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FACTORS INFLUENCING GROWTHS OF CULTURED JUVENILE CORALS IN A NURSERY SYSTEM

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ปฐพร เกื้อนุ้ย : ปัจจัยที่มีอิทธิพลต่อการเติบโตของปะการังวัยอ่อนเลี้ยงในระบบอนุบาลปะการัง (FACTORS INFLUENCING GROWTHS OF CULTURED JUVENILE CORALS IN A NURSERY SYSTEM) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.สุชนา ชวนิชย์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร.มะโคโตะ โอโมริ, หน้า.

ในปี 2010 เกิดปรากฏการณ์ปะการังฟอกขาวบริเวณหมู่เกาะแสมสาร จังหวัดชลบุรี ประเทศไทย ส่งผลให้ปะการังตายร้อยละ 32-78.8 ของประชากรทั้งหมด และส่งผลให้เกิดการฟอกขาวในดอกไม้ทะเลและหอยมือเสือ ภายหลังการเกิดเหตุการณ์นี้ ทำให้มีความพยายามในการฟื้นฟูแนวปะการังที่ได้รับผลกระทบ โดยในการศึกษาครั้งนี้ ทำการศึกษาปัจจัยทางกายภาพ (อุณหภูมิ ความเค็ม ความเข้มแสง และระยะเวลาในการได้รับแสง) และอาหาร (อัตราการจับอาหาร อัตราการย่อย ชนิดอาหารที่เลือกกิน และประสิทธิภาพของอาหาร) ที่มีผลต่อการเติบโต อัตรารอดของปะการัง และประสิทธิภาพในการสังเคราะห์แสงของสาหร่ายซูแซนเทลลี่โดยดูจากคลอโรฟิลด์ฟลูออเรสเซ้นต์ในเนื้อเยื่อของปะการังทั้ง 4 ชนิด คือ ปะการังเขากวาง *Acropora millepora* และ *Acropora nobilis* ปะการังดอกกะหล่ำ *Pocillopora damicornis* และปะการังสมองร่องยาว *Platygyra sinensis* ซึ่งเป็นปะการังที่ได้มาจากการเพาะขยายพันธุ์แบบอาศัยเพศ โดยผลการศึกษาพบว่า อุณหภูมิที่เปลี่ยนแปลงมีผลกระทบต่อปะการังทั้งอัตราการเติบโต อัตรารอด และประสิทธิภาพในการสังเคราะห์แสงของสาหร่ายซูแซนเทลลี่อย่างมีนัยสำคัญ ($p < 0.05$) เมื่อเทียบกับการเปลี่ยนแปลงของความเค็ม สำหรับการทดลองเรื่องแสง พบว่าปะการังสมองร่องยาว *Platygyra sinensis* สามารถทนต่อความเข้มแสงและระยะเวลาในการได้รับแสงในช่วงกว้างได้ดีกว่าปะการังชนิดอื่นๆ สำหรับการทดลองเรื่องการให้อาหารปะการัง พบว่าอัตราการจับอาร์ทีเมียของปะการังอยู่ระหว่าง 0.44-2.39 ตัวต่อโพลิปและสามารถย่อยอาร์ทีเมียจนหมดภายใน 2-2.5 ชั่วโมง นอกจากนี้ การเลือกกินอาหารและประสิทธิภาพของอาหารปะการัง พบว่าปะการังเขากวาง *A. millepora* ที่ให้กินอาร์ทีเมีย มีปริมาณโปรตีนในเนื้อเยื่อปะการังมากที่สุด สรุป ปัจจัยทางกายภาพ (อุณหภูมิ ความเค็ม ความเข้มแสง และระยะเวลาในการได้รับแสง) และอาหารล้วนมีผลต่อการเติบโต อัตรารอดของปะการัง และประสิทธิภาพในการสังเคราะห์แสงของสาหร่ายซูแซนเทลลี่ ซึ่งผลการศึกษาในครั้งนี้ สามารถนำไปประยุกต์ใช้ในการเพาะขยายพันธุ์ปะการังในโรงเพาะเลี้ยง หรือการฟื้นฟูแนวปะการัง

ภาควิชา	วิทยาศาสตร์ทางทะเล	ลายมือชื่อนิสิต .....
สาขาวิชา	วิทยาศาสตร์ทางทะเล	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
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PATAPORN KUANUI: FACTORS INFLUENCING GROWTHS OF CULTURED JUVENILE CORALS IN A NURSERY SYSTEM. ADVISOR: ASSOC. PROF. SUCHANA CHAVANICH, Ph.D., CO-ADVISOR: PROF. MAKOTO OMORI, Ph.D., pp.

In 2010, the hard coral mortality ranged between 32% to 78.8% of populations and other reef organisms, including sea anemones and giant clams were also affected by the bleaching at Samae san Island, Chon Buri Province, Thailand. After the bleaching events, there was an attempt to rehabilitate coral reefs in certain areas. In this study, the influence of physical factors (temperature, salinity, light intensity, and photoperiod) and food factors (capture rates, digestibility, feeding preferences, and feeding efficiency) on growth, survival, and photosynthetic efficiency of four coral species (staghorn corals, *Acropora millepora* and *Acropora nobilis*; cauliflower coral, *Pocillopora damicornis*; and brain coral, *Platygyra sinensis*) were used as an experimented corals. All corals used in the experiment were cultivated via sexual propagation. The results showed that temperature significantly affected growth, survival, and photosynthetic efficiency ($p < 0.05$) compared to salinity. For the light intensity experiment, *P. sinensis* seemed to have more tolerance to wider ranges of light intensities and photoperiods than other coral species. When feeding preference experiments of corals were conducted, the results showed that the capture rates on *Artemia salina* nauplii of corals ranged between 0.44 to 2.39 individuals per polyp and 2 hours to 2.5 hours to complete the prey digestion. Moreover, the feeding preferences and feeding efficiency showed that the protein concentrations were higher in *A. millepora* fed on *Artemia salina*. In conclusion, both physical (temperature, salinity, light intensity, and photoperiod) and food factors can have an influence on growth, survival, and photosynthetic rates of corals. The results of this study can be used for achievement of coral culturing technique for restoration of coral reefs

Department:	Marine Science	Student's Signature	.....
Field of Study:	Marine Science	Advisor's Signature	.....
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CHAPTER I
Introduction

Coral reef is a highly diverse and rich marine ecosystem. It acts as a nursery area as well as a source of food and refuge for marine organisms. Moreover, it prevents coastal erosion brought about by the scouring of natural waves. At present, coral reefs have continuously deteriorated throughout the world due to human activities and various natural phenomenal (Burke et al. 2011). As such, humans need to initiate efforts toward the recovery of coral reefs and find ways to ensure sustainable use. Recently, a new approach to coral reef restoration is to culture corals through a rearing system using sexual reproduction technique (Edwards and Gomez 2007). This method uses gametes or planula larvae from nature which are transferred to the rearing system for fertilizing and rearing inside a tank. Subsequently, the settled juveniles are released back to the sea (Raksasab 2007, Kuanui 2008). However, during the rearing, the growth of corals is slow, and the corals are required to rise at last 1 year before transferred back to the sea. Thus, this study aims to determine the impact of temperature on coral bleaching and coral population in the sea and the influence of physical factors (light intensity, photoperiod, temperature and salinity) and food on the growth and survival of the juvenile corals in the rearing system. The hypothesis is that, before being transferred back to the sea, the combination of both physical factors (light intensity, photoperiod, temperature and salinity) and food can increase the growth and survival rate of juvenile corals in a rearing system.

CHAPTER II
Literature review

Coral reef ecosystems exist in tropical and subtropical zones with suitable temperature and light for existence (Veron 2000b). Thus, to culture and rear corals, the artificial rearing system of juvenile corals on land should mimic natural factors, such as light (Schutter et al. 2010), temperature (Jokiel and Coles 1977), salinity (Hoegh-Guldbergl and Smith 1989), food quantity (Houlbrèque et al. 2004), and density of zooxanthellae (Houlbrèque et al. 2003).

Physical factors

Light intensity and photoperiod on coral growth

The intensity of light received by corals can effect on the photosynthesis of zooxanthellae. Different sizes of corals also require diverse light intensities (Babcock and Mundy 1996). Some corals, such as *Stylophora pistillata*, require light intensity at 140 µmol $m^{-2}s^{-1}$ in the rearing system (Rinkevich and Loya 1984, Stambler and Dubinsky 2005). By contrast, the rearing of *Galaxea fascicularis* requires light intensity at 280 µmol $m^{-2}s^{-1}$ (Schutter 2010). Some studies have shown that the varied positions of settlement with different light intensities also exert significant effects on both growth and survival rates of corals (Baird and Hughes 2000b, Richier et al. 2008). Zooxanthellae can adapt their capability of photoacclimation in different light intensities. For instance, in low light intensity, zooxanthellae increase accumulation of photosynthetic pigments and their light harvesting capability, thereby increasing the absorbed light per area of zooxanthellae (Dubinsky et al. 1984, Titlyanov 1991,

Stambler and Dubinsky 2005, Mass et al. 2010). Moreover, respiration is related to light intensity, as low light intensity requires minimal respiration, whereas high light intensity promotes higher respiration (Falkowski and Owens 1980, Mass et al. 2007). In low light intensity, corals exhibit darker tissues than those in high light intensity because they contain high pigment concentrations without changing the zooxanthellae density (Falkowski et al. 1984, Mass et al. 2007, Al-Hammady 2013).

However, other studies indicated that a high zooxanthellae density can occur in low light condition (Titlyanov et al. 2001). Zooxanthellae density can be associated with seasons. In fact, the zooxanthellae density is higher in summer than in autumn (Mass et al. 2007, Al-Hammady 2013). However, photoinhibition caused by excessive light can reduce the photosystem II (PSII) quantum efficiency, as well as the pigment concentration in zooxanthellae (Coles and Jokiel 1978, Krause and Weis 1991, Beer et al. 1998) (Franklin et al. 2006, Chow et al. 2009, Rocha et al. 2013). Thus, light is an important factor in photosynthesis followed the equilibrium:

$$CO_2 + 2H_2O + \text{LIGHT} \rightarrow (CH_2O) + O_2$$

For the photoperiod, Schutter (2010) found that exposing high light intensity did not affect the growth rates at 8, 12, and 16 hours, but the corals began to experience bleaching at 24 hours. Generally, normal light intensity for corals ranges from 140 µmol $m^{-2}s^{-1}$ to 280 µmol $m^{-2}s^{-1}$, with a light duration of 12:12 hours (Reynaud et al. 2002, Reynaud et al. 2004, Moya et al. 2006, Ferrier-Pagès et al. 2007, Chow et al. 2009). Moreover, the rate of coral calcification in light condition is approximately three times higher than that in dark condition, (Al-Horani et al. 2007, Mass et al. 2007, Herfort et al. 2008) and the calcification rates can eventually decrease by depths (Mass et al. 2007). The types of lighting for the coral rearing

system can be divided into three groups. The first group involves the metal halide lamp. The light from this lamp can penetrate deep into water, but demonstrates a short lifetime of light intensity for only six months; this light can also produce heat, causing potential damage to corals (Reynaud et al. 2002, Osinga et al. 2008, Fitzgerald 2010, Rocha et al. 2013). The second group uses a T5 fluorescent light, which can produce higher photon per watt than standard fluorescent types, without any problem regarding heat (Osinga et al. 2008, Petersen et al. 2008, Holcomb et al. 2010). The third group employs LED light, which requires high cost, but exerts longer lifetime than other light types, and cause no problem regarding heat. However, both T5 and LED light cannot penetrate deep into water (Osinga et al. 2008).

Water temperature on coral growth

In general, water temperature is related to light intensity and consequently affects the coral survivals. In the summer, sea water temperature is higher, which leads to coral bleaching because zooxanthellae cannot survive inside the corals (Jokiel and Coles 1990, Franklin et al. 2006, Chavanich et al. 2009, Chow et al. 2009). While several sources of stress can cause bleaching, ocean atmosphere phenomena, such as the El Nino-Southern Oscillation (ENSO), is one of major bleaching event related to raising ocean temperatures (Baker et al. 2008). The rate of coral tolerance bleaching can be varying depend on coral species and locations. For example, in Hawaii, coral bleached after they were exposed to 4^0C to 5^0C above normal ambient water temperature (Jokiel and Coles 1977). The higher temperature can decrease in effective quantum yield (fluorescence value/Variable fluorescence or Fv/Fm) of Photosystem II (Franklin et al. 2006, Ferrier-Pagès et al. 2007, Chow et al. 2009) and also lead to

decreasing photosynthesis and respiration rates (Agostini et al. 2013) and growth rates (Reynaud et al. 2003). However, in tropical corals, such as *Oculina arbuscula* have a high growth rate in summer (Miller 1995) and also found that *Stylophora pistillata* had a higher growth rate when it was in the temperatures higher than normal (Reynaud et al. 2004). Which water flow in high water temperature can result in increased survival rate and photosynthesis (Schutter 2010). This is because water flow can supply basic requirements, such as oxygen, inorganic carbon, inorganic nutrients, and food (Stambler et al. 1991, Lesser et al. 1994, Borell 2008). Which is different (Rodolfo-Metalpa et al. 2008) showed that *Cladocera caespitosa* had a high growth rate during the winter season (low temperature) because they had more active consumption behavior in the winter season than in summer season. Nevertheless, some corals have lower photosynthesis rates in low temperature, even if these corals are exposed to high light intensity (Sakami 2000, Saxby 2001).

Salinity on coral growth

As for the salinity factor, Sawatphira (1985) found that increasing salinity decreased the growth of *Pocillopora damicornis*. In addition, high temperature and low irradiation can reduce zooxanthellae tolerance against low salinity (Sakami 2000). Salinity within a range of 30–34 psu does not affect the survival of corals (Hoegh-Guldbergl and Smith 1989). In comparison, the lowest salinity level (<25 psu) reduced the survival ability of corals (Muthiga and Szmant 1987, Palaki 1998). In some corals, the growth rate decreases if the salinity changes by ± 2 psu from the normal salinity in more than 3 weeks (Ferrier-Pagès et al. 1999). This is due to the fact that water salinity has effect on the photosynthesis of the zooxanthellae. This

phenomenon decreases the energy transferred to the coral (Manzello and Lirman 2003), and thus, the coral needs to replace the missing energy (Borell 2008).

Water turbidity and sedimentation

The main cause of high water turbidity is terrestrial runoff which reduces light intensity and also increases sedimentation (Fabricius 2005b). The reduction of light intensity reduces the net photosynthesis of zooxanthellae in coral tissues (Anthony and Hoegh-Guldberg 2003, Junjie et al. 2014). The turbidity not only reduces the net photosynthesis but also causes the coral to use more energy to produce mucus and remove sediment (Kendall et al. 1985). However, zooxanthellae can adapt to different light intensities (Titlyanov 1991, Mass et al. 2010). Moreover, excessive sedimentation is an important factor that inhibits larval settling on a substrate, and the number of larval settling is also relative to the sedimentation rate (Babcock and Davies 1991, Birrell et al. 2005). Even though, some coral larvae were able to settle on the substrate, the post settlement mortality was high (Babcock and Davies 1991). Moreover, in unsuitable conditions such as high sedimentation rate, the settled *Pocillopora damicornis* planulae can revert from the metamorphosis stage to the planula stage (polyp bail-out) (Te 1992). In addition, sedimentation and turbidity can also induce coral diseases (Pollock et al. 2014).

Food on coral growth

Many studies showed that the density of zooxanthellae in the coral tissues is also higher in corals that received protein compared to those that did not receive protein (Ferrier-Pagès et al. 2003, Houlbrèque et al. 2003, Borell 2008). Clayton and

Lasker (1982) also showed that corals reared in dark condition lose some zooxanthellae density and their efficiency in catching food. Normally, zooxanthellae can transform carbon dioxide from coral respiration and nitrogen from ammonium form, and nutrients in the water for photosynthesis (Grottoli 2002). Also, approximately 80% of all outputs of photosynthesis, such as carbohydrates and oxygen, are transferred to the coral tissue, and around 10% to 22% are used for the respiration and growth of the zooxanthellae (Davies 1984a, Edmunds and Davies 1986). Corals use some energy to catch a prey; therefore, they need extra energy for the consumption. Corals use their tentacles to catch planktons (Ferrier-Pagès et al. 2003, Palardy et al. 2005), digestion takes place in stomach after the planktons pass the polyps. The stomach contents of natural corals consist of zooplanktons (e.g., copepod, nematode, polychaete, *Artemia*, juvenile marine animals, bacteria, dissolved organic, and particulate matter) (Anthony 1999, Houlbrèque et al. 2004). Zooplankton have been shown to be important for increasing growth and survivorship for a variety of scleractinian coral (Grottoli et al. 2006). Due to the different amounts of energy needed, different corals have different feeding rates (Sebens et al. 1996). Ferrier-Pagès et al. (2003) found that *Stylophora pistillata* captured zooplankton less than *Galaxea fascicularis*. In addition, the size of prey, density of prey, size of coral polyps, water flow rate, and ability of prey to avoid captured are all related to the corals' capability to capture prey (Sebens et al. 1996, Piniak 2002a, Palardy et al. 2006, Hii et al. 2009, Toh et al. 2014). *Artemia* spp. can be used for feeding corals as they are better than other juvenile marine organisms because they easily prepare, have high nutrient value and have a small size of about 0.5 mm in length (Leversee 1976, Helland et al. 2003, Reynaud et al. 2004, Schutter 2010). Petersen et al. (2008) and

Toh et al. (2014) found that corals feeding on *Artemia salina* nauplii had higher growth rates than no feed. In addition, damaging coral from bleaching recovered faster when fed with *Artemia* spp. (Grottoli et al. 2006). Moreover, recent artificial coral food, such as dried phytoplankton and zooplankton are commercially widely available. It is a cheaper and takes less time to prepare than live zooplankton (Forsman et al. 2012). Furthermore, cost-effectiveness analysis showed that the cost per unit of volumetric growth was reduced with increasing feed densities (Toh et al. 2014). Thus, in order to increase the coral growth in the hatchery, feeding corals with supplementary food would be an option.

Other factors

Calcification

The active transport of calcium ions received energy from photosynthesis (Al-Horani et al. 2005). Thus, the received energy increased, because of a better procedure (Tambutte et al. 1996), and this process can control the level of pH, which was considered suitable for the precipitation of calcium carbonate (Al-Horani et al. 2003, Ludwig et al. 2005, Moya et al. 2006). Moreover, a suitable temperature can increase coral calcification (Marshall and Clode 2004, Reynaud et al. 2004). Feeding corals by *Artemia salina* nauplii can also increase the calcification rate by 29% (Reynaud et al. 2002). Additionally, when the concentrations of carbonate and bicarbonate ions in the water column increase, the yield of calcium carbonate increases as follows:

$$Ca^{2+} + 2HCO^{-}_{3} \rightarrow CaCO_3 + H_2O + CO$$

(Rees et al. 2005, Borell 2008, Herfort et al. 2008). However, the variation of pH and increase of dissolved inorganic carbon affected the changes in carbon balance, leading to decreases in both aragonite saturation state and calcification rate (Ries et al. 2009).

Water flow

Water flow can influence coral growth and calcification. First, water flow can increase the oxygen diffusion, which enhances the dark calcification rate (Rinkevich and Loya 1984). Second, water flow controls the efflux rate of potentially toxic metabolic products such as oxygen and its radicals (Nakamura et al. 2005, Finelli et al. 2006). Third, water flow may indirectly promote coral growth by removing sediment (Fabricius 2005a). Fourth, water flow speed enhances the resistance of corals to high temperature (Smith 2004). Furthermore, water flow at 10 cm s^{-1} to 25 cm s^{-1} is the optimal condition for coral growth (Schutter 2010, Forsman et al. 2012).

Growth parameters

Coral growth rate can be measured using several methods, such as buoyant weight technique (Reynaud et al. 2002, Marubini et al. 2003, Reynaud et al. 2004, Schutter 2010). Specific growth rate can be determined using the changes in length and/or width of corals over time. The rate of changes occurring per day, month, or year can be calculated using the following equation:

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

Where SGR is the specific growth rate ($week^{-1}$), *BW*n is surface area at the end of the experiment, *BW*n-1 is surface area at the start of the experiment, and Δt is time between the measurement of surface area (Schutter et al. 2008, Schutter et al. 2010,

Osinga et al. 2011, Schutter et al. 2011). In the recent photographic analysis, a new method, called Coral Point Count with Excel extensions (CPCe), which take less time in the field, was introduced. This method is a visual basic program for the determination of coral and substrate coverage by scale on the photograph, which measures the changes in length and/or width of corals over time and calculates the specific growth rate (Kohler and Gill 2006, Kiel 2012). Furthermore, to determine the rate of photosynthesis, different oxygen levels were measured between light and dark conditions (Houlbrèque et al. 2003, Schutter et al. 2008). Nevertheless, the way to measure the responsibility of PSII effective quantum yield (Fv/Fm) by pulse-amplitude-modulated (PAM) fluorometry is faster. Initial fluorescence (Fo) was measured by applying a weak pulsed red light (LED 650 nm, 0.6 kHz, 3 μs). A saturating pulse of bright actinic light (8,000 μmol Photon m^{-2} s^{-1}, width 800 ms) was applied to yield the maximal fluorescence value (Fm). Variable fluorescence (Fv) was calculated as Fm-Fo and maximal quantum yield as Fv/Fm (Ferrier-Pagès et al. 2007, Frisch et al. 2007, Borell 2008, Piniak and Storlazzi 2008, Chow et al. 2009). However, in some conditions, the results of growth rate and PSII were not related (Sakami 2000).

Another method can also monitor the growth of corals by determining the proportion of biochemical compositions in coral tissues, including carbohydrates, lipids and proteins (Patton et al. 1977, Latyshev et al. 1991, Achituv et al. 1994). Several methods can measure these biochemical compositions; for example, carbohydrate extraction is measured by the phenol-sulfuric acid method of Dubois et al (1956) (Dubois et al. 1956, Szmant-Froelich and Pilson 1980, Rodrigues and Grottoli 2007). Lipid measurement is mostly determined by gas-liquid

chromatography, as described by Meyer et al (1974). Usually, the amounts of lipids range between 30% to 40% of dry weight (Stimson 1987, Rodrigues and Grottoli 2007). For protein quantification, several methods can be used, such as micro-Kjeldahl technique (Szmant-Froelich and Pilson 1980, Porter et al. 1989, Borell et al. 2008), microanalytical technique (Muscatine et al. 1989), BCA assay kit (Smith et al. 1987, Houlbrèque et al. 2003), and Lowry method with bovine serum albumin as standard and absorbance read at 750 nm. Lowry method provides advantages in small samples and takes less time. Thus, this method has become popular and widely used (Lowry et al. 1951, Marshall and Clode 2002, Marubini et al. 2003). The amount of protein ranges of 3 g to 8 g per gram of dry weight (Ferrier-Pagès et al. 1999, Rodrigues and Grottoli 2007). In case of coral bleaching, >50% of protein in coral tissues decreased (Porter et al. 1989).

Coral species in this study

Four coral species were used in the experiments: staghorn corals, *Acropora millepora* and *Acropora nobilis*; cauliflower coral, *Pocillopora damicornis*; and brain coral, *Platygyra sinensis*. All these species are hermatypic scleractinian corals with zooxanthellae (*Symbiodinium* sp.) living inside the coral tissues through symbiosis. *Acropora millepora* is a short branch spread growing in vertical direction (Hall 1997). *Pocillopora damicornis* is a small bush-shaped coral growing in radial direction (Le Tissier 1988). Both of these corals contain a small polyp measuring 1 mm to 2 mm in diameter. These corals are found in reef flat and reef slope in the Pacific and Indian Ocean (Veron and Pichon 1976). *Acropora nobilis* consist of small bushes, tending toward developing small tables. Its branches are slender. Radial corallites range from

being relatively long and slender. Axial corallites may not be larger than the radials. The corals are cream to brown, and the branch ends are pale (Veron 2000b). This species exists in shallow tropical reef environments, as well as in deep sandy lagoons to upper reef slopes from 3 m to 11 m, rarely from 12 m to 15 m, in the South China Sea, Southwestern Japan and Gulf of Thailand (Fujioka 1998, Titlyanov and Titlyanova 2002). *Platygyra sinensis* is massive or flat or dome-shaped coral, with thin walls. The septa of this species are thin and paliform lobes that are not developed. Columellae are weakly developed without columella centers. Tentacles are extending at night. The coral colonies are variably dull or bright (Veron 2000a). This species occurs in shallow between outer and inner reef flat, as well as in upper reef slopes from 1 m to 5 m (Fujioka 1998).

Objectives of this study

1. Impact of temperature on coral bleaching and coral population

2. To determine the influence of physical factors (light intensity, period of light receiving, temperature and salinity) on the growth and survival of juvenile corals.

3. To determine the influence of food on the growth and survival of juvenile corals.

CHAPTER III

2010 Mass bleaching of coral reef communities in the upper gulf of Thailand and the recovery

Introduction

In the past years, coral bleaching has been recognized to contribute to the loss of coral cover in many parts of the world (Brown 1997, Hoegh-Guldberg 1999, Wilkinson 2004, Whelan et al. 2007, Wilkinson 2008, Krishnan et al. 2011, Chavanich et al. 2012, Guest et al. 2012). Several environmental factors can influence coral bleaching and associated mortality (Hoegh-Guldbergl and Smith 1989, McClanahan et al. 2004). The examples of those environmental stresses included temperature, salinity, UV radiation, and bacterial infection (Hoegh-Guldbergl and Smith 1989, Drollet et al. 1994, Drollet et al. 1995, Brown 1997, Kushmaro et al. 1998, Chavanich et al. 2009). However, thermal anomaly is one of the important factors responsible to large scale bleaching (Hoegh-Guldberg 1999, Hoegh-Guldberg et al. 2007, Krishnan et al. 2011). Levels of bleaching and subsequently mortality of corals can differ among different coral species, depending on their size, range of tolerance, resilience, and symbiont types (Fabricius et al. 2004, McClanahan et al. 2004, Obura 2005, Bellwood et al. 2006). Adaptation of corals to elevated sea temperatures was also recently reported as a factor contributing to patterns of bleaching susceptibility among coral genera (Guest et al. 2012).

In Thailand, mass bleaching of corals has frequently been recorded in the past decade (Chavanich et al. 2009, Brown and Phongsuwan 2012, Chavanich et al. 2012, Phongsuwan and Chansang 2012). Recently, in 2010, an unusual warm water had caused mass coral bleaching both in the Andaman Sea and in the Gulf of Thailand

during the months of April-June (Brown and Phongsuwan 2012, Chavanich et al. 2012, Phongsuwan and Chansang 2012), Department of Marine and Coastal Resources, unpublished data). The bleaching on the Andaman Sea coast of Thailand usually occurs when the sea surface temperatures reach the annual maximum and exceed 30.4°C (Phongsuwan and Chansang 2012). In the upper Gulf of Thailand, the highest temperature recorded during the 2010 bleaching was 33.9°C (Chavanich et al. 2012). This 2010 bleaching event was reported to be more severe than the 1998 bleaching (Phongsuwan and Chansang 2012). In some areas in the Andaman Sea, more than 50% of coral bleached and later died (Phongsuwan and Chansang 2012). Other reefs organisms were also affected by the bleaching (Chavanich et al. 2012).

Extensive research on the 2010 coral bleaching in the Andaman Sea and the lower Gulf of Thailand was conducted; however, little was known in the upper Gulf of Thailand (Hoeksema and Matthews 2011, Brown and Phongsuwan 2012, Chavanich et al. 2012, Phongsuwan and Chansang 2012). The purpose of this study was to investigate the effect of mass bleaching on corals and reef organisms and their recovery in Chon Buri Province, upper Gulf of Thailand. In addition, the bleaching susceptibility of different coral taxa was also examined. The data also reflects the current status of coral communities in the areas. The hypothesis is that, the rising of water temperature could increase bleaching of the corals and other reef organisms, and result in a high mortality rate.

Materials and Methods

To evaluate the effect of the 2010 bleaching event on coral reef communities, the study was carried out at 6 reef sites of two locations, Samae San Island and Khram Island, in Chon Buri Province, the upper Gulf of Thailand (Fig. 3-1).

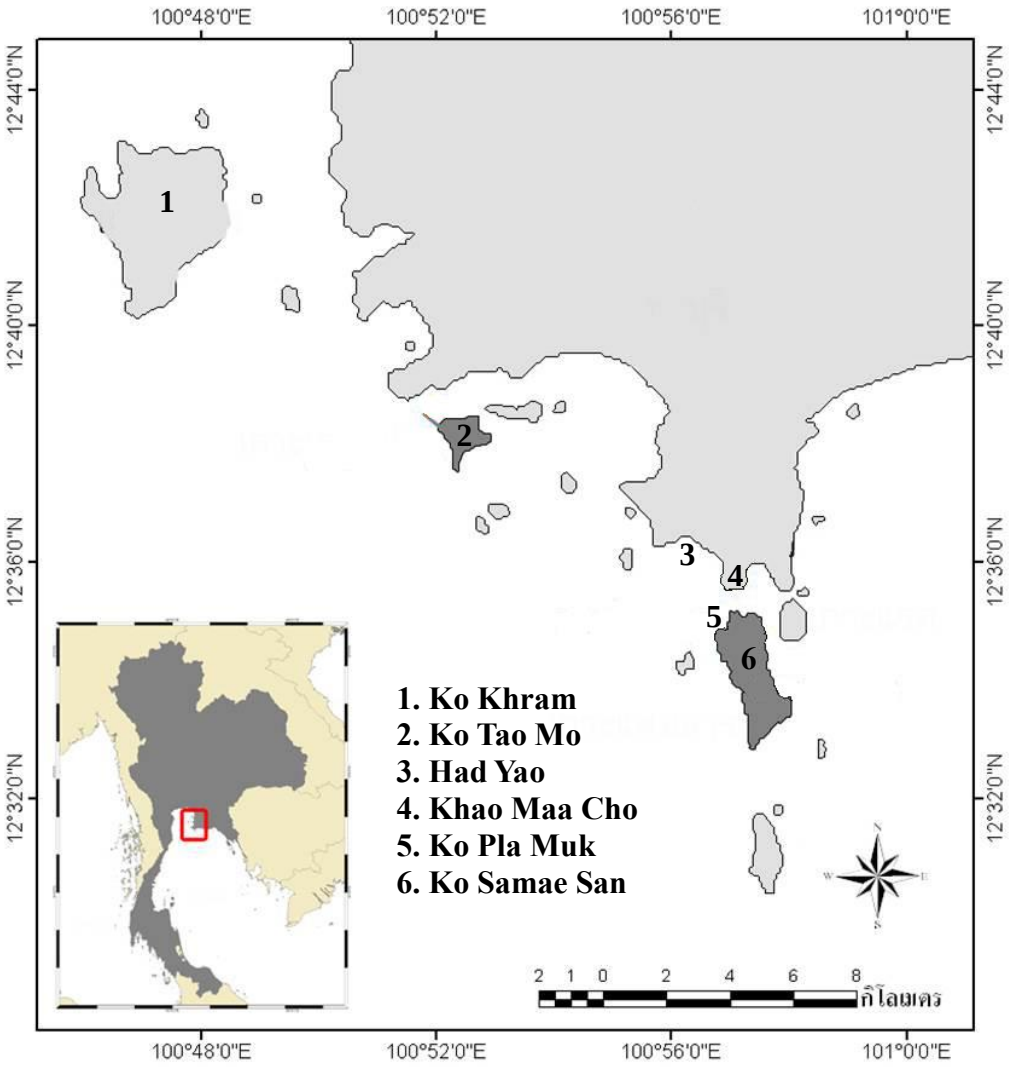

Figure 3-1. Study sites in Chon Buri Province, the upper Gulf of Thailand.

In each site, a permanent 100 m transect line was established on a reef flat parallel to the shore since 2009 to 2011. At least three line intercept transects (English et al. 1997) established the coral coverage at each site (Fig. 3-1). In addition, the

roving diver technique was applied (Munro 2005), covering the area as large as possible. Each coral found either in the line or during the roving diving technique was identified genus level, and the bleaching status that described the degree of bleaching of colony area of each colony was recorded. Bleaching status was categorized into the following five categories: 1) unbleached, 2) 0.1% to 25% of surface bleached, 3) 25.1% to 50% bleached, 4) 50.1% to 75% bleached, and 5) 75.1% to 100% bleached. The method of categorizing the bleaching status was adopted from Gleason (1993) and McClanahan (2004). The data were then analyzed by correlation analysis for comparison of the bleaching between sites. An equation was used to calculate the percentage of dead coral after bleaching:

% dead coral = ((coral coverage 2009 - coral coverage 2011)/coral coverage 2009)* 100

(Table 3-1).

In addition, sea anemones and giant clams (*Tridacna* spp.) affected by the bleaching were also investigated. Similar to the coral survey, sea anemones and giant clams encountered within a meter off the 100-m transect line or during the roving diving technique were recorded, and the bleaching status was identified either unbleached or bleached. Moreover, each individual of sea anemones and giant clams were tagged and monitored for one year for their mortality.

Recovery and mortality of corals due to the warm water were calculated from the permanent transect lines based on observations and decrease in coral coverage consequent on bleaching. Additional surveys were conducted 1 year before and after the 2010 bleaching event (2009 and 2011).

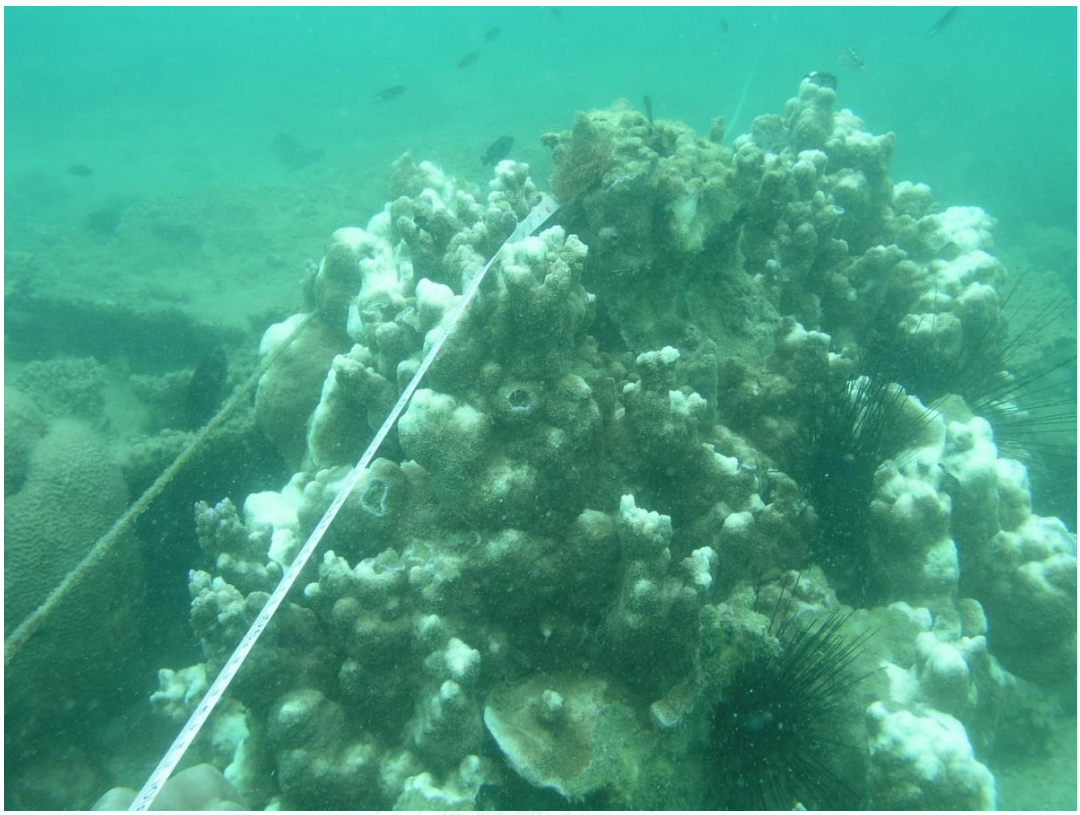

Figure 3-2. Line transect method.

Figure 3-3. Bleached massive and branching coral colonies during 2010.

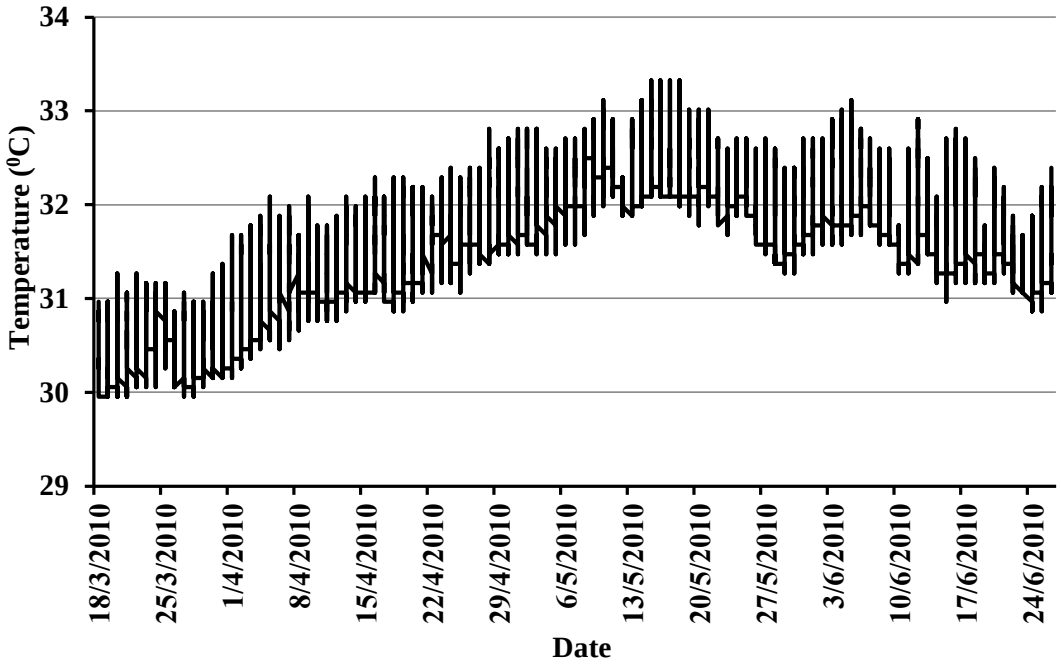

Figure 3-4. Average temperature on the study site.

Results

Anomalously warm temperatures that began in April 2010 caused extensive coral bleaching on reefs in Chon Buri Province, the upper Gulf of Thailand (Fig. 3-4). 70% to 100% of the hard coral populations in the study sites experienced extensive bleaching (Fig. 3-6). The proportion of bleaching on each colony differed between sites (Fig. 3-7). However, more than 40% of coral population in each site had 75.1% to 100% of colony bleaching (Fig. 3-7). Levels of colony bleaching varied among coral genera. When pooled data from all sites, the coral taxa, *Acropora, Goniastrea, Montipora, Pocillopora, Porites,* and *Symphyllia* were the most susceptible with all affected colonies completely bleached (Fig. 3-8). In contrast, *Turbinaria* colonies were unbleached (Fig. 3-8).

Table 3-1. Comparison of coral covers before and after the bleaching event between 6 study sites

Location	% Coral coverage (2009)	% Coral coverage (2011)	% dead coral* after bleaching
Samae San Location			
Maa Cho	53.0	34.3	35.3
Pla muk	35.0	23.8	32.0
Tao Mo	42.0	15.6	62.9
Had Yao	43.5	18.5	57.5
Khram Island			
Had Na Ban	40.1	18.2	54.6
Put Sa Wan	60.3	12.8	78.8

* An equation used to calculate a percentage of dead coral after bleaching was

% dead coral = ((coral coverage 2009 - coral coverage 2011)/coral coverage 2009)* 100

Monitoring after the bleaching event showed that by January 2011, coral mortality in all sites, which was calculated based on the percentage loss of hard coral cover, ranged between 32% to 78.8% (Table 3-1). Put Sa Wan had the highest percent of coral mortality (78.8%). The results from the surveys also showed that other reef invertebrates with zooxanthellae association, including sea anemones and giant clams, *Tridacna* spp., extensively bleached during the 2010 event (Fig. 3-5). Bleaching of sea anemones and *Tridacna* spp. from all sites was 73.3% to 100% and 50% to 100% respectively (Table 3-2). After the bleaching, 27.4% to 65.5% of bleached sea anemones and 11.1% to 60.8% of bleached *Tridacna* spp. had died (Table 3-2).

Table 3-2. Comparison of percent of bleaching and mortality of sea anemones and giant clams, *Tridacna* spp., between 6 study sites. (N of sea anemones and *Tridacna* spp. = 30 individual in each site; n/a represents no individual of sea anemone or giant clam was found in the area.

Location	Sea anemones		*Tridacna* spp.	
	% Bleaching	% Mortality	% Bleaching	% Mortality
Samae San Location				
Maa Cho	88.6 (27)	35.5 (10)	50.0 (15)	11.1 (2)
Pla muk	73.3 (22)	50.0 (11)	70.0 (21)	52.0 (11)
Tao Mo	n/a	n/a	n/a	n/a
Had Yao	100 (30)	65.5 (20)	100 (30)	60.8 (18)
Khram Island				
Had Na Ban	100 (30)	33.3 (10)	100 (30)	50.0 (15)
Put Sa Wan	93.3 (28)	27.4 (8)	75.5 (23)	33.5 (8)

Figure 3-5. Bleached sea anemone and giant clam during 2010**.**

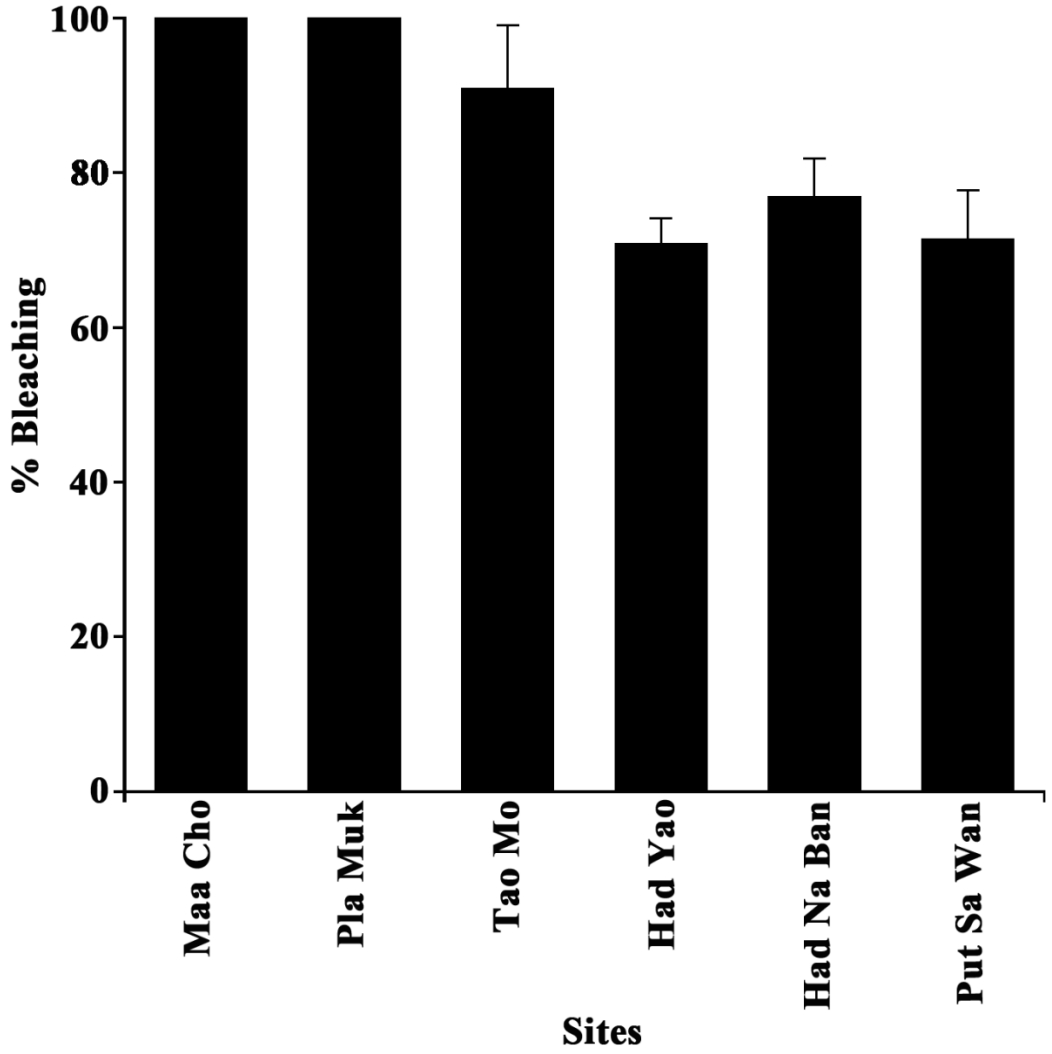

Figure 3-6. Average percentage of bleaching of coral populations from 6 sites.

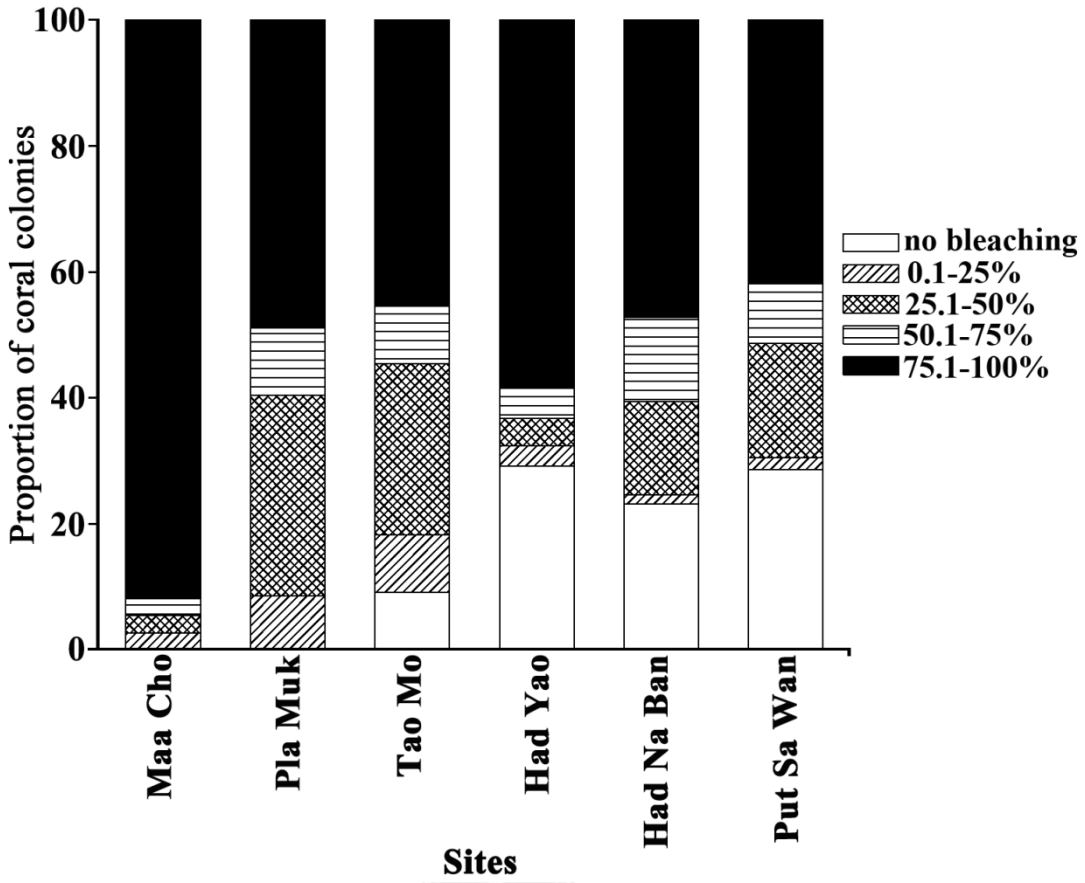

Figure 3-7. Proportion of coral colonies affected by each category of bleaching at 6 sites.

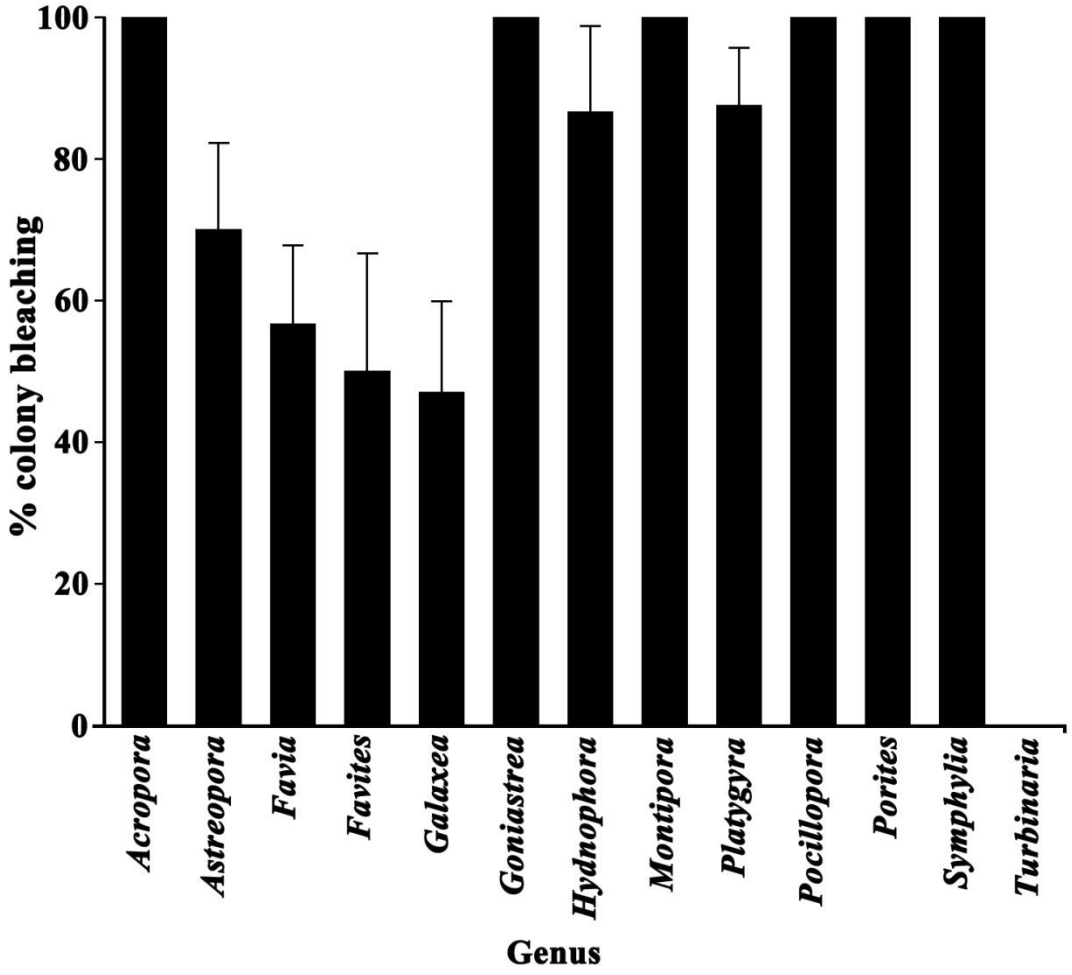

Figure 3-8. Average percentage of colony bleaching in each coral genus when pooled data from all sites.

Discussion

This study provides an overview of coral communities and their response to the 2010 anomalously warm water. In this study, there was a significant relationship in the bleaching response of the coral populations and taxa between and within study sites.

The susceptibility to bleaching and the mortality response of corals varies among genera (Kayanne et al. 2002, McClanahan et al. 2004). In this study, all of the *Turbinaria* corals and 30% to 50% of the corals species *Galaxea*, *Favia*, and *Favites* were not affected by bleaching. In 1998, a large-scale bleaching event occurred, and it was discovered that the *Turbinaria* and *Galaxea* corals were unaffected by bleaching and were consequently categorized as having low susceptibility, whereas the *Favia* and *Favites* were reported to have moderate susceptibility (Marshall and Baird 2000), While branching and plate-like acroporid and pocilloporid corals were reported to have high susceptibility to the bleaching, and the bleaching event led to selective coral mortality (Gleason 1993). Colonies that were completely bleached tended to have a lower rate of recovery than ones that partially bleached (Gleason 1993). From previous studies, complete recovery after mass bleaching events could take many months and highly dynamic (Goreau and Macfarlane 1990, McClanahan et al. 2001). In addition, the recovery of coral cover also depends on successful of larval recruitment (Baird and Marshall 2002).

It is also believed that size, resilience, and zooxanthellae types play an important role on the bleaching sensitivity (McClanahan et al. 2004, Obura 2005, Bellwood et al. 2006). McClanahan et al. (2004) reported that general patterns of susceptibility, coral change, and mortality were consistent for taxa within a region.

Bleaching was not only observed in hard corals, but also in other reef invertebrates such as sea anemones and giant clams, which have symbiotic zooxanthellae (in this study) (Krishnan et al. 2011, Chavanich et al. 2012). In this study, high mortality of sea anemones and giant clams occurred after the bleaching event. In addition to reef invertebrates, reef fish were reported to decline after the

coral bleaching through the reduction in live coral cover (Pratchett et al. 2006, Pratchett et al. 2008, Chavanich et al. 2012). Bleaching causes coral mortality and consequently can alter the abundance and community composition of reef organisms (Pratchett et al. 2006, Chavanich et al. 2012), and can affect the population dynamics of coral associates (Stella et al. 2011). As global ocean temperatures continues to increase, it is also important to examine and evaluate the effect of the bleaching event not only on corals but also on other reef organisms to understand the consequence of the bleaching on the population dynamics.

Coral reef community response to the 2010 bleaching event in this study suggests that recovery of populations is likely to slower than previous bleaching events since the mortality after the bleaching was high (Table 3-1). However, severely affected coral species such as *Acropora* spp. have shown that they can survive and reproduce (personal observations). Thus, their adaptation will be important for future bleaching events.

CHAPTER IV

Effects of temperature and salinity on survival rate of cultured corals and photosynthetic efficiency of zooxanthellae in coral tissues

Introduction

Physical environments such as salinity, temperature, and light can be major factors contributing to survival, growth and photosynthesis of corals (Ferrier-Pagès et al. 1999, Baird and Hughes 2000a, Ferrier-Pagès et al. 2007, Chow et al. 2009). Studies have shown that growth rates of coral decreases if the salinity changes $\pm$ 2 psu from the normal salinity (Ferrier-Pagès et al. 1999). Other studies also demonstrated that rapid change 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, Manzello and Lirman 2003). Approximately 80% of photosynthetic products such as carbohydrates and oxygen are transferred to the coral tissues; of these product, 10% to 22% are used for the respiration and growth of the zooxanthellae (Edmunds and Davies 1986, Davies 1984b).

Scleractinian corals are relatively stenohaline. These corals can tolerate only slight changes in salinity; however, these coral die if the salinity is < 25 ppt or > 45 ppt (Edmondson 1928, Jokiel et al. 1975). In the upper part of the Gulf of Thailand during the rainy season, it was discovered that in short period of time, the salinity was lower than 20 psu at the shallow coral reef area (Moberg et al. 1997). Changes in salinity affect not only coral photosynthesis but also reproduction and respiration (Richmond 1994, 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 temperature 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 the coral survival. However, when the water temperatures and light intensity exceed normal ranges, coral bleaching can occur (Franklin et al. 2006, Chavanich et al. 2009, Chow et al. 2009, Chavanich et al. 2012). Increased temperatures is also an important factor triggering to 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 a change 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 growth, survival, and photosynthetic efficiency in three corals, namely, *Pocillopora damicornis, Acropora millepora* and *Platygyra sinensis* of different ages (6-and 18-month old). The hypothesis is that, decreasing and increasing of

temperature and salinity negatively impact the coral growth, survival, and photosynthetic efficiency of zooxanthellae in the coral tissue. All experimental corals were cultured through sexual propagation; and thus, experimental coral in this study were age specific which no pervious study was done.

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 the hatchery, mixed, and fertilized. The gametes concentration used for mixing and fertilization were from 5 colonies in each species, and each colony had approximately more than 10,000 bundles (6 to 11 eggs/bundle and 2.7 to 3 x 10^6 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 these organisms reached the ages of 6 and 18 months. The experiments were done simultaneously on different cohorts that spawned on different dates (Fig. 4-1).

Experimental protocol

The colonies used in the experiments were approximately 1 and 2.5 cm in diameter of the ages of 6 and 18 months respectively. In each treatment, 30 x 60 x 30 cm^3 glass aquaria containing 54 L of water were used as experimental tanks. There were a total 3 experimental tanks in each treatment. Water in the aquaria was changed daily, and all of 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 12:12 h light and dark cycle.

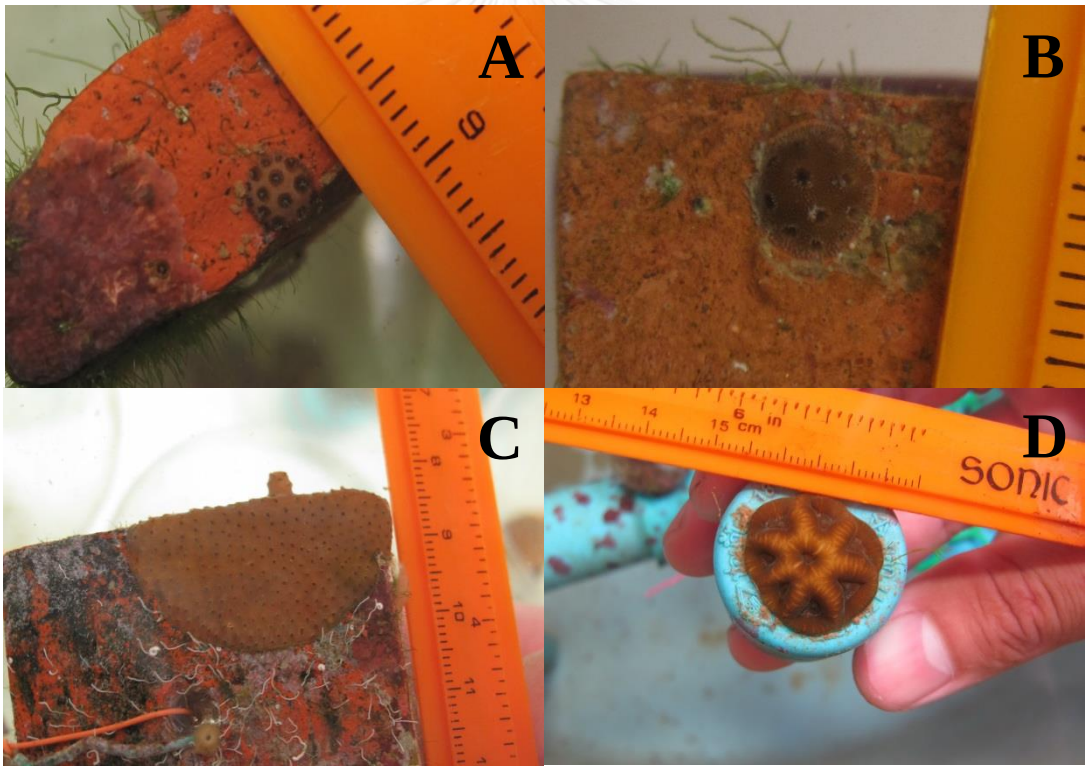

Figure 4- 1. A) 6-month old *Pocillopora damicornis*, B) 6-month old *Acropora millepora*, C) 18-month old *Acropora millepora*, and D) 18-month old *Platygyra sinensis*.

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 of 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 (Fig. 4-2). 28 °C (average ambient temperature measure in field) was used as a control in this experiment. The corals were maintained at ambient salinity (32 psu). Chiller and heater 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 experiment aquaria. Then, temperature was gradually increased or decreased (at a rate of 1 °C per 10 minute) 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 the coral surface areas using scale on photographs before and after experiments. An equation used to calculate a specific growth rate was

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

Where SGR is the specific growth rate ($week^{-1}$), *BW*n is surface area at the end of the experiment, *BW*n-1 is surface area at the start of the experiment, and Δt is 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 the darkness for 30 minutes, and measurement was set at 7 p.m. in each trial (Fig. 4-3).

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 trials were performed, salinity was gradually increased or decreased (at a rate of 1 psu per 10 minute) until salinity reached the experimental values. The corals were maintained at ambient temperature (28 °C) (Fig. 4-4). 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 experiments were set to be run for one month, temperature trials were terminated earlier, and run only for 13 days because most coral samples were died and could not tolerate with the experimental temperature conditions. At the end of the experiment, data on the survival rate and specific growth rates of corals from different salinity levels and temperatures were compared using the one-way ANOVA test, followed by Tukey's pairwise mean comparison, and the data on the photosynthetic efficiency of corals from different salinity levels and temperatures were compared using the paired samples t-test.

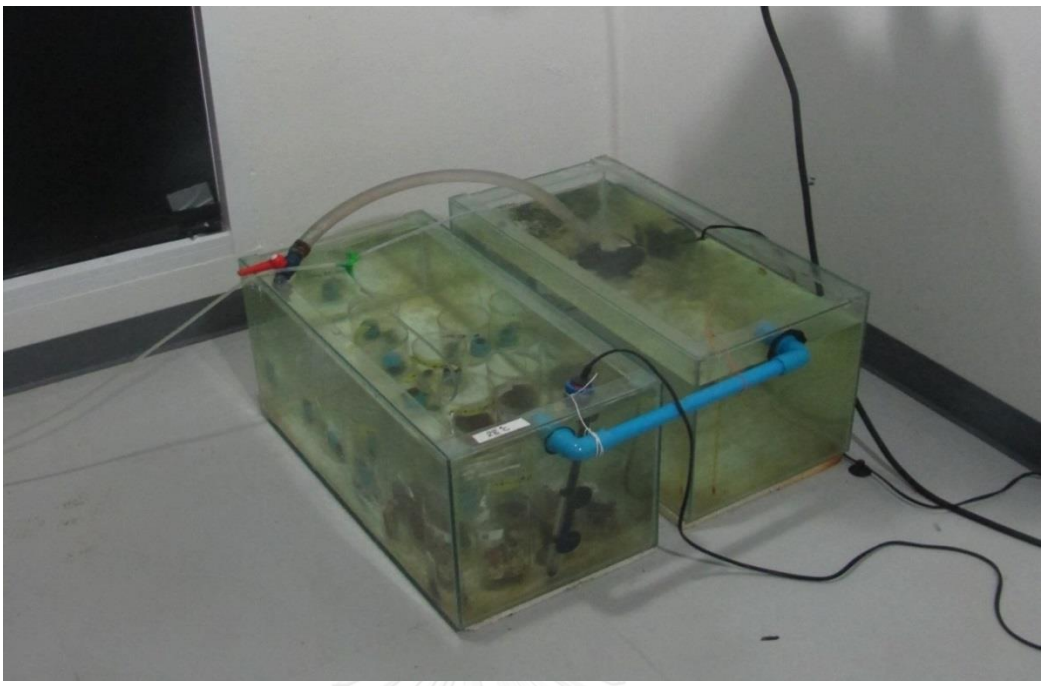

Figure 4- 2. A rearing system for temperature experiment.

Figure 4-3. Using underwater pulse amplitude modulation fluorometer (a DIVING PAM) to measured photosynthetic efficiency of zooxanthellae.

Figure 4-4. A rearing system for salinity experiment.

Results

The results showed that changes of both temperature and salinity affected growth, survival, and photosynthesis of *Pocillopora damicornis*, *Acropora millepora* and *Platygyra sinensis* of all ages. The changes in specific growth rate of different coral species in temperature trials are shown in Figure 4-5. Most coral samples did not increase their growth in any treatments, except at the ambient temperature (28 °C). Significant reductions in net photosynthesis of colonies exposed to 18 °C and 38 °C in all of coral species all ages were also documented ($p < 0.05$) (Fig. 4-6). In the day 3, it is important to note that photosynthesis was not detected, corals either died or the photosynthetic efficiency was very low to be detected. When comparing corals among different ages, the highest extent decrease of photosynthetic efficiency was

recorded in 18-month old *A. millepora* (Fig. 4-6). Therefore, 18-month old *A. millepora* could not survive under temperature changes (Table 4-1). 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 (Table 4-1).

Table 4-1. Survival rate of coral in different temperatures.

Species	Temperature				
	18 ^{0}C	23 ^{0}C	28 ^{0}C	33 ^{0}C	38 ^{0}C
6-month old *P. damicornis*	0	100±0	100±0	0	0
6-month old *A. millepora*	0	0	80±20	20±20	0
18-month old *A. millepora*	0	0	60±25	0	0
18-month old *P. sinensis*	0	100±0	100±0	100±0	0

Table 4-2. Survival rate of coral in different salinities.

Species	Salinity				
	22 psu	27 psu	32 psu	37 psu	42 psu
6-month old *P. damicornis*	100±0	100±0	100±0	100±0	80±20
6-month old *A. millepora*	100±0	100±0	100±0	80±20	0
18-month old *A. millepora*	100±0	100±0	100±0	100±0	0
18-month old *P. sinensis*	100±0	100±0	100±0	100±0	60±25

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 ranging from 22 psu to 37 psu (Fig. 4-7). 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* (Table 4-2). It was discovered that the corals started to bleach and lose tissue mass on the ninth day, and photosynthetic efficiency also decreased when salinity levels changed (Fig. 4-8). 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. 4-8).

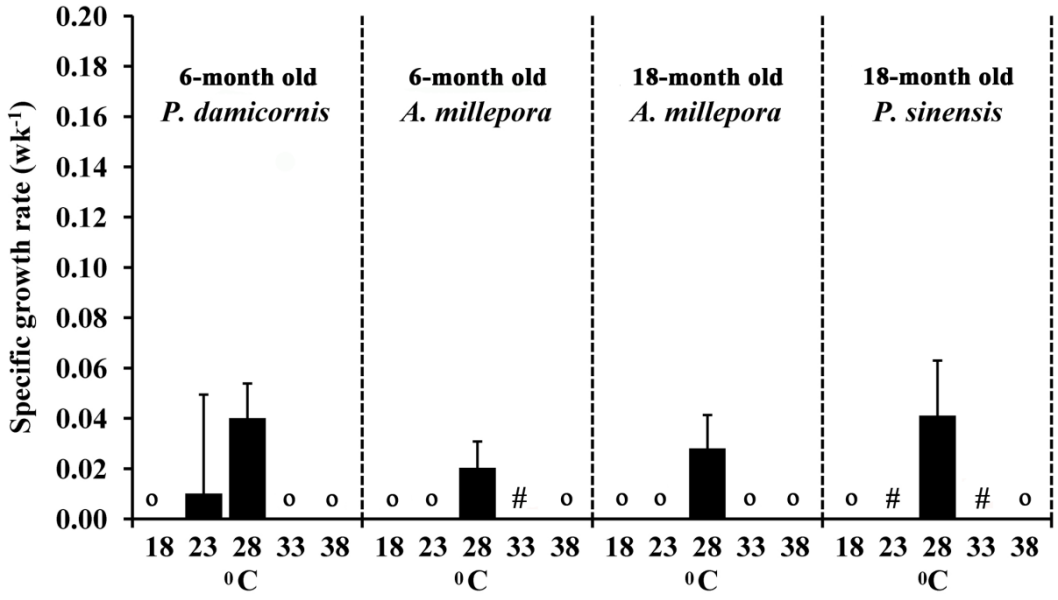

Figure 4- 5. Specific growth rates per week of three coral species in different temperature levels (o = die, # = not grow).

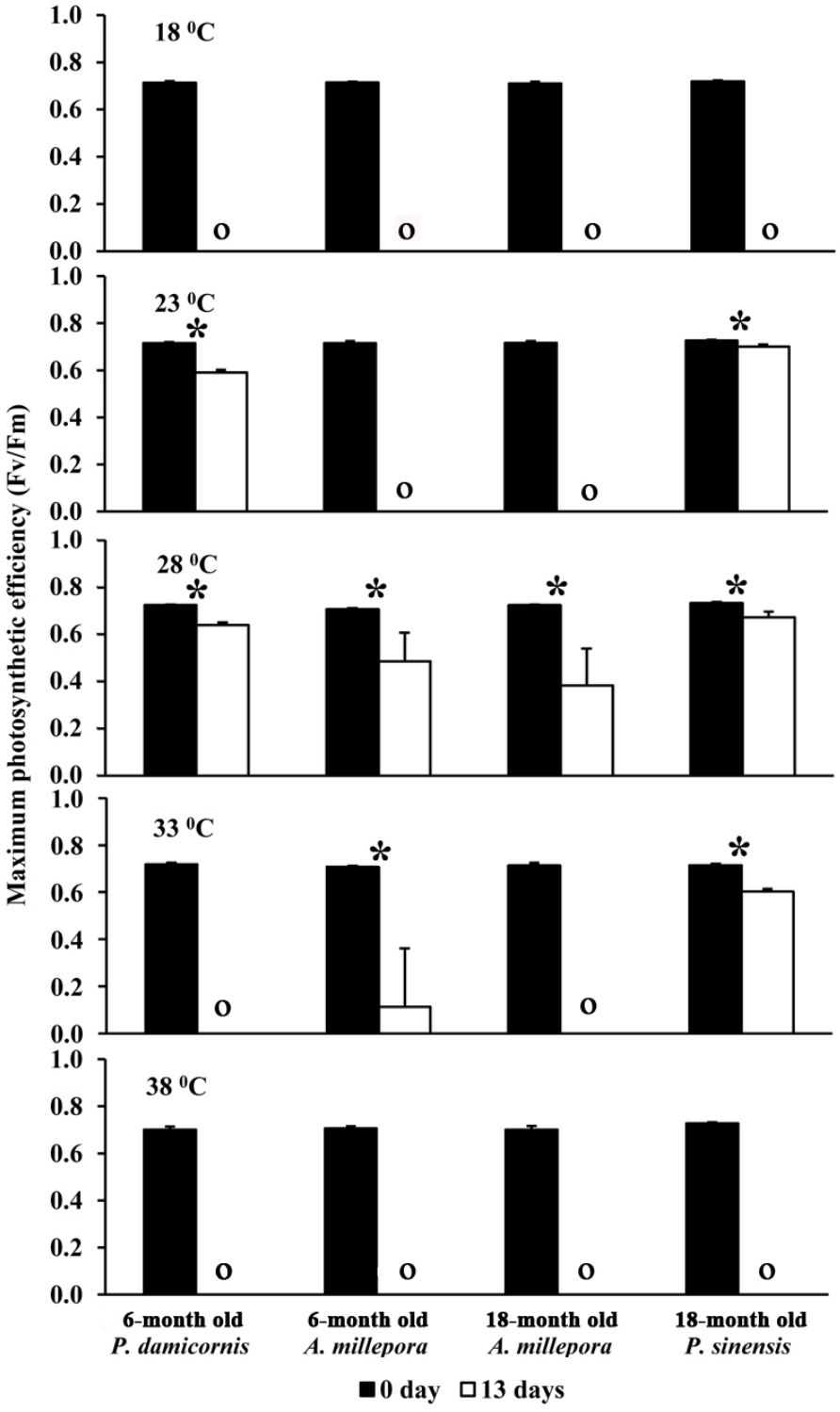

Figure 4-6. Maximum photosynthetic efficiency (Fv/Fm) of zooxanthellae associated with three coral species in different temperature levels (o = die and/or cannot be measured, *= significant difference).

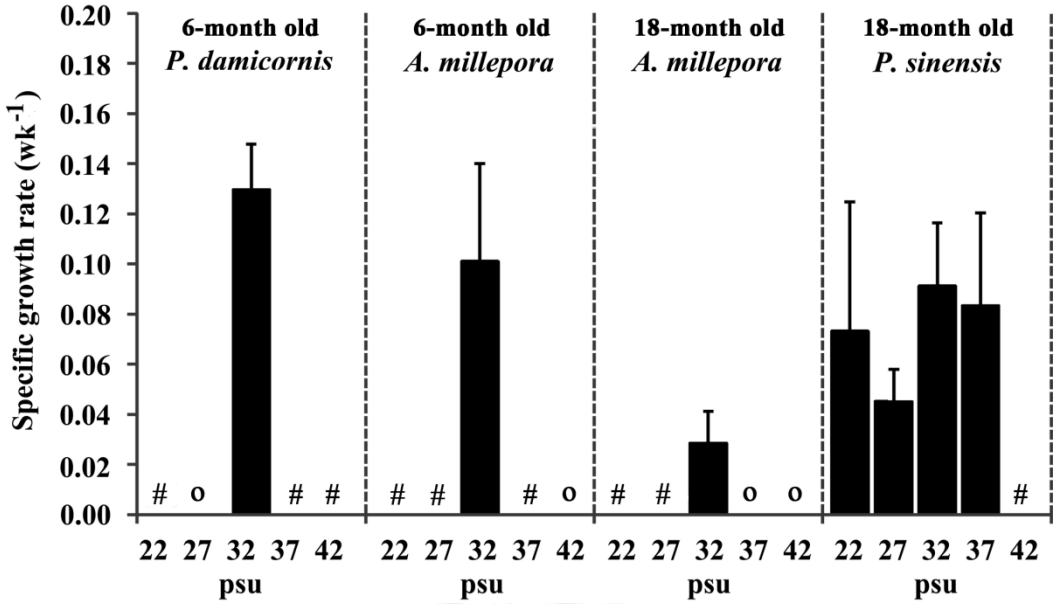

Figure 4-7. Specific growth rates per week of three coral species in different salinity levels (o = die, # = not grow).

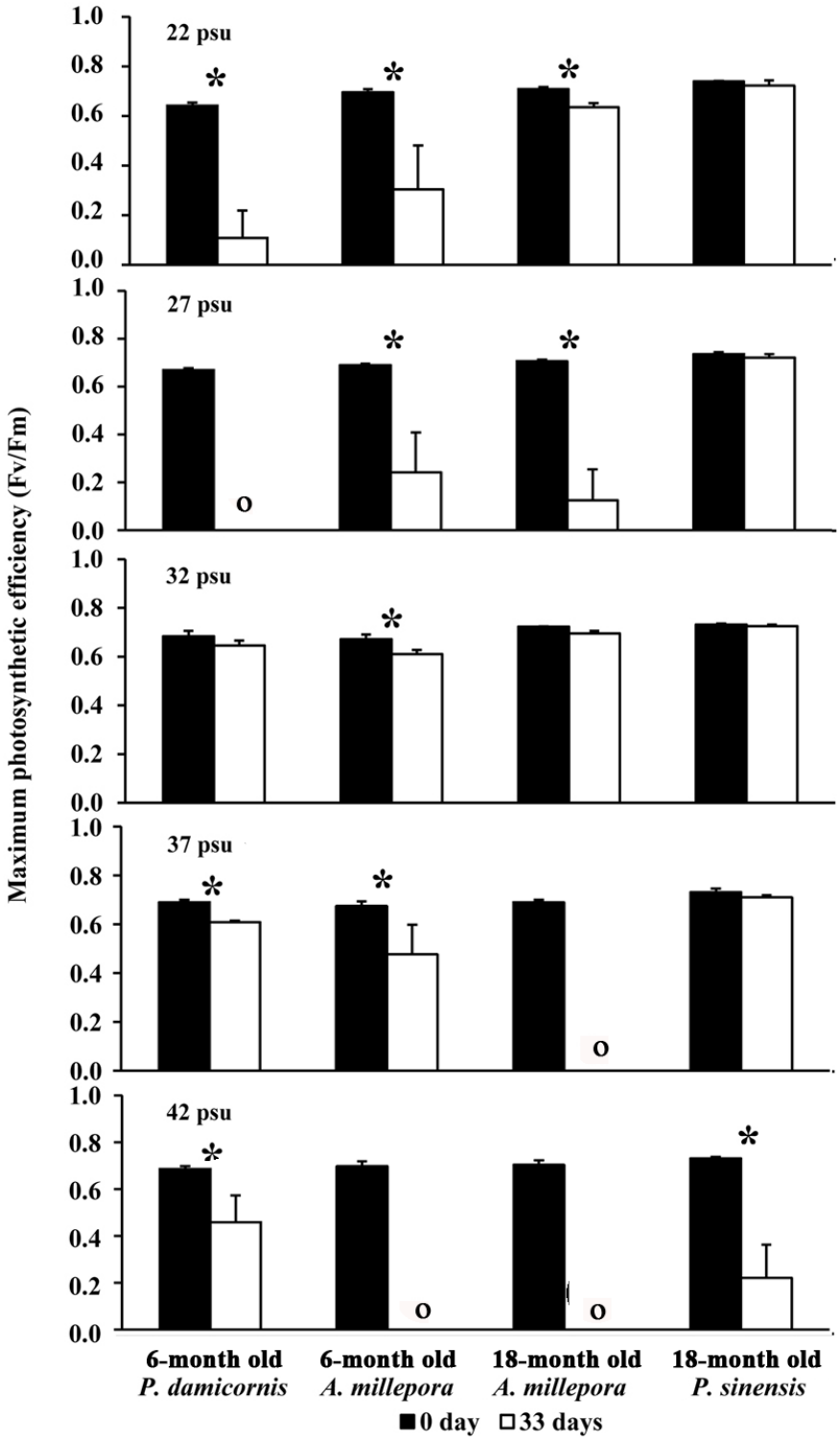

Figure 4-8. Maximum photosynthetic efficiency (Fv/Fm) of zooxanthellae associated with three coral species in different salinity levels (o = die and/or cannot be measured, * = significant difference).

Discussion

This study investigated the growth, survival, and photosynthetic response of corals and zooxanthellae associated with three coral species to changes in seawater temperature and salinity. The present study also confirmed that changed 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, which zooxanthellae were expelled from the coral host; 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 exposed to different temperature and salinity conditions. Under temperature stress, Fv/Fm of zooxanthellae remarkably decreased within 13 days and could not be detected thereafter (Fig. 4-6). However, under salinity stress, Fv/Fm of symbiotic dinoflagellates decreased, and Fv/Fm could be detected even at low and high salinity levels (Fig. 4-8). 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 to surrounding water (Muthiga and Szmant 1987). Corals can be both osmoconformer and 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 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 environment. However, coral of different ages and of different species did not elicit the same physiological response to changes in the environment factors. Furthermore, changes in temperature and salinity can negatively affect coral photobiology causing photoinhibition. Thus, more studies are needed to investigate a complex interaction between the environmental stress factors that can induce different responses among corals.

CHAPTER V

Effects of light intensity and photoperiod on survival rate of cultured corals and photosynthetic efficiency of zooxanthellae in coral tissue

Introduction

Light is a key factor for coral survival and physiology of corals that contain symbiotic zooxanthellae (Muscatine and Cernichiari 1969, Osinga et al. 2011). Zooxanthellae in corals use light and carbon dioxide through photosynthesis process to produce oxygen and organic compounds (Muscatine and Cernichiari 1969). When respiratory need of zooxanthellae is met with requirements, the excess photosynthetic products are transferred to coral host for growth and other activities (Muscatine and Cernichiari 1969).

Several studies have shown that light intensity or photon flux density have an influence on coral growth and photosynthesis (Rinkevich and Loya 1984, Stambler and Dubinsky 2005, Main and Goodbody-Gringley 2010). For example, *Acropora cervicornis* had the highest growth rate under light intensity at 38 µmol $m^{-2}s^{-1}$ to 44 µmol $m^{-2}s^{-1}$, but at ≥ 49 µmol $m^{-2}s^{-1}$ growth rate was reduced, while *Acropora plamata* had the highest growth rate under light intensity at 49 µmol $m^{-2}s^{-1}$ to 60 µmol $m^{-2}s^{-1}$ (Main and Goodbody-Gringley 2010). Light variation also affects zooxanthellae density and photosynthetic efficiency (Kuhl et al. 1995). However, excessive light intensity can cause a reduction of photosystem II quantum efficiency (Coles and Jokiel 1978, Krause and Weis 1991, Franklin et al. 2006, Chow et al. 2009) and lead to bleaching (Richier et al. 2008). In addition, the high solar radiation including photosynthetically active radiation (PAR) and ultraviolet radiation have shown to be linked to the DNA damage and the production of toxic reactive oxygen

species that cause the cellular damage in photosynthetic membranes (Lesser 1997, Lyons et al. 1998). In the environmental stress, organisms can also induce heat shock proteins, which play an important role in protective mechanisms and cellular repair (Lanneau et al. 2008). The heat shock proteins prevent protein aggregation and regulate stress-induced apoptosis (Chow et al. 2009).

Even though, light factor on corals has been studied intensively than any other environmental factors, it remains poorly understood in many coral species with different ages. In this present study, we investigated the effects of increase and decrease of light intensity and photoperiods on growth, survival, and photosynthetic efficiency in three different corals, *Pocillopora damicornis, Acropora millepora* and *Platygyra sinensis* with different ages (6, 8, 9, 11, 20 and 23-month old). All experimented corals were cultured from sexual propagation. The hypothesis is that, decreasing and increasing of light intensity and photoperiod negatively impact the coral growth, survival, and photosynthetic efficiency of zooxanthellae in the coral tissue.

Materials and Methods

Coral collection and maintenance

Gametes of corals, *Acropora millepora* and *Platygyra sinensis*, were collected during spawning periods from January-March at night around Sattahip, Chon Buri Province, Thailand. After collected, the gametes were transferred to the hatchery for artificial fertilization. Planulae were then maintained in the hatchery until they settled and metamorphosed to juvenile corals. Juveniles were raised in the hatchery until they became approximately 8, 11, 20, and 23-month old corals, and later were

introduced to the experimental trials. For a hermaphroditic brooder, *Pocillopora damicornis*, mature colonies were collected 1 week prior to the new moon and brought back to the hatchery. The colonies were kept in aerated tanks until larvae were released. Then, larvae were collected, and raised until they were approximately 6- and 9-month old.

Experimental protocol

Each treatment, 30 x 60 x 30 cm^3 glass aquaria with 54 liter of water volume was used as an experimental tank. During the experiments, water in the aquariums was exchanged daily, and was aerated continuously. Prior to the trials, corals were allowed to acclimate to aquaria conditions at the ambient light intensity for 2 days. In addition, throughout the experimental trials, the corals were maintained at the ambient salinity (32 psu).

Experimental trials

Experiments for both light intensity and photoperiod were conducted in the laboratory using different ages and different species of corals: 6- and 9-months old *P. damicornis*, 8, 11, 20, and 23-month old *A. millepora* and 20- and 23-month old *P. sinensis* (Fig. 5-1). For light intensity trials, juvenile corals were transferred and exposed to different treatments (0, 21, 42, 85, and 169 µmol m^{-2} s^{-1} provided by 400-W metal halide lamps) for 2 weeks. Twenty one µmol m^{-2} s^{-1} was served as a control. The average light intensity control was measured during the daytime at the coral reef site for one year. Chiller was used to control the temperatures at 28 °C. Each treatment, there were 10 replicate corals.

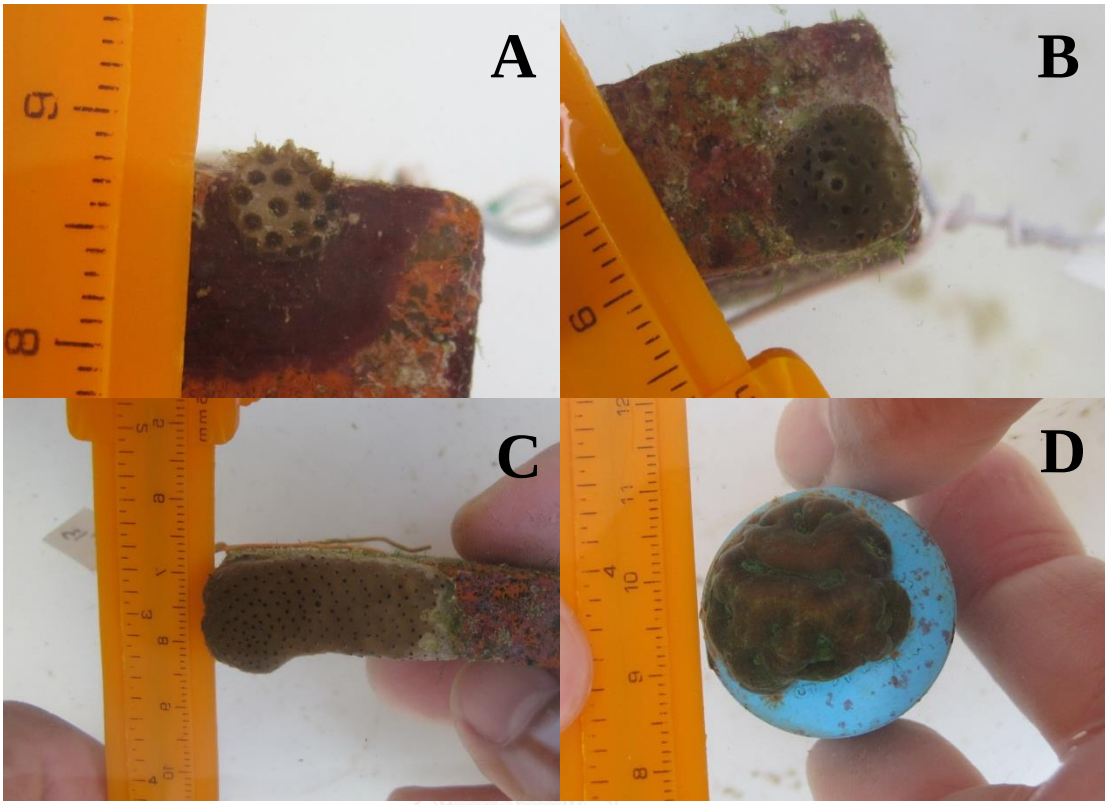

Figure 5-1. A) 6- and 9-month old *Pocillopora damicornis*, B) 6- and 11- month old *Acropora millepora*, C) 20- and 23-month old *Acropora millepora,* and D) 20- and 23-month old *Platygyra sinensis*.

At the beginning of the trials, the colonies were randomly selected to the experiment aquariums. Then, light intensity was gradually increased or decreased. The growth and survival rates of corals were measured every week for 2 weeks. To measure the growth, photographic analysis Coral Point Count with Excel extensions (CPCe) was used. This method is a visual basic program for determining coral cover by scaling on photographs and measuring the changes of length and/or width of corals over time (Kohler and Gill 2006, Main and Goodbody-Gringley 2010).

In addition, a pulse amplitude modulation fluorometer (a DIVING PAM, Walz, Germany) was used to assess the maximum quantum yield (Fv/Fm) of zooxanthellae within the coral tissues before and at the end of the experiment. This parameter gives information on photosynthetic efficiency. Before measuring, all corals were pre-adapted in the darkness for 30 minutes, and the measurement was set at 7 pm each time.

In the photoperiod experiment, the coral colonies were exposed to 5 different treatments (24/0, 18/6, 12/12, 6/18, and 0/24 h light-and-dark cycle) for 2 weeks. 12/12 h light-and-dark cycle was a control. Before the trials, period of light receiving was gradually increased or decreased until the value reached the experimented value. The corals were maintained at ambient temperature (28 °C) and at 21 μmol m^{-2} s^{-1} of light intensity. The growth, survival rates of corals, and maximum quantum yield (Fv/Fm) of zooxanthellae were measured before and at the end of the experiment followed the light intensity experimental protocol. A one-way ANOVA test, followed by a Tukey's pairwise mean comparison, was used to analyze the data for the survival rate and specific growth rate, and a Paired samples t-test was used to analyze the maximum quantum yield.

Figure 5-2. A rearing system for light intensity and photoperiod experiment.

Results

Light intensity and photoperiod had effects on the growth, survival and photosynthetic efficiency of corals. From the light intensity experiment, the results showed that 20-month old *Platygyra sinensis* survived (100%) under all light intensity levels, while the survival rates of other coral species decreased, ranging between 20% to 80%, when the light intensity levels changed from the ambient light condition (Table 5-1). From the observation, *Acropora millepora* tended to have a wider-range tolerance of light intensity than that of *P. damicornis*. While *P. damicornis* corals started to bleach, and tissue loss occurred during day 5 of the experiment. Moreover, when the growth rates were measured, the results showed that the controlled treatments in each species tended to have a higher growth rate than the experimental

treatments. But, there was no significant difference between the growths of corals under different light intensity levels ($P \geq 0.05$) (Fig. 5-3). The maximum quantum yields of zooxanthelllae in coral tissues exposed to different light intensities were presented in Figure 4. At the end of the experiments, corals reared under higher (42, 85, and 169 µmol m^{-2} s^{-1}) and lower light intensities (0 µmol m^{-2} s^{-1}) showed significantly reductions of the Fv/Fm values while the corals reared at ambient light intensity showed that increase of the Fv/Fm values (Fig. 5-4).

Table 5-1. Percentage of survival rates on coral in different light intensity.

Species	Light intensity µmol m^{-2} s^{-1}				
	0	21	42	85	169
6-month old *P. damicornis*	80±20	100±0	67±21	40±24	20±20
8-month old *A. millepora*	100±0	100±0	100±0	60±24	60±24
20-month old *A. millepora*	40±24	100±0	60±24	100±0	20±20
20-month old *P. sinensis*	100±0	100±0	100±0	100±0	100±0

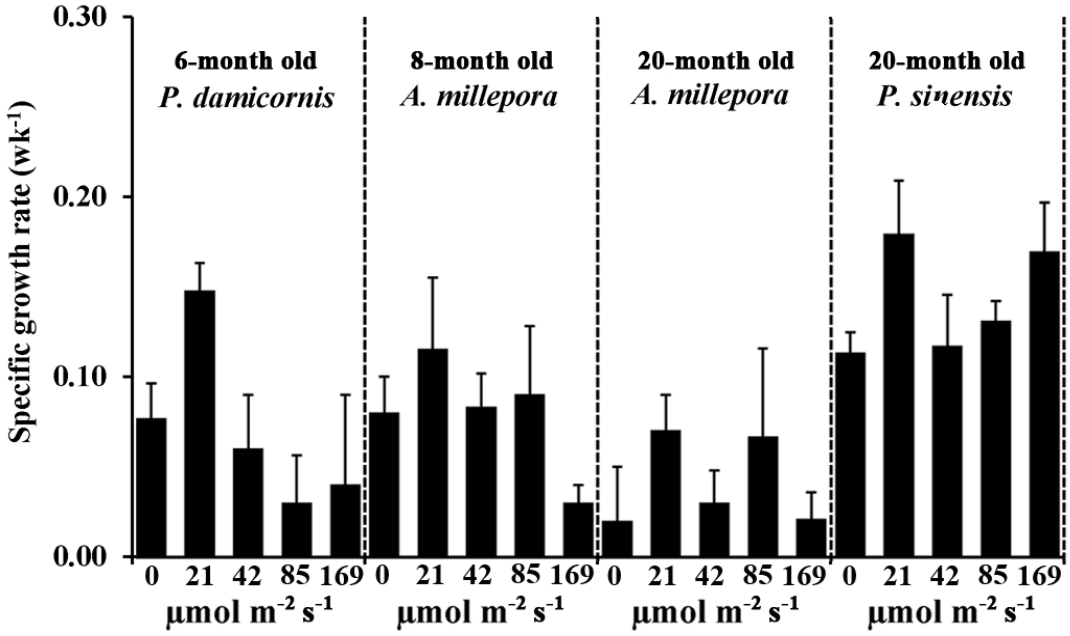

Figure 5-3. Specific growth rate per week of three coral species in different light intensity levels.

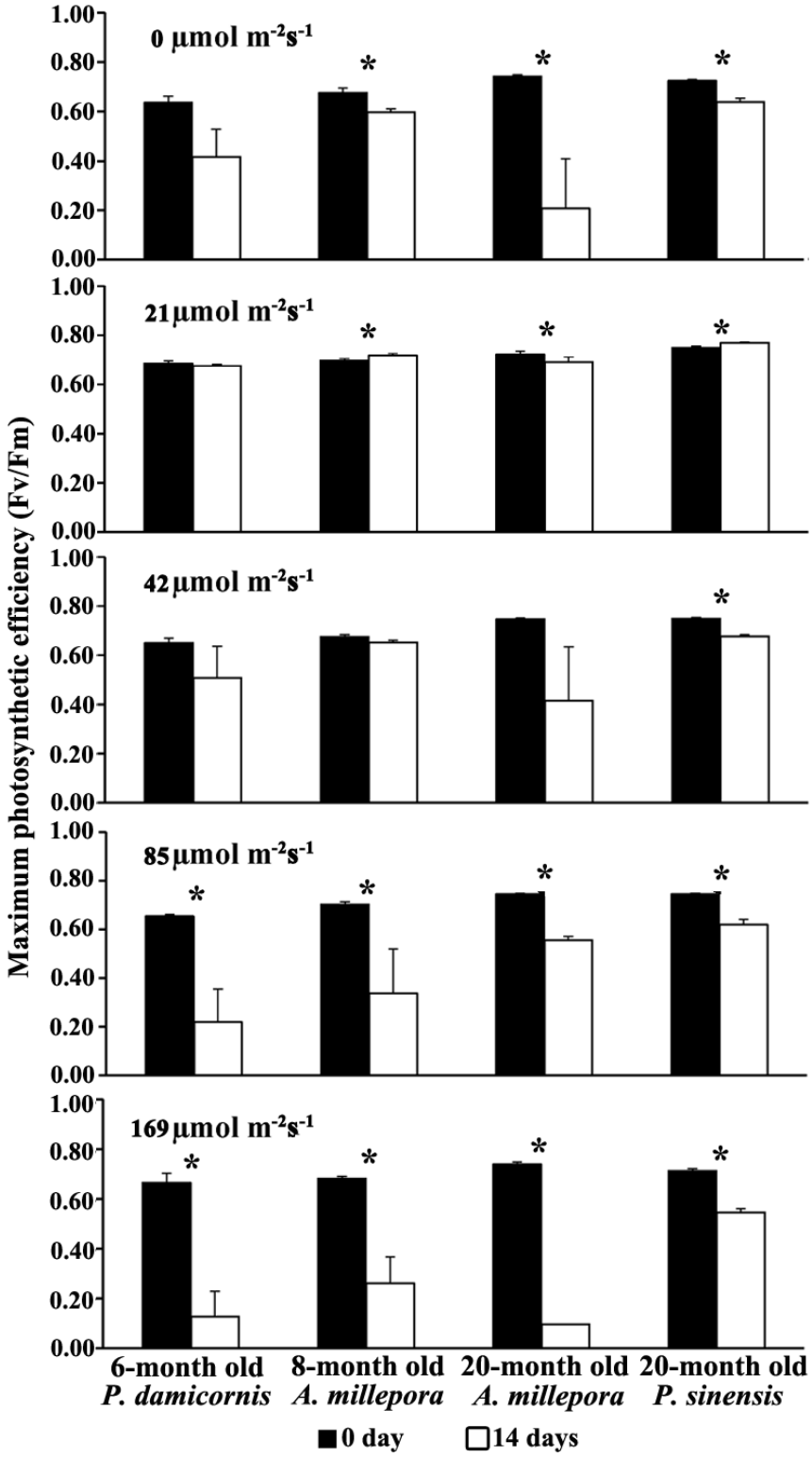

Figure 5-4. Maximum photosynthetic efficiency (Fv/Fm) of zooxanthellae associated with three coral species in different light intensity levels (* = significant difference).

For the photoperiod experiments, the survival rates of different coral species and ages were shown in Table 5-2. Similar to the light intensity experiment, 23-month old *Platygyra sinensis* survived (100%) under all photoperiod levels. In addition, 11-month old *Acropora millepora* showed 100% survival rate for all treatments. Overall, the percent of survival ranged between 80% to 100% (Table 5-2). However, studies showed that the *Acropora millepora* corals started to bleach and lose tissue mass on day 12 of the experiment and the results of the growth rates showed that there was no significant difference on the growth of corals between species, ages, and different light intensity levels ($P \geq 0.05$) (Fig. 5-5). When maximum quantum yields of zooxanthelllae were measured, corals reared under L 24h/D 0h and L 0h/D 24 h showed significantly reduction of the Fv/Fm values (Fig. 5-4). In addition, when photoperiods changed from normal (L 12h/D 12h), the photosynthetic efficiencies of 9-month old *P. damicornis*, 11-and 23-month old *Acropora millepora* and 23-month old *Platygyra sinensis* decreased significantly.

Table 5-2. Percentage of survival rates on coral in different light receiving periods.

Species	Receiving periods of light and dark (hours)				
	24/0	18/6	12/12	6/18	0/24
9-month old *P. damicornis*	80±20	100±0	100±0	80±20	100±0
11-month old *A. millepora*	100±0	100±0	100±0	100±0	100±0
23-month old *A. millepora*	100±0	100±0	100±0	100±0	80±20
23-month old *P. sinensis*	100±0	100±0	100±0	100±0	100±0

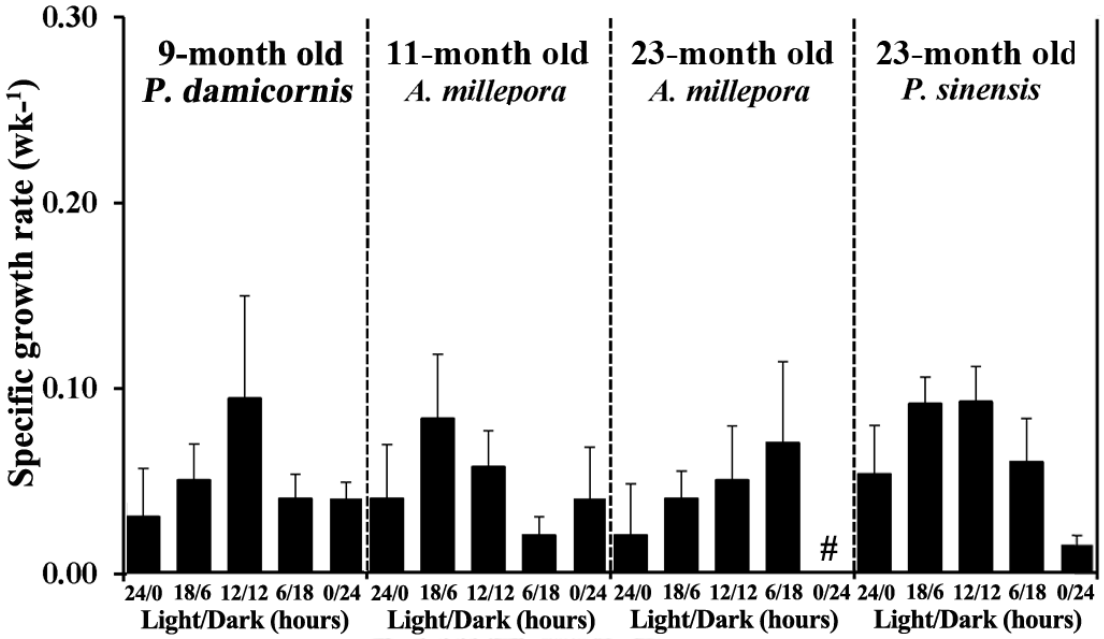

Figure 5-5. Specific growth rates per week of three coral species in different period of light receiving levels (# = not grow).

Discussion

Several factors have been reported to influence coral growth and photosynthesis of zooxanthellae in corals either positively or negatively (Houlbrèque et al. 2003, Rodolfo-Metalpa et al. 2008, Main and Goodbody-Gringley 2010, Wijgerde et al. 2012). There is no doubt that light can play an important role in the growth of both corals and zooxanthellae (Houlbrèque et al. 2003, Al-Horani et al. 2005, Main and Goodbody-Gringley 2010, Schutter et al. 2011). The proper light can also reflect properties of calcium carbonate skeletons in corals (Houlbrèque et al. 2003, Al-Horani et al. 2005, Wijgerde et al. 2012). This is the first study to demonstrate the effects of light intensity and photoperiod on different ages of corals.

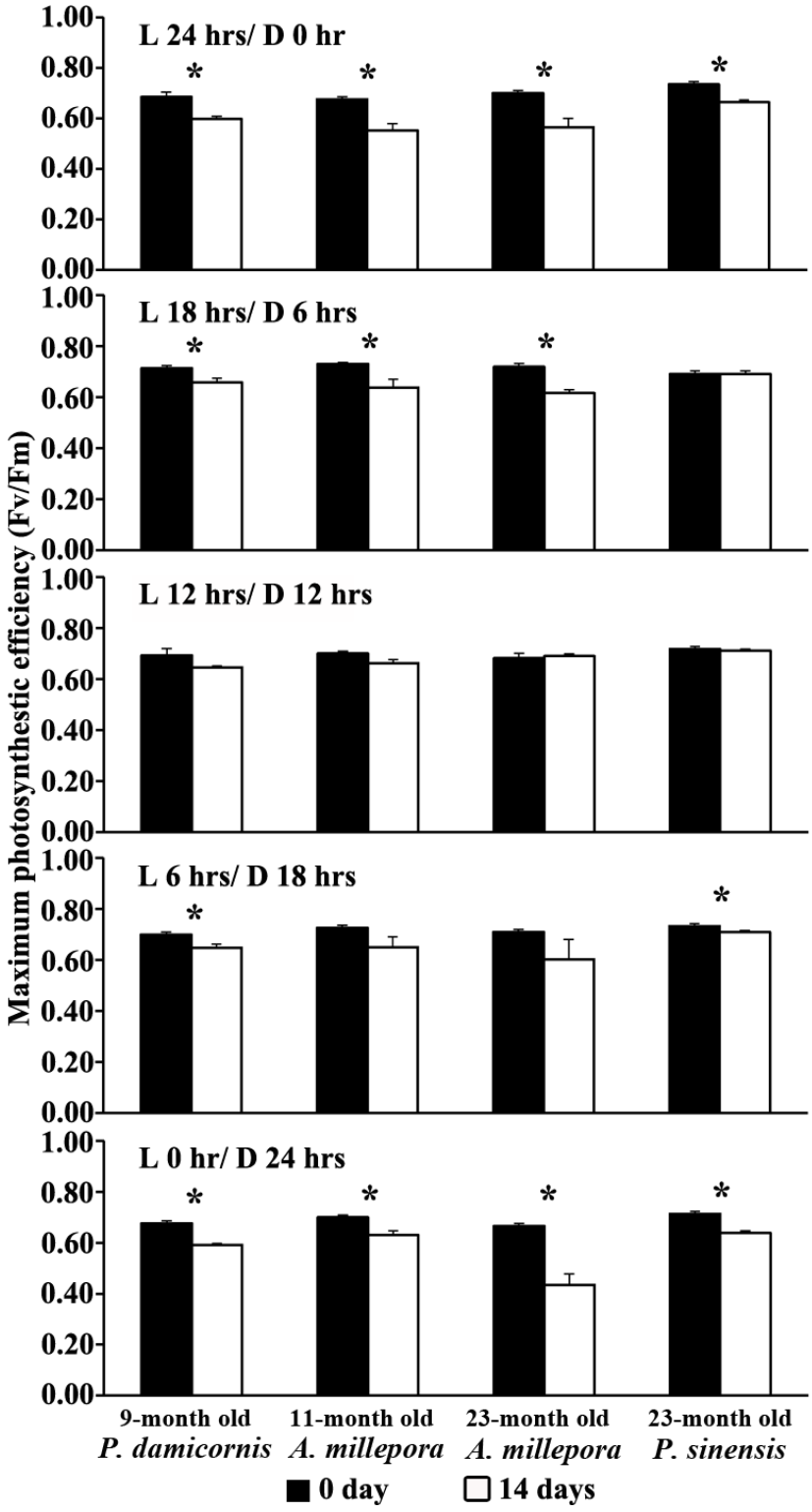

Figure 5-6. Maximum photosynthetic efficiency (Fv/Fm) of zooxanthellae associated with three coral species in different period of light receiving (* = significant difference).

In the present study, we measured the growth and photosynthetic efficiency of corals under different light intensities and photoperiods. Our results showed that there was no significant variation of coral growth between coral ages, species, different light intensities, and different photoperiods. Similar to our finding, Chalker (1977) and Moya et al. (2006) reported that even though, there was a tendency toward a decrease in calcification rates when photoperiods were changed, there was no significant difference. However, Chalker (1977) indicated that a circadian rhythm in photosynthesis can play a role in calcification rates, and this also depended on species.

The photosynthetic capacity varied between three coral species in this study (Fig. 5-4). This might be interpreted as being due to the difference in relative tissue biomass (Stambler and Dubinsky 2005, Borell et al. 2008). As a result, it can lead to differences in growth rates (Schutter et al. 2012, Rocha et al. 2013), in this study). From our experiments, *Acropora millepora* and *Pocillopora damicornis* grown in laboratory and aquaria settlings should be kept under light levels below 169 µmol m^{-2} s^{-1} while *Platygyra sinensis* can be exposed to light between 0 µmol m^{-2} s^{-1} to 169 µmol m^{-2} s^{-1}. Understanding the parameters that promote the highest growth, survival, and photosynthetic efficiency will benefit future coral aquaculture and restoration efforts. Excessive light intensity can damage the photosystem II (PSII) and D1 reaction center protein which can lead to heat-dependent photoinhibition (Warner et al. 1999). If the damage of the protein exceeds the capacity of repair of corals, zooxanthellae will enter a state of chronic photoinhibition, which lead to the decline in photosynthetic efficiency (Jones and Yellowlees 1997, Franklin et al. 2006).

Other studies have shown that calcification and photosynthesis are not as closely linked (Buddemeier and Kinzie 1976, Oliver et al. 1983, Rinkevich and Loya 1983). In addition, previous studies indicated that skeletal deposition was not correlated with the light intensities at different depths (Reed 1981, Oliver et al. 1983). Other factors such as an inherent factor and biochemical patterns can play a role in controlling rates and patterns of calcification in corals (Rinkevich 1982). Goreau (1963) and Rinkevich and Loya (1984) suggested that the high light VS dark ratios recorded in many investigations may be the outcome of a stress phenomenon caused by low pO_2 of the water, and different species had different sensitivity to low pO_2.

The most important finding of this study showed that *P. sinensis* seemed to have more tolerance to wider ranges of light intensities and photoperiods than other coral species. However, it is hard to give a clear cut reason on the most important factor influencing the growth of corals. Each species and each age required different values of light intensities to maximize their growth rates. Nevertheless, from this study, the results implied that corals were able to adapt to a prolonged light period and light intensity, which can be feasible for future coral aquaculture.

CHAPTER VI

Feeding behaviors of three tropical scleractinian corals in captivity

Introduction

At present, coral reefs around the world have been declined due to several natural and anthropogenic threats (Chavanich et al. 2005, Wilkinson 2008, Chavanich et al. 2009, Burke et al. 2011). Several restoration techniques have been developed to restore reefs and increase in coral coverage (Edwards and Gomez 2007, Edwards 2010). Restoration techniques include both active and passive restoration such as fragment transplantation, seeding production and larval rearing using sexual reproduction, and artificial reefs (Edwards and Gomez 2007, Edwards 2010). However, some techniques are at an experimental stage and some success at scales of up to a few hectares only (Edwards 2010). Recently, efforts to raise coral eggs to juvenile stage in a hatchery before being released to natural reefs have increased their popularity due to the results in high genetically diverse corals (Omori and Fujiwara 2004). However, nutrient requirement for maintaining corals in a hatchery or in captivity is still a major constraint (Petersen et al. 2008, Hii et al. 2009). In addition to restoration, there is a strong demand on culturing corals in captivity because live coral is one of the popular marine organisms in marine ornamental industry (Green 2003, Wabnitz et al. 2003). However, the culture method is not yet fully successful due to limitation of maintaining live organisms and the nutritional requirement in captivity (Arvedlund et al. 2003, Wabnitz et al. 2003).

To fulfill the nutritional need, scleractinian corals use both autotrophic through symbiotic zooxanthellae and heterotrophic mechanisms (Muscatine 1990,

Fabricius and Klumpp 1995, Houlbrèque and Ferrier-Pagès 2009). Majority of energy in corals are gained through zooxanthellae photosynthesis (Muscatine 1990, Fabricius and Klumpp 1995). However, several studies have shown that corals ingest varieties of food ranging from bacteria to zooplanktons as well as dissolved organic and particle matters (Sebens et al. 1996, Anthony 1999, Houlbrèque and Ferrier-Pagès 2009), and the amount can account for up to 66% of the fixed carbon into coral skeletons (Houlbrèque and Ferrier-Pagès 2009). Due to different amounts of energy needed, different corals have different feeding rates (Sebens et al. 1996, Ferrier-Pagès et al. 2003). Ferrier-Pagès et al. (2003) found that *Stylophora pistillata* captured zooplankton less than *Galaxea fascicularis*. In addition, the size of prey and size of coral polyps, water flow rate, and ability of prey to avoid captured are all related with the corals' potential to capture prey (Sebens et al. 1996, Piniak 2002a, Palardy et al. 2006). *Artemia* spp. can be used for feeding corals. They are more ideal than other juvenile marine organisms because they are very easy to prepare, have high nutrient value and have a small size of about 0.5 mm in length (Leversee 1976, Helland et al. 2003, Reynaud et al. 2004). Petersen et al. (2008) and Toh et al. (2014) found that corals feeding on *Artemia* spp. had higher growth rates than no feed. Thus, to increase the coral growth in a hatchery, feeding corals with supplementary food may be an option. However, so fare, few studies have measured the feeding rates and digestible capacity of corals in captivity and none was done on *Acropora millepora* and *A. nobilis* (Petersen et al. 2008, Hii et al. 2009, Wijgerde et al. 2011).

The aim of this study was to investigate capture rates and digestibility of three corals species in two genera, *Acropora* and *Pocillopora*, found dominantly in Thailand and Federated States of Micronesia by using *Artemia salina* nauplii as a

food source. In addition, the effect of day and night times on coral feeding was examined. The hypothesis is that, corals can capture *Artemia salina* and the capture rate is relative to the density of prey.

Materials and Methods

Coral specimens and Artemia salina nauplii

The experiments were run both in Thailand and in Federated States of Micronesia. In Thailand, specimens of common species of reef-building corals, *Acropora millepora* and *Pocillopora damicornis* were used in the experiments. All experimented corals were from aquaculture using sexual reproduction in the coral hatchery. *A. millepora* is a spawning coral while *P. damicornis* is a brooding coral. The colonies of *A. millepora* derived from cross fertilization of gametes from at least 3 colonies. Larvae were then allowed to settle on cotta tiles and grew in the coral hatchery located at Samae San Island until 1-year old before experimented. In case of *P. damicornis*, larvae were collected directly from parental colonies. Then, cotta tiles were provided for larval settlement and metamorphosis into the juvenile stage. Similar to *A. millepora*, juvenile *P. damicornis* were raised until 1-year old. The sizes of 1-year old cultured juvenile corals were approximately 2 cm in diameter and had about approximately 130 polyps (Fig. 6-1 and 6-2).

In Chuuk, Federated States of Micronesia, *Acropora nobilis* was chosen instead of *A. millepora* due to more abundance of the species in the area. Experimented corals both *A. nobilis* and *P. damicornis* were collected at 2 m to 4 m depths at a reef in front of Korea South Pacific Ocean Research Center. Collected corals were then broken into small fragments. The fragments were glued to small rocks using non toxic super glue, and were placed in a tank for acclimation at least 4

days prior to the experiment trials (Fig. 6-1 and 6-3). Sizes of fragments were approximately 2-3 cm in diameter similar to the experimented corals in Thailand.

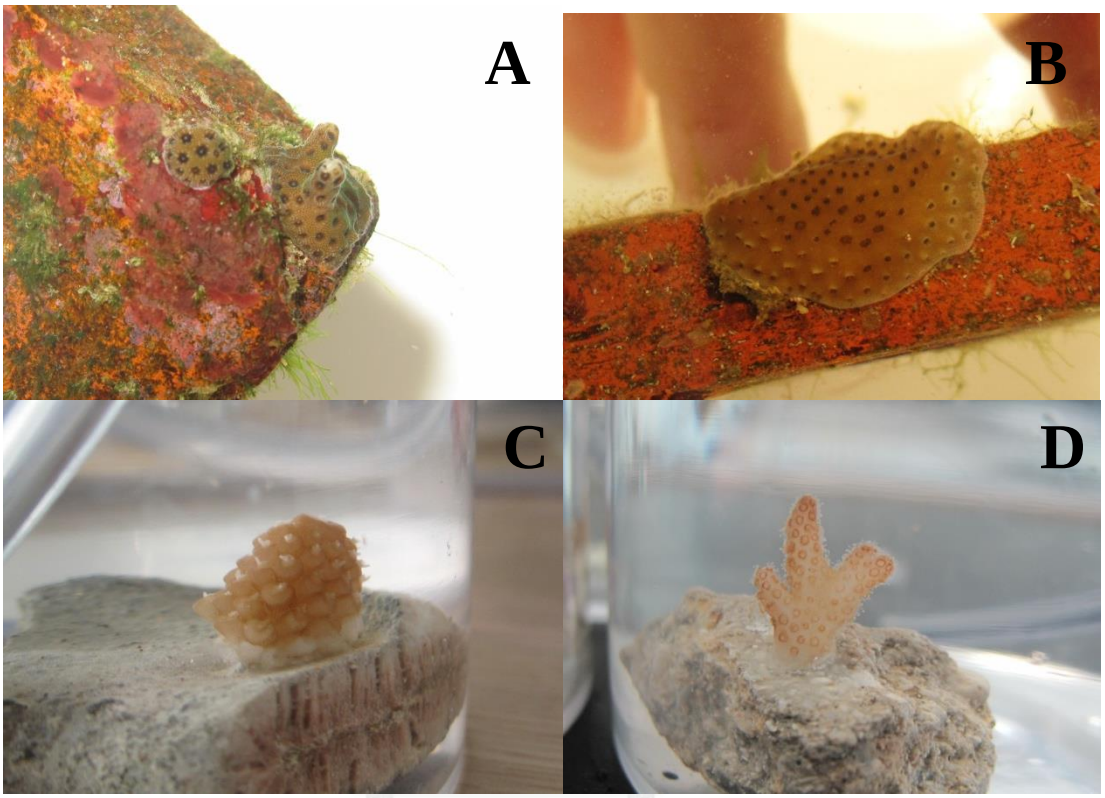

Figure 6- 1. A) 1-year old *Pocillopora damicornis* in Thailand, B) 1-year old *Acropora millepora* in Thailand, C) *Acropora nobilis* fragment in Chuuk and D) *Pocillopora damicornis* fragment in Chuuk.

Before starting the experiment trials, all corals were acclimated to experimental conditions at a temperature of approximately 29°C with light intensity 21 µmol m^{-2} s^{-1} in 12-h light and 12-h dark cycle for at least 4 days. In addition, newly cultured *Artemia salina* nauplii were hatched from commercial eggs, and were prepared 24 hours prior to the experiments (Fig. 6-4).

Figure 6- 2. A rearing system for feeding experiments in Thailand.

Figure 6-3. A rearing system for feeding experiments in Chuuk, Federated States of Micronesia.

Feeding behavior

The feeding experiments were carried out to investigate the feeding activity of juvenile corals both *Acropora* spp. and *Pocillopora damicornis.* Newly hatched *Artemia salina* was used a coral food. Each coral colony was assigned to feed in one of the following treatments: 1) early in the morning (0600), 2) late in the evening (1800), 3) both in the morning and evening (0600 and 1800). Five replicates in each treatment were employed in each coral species in each study site. In each replicate, a juvenile colony was placed in a 1- liter aquarium and 300 individuals of *A. salina*

were given to corals each time based on the experimental times. Corals were allowed to feed for 12 hours. The remaining densities of *A. salina* were counted 12 hours after the food were given each time (Fig. 6-4). Control corals of each species were at the same identical aquariums and environment but without *A. salina*. The experiments were run for 7 days. In addition, the numbers of coral polyps were counted at the beginning and at the end of the experiment (Fig. 6-5). Capture rate was measured by the number of *A. salina* eaten per polyp per 12 hours.

Digestion rates

To determine the digestion rates of both *Acropora* spp. and *Pocillopora damicornis*, laboratory experiment was conducted. A total of 108 coral fragments of 3 species were experimented. Each coral fragment (approximately 220 polyps) was fed by *A. salina* at 500 individuals per 1 liter aquarium. After corals were fed, every 9 fragments were withdrawn at 1.5, 2, and 2.5 hours after the initial food were given. Then, samples were preserved at 10% formalin, and were decalcified to analyze the stomach content of each coral polyp. The percentage of *A. salina* digestion by each coral species was calculated based on contents left in the gastrovascular cavity of each coral polyp. Gastrovascular cavity contents of 2 day starved corals were used as controls.

A one way ANOVA test followed by Tukey's pairwise mean comparison was performed to examine differences in capture rates and digestion rate of different corals between in Thailand and in Federated States of Micronesia and between day and night times.

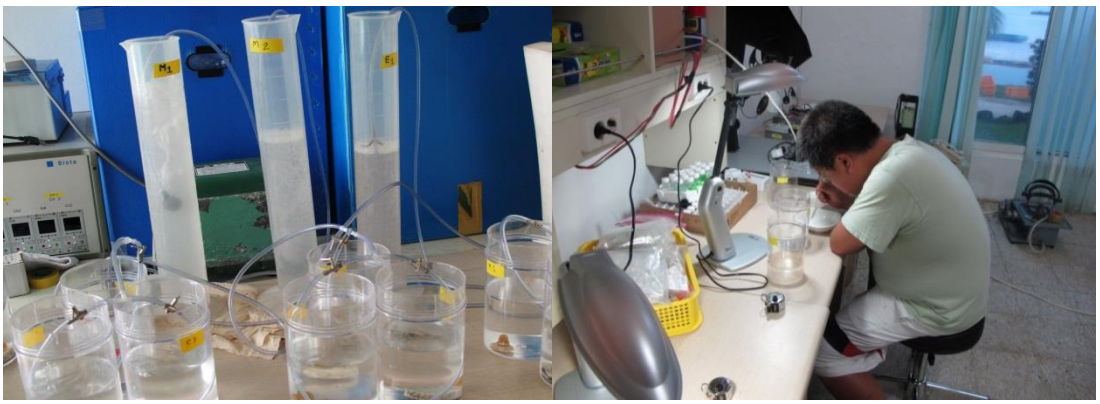

Figure 6- 4. Preparing and counting *Artemia salina* for feed coral.

Figure 6-5. Counting number of polyp coral by use Photoshop program.

Table 6-1. Comparison of capture rates of *Artemia salina* by scleractinian corals from various locations.

Places	Species	Concentration of food (ind.L^{-1})	Individual capture rate /polyp/hr	Sources
Aquarium, Netherland	*Galaxea fascicularis*	2,000	50.68	Osinga et al. (2008)
	Galaxea fascicularis	4,100	93	Wijgerde et al. (2011)
Mediterranean	*Turbinaria reniformis*	2,000	20	Ferrier-Pagès et al. (2010)
	Stylophora pistillata		27	
Pulau Bidong, Malaysia	*Galaxea fascicularis*	10,000	50	Hii et al. (2009)
Samae San Island, Thailand	*Pocillopora damicornis*	300	0.14	In this study
	Acropora millepora		0.13	
Chuuk Island, Micronesia	*Pocillopora damicornis*		0.05	
	Acropora nobilis		0.12	

Results

The experiments showed that all three coral species, *Acropora millepora*, *A. nobilis*, and *Pocillopora damicornis* captured and consumed *Artemia salina* nauplii (Fig. 6-6). The capture rates of all three species ranged between 0.44 to 2.39 individuals per polyp (Fig. 6-7 and 6-8). Overall, the capture rates were not significantly different between *Acropora* species in Thailand and in Chuuk, Federated States of Micronesia; however, *P. damicornis* in Thailand captured significantly more than ones in Chuuk. When comparing the feeding rates of coral species that were experimented in different parts of the world, corals in Thailand and in Federated Stated of Micronesia tended to have a lower capture rates (Table 6-1).

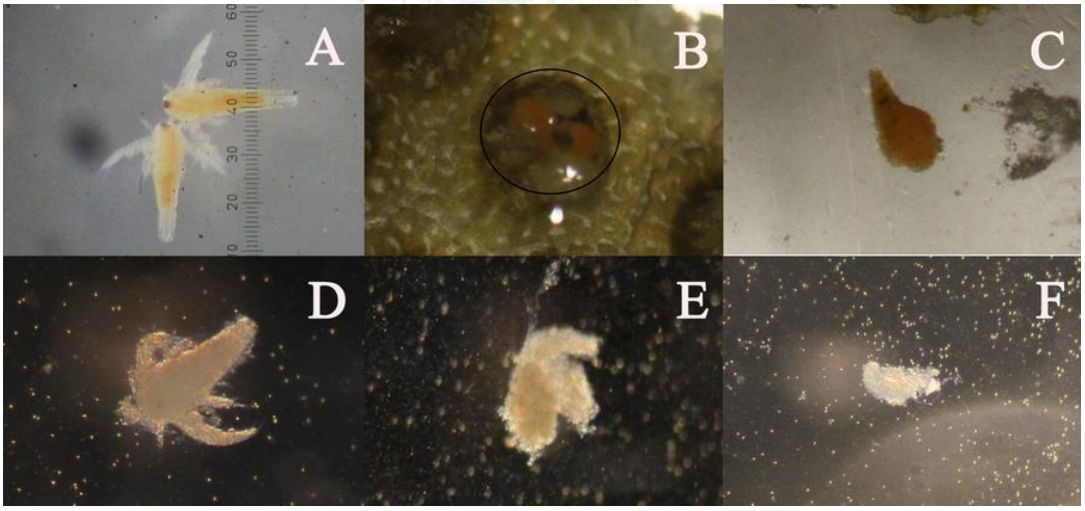

Figure 6- 6. *Artemia salina* nauplii from the gastrovascular cavity of corals at different time series A) Newly hatched *Artemia salina*, B) *Artemia salina* in gastrovascular cavity 1.5 hours after feeding, C and D) 1.5 hours after feeding, E) 2 hours after feeding, F) 2.5 hours after feeding.

Table 6-2. Percentage of prey remaining during the *Artemia salina* nauplii digestion by different corals at different sampling times (n=108).

Country	Coral species	% of prey remaining		
		1.5 hours	2.0 hours	2.5 hours
Thailand	*Acropora millepora*	62.14 ± 6.95	0 ± 0	0 ± 0
	Pocillopora damicornis	43.64±7.84	62.50 ± 22.5	0 ± 0
Chuuk	*Acropora nobilis*	58.44± 6.03	0 ± 0	0 ± 0
	Pocillopora damicornis	39.72±5.19	18.32±4.55	0 ± 0

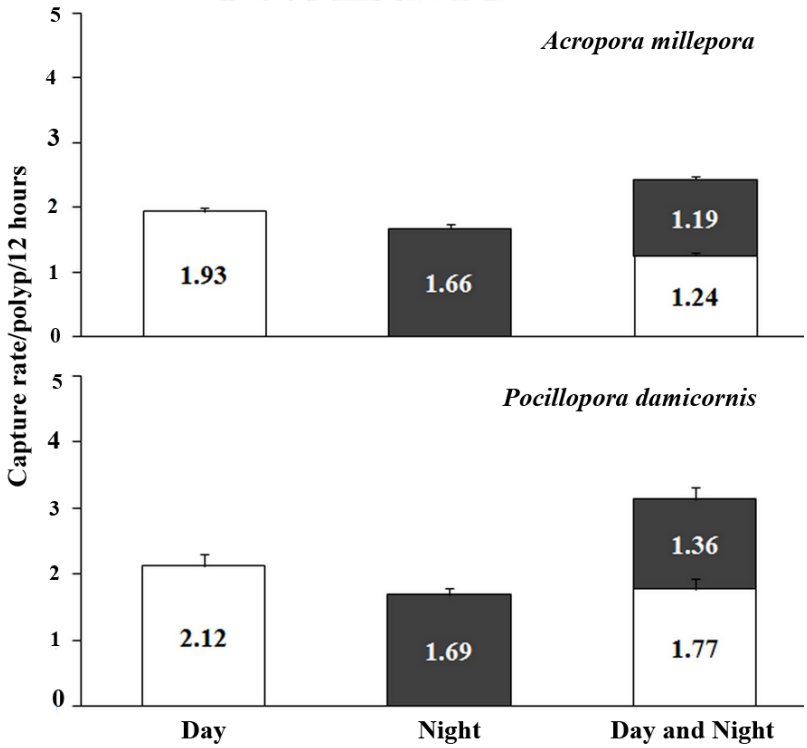

Figure 6-7. Capture rates of experimented *Acropora millepora* and *Pocillopora damicornis* fed on *Artemia salina* nauplii during day, night and both day and night in Samae San Island, Thailand.

In addition, from the experiments, there were significant differences on the capture rates of both corals in Thailand between treatments ($P < 0.05$). When food was given once a day, *P. damicornis* and *A. millepora* consumed more *A. salina* in the morning (average 2.12 ± 0.08 and 1.93 ± 0.08 individuals/polyp) than in the evening (average 1.69 ± 0.04 and 1.66 ± 0.07 individuals/polyp), respectively (Fig. 6-7). However, when *P. damicornis* and *A. millepora* were fed twice a day, *P. damicornis* consumed 3.13 ± 0.014 individuals of *A. salina* per polyp daily, and preferred to feed in the morning while *A. millepora* consumed only 2.43 ± 0.09 individuals per polyp, and showed no significant difference in its capture rates between day and night times (Fig. 6-7).

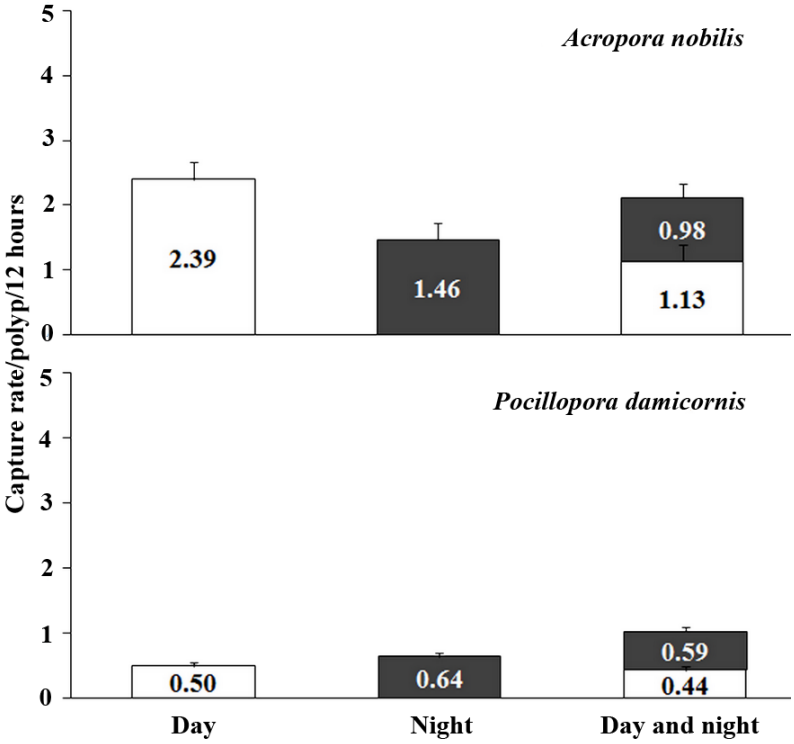

Figure 6-8. Capture rates of experimented *Acropora nobilis* and *Pocillopora damicornis* fed on *Artemia salina* nauplii during day, night and both day and night in Chuuk, Federated States of Micronesia.

In Chuuk, the results showed that there was a significant difference on the capture rates of *A. nobilis* between days and nights ($P < 0.05$), while *P. damicornis* showed no significant difference in the capture rates ($P > 0.05$). When *A. nobilis* was fed once a day, it consumed more *A. salina* during the day (average 2.39 $\pm$ 0.28 individuals/polyp) (Fig. 6-8). Table 6-2 showed the digestive rates of the three coral species in Thailand and Federated States of Micronesia. Complete digestions of *A. salina* nauplii by *A. millepora* and *A. nobilis* were observed after 2 hours while *P. damicornis* took at least 2.5 hours to complete the prey digestion.

Discussion

Our results revealed that all three corals species *Acropora millepora*, *A. nobilis*, and *Pocillopora damicornis* in Thailand and in Federated States of Micronesia were able to feed on and digest *Artemia salina* nauplii. Capability of feeding of coral species depends on feeding mechanism, polyp size, number of tentacles, prey size, prey density, water flow, temperature, and light (Lasker 1981, Fabricius and Klumpp 1995, Sebens et al. 1998, Anthony 1999, Piniak 2002b, Houlbrèque and Ferrier-Pagès 2009, Toh et al. 2014). Table 6-1 shows that the capture rate of the coral *Galaxea fascicularis* coral is higher than that of the *Acropora* spp. and *Pocillopora damicornis* corals, because the polyp size of the *Galaxea fascicularis* is larger than the other species (Wijgerde et al. 2011). Light and dark conditions can have an effect on the feeding behavior of corals (Ferrier-Pagès et al. 1998, Hii et al. 2009). For example, under the light condition, *Stylophora pistillata*'s polyps were closed and had low ingestion rates compared to under the dark condition (Ferrier-Pagès et al. 1998). Capacity of coral feeding also depend on coral's feeding

effort in which corals are able to control their feeding rate when the environments such as light intensity change (Anthony and Fabricius 2000, Ferrier-Pagès et al. 2010). In this study, corals fed both during the day and at night; thus, it seemed that light condition had no effect on the feeding of *Acropora* and *Pocillopora* species.

In addition, the feeding behaviors of coral species vary and depend on prey density (Clayton and Lasker 1982, Hii et al. 2009). Hii et al. (2009) reported that when a coral, *Galaxea fascicularis*, were fed with high *Artemia salina* nauplii density, its feeding rate was 50 times higher than those fed with low density. The results from the experiments both in Thailand and in Chuuk demonstrated that when experimented corals were fed twice a day, most experimented coral species, except *A. nobilis*, tended to consume more number of nauplii compared with ones fed only once a day.

From our observation, all ingested food in experimented corals was cleared from their gastrovascular gut within 2.5 hours (Table 6-2). Depending on coral and soft corals species, the time to complete zooplankton digestion are varied ranging between 2.5 hours to 24 hours (in this study) (Coffroth 1984, Lewis 1992, Hii et al. 2009). Several factors such as prey size and polyp size can play an important role (Houlbrèque and Ferrier-Pagès 2009). Yet, Wijgerde et al. (2011) pointed that in *Galaxea fascicularis*, 98.6% of prey captured was not digested in the gastrovascular, but externally digested by mesenterial filaments. Thus, extracoelenteric feeding is also an important mechanism for nutrient acquisition of corals (Wijgerde et al. 2011).

Feeding enhances not only the calcification rates of corals (Houlbrèque et al. 2003), but also there is an evidence showed that corals with the increase of their heterotrophic carbon input recovered through the bleaching event faster than ones depending mainly on photosynthesis (Grottoli et al. 2006). Additional to physical

factors such as light and temperature, enhancing coral growth in captivity through feeding with supplementary food such as zooplankton may be necessary to overcome nutrient deficiency since the food increase the protein levels of host tissues (Ferrier-Pagès et al. 2003, Houlbrèque et al. 2003). This study showed that *Acropora millepora*, *A. nobilis*, and *Pocillopora damicornis* can be fed by *Artemia salina* nauplii under light and dark conditions, even though, the feeding rates were different under different conditions depending on coral species. More works related to improving feeding methods for corals in captivity are needed.

CHAPTER VII

Feeding efficiency of corals in several types of foods

Introduction

Live coral is one of the most favored marine ornamental organisms (Lovell 2001, Bruckner and Borneman 2004, Wood et al. 2012). Over the past few years, maintaining scleractinian corals in aquaria has become popular (Wood et al. 2012). Harvesting corals from the wild can be illegal and unsustainable; thus, several technologies have been developed to improve methods for coral husbandry and coral culture in captivity (Delbeek and Sprung 2005, Calfo 2007).

Considering that corals use an autotrophic mechanism via symbiotic zooxanthellae, lighting technology has received considerable attention in the past decade, and has been considered a mean of improving coral breeding and husbandry. The energy in corals primarily comes from zooxanthellae photosynthesis (Muscatine 1990, Fabricius and Klumpp 1995, Calfo 2007). Approximately 80% of all outputs of photosynthesis such as carbohydrates and oxygen are transferred to the coral tissues, and around 10% to 22% are used for the respiration and growth of the zooxanthellae (Davies 1984a, Edmunds and Davies 1986). A second possible technique to improve coral husbandry and breeding is through heterotrophic mechanism (Anthony 2000, Houlbrèque et al. 2004). Corals use their tentacles to catch planktons; once the planktons pass the polyps, digestion takes place at the stomach of the former (Ferrier-Pagès et al. 2003, Palardy et al. 2005). Several studies have shown that the stomach contents of natural corals can compose of zooplanktons (e.g., copepod, nematode, polychaete, and amphipod), juvenile marine animals, bacteria, as well as dissolved

organic and particulate matter (Sorokin 1973, Porter 1974, Sebens et al. 1996, Ferrier-Pagès et al. 1998, Anthony 1999, 2000, Houlbrèque et al. 2004).

Heterotrophic mechanism increases both the tissue and skeleton growth of corals (Borell et al. 2008). However, the need of corals to be fed as a supplemental source of food depends on environmental parameters such as light or sedimentation (Anthony 2000, Forsman et al. 2012). Corals in captivity can experience in limitation of light and food; thus, most corals in aquariums require some kind of feeding to meet their specific nutrient requirement (Calfo 2007). Live food such as *Artemia salina* has been considered an important additional food for coral growth in aquariums (Goldman 2007, Toh et al. 2014). *Artemia salina* comprises of 53% protein, 17% lipid, and 18% carbohydrate (Coles 1969), of which protein concentration is slightly higher than that in natural zooplanktons such as copepod (Helland et al. 2003). However, the active culture of live food can be laboring (Calfo 2007). Therefore, artificial, dry, and frozen food have remarkably attracted the interest of aquarists because their production methods are less labor extensive and less expensive than live food (Calfo 2007, Goldman 2007). Recently, commercial artificial food such as dead pasteurized *Artemia* nauplii, Nori Micro (Zoolife, UK), Reef Chili (contains zooplankton, copepods and rotifers), Reef-Roids (contain zooplankton, ingredient; protein 60%), Roti-Feast (contains rotifers and rotifer eggs), and oyster eggs (contain actual oyster eggs) are used (Osinga et al. 2008, Petersen et al. 2008, Forsman et al. 2012). Other artificial food such as chitin, which is extracted to chitosan, is also used in aquaculture (Rinaudo 2006).

Usually, when corals capture food, their polyps normally expand (Porter 1974). Many coral species such as *Montastrea cavernosa* and *Meandrina meandrites* contract polyps in the daytime and expand polyps for feeding at night (Coles 1969, Porter 1974, Heidelberg et al. 1996). By contrast, the polyps of *Acropora* spp. and *Pocillopora damicornis* expand and are ready for feeding both day and night times (Lewis and Price 1974, Anthony and Fabricius 2000).

The purpose of this study was to examine the possible food supplements, both live and commercially artificial food, available to corals in aquariums, and to investigate the effects of four different food sources on growth, survival, photosynthesis, and tissue components of corals. Specifically, the study addressed 1) Does feeding influence the coral growth and protein components in coral tissues? and 2) Does feeding directly affect the photosynthesis of corals ? The hypothesis is that, corals fed on any type of food will have a higher growth rate and contain more protein in their tissues.

Materials and Methods

Coral collections and maintenance

Two groups of corals (broadcaster and brooder) were collected. A staghorn coral, *Acropora millepora* (Ehrenberg, 1834) is a model species of broadcaster while a cauliflower coral, *Pocillopora damicornis* (Linnaeus, 1758) is a model species of brooder. Coral fragments (2 cm to 3 cm in length or approximately 130$\pm$70 polyps) of *A. millepora* and *P. damicornis* were collected from natural colonies in the reef around Samae San Island, Chon Buri Province in the upper Gulf of Thailand, and were glued to pvc pipe caps with nontoxic super glue. Prior to the experimental trials,

these fragments were placed in aquariums for acclimation at least 2 weeks at the coral hatchery located on Samae San Island. (Fig. 7-1 and 7-2). All corals were acclimated to experimental conditions at temperature 29 C, salinity 32 psu, and 12:12 h light and dark cycle, light intensity 21 µmol m^{-2} s^{-1} .

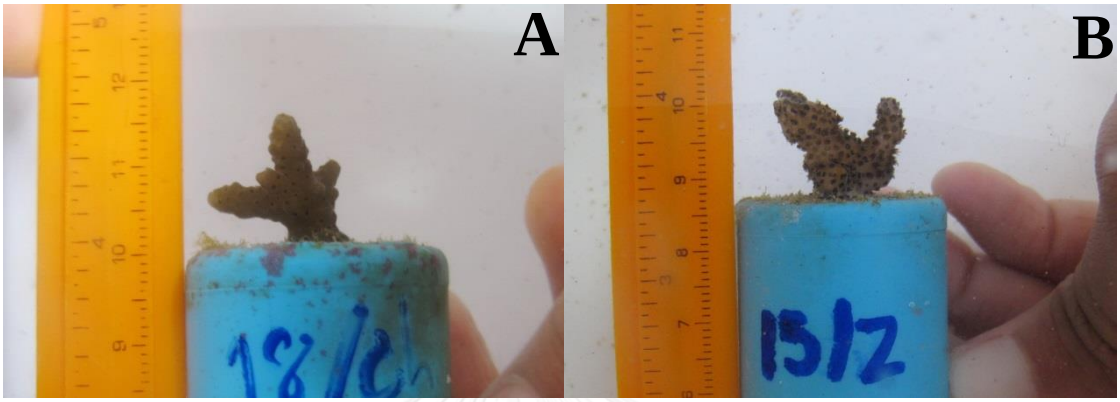

Figure 7-1. Coral preparation for the experiment. A) *Acropora millepora* fragment, B) *Pocillopora damicornis* fragment.

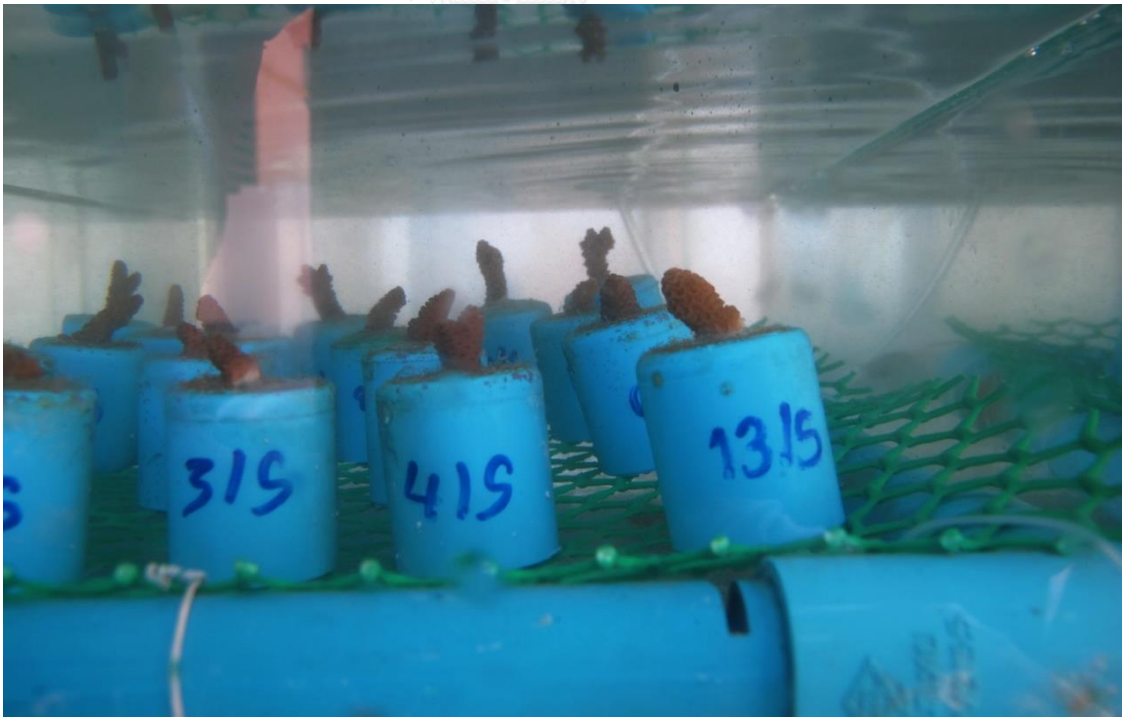

Figure 7-2. A rearing system for feeding experiments.

Feeding experiments

The experiments were conducted to investigate the feeding preferences and feeding efficiency of two coral species. The coral fragments were offered four different types of food (newly hatched *Artemia salina*, dried zooplankton, dried *Spirulina* sp. (Fig. 7-3), and chitosan), which most are commonly used in aquaculture, except chitosan. Control corals (starved corals) were also tested. There were a total of five treatments with 15 replicates each. In each treatment, the coral fragments were placed in a 30 L glass aquarium. The corals were offered approximately an equal weight (0.16 g of dry weight) of either one of the three foods or 10 ppm of chitosan. The corals were fed one time a day for four consecutive weeks. Each day, the corals were allowed to feed for 6 hours, during which the water flow was turned off whereas the air pump was maintained to keep the water well oxygenated and to allow food to circulate in the water column. During the experiments, the growth and survival corals were observed every week. The growth of corals was measured by photographing corals, and changes of coral covers per week were calculated using the Coral Point Count with Excel extensions (CPCe) software (Kohler and Gill 2006). In addition, the maximum quantum yield (Fv/Fm) of photosynthetic efficiency of each coral colony was measured every week using an underwater pulse amplitude modulation fluorometer (a DIVING PAM; Heinz Walz Company, Germany). At the end of the four-week experiment, the corals were measured for protein concentrations following the procedure described by Lowry et al. (1951), and the standard curve was established with bovine serum albumin standard (Fig. 7-4 to Fig. 7-6). A one way ANOVA test followed by Tukey's pairwise mean comparison and Paired samples t-test was performed to examine differences between specific growth rates, the survival

rates, the maximum quantum yield of photosynthesis, and protein concentrations among the treatments and before and after the experiments.

Figure 7-3. Dried zooplankton and dried phytoplankton (*Spirulina* sp.).

Figure 7-4. Using water jet for extracting coral tissues from hard structure and filtering tissue on GF paper.

Figure 7-5. Hydrolyze at 100°C in a water bath and add solution for extracting protein.

Figure 7-6. Centrifuge and measure with spectophotometer 750 nm.

Results

The results showed that the survival rates of both corals, *Acropora millepora* and *Pocillopora damicornis,* during the experiments were over 90%, and the survival of corals in each treatment did not differ significantly among any feeding regimes (Table 7-1).

Table 7-1. Survival rates of corals, *Acropora millepora* and *Pocillopora damicornis* in different types of food.

Treatments	*Acropora millepora* (28 days)	*Pocillopora damicornis* (28 days)
Control	100.0 ± 0.0	100.0 ± 0.0
Artemia salina	90.0 ± 10.0	100.0 ± 0.0
Dried zooplankton	100.0 ± 0.0	100.0 ± 0.0
Dried *Spirulina* sp.	100.0 ± 0.0	100.0 ± 0.0
Chitosan	90.0 ± 10.0	100.0 ± 0.0

In addition, no significant difference was observed in the specific growth rates of both coral species between trials (Fig. 7-7). Overall the growth rates of *A. millepora* and *P. damicornis* ranged between 0.032 cm^2 to 0.044 cm^2 and from 0.035 cm^2 to 0.081 cm^2 per week respectively.

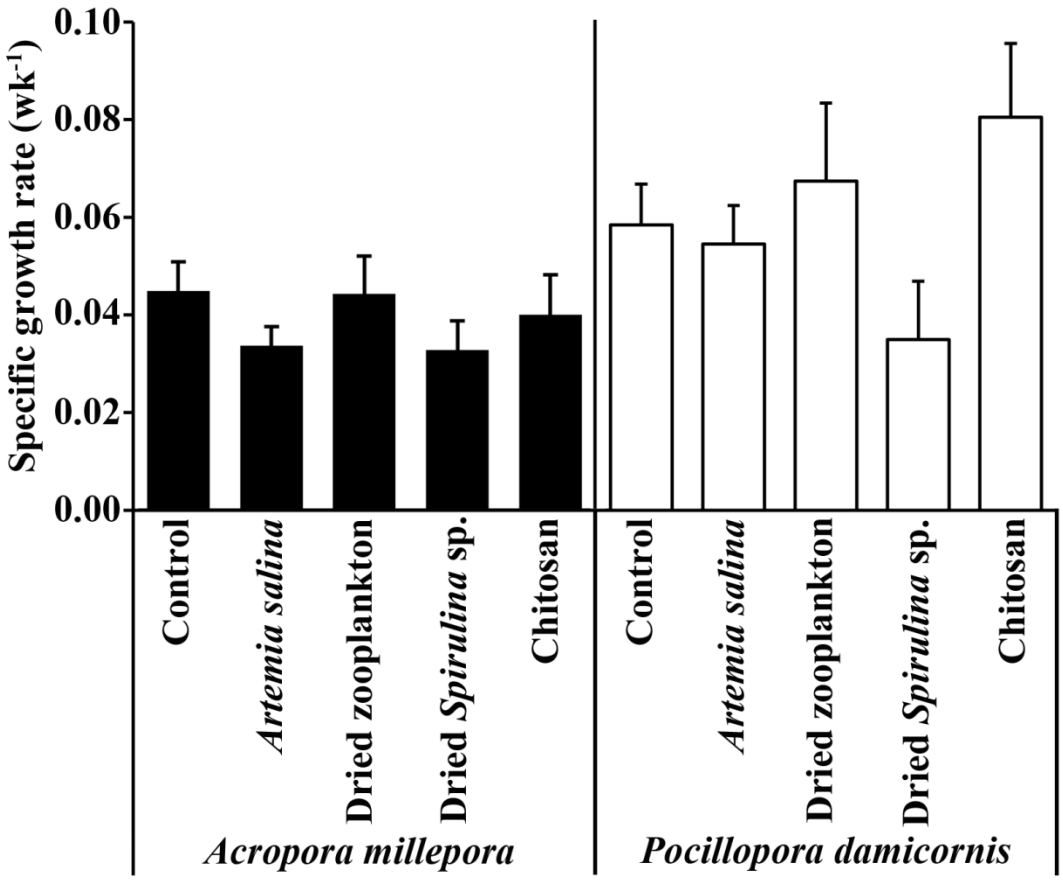

Figure 7-7. Specific growth rates per week of two corals species fed on different types of food.

After one month, Fv/Fm values were measured, and showed significantly increase in net photosynthesis ($P \leq 0.05$). The results indicated that Fv/Fm was higher in *A. millepora* fed on *Artemia salina*, dried *Spirulina* sp., and chitosan, while *P. damicornis* fed on chitosan (Fig. 7-8).

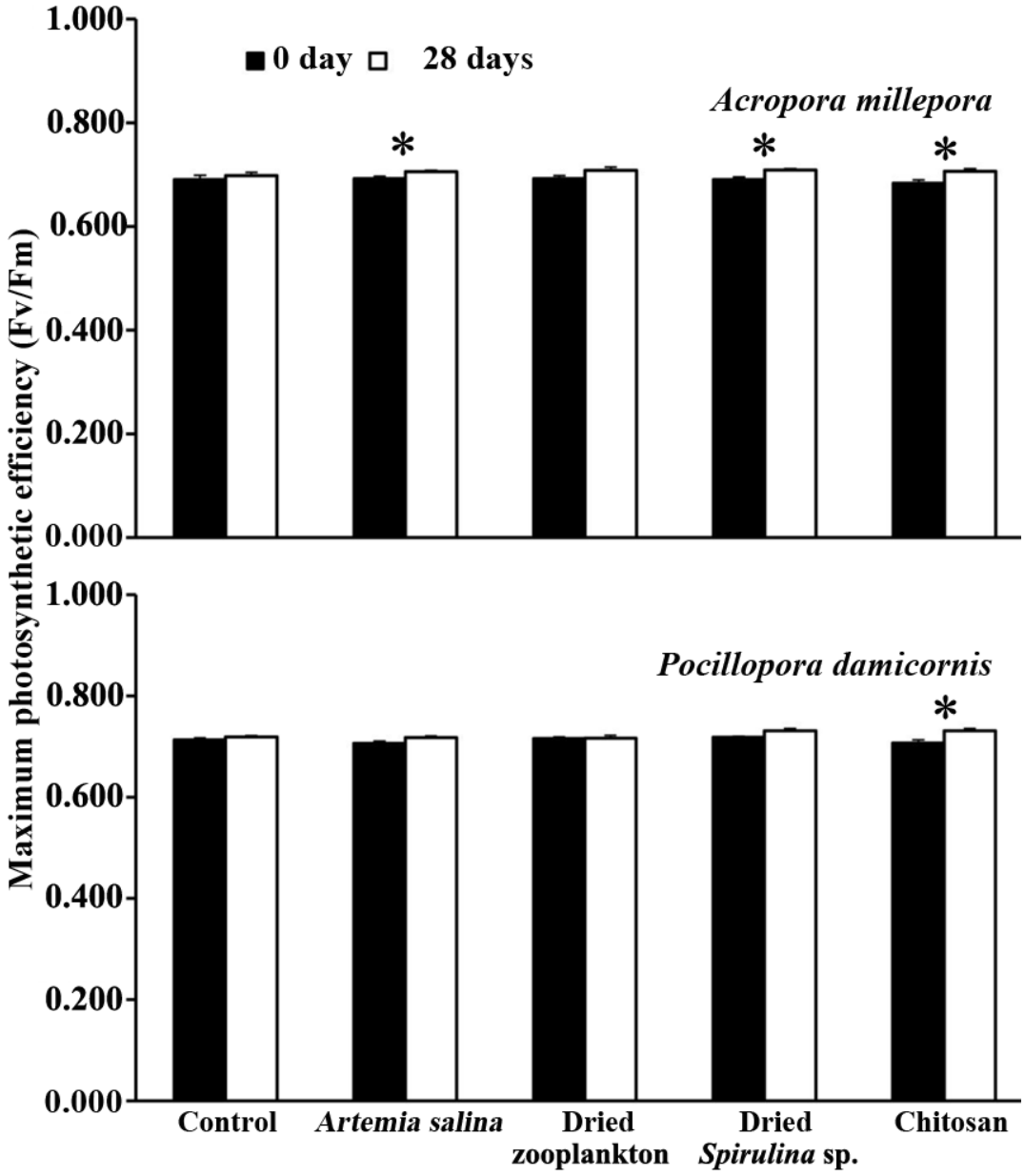

Figure 7- 8. Maximum photosynthetic efficiency (Fv/Fm) of zooxanthellae associated with two coral species fed on different types of food (* = significant difference).

At the end of the four-week experimental trails, protein concentrations in coral tissues were measured. The average protein concentrations of the tissues were significantly different between diets ($P \leq 0.05$) (Fig. 7-9). The results indicated that the protein concentrations were higher in *A. millepora* fed on *Artemia salina*, while *P. damicornis* fed on *A. salina*, dried zooplankton, and dried *Spirulina* sp. showed the higher protein concentrations than the ones fed on chitosan. Conversely, the protein concentrations in the starved corals were significantly lower by 47% (Fig. 7-9).

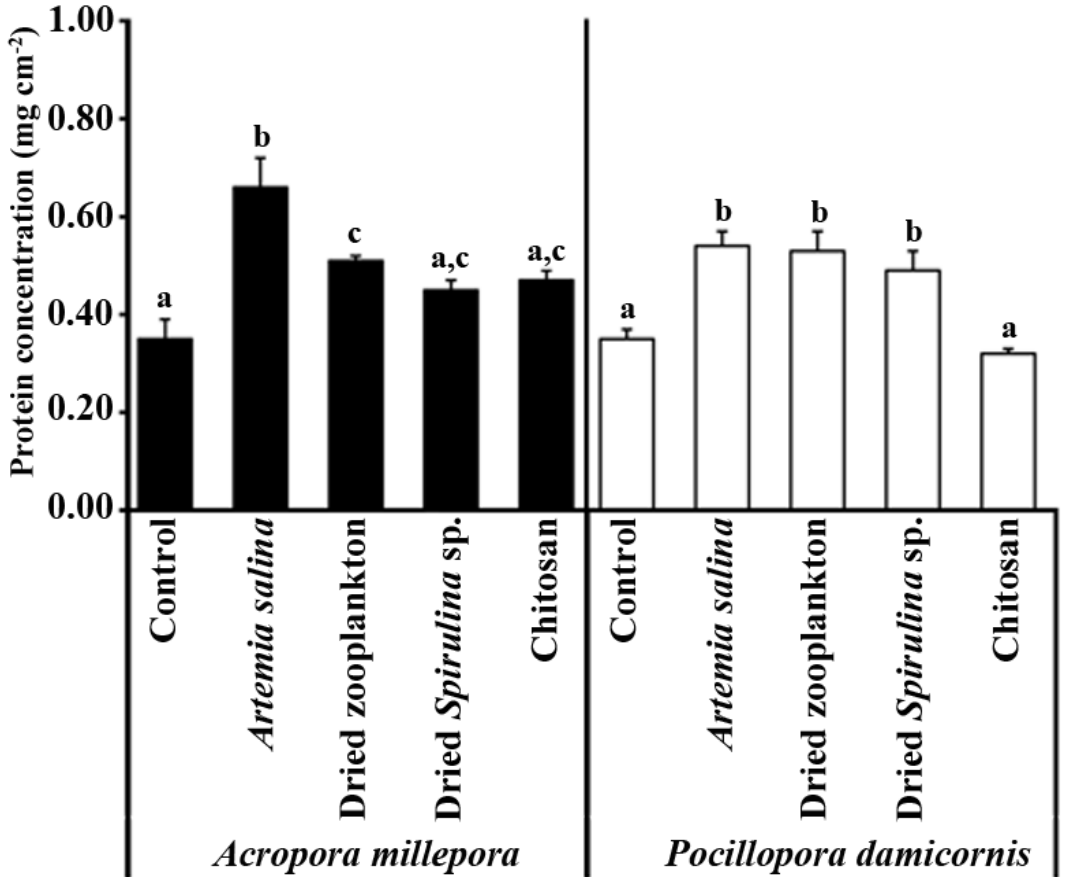

Figure 7-9. Protein concentrations in two coral species fed on different types of food. Letters above each histogram designate protein concentrations that differ significantly among diets ($P \leq 0.05$).

Table 7-2. Comparison of protein concentrations in different types of food from various locations.

Species	Food types	Concentrates	Protein (mg cm^2)	Time (wks)	Locations	Sources
Stylophora pistillata	starved	-	0.30±0.02	-	Gulf of Aqaba	Ferrier-Pagès et al. (2003)
Stylophora pistillata	starved	-	0.80±0.20	5-9	Gulf of Aqaba	Houlbrèque et al. (2003)
	Artemia sp.+ zooplankton	3000 nauphii/L	2.30±0.20			
Stylophora pistillata	starved	-	0.40±0.02	4	Gulf of Aqaba	Grover et al. (2002)
	Artemia sp.	5 g	0.90±0.09			
Pocillopora damicornis	starved	-	1.50±0.35	8	Hawaii Island	Achituv et al. (1994)
	Ammonium	20 µM	1.69±0.13			
		50 µM	2.10±0.42			
Porites porites	starved	-	4.23±0.57	4	Barbados Island	Marubini and Davies (1996)
	Nitrate	20 µmol L	4.96±0.20			
Astrangia danae	starved	-	5.90±0.30	-	Rhode Island, USA	Szmant-Froelich and Pilson (1980)
	frozen *Artemia* sp.	3 time/wk	11.30±0.50			
Acropora millepora	starved	-	0.35±0.04	4	upper Gulf of Thailand	In this study
	Artemia	3000 nauphii/L	0.66±0.06			
	Dried zooplankton	0.160 g/dry weight	0.51±0.01			
	Dried *Spirulina* sp.	0.160 g/dry weight	0.45±0.02			
	Chitosan	10 ppm	0.47±0.02			
Pocillopora damicornis	starved	-	0.35±0.02			
	Artemia sp.	3000 nauphii/L	0.54±0.03			
	Dried zooplankton	0.160 g/dry weight	0.53±0.04			
	Dried *Spirulina* sp.	0.160 g/dry weight	0.49±0.04			
	Chitosan	10 ppm	0.32±0.01			

Discussion

This paper presents the effect of feeding on growth, survival, photosynthetic efficiency, and protein concentrations of the scleractinian corals, *Acropora millepora* and *Pocillopora damicornis*. The present study confirms that feeding can enhance the growth and photosynthetic rates of corals. The results of this study complements those obtained by previous studies (Ferrier-Pagès et al. 2003, Petersen et al. 2008, Forsman et al. 2012) on influence of different food sources on corals. The corals fed on *Artemia salina* had higher growth and calcification rates than that of starved corals, and food had species-specific effects on coral growth (Ferrier-Pagès et al. 2003, Forsman et al. 2012, Toh et al. 2014). Moreover, food supplements can help in sustaining coral photosynthesis and preventing damage to the photosynthetic apparatus of the zooxanthellae in stressed corals (Ferrier-Pagès et al. 2010).

Several types of diets including live and artificial food were experimented in corals (Osinga et al. 2008, Petersen et al. 2008, Forsman et al. 2012, Toh et al. 2014). However, no previous study was done using chitosan as one of the selected diets for corals. In this study, corals fed on chitosan exhibited an increase of photosynthesis. Chitosan, an element of hard structures of marine arthropods, mainly comprises of carbohydrate (Jackson et al. 1992), is widely used for food and other applications such as biosensors (Rinaudo 2006).

Most corals are mixtrophic (between autotrophs and heterotrophs) (Calfo 2007). Some coral species gather enough nutrition from photosynthesis; however, in circumstances such as growth and reproduction may require additional nutritive supplementation from feeding or absorption (Calfo 2007). The present study clearly shows that supplementary diets such as *Artemia salina* and dried zooplankton,

increased the growth and protein concentrations of corals. *Artemia* sp. is commonly used in aquaculture because it is easy to culture and low cost (Calfo 2007, Goldman 2007, Toh et al. 2014). They also act as biocarriers to supply specific nutrients to corals (Helland et al. 2003, Osinga et al. 2008). Feeding rates of corals can be varied depending on species, feeding mechanisms, morphology, polyp sizes, number of tentacles, prey types and sizes, and environmental parameters (Sebens et al. 1998, Palardy et al. 2006, Hii et al. 2009, Houlbrèque and Ferrier-Pagès 2009). Any changes of the environmental parameters can affect coral feeding rates (Dai and Lin 1993, Piniak 2002b). To enhance the capture capacity, some corals produce mucus on their tentacles for better prey entrapment (Hii et al. 2009).

Live or instant food can have an influence on food capture of corals. Live zooplanktons have been reported to be captured more easily by corals than instant food (Heidelberg et al. 1996). Corals kill their preys through nematocyst discharge; thus, prey approaching to tentacles with high velocity would be captured easily (Heidelberg et al. 1996).

Feeding also enhance both lipid and protein concentrations (Grover et al. 2002, Houlbrèque et al. 2003, Treignier et al. 2008). The concentrations of fatty acids, sterol, and alcohols increased when corals fed on natural zooplanktons (Treignier et al. 2008, Tolosa et al. 2011). In addition, there were studies showed that fed corals could increase their zooxanthellae concentrations, which led to an increase of pigments per cell and tissue color (Hoegh-Guldbergl and Smith 1989, Dubinsky and Stambler 1996). In this study, the protein concentrations differed depending on coral species and types of diets fed by corals. To increase protein concentrations in the coral tissue, feeding to corals is another option (Table 7-2). Several studies

showed that when corals were fed with different food types, protein concentrations would be higher than that of starved corals (Table 7-2).

In conclusion, the heterotrophic mechanism is an important feeding behavior for corals to overcome any nutrient deficiency in special circumstance such as in captivity or in aquariums. Thus, supplementary food or artificial diets can play a major role in increasing growth, photosynthetic rates, and tissue components of corals. In addition, it can reduce the coral mortality rates in the captivity.

CHAPTER VIII
Conclusions

In 2010, an usual warm water developed in the Gulf of Thailand and the Andaman Sea. This study examined patterns of susceptibility and recovery of corals and other reef organisms from bleaching on reefs in Chon Buri Province, the upper Gulf of Thailand. 70% to 100% of hard coral populations experienced extensive bleaching, and more than 40% of populations had 75% to 100% of colony bleaching. However, there was no significant relationship in the bleaching response of coral populations and taxa between and within study sites. After the bleaching, coral mortality ranged between 32% to 78.8% of the populations depending on sites. Other reef organisms, including sea anemones and giant clams were also affected by the bleaching. The mortalities of sea anemones and giant clams after the bleaching event were between 27.4% to 65.5% and 11.1% to 60.8% respectively. The results from this study suggest that the susceptibility pattern of bleaching corals was not uniformed.

In this study, 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-month old) were investigated. 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) ($p < 0.05$). 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. Results in this study also showed that corals of different ages and of different species did not elicit the same physiological response to changes in environmental factors. Thus, the ability of corals to tolerate to salinity and temperature stresses depends on several factors such as ages and species.

For the effects of increase and decrease of light intensity and photoperiods on juvenile corals were transferred and exposed to different treatments (0, 21, 42, 85, and 169 µmol m^{-2} s^{-1} provided by 400-W metal halide lamps) for 2 weeks, while in the photoperiod experiment, the coral colonies were exposed to 5 different treatments (24/0, 18/6, 12/12, 6/18, and 0/24 h light-and-dark cycle). The results from the light intensity experiment showed that 20-month old *Platygyra sinensis* survived (100%) under all light intensity levels, while the survival rates of other coral species decreased, ranging between 20% to 80%, when the light intensity levels changed from the ambient light condition. For the photoperiod experiments, the results showed that 23-month old *Platygyra sinensis* and 20-month old *Acropora millepora* survived (100%) under all photoperiod levels. However, there was no significant difference on the growth of corals between different light intensity levels and different photoperiods. From this study, *P. sinensis* seemed to have more tolerance to wider ranges of light intensities and photoperiods than other coral species. In addition, the results implied that corals were able to adapt to a prolonged light period and light intensity, should the best condition for rearing coral.

Coral can capture planktons in water columns. In this study, capture rates of *Acropora* spp. and *Pocillopora damicornis* in Thailand and Federated States of Micronesia were examined. In addition, the effect of day and night times on coral feeding was investigated. The results showed that all three coral species, *Acopora*

millepora, A. nobilis, and *Pocillopora damicornis* captured and consumed *Artemia salina* nauplii both light and dark conditions. The results showed that the capture rates of all three species ranged between 0.44 to 2.39 individuals per polyp. The results also showed complete digestions of *A. salina* nauplii by *A. millepora* and *A. nobilis* were observed after 2 hours while *P. damicornis* took at least 2.5 hours to complete the prey digestion. Feeding corals with supplementary food such as *Artemia salina* nauplii may be an option for corals in captivity or aquarium.

In addition, feeding preferences and feeding efficiency of two coral species were investigated. The results showed that the survival rates of corals, *Acropora millepora* and *Pocillopora damicornis*, during the experiments were over 90%, and the survival of corals in each treatment did not differ significantly among any feeding regimes. In addition, no significant difference was observed in the specific growth rates of both coral species between trials. At the end of the four-week experimental trails, the protein concentrations in coral tissues were measured. The average protein concentrations of the tissues were significantly different between diets ($P < 0.05$). The results indicated that the protein concentrations were higher in *A. millepora* fed on *Artemia salina*. In conclusion, the heterotrophic mechanism is an important feeding behavior for corals to overcome any nutrient deficiency in special circumstance such as in captivity or in aquariums.

In conclusion, both physical (temperature, salinity, light intensity, and photoperiod) and biological (food) factors can have an influence on growth, survival, and photosynthetic rates of corals. However, corals of different ages and different species may have difference physiological response to changes in different factors.

The results of this study can be used for achievement of coral culturing technique for restoration of coral reefs.

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APPENDIX

VITA

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List of Publications:

1. Kuanui P, Chavanich S, Raksasab C, Viyakarn V. 2009. Lunar periodicity of larval release and larval development of Pocillopora damicornis in Thailand. Proceedings of the 11th International Coral Reef Symposium, Florida, USA, 375-377.
2. Songploy S, Kuanui P, Chavanich S, Viyakarn V, Hemachandra W. 2015. Diversity of reef fish at Sattahip Area, Chonburi Province, Thailand. Indian Journal of Marine Science. (Accepted)
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6. Kuanui P, Chavanich S, Viyakarn V, Omori M, Lin C. 2015. Effect of light intensity on survival rate of cultured corals and photosynthetic efficiency in corals. (In review)
7. Kuanui P, Chavanich S, Viyakarn V, Omori M, Lin C, Pichyangkura R. 2015. Feeding of corals in several types of foods in captivity. (In review)