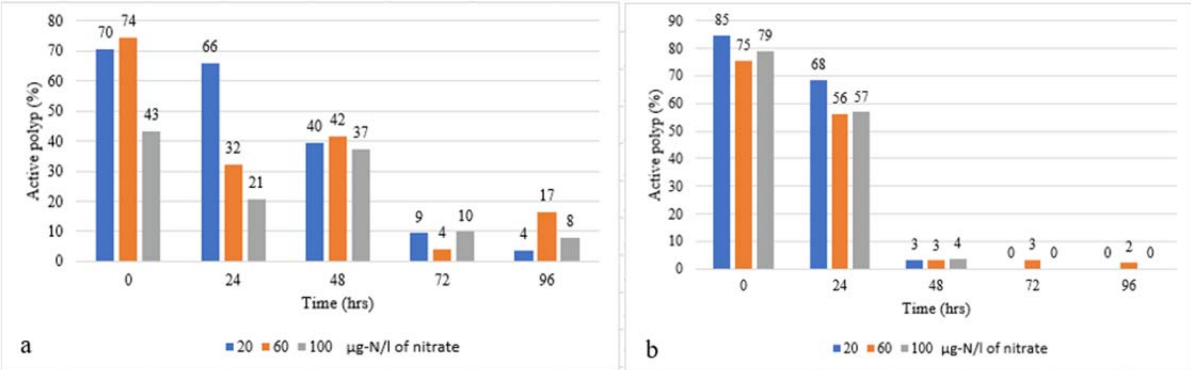

Figure 4 Active polyp percentages for *Acropora sp.* a) Salinity at 30 psu b) Salinity at 15 psu

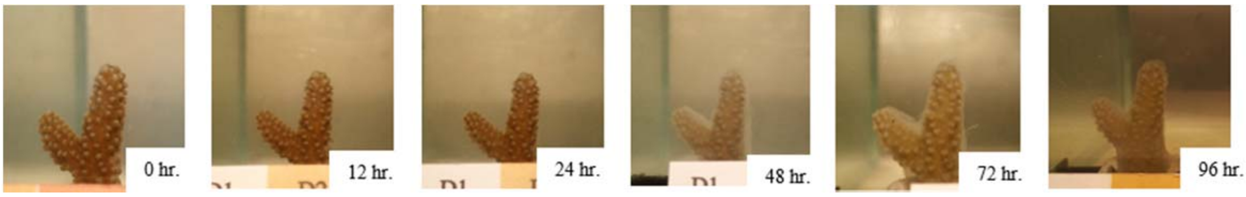

Figure 5 *Acropora sp*. at nitrate concentrations of 100 μg-N/l with salinity 15 psu in 0 to 96 hrs

Though, extremely releasing mucus can cause to tissue sloughed-off which coral can unable to recovery themselves in natural seawater, unlike to paled coral which can recovery themselves completely under natural condition [12] shown in 72 and 96 hour relating with color health level into poor to fair health (in levels of 2 and 3) or healthy status percent in 44.44 to 77.78 which shown in Table 3. Thus, nitrate is one of the severe factors that threatening coral health.

Conclusion

In this study, the result of the salinity and nitrate effects on the coral health status of branching coral (*Acropora sp.*) at the salinity varied in 15, 20, 25, and 30 psu and the concentration of nitrate varied in 20, 60, and 100 μg-N/l, during 0-96 hrs. the result showed that at salinity 15-30 psu along with nitrate concentrations increasingly caused mortality or trigged to bleaching coral. Mortality in coral related to higher nitrate concentrations, affected on more paling color and stimulating to release mucus. At salinity 15 psu with a nitrate concentration of 100 µg-N/l and 96 hrs, coral had the highest mortality percentages (33.33-55.56% in mortality) which contrarily related to the lowest active polyp percentages (0-2% in active polyp). Indicating that coral has a defense mechanism, trigged from stress or irritating in severe environmental conditions by reduced polyp activity and using mucus to cover itself to unexposed these conditions. Therefore, at salinity 15 psu with a nitrate concentration of 100 µg-N/l and 96 hrs had the highest mortality percentages, more than 50% in mortality, which could be calculated into LC50 using Probit analysis. Then, nitrate concentrations that effect on coral bleaching or 50-percent coral mortality at 96 hrs was equal to 89.50 µg-N/l.

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References

[1] Connell, D. W. and Hawker, D. W. 1992. Pollution in tropical aquatic systems. 1, Boca Raton: CRC Press.

[2] Zhu, B. H., Wang, G. C., Huang, B. 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.

[3] 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. *International Conference on Information Modelling and Knowledge Bases XXIX,* 301, IOS Press, 137-148.

[4] CoralWatch. 2002. Coral Health Chart [Online]. https://coralwatchold.org/web/guest/coral-health-chart.

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

[6] Finney, D. J. 1971. Probit Analysis. 3rd edition. Cambridge University: Cambridge University Press.

[7] Kerswell, A. and Jones, R. 2003. Effects of hypo-osmosis on the coral Stylophora pistillata: Nature and cause of 'low-salinity bleaching'. Marine Ecology-progress Series - MAR ECOL-PROGR SER, 253, 145-154.

[8] Nordemar, I., Nystrom, M. and Dizon, R. 2003. Effects of elevated seawater temperature and nitrate enrichment on the branching coral Porites cylindrica in the absence of particulate food. *Marine Biology,* 142(4): 669-677.

[9] Schloder, C. and D'Croz, L. 2004. Responses of massive and branching coral species to the combined effects of water temperature and nitrate enrichment. Journal of Experimental Marine Biology and Ecology, 313(2): 255-268.

[10] Marubini, F. and Davies, P. S. 1996. Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. Marine Biology, 127(2): 319-328.

[11] Udomsap, B., Chawakitchareon, P. and Rungsupa S. 2018. The effects of temperature and ammonia to coral health level: A case study of hump coral (Porites sp.) at Sichang island, Thailand. The Proceedings of 56th Kasetsart University Annual Conference. 30 January - 2 February, 2018. Kasetsart University. 279-286.

[12] Jandang, S. 2015. Effect of chronic salinity stress on bleaching and recovery of a Staghorn coral Acropora millepora. The Proceedings of 6th Hatyai National Conference. 26 June 2015. Hatyai University. 1544-1553.