Comparative Growth Rates of Cultured Zooxanthellae and the Effects of Temperature and Salinity

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ABSTRACT

Dinoflagellates of the genus *Symbiodinium* form symbioses with a variety of hosts including hard corals, sea anemones, soft corals, and giant clams. Environmental stresses, especially elevated temperature and decreased water salinity, impair zooxanthellae (*Symbiodinium* sp.) and cause coral bleaching. To determine the role of temperature and salinity on zooxanthellae, this study was conducted on zooxanthellae isolated from seven marine invertebrate hosts: mushroom coral (*Fungia fungites*), cauliflower coral (*Pocillopora damicornis*), sea anemone (*Epiactis* sp.), soft coral (*unidentified*), staghorn coral (*Acropora millepora*), honeycomb coral (*Goniastrea* sp.) and giant clam (*Tridacna squamosa*). The growth response of axenic culture was observed at three levels of temperature (27 ºC [control], 30 ºC and 33 ºC) in combination with three levels of salinity: 10, 20, and 30 (control) psu. Cells were sampled and enumerated every two days for 14 days. The results showed that the main effects of host, salinity and temperature each significantly determined growth of zooxanthellae while the interactions between host and temperature and between salinity and temperature were significant. Specific growth rates of zooxanthellae grown in four different combinations of temperature and salinity ranged between 0.371±0.198 and 0.683±0.074 day^{-1}. At 27 ºC and 30 ºC, with a salinity of 10 psu, and at 33 ºC with all salinity levels, most cells died before the end of the experiment. These results suggest that high temperature (33 ºC) and low salinity (10 psu) strongly affect the growth rate of zooxanthellae, and that the combination of high temperature and low salinity also have synergistic effects on the growth of zooxanthellae.

Keywords: Growth rate, Salinity, Temperature, Zooxanthellae

INTRODUCTION

Dinoflagellates of the genus *Symbiodinium*, called “zooxanthellae,” form symbioses with a variety of hosts such as corals, sea anemones, and giant clams. (Klueter *et al*., 2017) Zooxanthellae have a yellow-brown color and live in the tissue of their hosts. The hosts provide a favorable environment and nutrients for growth of zooxanthellae, while zooxanthellae provide oxygen and organic compounds (derived from their photosynthesis) to the hosts accounting for up to 90 % of the host’s energy and nutrients (Trench, 1979).

Stress caused by changes in environmental conditions such as temperature, light intensity, salinity, nutrients, or a combination of these factors impairs zooxanthellae and causes coral bleaching. However, the frequency and severity of bleaching events has been reported mostly when corals were under thermal stress. Baird *et al*. (2006) exposed symbiotic and non-symbiotic larvae of staghorn coral

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Received 7 March 2022 / Accepted 24 August 2022

(*Acropora muricata*) to three different temperatures under a normal level of light for seven days. Many larvae survived well at 28 and 32 ºC, while all larvae died within 40 h at the highest temperature of 36 ºC. Moreover, an increase in water temperature was shown to cause decreases in growth rate, density, and photosynthetic efficiency of cushion coral (*Cladocora caespitosa*) and compact ivory bush coral (*Oculina patagonica*) (Rodolfo-Metalpa *et al*., 2006). Water temperatures of 32-34 ºC were also reported to affect photosynthesis of zooxanthellae (Warner *et al*., 1996). A study by Kemp *et al*. (2014) also showed that maximum quantum yield of photosystem II was significantly lower during bleaching compared to before and after bleaching events. Moreover, elevated temperature (above summer ambient levels, but still below the bleaching threshold) can also impair growth and reproduction of zooxanthellae (Jokiel and Coles, 1990). Under thermal stress conditions, corals more actively digest and expel damaged zooxanthellae. This is probably a response to the environmental stressor (Fujise *et al*., 2014). Increased temperature and light have been well documented as causes of bleaching, but other factors like salinity can also stress the holobiont. Under salinity stress, maximum photosynthetic efficiency (Fv/Fm) of zooxanthellae is decreased, although some degree of photosynthetic efficiency has been detected even at low and high salinity levels (Kuanui *et al*., 2015).

Abnormal temperature and salinity have been considered threats to coral reefs and their symbionts. However, few laboratory experiments have demonstrated the combined effects of seawater temperature and salinity on cultured zooxanthellae, especially those isolated from different hosts.

Therefore, this study examines the effects of temperature and salinity on zooxanthellae isolated from seven marine invertebrate hosts collected from Samaesan Island, Chonburi, Eastern Thailand. Differences in growth rates among zooxanthellae collected from different host are also emphasized. All zooxanthellae cultured in this study were *Durusdinium* spp. (D1).

MATERIALS AND METHODS

Sample collection and culturing

Marine invertebrate samples were randomly collected at depths of 3-4 m from three sites around Samaesan Island in Chonburi Province, Eastern Thailand: Khao Mha Jor (12º35’55.0” N 100º56’ 47.4” E), Pla Muek Island (12º35’13.6” N 100º56’ 37.7” E), and Had Tien diving point (12º34’07.7” N 100º57’36.7” E) (Figure 1). All samples were brought back to the laboratory for zooxanthellae cell isolation. The Pasteur pipette single-cell isolation technique was applied to obtain zooxanthellae cells. We isolated zooxanthellae from seven marine invertebrate hosts, including mushroom coral (*Fungia fungites*), cauliflower coral (*Pocillopora damicornis*), sea anemone (*Epiactis* sp.), soft coral (unidentified), staghorn coral (*Acropora millepora*), honeycomb coral (*Goniastrea* sp.) and giant clam (*Tridacna squamosa*). The established axenic cultures of all isolates were maintained in Daigo’s IMK Medium (Nihon Pharmaceutical Co., Ltd) under the following conditions: temperature of 27 ºC; salinity 30 psu; and light intensity of 4,000 lux with 12 h light: 12 h dark photoperiod.

Experimental setup

Seven zooxanthellae isolates were cultured at different levels of temperature and salinity. The growth response of zooxanthellae isolated in axenic culture was observed at three temperatures (27 ºC [control], 30 ºC, and 33 ºC) and three levels of salinity (10, 20, and 30 [control] psu). Three replications were made for each combination of temperature and salinity. Stock cell cultures of zooxanthellae were acclimated for 24 h at each temperature and salinity combination prior to the experiments. Glass flasks (250 mL) containing 150 mL of Daigo’s IMK Medium with 5,000 cells·mL^{-1} as the initial cell density were used for all experimental treatments. Cells were sampled and enumerated every two days for 14 days; then the specific growth rate was determined by the equation

$$N_t = N_o e^{K} e^{t} \quad \text{(Guillard, 1973)}$$

Where N is cell concentration (cells·mL^{-1}), K_e is the growth constant in base e units, t is time (days), and $N_t = N_o$ at t = 0. The transformation and conversion of units are accomplished by first taking logarithms to the base 10:

$$\log N = \log N_o + K_e t \log(e)$$

$$= \log N_o + (0.4343)\, K_e t$$

Cell morphology of zooxanthellae was also observed during the experimental period whereby shape, size (diameters), color, organelles, and other factors were recorded.

Statistical analysis

In this study, effects of algal host and culture conditions (salinity and temperature) on zooxanthellae density were analyzed using 3-way analysis of variance (7×3×3 factorial experiment in CRD). Means were then compared using Duncan's new multiple range test. In addition, Pearson's correlations between zooxanthellae densities and salinity or temperature were analyzed.

Figure 1. Map showing three sites for collection of zooxanthellae host specimens in Chonburi Province, Thailand.

RESULTS AND DISCUSSION

Growth characteristics of zooxanthellae

Zooxanthellae isolated from seven marine invertebrates showed similar growth under the control conditions: temperature at 27 ºC; salinity of 30 psu and light intensity of about 4,000 lux with 12:12 h (light: dark) photoperiod (Figure 2). In general, zooxanthellae cells gradually decreased in number on day 2 (lag phase), rapidly increased from day 4 to day 10 (logarithmic phase), then decreased slightly (stationary phase), and finally decreased more sharply at the end of the experiment (death phase) (Figure 2).

Effects of algal host, temperature and salinity on zooxanthellae growth

The experiments clearly showed that zooxanthellae density (estimated at days 0-14) varied significantly by algal host, salinity and temperature ($p = 0.000$; Table 1). In the control condition (27 ºC and 30 psu), zooxanthellae isolated from mushroom coral, cauliflower coral and soft coral showed highest density at day 14 (Figure 2). Zooxanthellae were successfully grown in four out of the nine combinations of temperature and salinity. At a temperature of 27 ºC or 30 ºC and salinity of 20 or 30 psu, specific growth rates ranged from 0.371±0.198 to 0.683±0.074 day^{-1}.

In addition, interactions between algal host and temperature ($p = 0.002$), and temperature and salinity ($p = 0.000$) had significant effects on cell density, whereas neither the interaction between algal host and salinity nor the interaction among the three factors were significant ($p = 0.146, 0.513$).

The correlation between salinity and zooxanthellae density was positive and significant ($p = 0.000$) although relatively weak ($r = 0.267$), suggesting an increase of zooxanthellae at higher salinity (Figure 3). On the other hand, temperature and zooxanthellae density had a significantly negative relationship ($p = 0.000$), with $r = -0.318$,

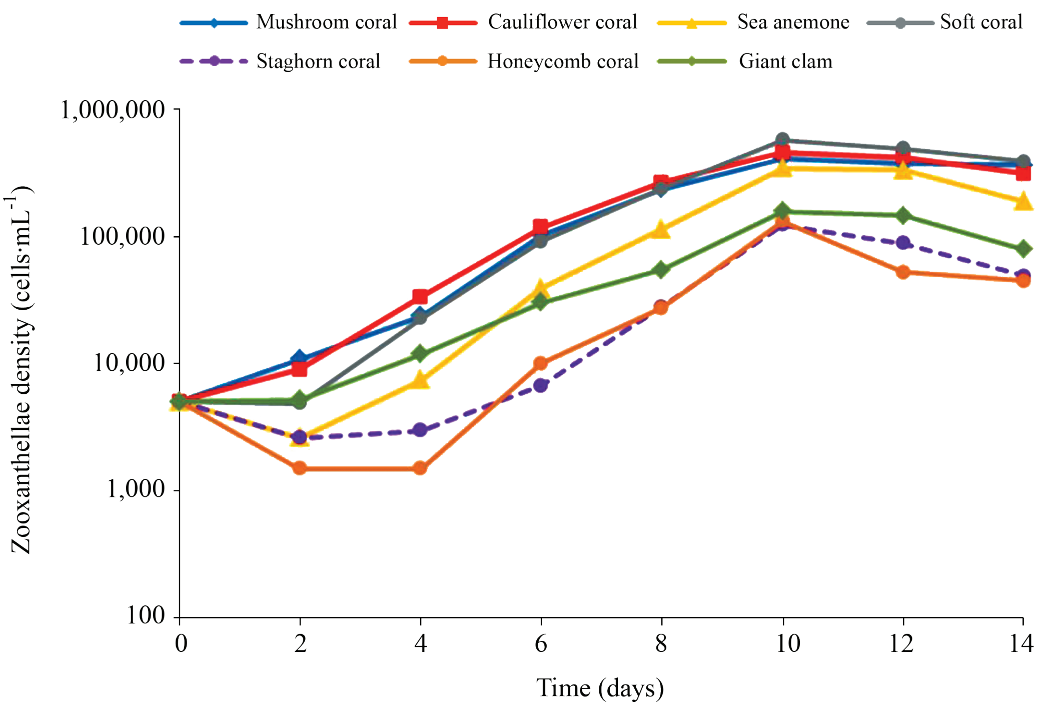

Figure 2. Densities of zooxanthellae cultured under control conditions (27 ºC and 30 psu).

suggesting a decrease of zooxanthellae density at high temperatures (Figure 4).

Zooxanthellae could not grow at the highest temperature of 33 ºC (at all salinity levels) or at the lowest salinity level of 10 psu (at all temperature levels). These results suggest that the highest temperature level (33 ºC) and lowest salinity level (10 psu) negatively affected the growth of zooxanthellae. Elevated temperatures can disrupt *Symbiodinium* photosynthesis by hindering the repair of damaged photosystems (Takahashi *et al*., 2009), and the salinity stress can cause the decrease of maximum photosynthetic efficiency (Fv/Fm) of *Symbiodinium* (Kuanui *et al*., 2015).

When considering the interaction of temperature and zooxanthellae host type and their effect on zooxanthellae density, the experiments showed that zooxanthellae (*Durusdinium* spp. (D1)) isolated from different host types responded differently to temperature (Figure 4). Among the seven algal hosts, zooxanthellae from giant clam responded differently to higher temperature than those from other hosts. It was the only treatment with a significantly higher zooxanthellae density at 30 ºC than at 27 ºC.

When considering the interaction effect of temperature and salinity on zooxanthellae densities, it was found that increasing salinity and temperature enhanced zooxanthellae growth with two exceptions: at salinity of 10 psu, growth was not enhanced although temperature increased; similarly, growth retardation was seen at temperature of 33 ºC across three salinity levels (Figure 3).

This study showed that zooxanthellae isolated from different host types responded differently to temperature, as indicated by the significant ($p = 0.002$) interaction between algal host and temperature (Table 1; Figure 4). For instance, growth of zooxanthellae from mushroom coral, cauliflower coral and soft coral did not differ between 27 ºC and 30 ºC treatments, while growth was significantly increased at the higher temperature in zooxanthellae from giant clam (Figure 4). However, it was apparent that a temperature of 33 ºC was too high for zooxanthellae of all algal hosts. Symbiodiniacean species have different

Table 1. Results of 3-way ANOVA on effects of algal host, temperature and salinity on density of zooxanthellae.

ANOVA				
Source	**df**	**Mean Square**	**F**	**p value**
Corrected Model	62	2051830991.804	4.649	0.000
Intercept	1	591395427198.942	134.008	0.000
Algal host	6	23382579183.386	5.298	0.000
Temperature	2	163830217093.526	37.123	0.000
Salinity	2	124297380865.922	28.165	0.000
Algal host*Temperature	12	11484779283.392	2.602	0.002
Algal host*Salinity	12	6340798268.075	1.437	0.146
Temperature*Salinity	4	59875153229.210	13.567	0.000
Algal host*Temperature*Salinity	24	4257370039.090	0.965	0.513
Error	441	4413143843.854		
Total	504			
Corrected Total	503			

Note: $r^2 = 0.395$ (Adjusted $r^2 = 0.310$); the tests were considered significant at $p<0.05$

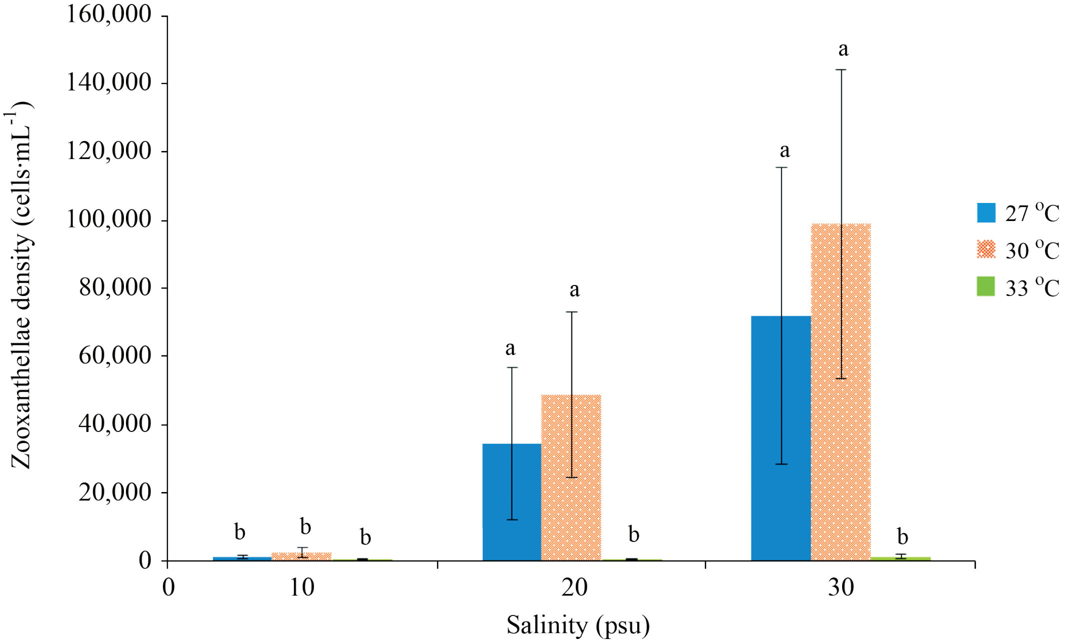

Figure 3. Combined mean densities of all zooxanthellae isolates cultured at three salinities (10, 20 and 30 [control] psu) and three temperatures (27 [control], 30 and 33 °C).

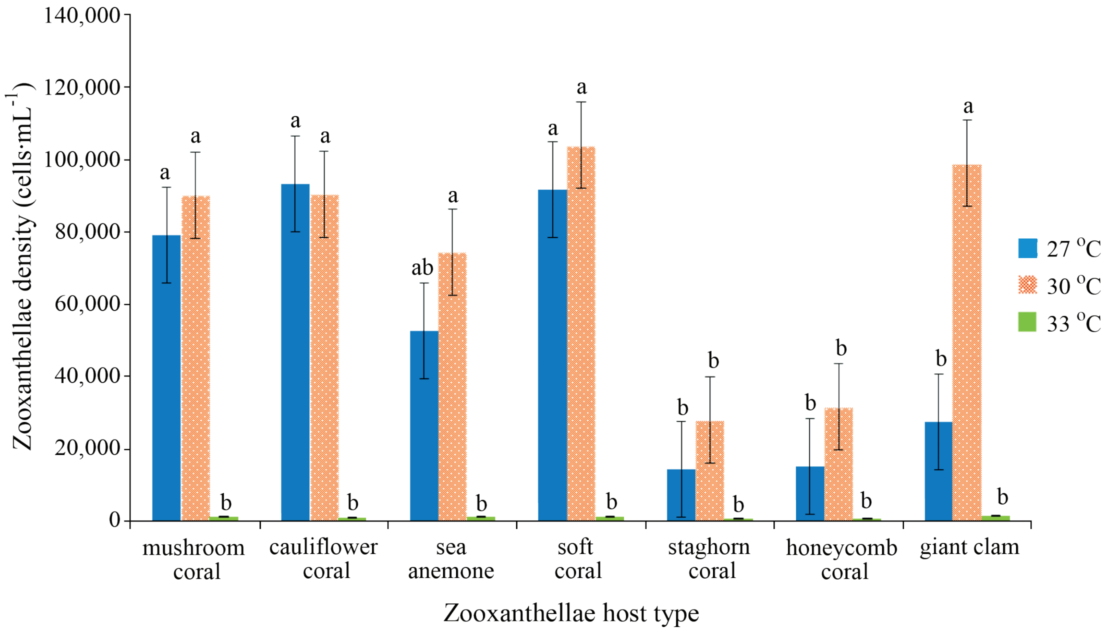

Figure 4. Densities of zooxanthellae from various host types cultured at three levels of temperature (27, 30 and 33 °C).

photokinetic and thermal optima, which may affect their growth-related nutritional physiology and allow them to modify their response to environmental stresses (Klueter *et al.*, 2017). Moreover, zooxanthellae from different genera exhibit differences in their ability to prevent over-excitation of the photosynthetic apparatus (Lesser, 2011).

Zooxanthellae isolated from different host types responded similarly to the changes of salinity, but differed when subjected to increased temperature. It is obvious that temperature strongly affects the growth rate of zooxanthellae, and this is consistent with several previous studies (Jokiel and Coles, 1990; Warner *et al*., 1996; Baird *et al*., 2006; Rodolfo-Metalpa *et al*., 2006). Most of the studies showed that the effect of temperature on coral has been widespread, whereas the effect of lower salinity has been localized to reefs organisms and their symbionts (Rogers and Davis, 2006). However, few studies (Hoegh-Guldberg and Smith, 1989; Sakami, 2000; Rogers and Davis, 2006) have examined the interaction of temperature and salinity as it affects zooxanthellae density. Those studies that mentioned this interaction considered different algal hosts and a different suite of environmental factors.

Cell morphology of zooxanthellae isolates

Zooxanthellae cells maintained under control conditions (27 ºC, 30 psu) were round in shape with a diameter of about 10 µm; they were yellow-brown in color with green pigments inside the cell. This is the normal cell morphology of zooxanthellae that can be found in the natural tissue of marine invertebrates (Figure 5a).

On the other hand, the cells cultured in all treatments at the highest temperature level (33 ºC) and in all treatments under the lowest salinity level (10 psu) were different from the control. They appeared similar to normal cells in shape and size but were clearly paler in color with fewer of cytoplasmic organelles (Figure 5b).

These results are consistent with previous study of Rodolfo-Metalpa *et al*. (2006), who reported that pale zooxanthellae cells with a low number of cytoplasmic organelles occurred at high temperature (more than 32 ºC) and low salinity. These stressful conditions were shown to affect the organelles of zooxanthellae cells, especially chlorophyll, and the cells died when exposed to a temperature of 34 ºC (Sammarco and Strychar, 2009).

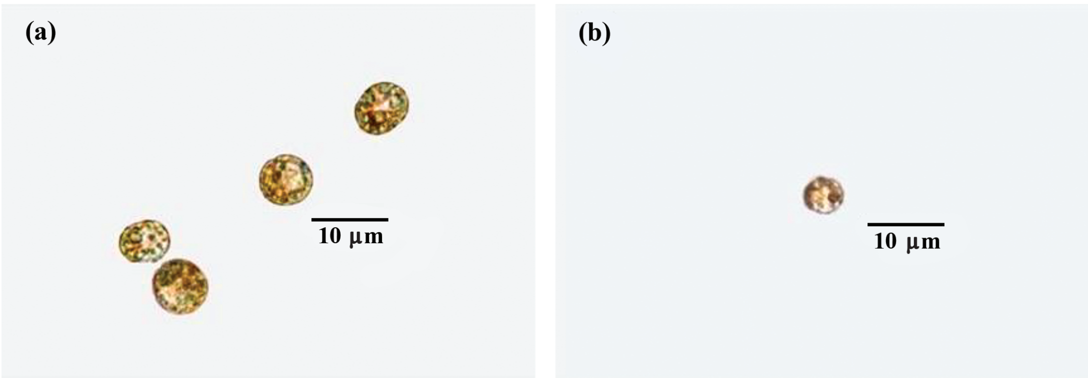

Figure 5. Normal (a) and Abnormal (b) zooxanthellae cells under axenic culture.

CONCLUSION

The following conclusions can be made from this study:

(1) The zooxanthellae isolated from mushroom coral (*Fungia fungites*), cauliflower coral (*Pocillopora damicornis*) and soft coral (unidentified) showed the highest densities when cultured in control conditions (27 ºC and 30 psu).

(2) Zooxanthellae isolated from different host types responded differently to temperature, whereby zooxanthellae from giant clam significantly increased growth when temperature increased from 27 ºC to 30 ºC, while growth of the others was not significantly changed.

(3) Salinity of 10 psu and temperature of 33 ºC each had a detrimental effect on growth of zooxanthellae regardless of source.

RECOMMENDATION

In recent decades, coral bleaching has increased in frequency, intensity and geographic scale, resulting in mass mortality of corals. The frequency and severity of bleaching events has occurred under thermal stress and sometimes under decreased salinity.

However, coral can adapt to these environmental stresses, probably as a mechanism for survival. Sometimes corals can select zooxanthellae by alternative discharge. Moreover, bleached hosts can acquire new symbionts for re-establishment.

This study has shown that zooxanthellae from different sources react differently to temperature elevation, and further studies are warranted to understand the mechanism underlying this phenomenon. In addition, it is worth investigating zooxanthellae from unexplored hosts which might tolerate wider ranges of temperature and salinity. This study was only the first part of a project aimed at selecting strains of zooxanthellae that exhibit the thermal and salinity tolerance for bleaching recovery. Hence, future studies should consider a wider range of zooxanthellae strains, as well as other host collection sites and other levels of temperature and salinity.

ACKNOWLEDGEMENTS

We would like to thank the Royal Thai Marine Corps for their assistance with sample collecting. We are also grateful to Assistant Professor Dr. Sirichai Dharmvanij and Mr. Christan Michael Voss for help with proofreading. 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 of Chulalongkorn University, and the Science Achievement Scholarship of Thailand.

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