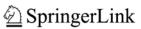
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# Effects of Temperature and Salinity on Survival Rate of Cultured Corals and Photosynthetic Efficiency of Zooxanthellae in Coral Tissues

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Abstract - This study investigated the effects of temperature and salinity on growth, survival, and photosynthetic efficiency of three coral species, namely, Pocillopora damicornis, Acropora millepora and Platygyra sinensis of different ages (6 and 18 months old). The experimental corals were cultivated via sexual propagation. Colonies were exposed to 5 different temperatures (18, 23, 28, 33, and 38°C) and 5 different salinities (22, 27, 32, 37, and 42 psu). Results showed that temperature significantly affected photosynthetic efficiency (Fv/Fm) (p < 0.05) compared to salinity. The maximum quantum yield of corals decreased ranging from 5% to 100% when these corals were exposed to different temperatures and salinities. Temperature also significantly affected coral growth and survival. However, corals exposed to changes in salinity showed higher survivorship than those exposed to changes in temperature. Results in this study also showed that corals of different ages and of different species did not display the same physiological responses to changes in environmental conditions. Thus, the ability of corals to tolerate salinity and temperature stresses depends on several factors.

**Key words** – temperature, salinity, specific growth rate, survival rate, maximum quantum yield

# 1. Introduction

Physical environments such as salinity, temperature, and light are major factors contributing to the survival, growth and photosynthesis of corals (Ferrier-Pagès et al. 1999; Baird and Hughes 2000; Ferrier-Pagès et al. 2007; Chow et al. 2009). Studies have shown that growth rates of coral decrease if salinity changes by  $\pm 2$  psu from normal levels (Ferrier-Pagès et al. 1999). Other studies also demonstrated that rapid changes in salinity may induce coral death (Sakai et al. 1989; Jokiel et al. 1993). Such death is attributed to the effects of salinity on the photosynthesis of zooxanthellae, resulting in a decrease in the amount of energy transferred to corals (Muthiga and Szmant 1987; Manzell and Lirman 2003). Approximately 80% of photosynthetic products such as carbohydrates and oxygen are transferred to coral tissues; of these products, 10% to 22% are used for the respiration and growth of zooxanthellae (Davies 1984; Edmunds and Davies 1986).

Scleractenian corals are relatively stenohaline. These corals can tolerate only slight changes in salinity; however, they die if the salinity is < 25 ppt or > 45 ppt (Edmondson 1928; Jokiel et al. 1974). Changes in salinity affect not only coral photosynthesis but also reproduction and respiration (Richmond 1993; Porter et al. 1999). It is also known that changes in salinity disrupt normal cellular electrochemical processes and lead to a metabolic drain in marine organisms (Vernberg and Vernberg 1972).

Suitable temperatures can increase calcification rates (Jacques et al. 1983; Marshall and Clode 2004; Reynaud et al. 2004). In general, water temperature is related to light quantity, upwelling, and periods of calm water, and consequently affects coral survival. However, when water temperatures and light intensity exceed normal ranges, coral bleaching can occur (Franklin et al. 2006; Chavanich et al. 2009; Chow et al. 2009;



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Chavanich et al. 2012). Increased temperature is also an important factor triggering coral bleaching and decreasing photosynthetic efficiency of symbiotic dinoflagellates (Hoegh-Guldberg 1999). Photoinhibition by excessive temperature and light is a well-known cause of a reduction of photosystem II quantum efficiency (Coles and Jokiel 1978; Krause and Weis 1991; Beer et al. 1998; Franklin et al. 2006; Chow et al. 2009). Damage in D1 protein of photosystem II also results in the loss of photosynthetic efficiency (Warner et al. 1999). To measure the response of photosystem II (PSII), effective quantum yield (Fv/Fm) through pulse-amplitude-modulated (PAM) fluorometry is used (Franklin et al. 2006; Ulstrup et al. 2006; Ferrier-Pagès et al. 2007; Frisch et al. 2007; Piniak and Storlazzi 2008; Chow et al. 2009). In addition, different coral species may react to changes in temperature differently because of different levels of oxidative stress, tissue thickness, and zooxanthellae clades (Lesser 1996; Loya et al. 2001; Franklin et al. 2006).

The aim of this study was to evaluate the effects of changes in temperature and salinity on the growth, survival, and photosynthetic efficiency of three species, namely, *Pocillopora damicornis, Acropora millepora* and *Platygyra sinensis* of different ages (6 and 18 months old). We hypothesized that both decreases and increases in temperature and increases and decreases in salinity would have negative effects on coral growth, survival, and photosynthetic efficiency. All experimental corals were cultured through sexual propagation; and thus, experimental corals in this study were age specific, something which no previous study of this type has done.

## 2. Materials and Methods

## Coral collection and maintenance

The gametes of the spawning corals, *Acropora millepora* and *Platygyra sinensis*, were collected during spawning periods from January to March at nighttime in Sattahip Bay, Chon Buri Province, Thailand. The gametes were then transferred to a hatchery, mixed, and fertilized. The gamete concentrations used for mixing and fertilization were from 5 colonies in each species, and each colony had approximately more than 10,000 bundles (6-11 eggs/bundle and 2.7-3  $\times$  10<sup>6</sup> sperms/ml). Planulae were maintained in the hatchery until they settled on cotta tiles and metamorphosed to juvenile corals. Juveniles were also raised in the hatchery on Samae San Island until they reached the ages of 6 and 18 months. For the hermaphroditic brooder, *Pocillopora damicornis*, mature colonies were collected

one week before the new moon and brought back to the hatchery. The colonies were maintained in aerated tanks until larvae were released. The larvae were collected, settled, and raised until they reached the ages of 6 and 18 months. The experiments were done simultaneously on different cohorts that spawned on different dates.

## **Experimental protocol**

The colonies used in the experiments were approximately 1 and 2.5 cm in diameter for the ages of 6 and 18 months respectively. In each treatment,  $30 \times 60 \times 30$  cm<sup>3</sup> glass aquaria containing 54 L of water were used as experimental tanks. There were a total of 3 experiment tanks in each treatment. Water in the aquaria was changed daily, and all the aquaria were aerated continuously. Before the trials were performed, the corals were acclimated to experimental conditions for 2 days. All experimental aquaria were exposed to a 12 h light and dark cycle.

#### **Experimental trials**

Experiments under different salinity and temperature conditions were conducted in the laboratory by using juvenile coral colonies (6-month old P. damicornis, 6-and 18-month old A. millepora, and 18-month old P. sinensis). Because of availability issues regarding corals in the hatchery, there were no 18-month old P. damicornis and 6-month old P. sinensis. In temperature trials, juvenile corals were transferred and exposed to different treatments (18, 23, 28, 33, and 38°C) for one month. The average ambient temperature of 28°C as measured in the field was used as a control in this experiment. The corals were maintained at ambient salinity (32 psu). Chillers and heaters were used to control temperatures. Ten replicate corals were prepared in each treatment. At the beginning of the trials, the colonies were transferred randomly to the experimental aquaria. Temperature was then gradually increased or decreased (at a rate of 1°C per 10 minutes) until temperature reached the experimental values. During the experiments, the specific growth rates were measured weekly by using the Coral Point Count with Excel extensions program (CPCe) (Kohler and Gill 2006) to determine coral surface areas using a scale on the photographs before and after the experiments. An equation used to calculate a specific growth rate was

 $SGR = (In(BWn/BWn-1))/\Delta t [week^{-1}]$ 

where SGR is the specific growth rate (week<sup>-1</sup>), BWn is the surface area at the end of the experiment, BWn-1 is the

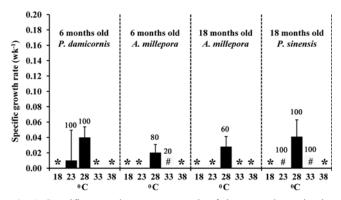
surface area at the start of the experiment, and  $\Delta t$  is the time between the measurement of surface area (Schutter et al. 2011).

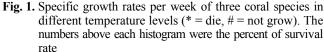
In addition, survival rates of corals were measured every week. The maximum quantum yield (Fv/Fm) of zooxanthellae or photosynthetic efficiency was also measured using an underwater pulse amplitude modulation fluorometer (a DIVING PAM) in each coral colony before and after the experiment. Before the maximum quantum yield was measured, the corals were kept in darkness for 30 minutes, and measurement was set at 7 pm. in each trial.

In the salinity experiment, the coral colonies were exposed to five different treatments (22, 27, 32, 37, and 42 psu) for one month. Before the trails were performed, salinity was gradually increased or decreased (at a rate of 1 psu per 10 minutes) until salinity reached the experimental values. The corals were maintained at ambient temperature (28°C). In the experimental trials, the growth and survival rates of corals were measured every week, and photosynthetic efficiency was measured before and after the experiment, following the temperature experimental protocol. Although all of the experiments were set to be run for one month, temperature trials were terminated earlier, and ran only for 13 days because most coral samples died and could not tolerate the experimental temperature conditions. At the end of the trials, data on specific growth rates and photosynthetic efficiency of corals from different salinity levels and temperatures were compared using a one-way ANOVA test following Tukey's pairwise mean comparison.

### 3. Results

The results showed that changes in temperature and salinity affected growth, survival, and photosynthesis of *Pocillopora damicornis, Acropora millepora* and *Platygyra sinensis* of all ages. The changes in specific growth rates of different coral species in temperature trials are shown in Fig. 1. Most coral samples did not increase their growth in any of the treatments, except at the ambient temperature (28°C). Significant reductions in the net photosynthesis of colonies exposed to 18°C and 38°C in all of the coral species at all ages were also documented (p<0.05) (Fig. 2). It is important to note that when photosynthesis was not detected, corals either died or the photosynthetic efficiency was too low to be detected. When comparing corals among different ages, the highest extent of decrease of photosynthetic efficiency was





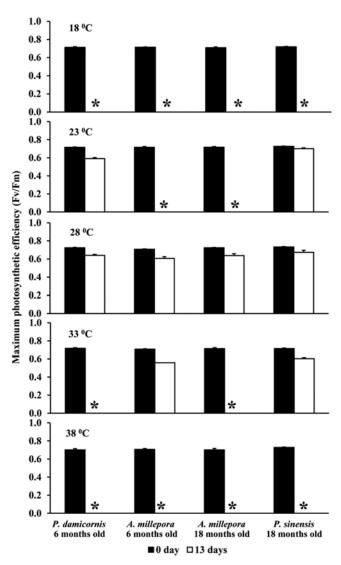


Fig. 2. Maximum photosynthetic efficiency (Fv/Fm) of zooxanthellae associated with three coral species in different temperature levels. (\* = die and/or cannot be measured)



0.20 6 months old 18 months old 6 months old 18 months old P. damicornis A. millepora A. millepora 0.18 P. sinensis Specific growth rate (wk<sup>-1</sup>) 0.16 0.14 0.12 0.10 0.08 0.06 0.04 0.02 0.00 22 27 32 37 42 22 27 32 37 42 22 27 32 37 42 22 27 32 37 42 psu psu psu psu

**Fig. 3.** Specific growth rates per week of three coral species in different salinity levels (\* = die, # = not grow). The numbers above each histogram were the percent of survival rate

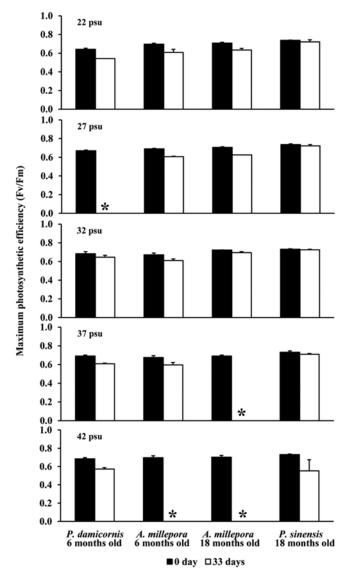


Fig. 4. Maximum photosynthetic efficiency (Fv/Fm) of zooxanthellae associated with three coral species in different salinity levels. (\* = die and/or cannot be measured)

recorded for 18-month old *A. millepora* (Fig. 2). Therefore, 18-month old *A. millepora* could not survive under temperature changes (Fig. 1). However, 6-month old *P. damicornis* survived at 23°C, but not at 33°C; by contrast, 6-month old *A. millepora* survived at 33°C but not at 23°C (Fig. 2).

For the salinity experiments, the results showed that growth rates were only detected in corals exposed to 32 psu, except 18-month old *P. sinensis*, which could grow at salinity levels ranging from 22 psu to 37 psu (Fig. 3). In contrast to corals exposed to different temperature conditions, corals exposed to different salinity levels (from 22 psu to 42 psu) could survive, except 6-and 18-month old *A. millepora* (Fig. 3). Photosynthetic efficiency also decreased when salinity levels changed (Fig. 4). Fv/Fm was not detected when 6-and 18-month old *A. millepora* were exposed to 42 psu; likewise, Fv/Fm was not detected when 6-month old *P. damicornis* was exposed to 27 psu (Fig. 4).

# 4. Discussion

This study investigated the growth, survival, and photosynthetic responses of corals and zooxanthellae associated with three coral species to changes in seawater temperature and salinity. The present study also confirmed that changes in temperature and salinity negatively affected *Pocillopora damicornis, Acropora millepora* and *Platygyra sinensis* regardless of age. It was observed that an increase or decrease in seawater temperature caused coral bleaching, in which zooxanthellae were expelled from coral hosts; as a result, the hosts became white.

The effects of 2 stressors, salinity and temperature on photosynthetic efficiency can be seen in this study. A decrease in the maximum quantum yield of all corals ranged from 5% to 100% when these corals were exposed to different temperature and salinity conditions. Under temperature stress, Fv/Fm of zooxanthellae decreased remarkably within 13 days and could not be detected thereafter (Fig. 2). However, under salinity stress, Fv/Fm of symbiotic dinoflagellates decreased, and Fv/Fm could be detected even at low and high salinity levels (Fig. 4). The survival of corals exposed to salinity stress was prolonged compared with that of corals exposed to temperature stress. Lesser (1996) also observed a similar reaction in symbiotic dinoflagellates exposed to increased temperature and ultraviolet radiation. Several studies have shown that corals tended to be more tolerant to salinity than temperature stress (Buddemeier and Fautin 1993; Reynaud

et al. 2004; Chavanich et al. 2009). The ability of corals to tolerate salinity stress or other stresses depends on species, shapes and size of colonies, range of stress tolerance, zooxanthellae clades, and ability to regenerate after disturbance (Buddemeier and Fautin 1993; Fabricius et al. 2004). In addition, it can depend on its polyp retraction response under osmotic stress, which reduces the tissue surface area in contact with surrounding water (Muthiga and Szmant 1987). Corals can be both an osmoconformer and an osmoregulator; thus, corals can physiologically acclimate and tolerate osmotic stress (Vernberg and Vernberg 1972).

In addition to having direct measurements of coral growth and counting of the number of coral survivals under stress conditions, measurement of lipid concentrations can be a good indicator in predicting coral survivorship (Anthony et al. 2007; Lin et al. 2012). Lin et al. (2012) found that thermal stress-induced changes in membrane lipid content and composition of coral oocytes led to alterations in the ratio of saturated/unsaturated fatty acids. These changes in lipid profiles shifted not only the overall lipid phase transition temperature, but also could influence the development and survivorship of the coral (Lin et al. 2014). Further studies on the effects of stressors on coral physiology are needed.

In summary, this study revealed that *P. sinensis, A. millepora,* and *P. damicornis* could highly tolerate changes in salinity; this characteristic allows corals to survive in stressful environments. However, corals of different ages and of different species did not display the same physiological response to changes in other environmental factors. Furthermore, changes in temperature and salinity can negatively affect coral photobiology causing photoinhibition. Thus, more studies are needed to investigate the complex interaction between environmental stress factors that can induce different responses among corals.

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