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EFFECTS OF SALINITY AND NITRATE ON CORAL HEALTH LEVELS: A CASE STUDY OF HUMP CORAL (*Porites sp.*)

Kittiya Samlansin [1], Petchporn Chawakitchareon [1]*, and Sompop Rungsupa [2]

[1] *Department of Environmental Engineering, Chulalongkorn University, Bangkok 10330, THAILAND.*
[2] *Aquatic Resources Research Institute, Chulalongkorn University, Bangkok 10330, THAILAND.*

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ABSTRACT

This research focused on the effect of salinity and nitrate on coral health levels in hump coral (*Porites sp.*). The corals incubated under salinity vary in 15, 20, 25, and 30 psu, and nitrate vary in 5, 20, 60, and 100 µg-N/l with triplicate experiments. The experimental results showed that the range of salinity in 20, 25 and 30 psu at 120 hours was unable to calculate LC_{50} (Lethal Concentration) because of insufficient in motility percentage. However, the results showed that 15 psu of salinity at 120 hours was able to calculate LC_{50} using Probit analysis. LC_{50} in 15 psu of salinity at 120 hours was equally to 105.40 µg-N/l. The loss of Zooxanthellae from coral tissues could observe by the change in color as coral slowly turning into paling color but there were no releasing mucus and tissue damage as tissue sloughed-off observed in any treatments in this study because of the highly tolerant to reduced salinity and high nitrate in *Porites sp*. The experimental results showed that the corals incubated under separately high nitrate or reduced salinity treatment indicated an increased amount of loss of Zooxanthellae. Then, the combination of salinity and nitrate exposure in coral can create more stressful conditions and adversely affect coral health status than exposure to salinity or nitrate alone.

Disciplinary: Environmental Science and Engineering, Marine Sciences.

1. INTRODUCTION

Corals and coral reefs play an important role both direct and indirect way as providing vital ecosystem services as the main 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 tourism that increased revenue to the 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

*Corresponding author (P.Chawakitchareon) Tel +66-22186674, +66-22186666 petchporn.c@chula.ac.th

continually declining in both the Gulf of Thailand and the 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 very 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 reduced 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 photosynthesis, respiration (Moberg et al., 1997), growth, reproduction (Richmond, 1996), and to severely result in coral bleaching (Hoegh-Guldberg and Smith, 1989) (Connell and Hawker, 1992). 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 (Zhu et al., 2004) (Rungsupa et al., 2016). Moreover, photographic assessment is developed for monitoring and analyzing coral health such as the change in coral color (Siebeck et al., 2006) or the change in coral's activity (Rungsupa et al., 2018) (Hansuebsai et al., 2018). Therefore, this study is focused on the effects of salinity and nitrate on the health status of hump coral (*Porites sp.*) with an acute toxicity test. Coral health evaluation conducted a coral health chart, used for calculating health status and mortality percentages. The acute toxicity of nitrate concentrations resulting in bleached coral at more than 50% (50% Lethal Concentration: LC_{50}) 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.

2. METHODOLOGY

2.1 ACUTE EFFECTS OF SALINITY AND NITRATE

Hump coral or *Porites sp.*, colony size 15 centimeters 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 the coral health chart at 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 5, 20, 60, and 100 µg-N/l respectively as shown in Figure 1. Temperature and pH were constantly controlled at 30ºC and pH 8. In monitoring, coral health status was recorded and photographed by using Olympus stylus TG-4 in Macro mode in 120 hours.

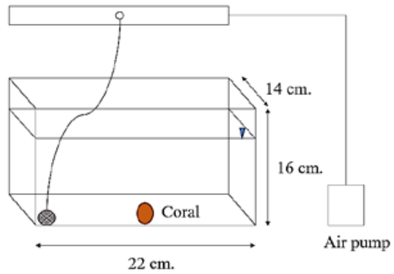


Figure 1: Experiment kit example

2.2 CORAL HEALTH STATUS EVALUATION

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

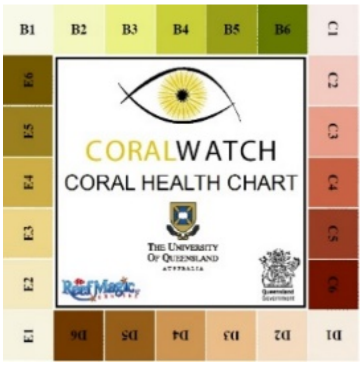


Figure 2: Coral health chart (Siebeck et al., 2006)

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

Level	Remark	Health status percentages	Mortality percentages
1	Unhealth	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

2.3 DATA ANALYSIS

Pictures and amounts of color in 120 hours were used for converting into health status and mortality percentages with Table 1. 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.

3. RESULTS AND DISCUSSION

After culturing *Porites sp.* under several seawater conditions. Table 2 shows the percentages of coral health status and mortality within 120 hr. At ambient salinity (30 psu) at 120 hrs showed a small change in health 83.33-100.00% as remarked on good to best health. This ambient salinity also found the lowest mortality percentage which was in ambient nitrate (5 µg-N/l). At reduced salinity (15 psu) found the highest mortality percentage in 100 µg-N/l of nitrate concentration at 120 hrs. Its coral mortality was equal to 50.00%. When the mortality percentage lower than 50, it would not enable in the calculation of nitrate concentrations and their effects on coral bleaching or LC_{50}. Therefore, at 120 hrs with 50.00% in mortality could be calculated into LC_{50} using Probit analysis. Nitrate concentration that affected on coral bleaching or 50-percent coral mortality at 120 hrs was equal to 105.40 µg-N/l, shown in Figure 3.

*Corresponding author (P.Chawakitchareon) Tel +66-22186674, +66-22186666 petchporn.c@chula.ac.th

Table 2: Health status and mortality percentages at salinity 15, 20, 25 and 30 psu

Salinity 15 psu			Health Status (%)				Mortality (%)
Nitrate (µg-N/l)	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.	120 hr
5	100.00	100.00	88.89	88.89	88.89	88.89	11.11
20	100.00	100.00	100.00	83.33	83.33	72.22	27.78
60	94.44	94.44	88.89	72.22	72.22	61.11	38.89
100	100.00	100.00	94.44	83.33	72.22	50.00	50.00
Salinity 20 psu							
Nitrate (µg-N/l)	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.	120 hr.
5	100.00	100.00	100.00	94.44	94.44	94.44	5.56
20	100.00	100.00	100.00	94.44	94.44	94.44	5.56
60	100.00	100.00	100.00	94.44	83.33	83.33	16.67
100	100.00	94.44	94.44	94.44	83.33	72.22	27.78
Salinity 25 psu							
Nitrate (µg-N/l)	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.	120 hr.
5	100.00	100.00	100.00	100.00	94.44	94.44	5.56
20	94.44	94.44	88.89	88.89	88.89	88.89	11.11
60	100.00	100.00	100.00	100.00	100.00	88.89	11.11
100	94.44	94.44	94.44	94.44	94.44	77.78	22.22
Salinity 30 psu							
Nitrate (µg-N/l)	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.	120 hr.
5	100.00	100.00	100.00	100.00	100.00	100.00	0.00
20	100.00	100.00	100.00	100.00	94.44	94.44	5.56
60	100.00	100.00	100.00	100.00	94.44	88.89	11.11
100	88.89	88.89	88.89	88.89	88.89	83.33	16.67

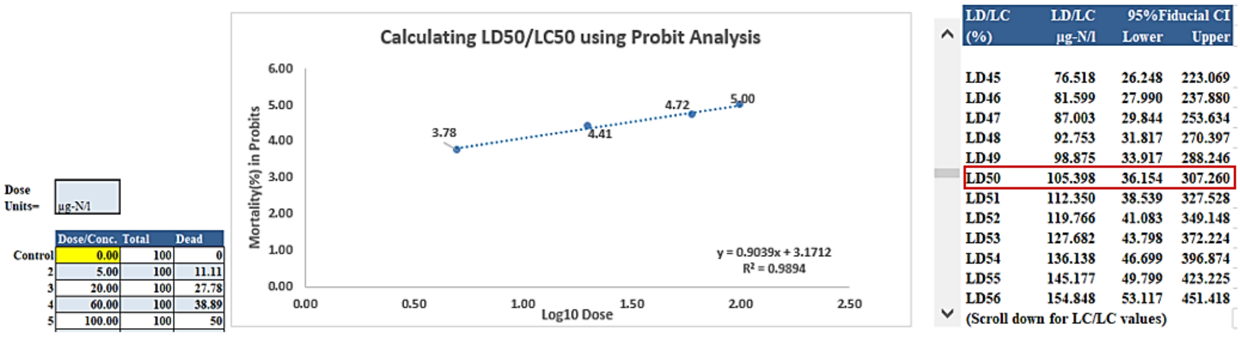
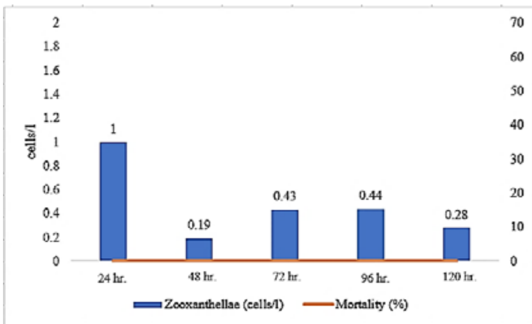
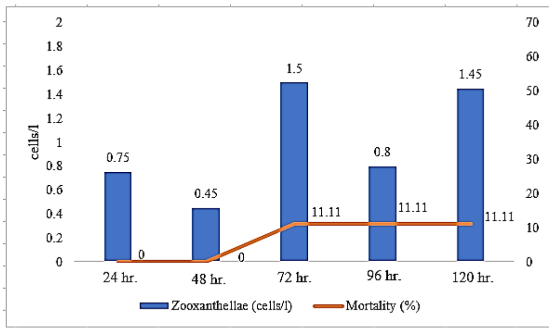


Figure 3: Calculating LC_{50} using Probit analysis at salinity 15 psu

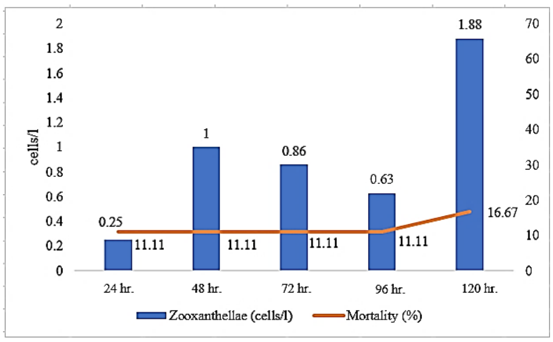
In this study, the loss of Zooxanthellae (algal expulsion) from coral tissue can be measured by Zooxanthellae density in seawater shown in Figure 4. At ambient condition in 120 hrs, at 30 psu in salinity and 5 µg-N/l in nitrate concentration in Figure 4 a), the Zooxanthellae density was 0.19-1.00 cells/l while at reduced salinity (15 psu), the Zooxanthellae density was higher than ambient salinity (30 psu). In Figure 4 b), at 15 psu in salinity and 5 µg-N/l in nitrate concentration the Zooxanthellae density was 0.45-1.50 cells/l and the highest Zooxanthellae density was equal 0.59-1.76 cells/l at15 psu in salinity and 100 µg-N/l in nitrate concentration in Figure 4 d). Then, there was a positive association between the loss of Zooxanthellae and the percentage of mortality in coral. Loss of Zooxanthellae from coral tissues can be caused by the disfunction of energy transfer from Zooxanthellae to coral tissues. The photosynthetic products, provided by Zooxanthellae as carbohydrates and oxygen are required for growth in coral (Muthiga and Szmant, 1987).



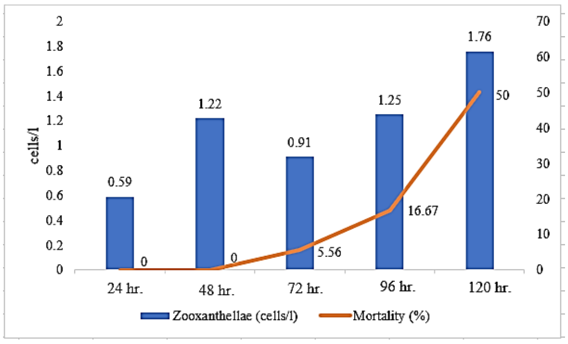
a) Nitrate concentration of 5 µg-N/l, 30 psu



b) Nitrate concentration of 5 µg-N/l, 15 psu



c) Nitrate concentration of 100 µg-N/l, 30 psu



d) Nitrate concentration of 100 µg-N/l, 15 psu

Figure 4: Zooxanthellae density and mortality percentage in *Porites sp.*

In Figure 5, change in coral color was also related to loss in Zooxanthellae in coral tissue. Because of coral slowly turning into paling color. Moreover, there was acclimation or short-term recovery in coral within 120 hrs. *Porites sp*. showed fluctuating in Zooxanthellae density when they were in abnormal conditions as reduced salinity and/or high nitrate concentration in seawater. Apparently, at high nitrate condition in Figure 4 a), acclimating coral could be detected within 48 to 96 hrs when Zooxanthellae density gradually deceased to show those coal tried to adjust themselves to the new condition. However, in this study, *Porites sp*. could survive throughout 120 hrs and had no mucus secretion under any treatments. The report of Manzello and Lirman (2003), noted that *Porites furcate* with 20 psu displayed mucus excretion and complete retraction of polyps but no tissue mortality in 24 hr of exposure Manzello and Lirman (2003). The coral defense mechanism, the corals under changes in environment or unstable conditions would release mucus covering their surface to protect themselves from all stimuli (Brown and Bythell, 2005). Though, extremely releasing mucus can cause tissue sloughed-off which coral is unable to recovery themselves in natural seawater, unlike to paled coral which can recovery themselves completely under natural conditions (Jandang, 2015).

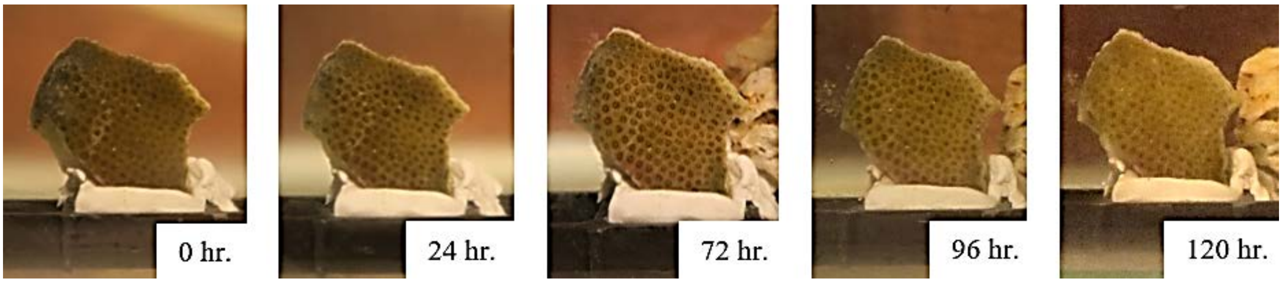


Figure 5: Change in *Porites sp* at nitrate 100 µg-N/l with salinity 15 psu within 120 hrs

According to the results in Figure 6, the association between nitrate and the loss of Zooxanthellae under ambient salinity level within 120 hrs. At ambient nitrate (5 µg-N/l) in seawater (30 psu), the upper and lower of Zooxanthellae density in seawater was equal to 0.19-1.00 cells/l while at increased nitrate concentration (100 µg-N/l) showed an increase in Zooxanthellae density as the range between 0.25-1.88 cells/l. Therefore, this study indicated that loss of Zooxanthellae increased under increased nitrate relative to ambient nitrate in seawater. Similar to Schloder and D'Croz (2004), found that the branching corals incubated under nitrate enrichment resulting in the significant loss of Zooxanthellae and increased in size of algae and increased chlorophyll concentration (Marubini and Davies, 1996). Its effect on the loss of Zooxanthellae also caused paling in color and bleaching.

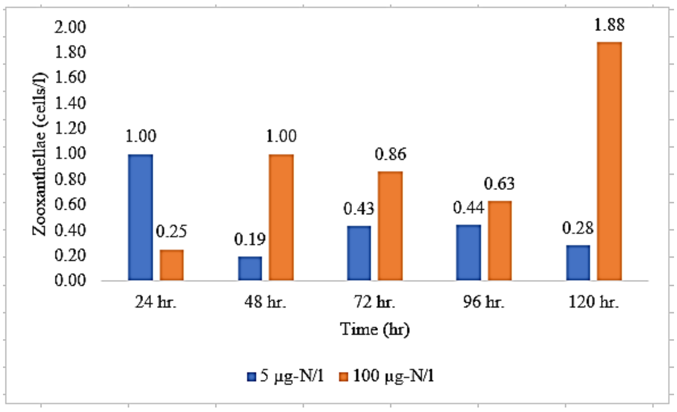


Figure 6: Zooxanthellae density in salinity at 30 psu at nitrate 5 and 100 µg-N/l

In conversely, Tanaka et al. (2014) and Samlansin et al. (2018) also found the same results in the coral *Acropora tenuis*, loss of Zooxanthellae under high nutrient condition (both high in nitrate and phosphate) but the percentage rate of Zooxanthellae loss was not significant in the statistic. Loss of Zooxanthellae due to excess of algal symbionts that increased competition between algae and coral host, those algae generated more stress in coral. To control symbiont density in coral, this coral response to detoxification or algal expulsion to decreases their stress (Cunning and Baker, 2013). But there is a process of Zooxanthellae loss from coral, which is also possible. This is an internal degradation as coral apoptosis or coral cell dead (Tissier and Brown, 1996). There were some studies confirmed that nitrate can adversely inhibit calcification on *Porites compressa* and *Montipora caiptata* by increasing competition between Zooxanthellae and coral host (Marubini and Brenda, 1999) (Silbiger et al., 2018). Moreover, an increased loss of Zooxanthellae was similarly found in the effect of ammonia on dice coral (*Turbinaria peltate)* that ammonia resulted in increased Zooxanthellae density at 48 hr (Udomsap et al., 2018). It was also correlated with a decline in coral health status (Udomsap et al., 2019).

The result in Figure 7 showed the association between salinity and the loss of Zooxanthellae under ambient. nitrate concentration within 120 hrs. The corals cultured at ambient salinity (30 psu), the Zooxanthellae density in seawater was equal to 0.19-1.00 cells/l while at reduced salinity condition (15 psu), the range of Zooxanthellae density slightly increased to 0.45-1.50 cells/l. According to the result of the reduced salinity condition, the Zooxanthellae density in seawater was higher than the ambient salinity condition within 120 hr. Thus, indicating that loss of Zooxanthellae in reduced salinity also was higher than the ambient salinity.

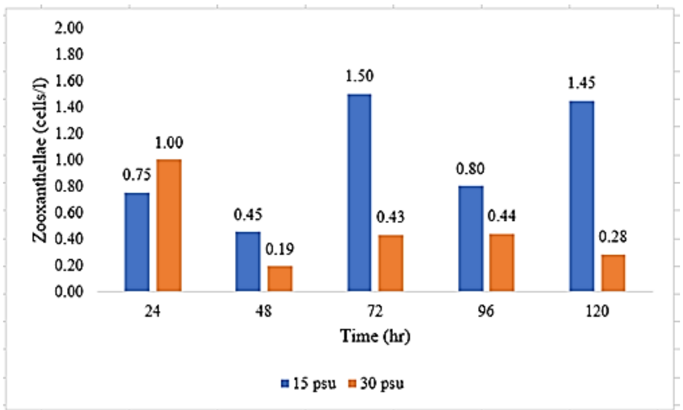


Figure 7: Zooxanthellae density at nitrate 5 µg-N/l in salinity at 15 and 30 psu

The other study found in a similar way as *Sarcophyton spp* turned into completely bleaching at 20 psu within 204 hr in the acute salinity test, and in the chronic test, this coral was decreased significantly in live tissue at 16 psu within 120 h (Nakano et al., 2009). The tissue damage is affected by the change in salinity (Van Woesik et al., 1995) and can mainly reduce photosynthesis and respiration rates on coral (Moberg et al., 1997). However, *Porites sp* used in our study could not show tissue damage or tissue sloughed-off because the other report confirmed that the species of *Porites* is highly tolerant of reduced salinity (survival 120 hrs in 17 psu) (Nakano et al., 2009).

Moreover, massive corals such as hump coral have large tissue layers using to provide more energy available and photosynthetic products. Those corals are slow in growth rate but long in living as an increase in regeneration and adaptability to the changes in the environment (Jimenez et al., 2001). Whereas, branching corals have thinner tissue layer compared with massive corals. These corals are considerably sensitive to the changes in the environment. The finding by Samlansin et al. (2018) showed that *Acropora sp.* rapidly responded to the combination of reduced salinity and high nitrate by showing tissue damage or tissue sloughed-off and decreasingly in coral health level within 96 hours while hump coral (*Porites sp.*) in this study showed its effect in 120 hours. Consequently, the above findings of *Acropora sp.* can confirm that branching coral is more sensitive than hump coral observed in this study.

4. CONCLUSION

The combination of reduced salinity (15 psu) with high nitrate (100 µg-N/l) in 120 hrs showed the highest mortality percentages. High nitrate in seawater cause to increase in size and density of Zooxanthellae, living in coral tissue. Excess of Zooxanthellae in coral tissue leads to stressful conditions by increasing the competition between algal symbionts and coral hosts. To control those algal symbionts, these coral responses to expulsion or loss of algae that can physically indicate by paling in color or bleaching. In similar, reduced salinity affected the loss of Zooxanthellae and adversely affected tissue damage as tissue sloughed-off. Thought, there were no releasing mucus and tissue sloughed-off observed in this study.

Hence, the combination of salinity and nitrate exposure in coral can affect adversely coral health status than exposure to salinity or nitrate alone. However, the bleaching sensitive in coral depending on species of coral. Hump corals have larger layers of tissue more than branching corals.

Its tissue can provide more energy and photosynthetic products for their growth resulting in good resistance and adaptability under an unsuitable environment.

5. AVAILABILITY OF DATA AND MATERIAL

All relevant data are already included in this article.

6. ACKNOWLEDGEMENT

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Kittiya Samlansin is a graduate student at Department of Environmental Engineering, Chulalongkorn University. She received her Bachelor's degree in Environmental Sciences from Faculty of Science, Chulalongkorn University. Her current researches involve Coral Health related to Environmental Changes and Coral Health Monitoring.

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 the Utilization of Industrial Waste for Environmental Engineering Applications, Coral Health Monitoring and Environmental Data Mining Applications.

Dr.Sompop Rungsupa was 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 related to Bio-aquatic Plants and Animals. Dr.Sompop also focused on the Impact of Water Quality related to the Circulation System in Thai Abalone (Haliotis asinina).