VARIATION OF SYMBIODINIACEAE IN BROADCASTER *Acropora humilis* AND BROODER *Pocillopora damicornis* CORALS

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ความแปรปรวนของสาหร่ายซูแซนเทลลีในปะการังเขากวาง *Acropora humilis* และ ปะการังดอกกะหล่ำ *Pocillopora damicornis*

น.ส.ศุภกาญจน์ จันทร์แดง

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ศุภกาญจน์ จันทร์แดง : ความแปรปรวนของสาหร่ายซูแซนเทลลีในปะการังเขากวาง *Acropora humilis* และ ปะการังดอกกะหล่ำ *Pocillopora damicornis* . (VARIATION OF SYMBIODINIACEAE IN BROADCASTER *Acropora humilis* AND BROODER *Pocillopora damicornis* CORALS) อ.ที่ปรึกษาหลัก : ศ. ดร.สุชนา ชวนิชย์, อ.ที่ปรึกษาร่วม : รศ. ดร.ชูยะ ชินซาโตะ

ปะการังอาศัยอยู่ร่วมกับสาหร่ายเซลล์เดียวครอบครัว Symbiodiniaceae ในลักษณะพึ่งพาอาศัยซึ่งกันและกัน โดยสาหร่ายสามารถสังเคราะห์แสงและส่งถ่ายพลังงานให้กับปะการังกว่า 50-95 เปอร์เซ็นต์ เพื่อใช้ในการเติบโตและดำรงชีวิต ปัจจุบัน ผลกระทบจากการเปลี่ยนแปลงสภาพแวดล้อม ส่งผลให้แนวปะการังในหลายพื้นที่ทั้งในและต่างประเทศเสื่อมโทรมลง ด้วยเหตุนี้ จึงมีหน่วยงานในหลายภาคส่วนได้ตระหนักถึงการอนุรักษ์ทรัพยากรปะการังมากขึ้น กลุ่มการวิจัยชีววิทยาแนวปะการัง ภาควิชาวิทยาศาสตร์ทางทะเล จุฬาลงกรณ์มหาวิทยาลัย เป็นหน่วยงานที่สามารถผลิตลูกพันธุ์ปะการังแบบอาศัยเพศเพื่อนำไปใช้ในการอนุรักษ์ตั้งแต่ปี พ.ศ. 2546 อย่างไรก็ตาม การวางแผนนโยบายเพื่อการอนุรักษ์และฟื้นฟูแนวปะการังนั้น มีความจำเป็นต้องอาศัยข้อมูลพื้นฐานทางชีววิทยาของปะการัง การศึกษาครั้งนี้ จึงได้ออกแบบการทดลองรวมทั้งสิ้น 3 การทดลอง เพื่อรวบรวมข้อมูลที่เกี่ยวข้อง โดยเฉพาะข้อมูลการปล่อยเซลล์สืบพันธุ์ปะการังบริเวณอ่าวแสมสาร และความหลากหลายชนิดของสาหร่ายที่อาศัยอยู่ร่วมกับปะการังบริเวณอ่าวไทยตอนบน การทดลองที่ 1: จากการเก็บข้อมูลการปล่อยเซลล์สืบพันธุ์ของกลุ่มปะการังเขากวาง (*Acropora* spp.) พบปะการังปล่อยเซลล์สืบพันธุ์เฉลี่ยในช่วงเวลาระหว่าง 20.00-21.00 น. ทั้งในช่วงข้างขึ้นและข้างแรม การติดตามผลบ่งชี้ว่า ในบางปี ปะการังมีการปล่อยเซลล์สืบพันธุ์ติดกันทุกคืน ทำให้เซลล์สืบพันธุ์ที่ปล่อยออกมามีปริมาณน้อย ด้วยเหตุนี้ อาจส่งผลต่ออัตราการปฏิสนธิและจำนวนลูกพันธุ์ปะการังในธรรมชาติที่อาจลดลงได้ การทดลองที่ 2: ปะการังเขากวางและปะการังดอกกะหล่ำมีการเปลี่ยนแปลงประชาคมของสาหร่ายซูแซนเทลลีในระบบโรงเพาะฟักฯ ซึ่งมีความแตกต่างกันกับโคโลนีแม่พันธุ์ในธรรมชาติ อย่างไรก็ตาม ในการทดลองนี้ได้ตรวจพบสาหร่ายสกุล *Symbiodinium* ซึ่งเป็นกลุ่มที่ยังไม่เคยมีรายงานมาก่อนในประเทศไทย การทดลองที่ 3: จากการติดตามปะการังที่เติบโตตามธรรมชาติในแต่ละฤดูกาล พบว่าความหนาแน่นของเซลล์สาหร่ายเซลล์เดียวลดลงอย่างมีนัยสำคัญเมื่อความเข้มแสงสูงขึ้นในช่วงฤดูร้อน แต่ไม่พบการเปลี่ยนแปลงของชนิดและสัดส่วนประชาคมสาหร่ายภายในเนื้อเยื่อปะการัง ผลที่ได้จากการศึกษาในแต่ละการทดลอง สามารถนำไปใช้เพื่อประเมินแนวโน้มการปรับตัวของปะการังและสถานภาพแนวปะการังในประเทศ รวมถึงเป็นแนวทางในการคัดเลือกชนิดของปะการังที่จะนำไปใช้ในการอนุรักษ์แนวปะการังอย่างเหมาะสมและประสิทธิภาพต่อไป

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Suppakarn Jandang : VARIATION OF SYMBIODINIACEAE IN BROADCASTER *Acropora humilis* AND BROODER *Pocillopora damicornis* CORALS. Advisor: Prof. Suchana Chavanich, Ph.D. Co-advisor: Assoc. Prof. Chuya Shinzato, Ph.D.

Reef-building corals sustain a symbiotic relationship with single-cell algae belonging to family Symbiodiniaceae. Symbiotic algae contribute up to 50-95% of the metabolic needs by supplying photosynthetic products to the coral host. Therefore, the symbiosis between corals and Symbiodiniaceae is essential for the development and survival of coral reefs. Over the past years, significant losses and changes in coral reef ecosystems have been caused by anthropogenic activities and natural phenomena. Thus, relevant national organizations have raised awareness regarding the conservation of coral populations. Our Reef Biology Research Group, Department of Marine Science, Chulalongkorn University have been producing corals using a sexual propagation technique for coral reef rehabilitation since 2003. Nevertheless, studies on coral background, including their biology and adaptation mechanism, are required for policy and/or action plan of coral reef management and conservation. This study consisted of three experiments that aimed to collect the related information on corals, especially coral spawning, and investigate the diversity of Symbiodiniaceae in corals from the upper GoT. Two *Acropora* species spawn their gametes around 8 PM to 9 PM across all lunar periods with no clear indication of lunar-associated cue. According to year data, asynchronous spawning occurs and may lead to the low success of fertilization and the decreased number of coral recruitments (Experiment 1). *A. humilis* and *P. damicornis* exhibited the shuffling and switching of Symbiodiniaceae community under hatchery conditions. However, we recorded the association of Symbiodiniaceae genus *Symbiodininium* with coral, which has never been reported before in Thailand (Experiment 2). Tagged colonies of wild *Acropora* and *Pocillopora* corals showed a significant decline in zooxanthellae cells during summer, but the community of Symbiodiniaceae exhibited no change throughout the year (Experiment 3). The results from this study are useful for understanding the coral life cycle and coral-Symbiodiniaceae relationship, which can be applied to the prediction of potential adaptation of corals in localized reef environments and improve coral cultivation and conservation in Thailand.

Field of Study: Marine Science

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Student's Signature .....

Advisor's Signature .....

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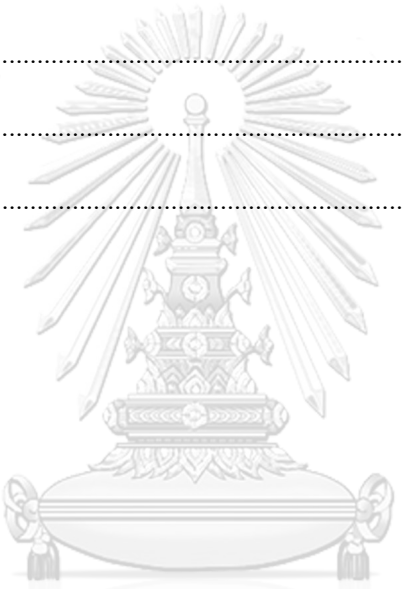

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CHAPTER 1

INTRODUCTION

1.1 Overview

The mutualistic relationship between coral and Symbiodiniaceae is an important key to the success of reef-building corals. Worldwide studies on coral-Symbiodiniaceae association have been carried out across the Indo-Pacific to Atlantic Ocean. However, information on the relationship of corals and their symbionts in Thailand is still limited. Our research group conducted coral conservation in Thailand by producing corals using sexual propagation technique. The investigation of coral-Symbiodiniaceae relationship in these corals may enhance the understanding of coral adaptation mechanism and improve coral cultivation and conservation in Thailand. The flexibility of Symbiodiniaceae changes in coral is important to predict future survival in response to climate change. This study, we investigated the ability of corals for adaptation by monitoring and examination with a high-accuracy molecular technique.

1.2 Biology of coral

1.2.1 Coral physiology

Corals are marine invertebrates belonging to class Anthozoa of phylum Cnidaria and typically form colonies consisting of numerous individual polyps (Veron, 2011). Scleractinian corals deposit calcium carbonate from sea water to build an outer calcareous skeleton ($Ca^{2+} + 2\ HCO_3^- \longrightarrow Ca\ (HCO_3)_2 \longrightarrow Ca\ CO_3 + CO_2 + H_2O$) (Constantz, 1986; Veron, 2011). In general, coral body (soft part) or polyps consist of epidermis and gastrodermis tissues (diploblastic). These layers are separated by a matrix material called mesoglea. Coral polyps are usually tube-shaped with a bilateral symmetry. The apical polyp side has tentacles surrounding the hyposome (mouth), whereas the basal part is connected with other polyps by the coenosarc. Given the simple structure and lack of excretory organ, all processes, including respiratory,

nutrient, and waste circulation, occur only in the gastrovascular cavity. Waste and other digested material are excreted through the mouth (Fig 1.1) (Berking, 2007; Peng et al., 2007; Veron, 2011). Scleractinian corals with polyp-maintaining symbionts have the characteristics of heterotrophs and autotrophs (Burmester et al., 2018; Pacherres et al., 2013). These corals can ingest various kinds of food, such as bacteria, sediments, zooplanktons, and particle organic matter (Anthony, 1999; Ferrier-Pagès et al., 1998), or derive nutrition from photosynthesis translocated by symbiotic algae that live in their tissues (Muscatine, 1990). Reef-building corals are a major contributor to the formation of complex reef habitats (Pratchett et al., 2008). In general, a common coral form can be divided into nine groups based on the appearance of a calcium carbonate structure (Fig 1.2). However, different environments and habitat conditions, including depth, temperature, light, etc., may drive the variation in their form (Pratchett et al., 2015).

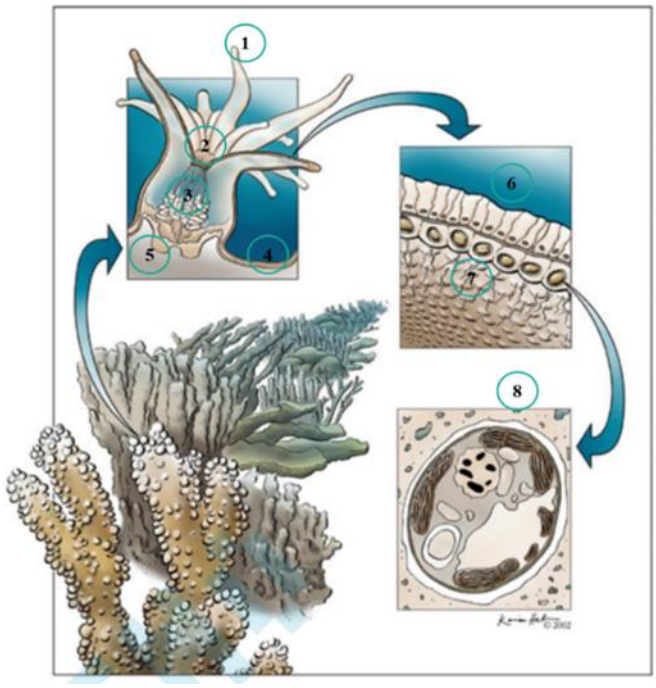

Figure 1.1 An individual coral polyp illustration ;1) tentacles 2) mouth 3) gastrovascular cavity 4) coenosarc 5) skeleton 6) epidermis 7) endodermis and 8) zooxanthellae cell (adapted from Muller-Parker et al. (2015)

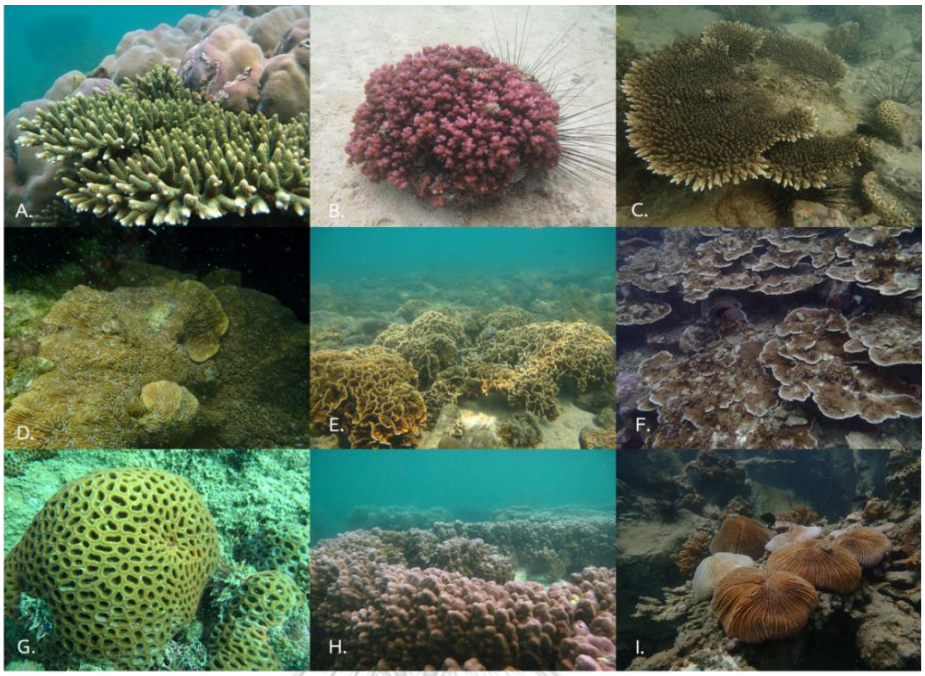

Figure 1.2 Coral growth forms ;A) branching B) corymbose C) digitate D) encrusting E) foliose F) laminar G) massive H) submassive and I) solitary/free living.

1.2.2 Coral reproduction

Corals are marine sessile organisms that evolved a remarkable range of reproductive strategies to survive under environmental stresses (Johnston et al., 2020). Most scleractinian corals reproduce asexually (budding or fragmentation) and sexually (producing gametes) throughout their lifetime (Harrison, 2011). Asexual reproduction is widely known in scleractinian corals. Coral budding often occurs when a parent polyp requires an accretive growth form by producing a new clonal tissue connected with a parent polyp (Gateño & Rinkevich, 2003) whereas coral fragmentation is produced by entire branches or fragments broken off from the parent to form a new colony (Lirman, 2000). Several benefits of asexual reproduction in corals have been reported; such benefits include the higher growth and survival rate compared with the planula produced from sexual reproduction (Jackson, 1997). On the other hand, sexual reproduction is established by either gonochoric (separate sex) or hermaphroditic

(production of eggs and sperm) within a single colony (Kerr et al., 2011). Approximately 70%-75% of sexually reproducing corals are hermaphroditic, whereas 25%-30% are considered gonochoric (Kerr et al., 2011; Padilla-Gamiño et al., 2011). Sexually reproducing corals are divided into two groups in accordance with the following fertilization strategies: (1) broadcaster/spawner: a coral spawns gametes freely (eggs and sperm) into the water column where external fertilization occurs; (2) brooder: a coral produces the embryo inside the parent polyp (Harrison, 2011) (Fig 1.3).

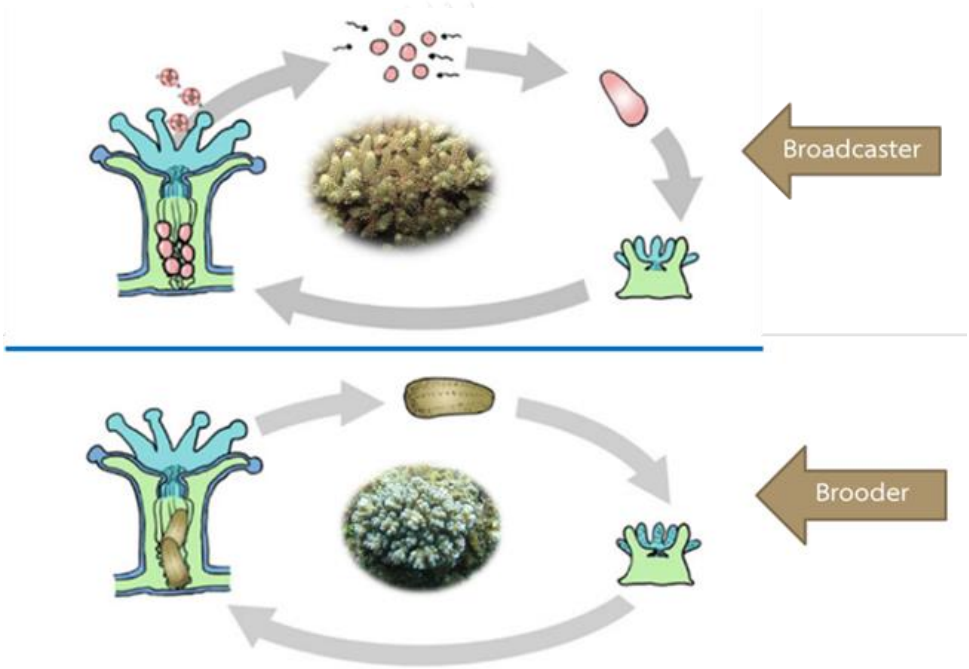

Figure 1.3 Coral reproductive and symbiont transmission modes **(**adapted from Dwi Haryanti).

Broadcast spawner coral

The majority of reef-building corals belong to a group of simultaneously hermaphrodite broadcasters (Harrison & Wallace, 1990). Mass spawning events can involve over 100 species of corals in a given reef spawning in a single night (Babcock et al., 1986). Mass spawning is an annual natural phenomenon in which multi species of corals synchronize their gametes into the water column to promote successful

fertilization (Gouezo et al., 2020; McEdward, 1995). Spawner corals have a higher variation in spawning pattern in reefs than brooding corals (Gilmour et al., 2015). Broadcast spawning allows cross-fertilization between coral colonies and the development of planktonic larva that disperse to wide distances (Graham et al., 2008). Most broadcasting coral species spawn once a year (Harrison & Wallace, 1990). However, a group of staghorn corals around equatorial reefs in Kenya was discovered to undergo asynchronous spawning due to the variation in local environment, causing difficulty in the determination of the spawning pattern (Mangubhai & Harrison, 2008). A wide array of conditions and cues have been linked to the synchronicity of such events, (Keith et al., 2016; Mendes & Woodley, 2002; Van Woesik, 2009), most predominantly the effect of temperature and lunar cycle (Babcock et al., 1986; Gorbunov & Falkowski, 2002). The variation in the average sea surface temperature has a large influence on coral physiological processes (Baird & Guest, 2009). Numerous examples of broadcaster coral spawns were observed under warm water temperature in several reef locations (Harrison & Wallace, 1990). *Acropora hyacinthus* and *A. japonica* corals at high latitudes exhibit delayed spawning at low temperatures (Nozawa, 2012). Similarly, studies on *Acropora* spp. spawning in Gulf of Oman showed that 75%–100% of mature gametes developed in April during full moon in 2013, whereas a year later, the matured gametes were observed around May, which correlated with a lower temperature (Howells et al., 2014). Most *Acropora* spp. from Indian and Pacific Oceans showed a similar trend; the highest peak of spawning occurred when the sea surface temperature increased, suggesting that seasonal rapid increase in the sea surface temperature is a strong cue of *Acropora* coral spawning (Keith et al., 2016). However, the dramatic increase in sea-water temperature can cause coral bleaching and may affect the number of gamete/larva production as well (Chen et al., 2020).

Brooder coral

Most brooder corals are considered hermatypic self-fertilization corals, which produce egg and sperm and are fertilized internally in the gastrovascular cavity (Okubo et al., 2007; Permata et al., 2000). Corals allow the embryo to develop inside

their polyp until the planula stage (Baird & Morse, 2004). This type of reproduction is observed as a minority in hermaphroditic reef-building corals (Sherman, 2008). However, several corals retained a self-fertilization strategy due to the limitation of male colonies nearby or unstable environmental conditions (Goodwillie et al., 2005; Jarne & Auld, 2006). In addition, brooder corals have several strategies for maintaining their population. For example, brooder larvae usually contain zooxanthellae cells from the maternal symbiont transmission process during embryo development and tend to settle within a short time after larva released (Baird & Guest, 2009; Hirose et al., 2008). The shorter time period of settlement and metamorphosis of brooder larva, which can minimize the risk or avoid predators while larvae are swimming in the water column, correlates with symbiont algae in their tissue compared with the spawner coral process (Cumbo et al., 2012; Keith et al., 2016; Kuanui et al., 2008). Typically, brooder corals produce larvae over several consecutive months within a year (Gilmour et al., 2015; Kuanui et al., 2008). In normal condition, the larvae will settle close to mother colony, which consequently maintains their local recruitment and population (Prasetia et al., 2017). By contrast, several studies showed that the brooder larvae of *Pocillopora damicornis* can remain in the water column for more than three months and possibly have a high potential for coral recruitment to distant areas (Richmond, 1987; Schmidt-Roach et al., 2012). However, it has been argued that self-fertilization may generate a lower fitness than broadcaster corals because of inbreeding depression and exhibit the low variation genotype due to the absence of gene combination between parent colony (Hedrick & Kalinowski, 2000; Sherman, 2008).

The timing of larval release in scleractinian corals plays a key role in determining the reproductive and larval dispersal success (Fan et al., 2006). Most brooder corals release their larva at nighttime or early morning (Edmunds et al., 2012; Schmidt-Roach et al., 2012). The brooder coral *Stylophora* spp. in Taiwan releases planula only in the early morning, whereas *P. damicornis* and *Euphyllia glabrescens* release planula in both early morning and nighttime (Fan et al., 2006). Similarly, *P. damicornis* in the upper Gulf of Thailand (GoT) consistently exhibited the maximum larva release at nighttime, suggesting that the light-dark cycle is a cue on the timing of

coral release (Kuanui et al., 2008). Possibly, the majority of pocilloporid corals release their planula in the dark to minimize predators (Fan et al., 2006). However, several daytime spawning had been reported. *Pocillopora verrucosa* at central Red Sea reef spawn their gametes one day before new moon between 8:40-9:10 AM (Bouwmeester et al., 2011). Daytime spawning suggests that several scleractinian corals are affected by selective pressures other than predation (Glynn, 1996). Moreover, the number of larvae released from brooder parents vary with the colony size and/or the lunar periodicity (Combosch & Vollmer, 2013).

1.2.3 Coral species in this study

Acropora humilis and *Pocillopora damicornis* are selected to represent of broadcast spawner and brooder coral, respectively. *A. humilis* is distributed in shallow reef regions of the Pacific and Indian Oceans (Sheppard, 1979; Wallace, 1999). In Sattahip, Thailand, *A. humilis* colonies have a green or brown appearance with large and obvious axial corallites. This species, which is found at a depth of 4–7 m, is among the species with the highest abundance at Ko Tao Mo in the upper GoT. *Acropora* spp. exhibited a distinct spawning pattern along biogeographic. For example, *Acropora* spp. in western of Great Barrier Reef were exhibited a biannual spawning (spring and autumn) (Foster & Gilmour, 2020). In contrast, most of *Acropora* spp. in Indo-Pacific have been showed a single gametogenesis or annual spawning, which release the gametes in the water column (i.e., external fertilization) (Chelliah et al., 2015; Nozawa, 2012). However, no information of spawning pattern in Thailand are recorded especially a group of *Acropora* coral. Therefore, this study is provided the data of *Acropora* spp. spawning including *A. humilis* in particular area, which would be explain in the next chapter.

Cauliflower coral or *P. damicornis* is among the species with the highest abundance in Thai waters. *P. damicornis* has been reported one of the most morphologically plastic in scleractinian coral species (Smith et al., 2017) Colonies of *P. damicornis* appear as a compact clump and may reach to several meters. Most colonies are usually brown, greenish, or pink. *P. damicornis* act as both a spawner and a brooder (i.e., mixed–mode reproduction) (Smith et al., 2019). However, the samples

of *P. damicornis* collected from our study site were found to be hermaphroditic brooders only (i.e., internal fertilization); regarding to previous study that parent colony produces both eggs and sperms and then releases planula in the water column throughout the year (Kuanui et al., 2008).

1.3 Coral-Symbiodiniaceae association

1.3.1 Coral-Symbiodiniaceae

Endosymbiotic dinoflagellates are associated with diverse marine organisms, such as bivalve mollusks (giant clam and heart cockles), foraminifera, sponges, and Cnidarian species (sea anemones, jellyfish, scleractinian corals, sea fans, and Alcyonacean corals) (Carlos et al., 1999). Reef corals rely on their symbiotic relationship with unicellular algae called zooxanthellae, which belong to family Symbiodiniaceae (Goulet, 2006; LaJeunesse et al., 2018). A paleontological evidence has confirmed that corals and Symbiodiniaceae emerged since the mid-Triassic period (Jingfang et al., 2017; Wang et al., 2020). The relationship between corals and zooxanthellae is mutualistic, that is, both partners benefit from the association (Harrison & Wallace, 1990; Lajeunesse & Trench, 2000). Coral colonies are built from calcium carbonate and can be maintained to protect zooxanthellae cells from predators. Coral provide an importance sources such as CO_2, phosphorus, and sulfur, which will be used in the photosynthesis by zooxanthellae (Veron, 2011). On the one hand, this symbiont contributes up to 50-95% of nutrient and organic compounds to supply coral activity through photosynthesis (Muscatine, 1990; Fabricius and Klumpp, 1995). Therefore, symbiosis between corals and endosymbionts is essential for the development and survival of coral reefs (Barton et al., 2017; Wilkinson et al., 2015). The average size of zooxanthellae cell ranges from 6 μm to 11 μm. However, the zooxanthellae cell size depends on the zooxanthellae species (Biquand et al., 2017). These small algal cells live in the host gastrodermis, which provides an optimum photosynthesis condition to zooxanthellae (Stambler, 2010). However, zooxanthellae cells can live and remain viable in the water column as a phytoplankton (free-living) when they are released by their hosts (Pasaribu et al., 2015; Pochon et al., 2012).

The coloration of corals is assumed to be produce by fluorescence proteins in the coral tissue and pigments from zooxanthellae (i.e., chlorophyll *a*, peridinin, and chlorophyll c_2) (Dove et al., 2001; Frade et al., 2008). However, several possibilities can cause the variability of coral color morphs (Quick et al., 2018). Different color morphs are frequently observed in coral reefs, with the changes in coral pigmentation caused by biotic and abiotic factors, such as the presence of different dominant groups of microbiomes (various pigment type), pigment concentration/symbiont density, and variable environment conditions (Jarett et al., 2017; Scheufen, Iglesias-Prieto, et al., 2017). Different coral species contain varied densities of zooxanthellae cells (Pillay et al., 2005). Most branching corals exhibit a lower number of zooxanthellae cells compared with other coral forms possibly due to the varied capacity for light absorption of each coral (A_{max}) (Scheufen, Krämer, et al., 2017). In addition, not all corals are associated with zooxanthellae. *Tubasstrea* spp. are considered ahermatypic/azooxanthellae corals, similar to most species of deep-sea corals, due to their limited light factor (Fosså et al., 2002; Precht et al., 2014). The different acquisitions of zooxanthellae cells in scleractinian corals depend on the reproductive mode (Hirose & Hidaka, 2006; Kayanne, 2016). The offspring of most broadcaster spawner corals acquire zooxanthellae from the ambient environment after fertilization (horizontal zooxanthellae transmission), whereas the offspring of brooder corals inherit algae from their parents (vertical zooxanthellae transmission) (Baird & Guest, 2009; Hirose et al., 2008).

1.3.2 Diversity of Symbiodiniaceae

Stony corals possess symbiotic relationships with a genetically diverse range of zooxanthellae, which have been attributed to coping with environmental stressors (Rouze et al., 2019; van Oppen et al., 2005). One genus (*Symbiodinium*) and nine clades (A–I) of zooxanthellae were identified based on nuclear ribosomal DNA and internal transcribed spacer region (ITS) (Baker, 2003; Pochon et al., 2007; Stat et al., 2011). However, the revised systematics of zooxanthellae were recently defined based on the new evidence of molecular analysis. Seven genera of zooxanthellae, including *Symbiodinium*, *Breviolum*, *Cladocopium*, *Durusdinium*, *Effrenium*, *Fugacium*, and *Greakladium* (formerly clades A, B, C, D, E, F, and G, respectively), were described, but other novel genera and species have not been yet classified (LaJeunesse et al., 2018) (Fig. 1.4).

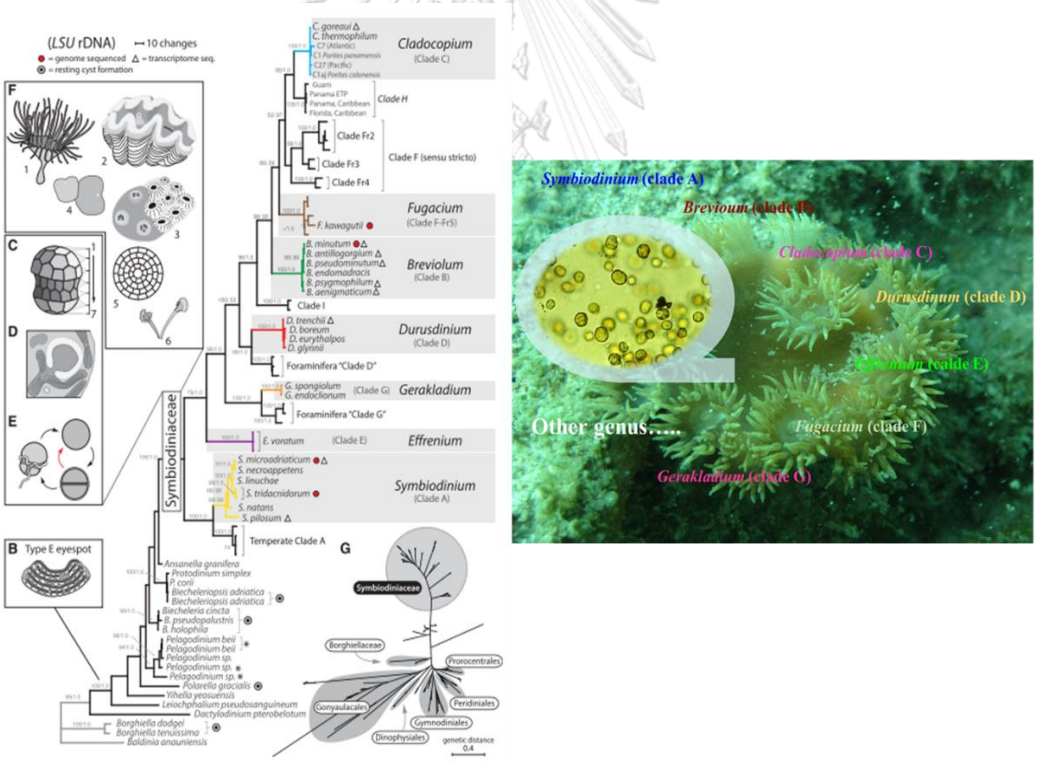

Figure 1.4 Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts LaJeunesse et al. (2018).

Different patterns of coral association with a genus/type of Symbiodiniaceae occur across the local scale to a wide geographic zone (Rodriguez-Lanetty et al., 2001; van Oppen et al., 2005). The community of Symbiodiniaceae between the Atlantic and Pacific oceans showed differences, in which *Cladocopium* was determined a common genus in the Indo-Pacific (Baker, 2003; LaJeunesse, 2005), whereas the Caribbean corals were associated with *Symbiodinium* and *Breviolum* (LaJeunesse, 2002). Distinct Symbiodiniaceae genera/types perform different responses when experiencing environmental changes (Ayalon et al., 2021; Liang et al., 2021). *Durusdinium* is one of the most tolerant to environmental changes and frequently associated with hosts living at /shallow reefs, where high temperature, light, and turbidity are present (LaJeunesse et al., 2010; Wham et al., 2017). *Brevilolum* is commonly distributed around higher-latitude reefs due to the adaptation after experiences of cold temperature (Silverstein et al., 2011). *Cladocopium* contains the highest number of clades/types and is wildly associated with corals across biogeographic regions (LaJeunesse, 2005). However, several studies have affirmed that this genus, excluding C15 and C55, is susceptible to environmental changes (Pootakham et al., 2018; Tan et al., 2020). On the other hand, *Cladocopium* translocates large amounts of nutrients and organic compounds to the host through photosynthesis compared with other genera (Cantin et al., 2009). In addition, community variation in Symbiodiniaceae in coral hosts may be driven by several factors, such as the local environmental condition, coral reproduction background, and stage of coral development (Hirose et al., 2008; Thornhill et al., 2014).

Scleractinian corals can be classified into two groups based on their specific association with Symbiodiniaceae: 1) generalists (corals harbored with distinct Symbiodiniaceae genus/type across a geography/region) and 2) specialists (corals harbored with particular Symbiodiniaceae genus/type) (Putnam et al., 2012). The brooder coral *Porites lutea* is one of the narrowest Symbiodiniaceae flexibility (specialist) across regions, including in Thailand. Almost 100% of *P. lutea* associated *Cladocopium* (C15), which are considered thermally tolerant types (Qin et al., 2019; Tan et al., 2020) . Corals maintaining a specific Symbiodiniaceae may have a beneficial response coral to uncertain environment (Qin et al., 2019; Rouze et al., 2019). By

contrast, most broadcast spawner corals acquire symbiont cells from environment that are more likely to be a generalist, such as *Acropora*, *Platygyra*, and some *Pocillopora* corals (Baker, 2003; LaJeunesse et al., 2010). However, a group of generalist corals is contented by these Symbiodiniaceae type under non-stress condition but is not helpful to the host under environmental stress (Putnam et al., 2012). Although coral-Symbiodiniaceae relationship has been studied in various countries, but a limited number of investigations were reported in Thailand (Chankong et al., 2020; Pootakham et al., 2021). According to the Department of Marine and Coastal Resources (DMCR) database, GoT and Andaman sea comprised of up to 380 species coral species whereas only seven species have been investigated on their Symbiodiniaceae association (Table 1.1).

1.4 Status of coral reef

Coral reefs are species-rich habitats with a high primary production (Blackall et al., 2015; Veron, 2011). They act as a home and nursery ground for numerous economically important marine species, protect coastlines from erosion, and provide food sources and income to millions of people living along coastlines (Harrison & Wallace, 1990; Hoegh-Guldberg, 1999). Over the last decade, significant losses and changes in marine biodiversity in coral reef ecosystems have been caused by anthropogenic activities and natural phenomena (Wilkinson, 2004). Such events include overfishing, destructive fishing practices, coastal development, non-indigenous species introduction, unsustainable tourism, climate change, ocean acidification, and tsunami (Chavanich et al., 2009; Chavanich et al., 2008). Elevated temperatures are the main contributing factor in mass coral bleaching events, which leads to widespread coral mortality (Chavanich et al., 2012; Hoegh-Guldberg et al., 2008). The increase of approximately 1.5 °C in temperature resulted in the destabilization of symbiosis between algae and corals, causing the expulsion of zooxanthellae (Baird et al., 2009; Brown, 1997; Thinesh et al., 2019). Studies on the coral cover loss in 1985–2012 included 214 reefs around the Great Barrier Reef, with 50.7% of corals declining from the initial coral cover and 3.38% year^{-1} mortality (De'ath et al., 2012).

Acropora spp. are common genera distributed in the Pacific and Atlantic oceans (Bottjer, 1980). Although *Acropora* corals provide complex habitats to marine organisms, several studies have reported that these corals are the most susceptible to bleaching (Hoogenboom et al., 2017; Kramer et al., 2012). By contrast, other research revealed a different trend; *Porites* spp. were used to be called "winners" because of their resistance potential to thermal stress (Edmunds et al., 2012) but after consecutive severe bleaching, these corals became "losers" (Grottoli et al., 2014; Putchim et al., 2017). However, the recent related information on coral adaptation under climate change has shown the opposite trend compared with that of previous studies which *Acropora* spp. have been reported to be more resistant during high thermal stress in some area. Thus, more observations and investigations at the localized scale are needed.

The coral reef status in Thailand varies among sites due to local environment conditions, such as monsoon, coral diversity, mass bleaching experience, etc. (Heery et al., 2018; Pengsakun et al., 2019). Different oceanographic backgrounds between Andaman sea and GoT showed varied coral distributions (Phongsuwan et al., 2013). Corals in Andaman live at a great depth possibly due to the sea water transparency while corals in GoT facing higher turbidity. The upper GoT is connected to four dominant rivers that can influence the river flow and sedimentation around the coastal area (Yeemin et al., 2009). Sedimentation is a significant factor for coral growth and survival because it is directly related with the photosynthesis process of coral symbionts (Junjie et al., 2014; Tuttle et al., 2020). Therefore, coral communities in this particular area, such as *Porites lutea*, *Turbinaria* spp., *Platygyra* spp., and *Acropora* spp., are the most stress-resistant corals (Morgan et al., 2017; Ng & Ang, 2016). Similar to other reefs, coral populations in Thai waters revealed a decrease in coral cover after severe bleaching events (1998 and 2010) (Chavanich et al., 2009; Yucharoen et al., 2020). Therefore, certain management tools and strategies can be employed to enhance coral reef resilience and reduce the decline in coral reefs (Omori, 2019). In general, coral asexual reproduction technique is widely applied for reef restoration and

rehabilitation given its low cost and fast preparation process (Omori, 2019; Omori et al., 2016). However, this technique strongly requires different donor colonies; otherwise, similar donors may result in coral inbreeding and/or mortality due to the low genetic diversity of the host (Poudel et al., 2014; Zayasu et al., 2018). Another active technique for increasing the abundance of coral populations is cultivation using sexual reproduction (eggs and sperms are fertilized) followed by transplantation of juvenile corals into degraded reefs (Nakamura et al., 2011; Omori, 2011). In Thailand, this method is initiated by the Reef Biology Research Group (Department of Marine Science, Faculty of Science, Chulalongkorn University) in collaboration with the Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, the Royal Thai Navy (the Naval Special Warfare Command), and Akajima Marine Science Laboratory in Japan. This technique has been successfully used in the production of several coral species, such as *Acropora* spp., *Platygyra* spp., and *Lobophyllia* sp. The survival of cultured corals after transplantation was up to 60%, with approximate growth rates of 2 and 5 cm/year for massive and branching corals, respectively (unpublished data).

1.5 Coral adaptation/Coral reef resilience

Reef-building corals must exhibit a flexibility mechanism to live and survive under uncertain environment conditions (Jones & Berkelmans, 2010; Ziegler et al., 2018). The expulsion of zooxanthellae cells is a simple mechanism employed by corals to maintain their intracellular system during high temperature and irradiance (Fujise et al., 2014; Kuroki & Van Woesik, 1999). The decrease in zooxanthellae density in coral tissue may prevent cell damage due to the reduction of reactive oxygen species during photosynthesis by zooxanthellae (Gardner et al., 2017).

Table 1.1 Previous studied of Symbiodiniaceae diversity in Thailand.

Species	Symbiodiniaceae genus	Symbiodiniaceae type	Reference	Remark
Pavona decussata	*Cladocopium, Durusdinium*	D17, D6, C116	Pootakham et.al., 2021	NGS
Pavona frondifera	*Cladocopium, Durusdinium*	D17*, D6, C117	Pootakham et.al., 2021	NGS
Platygyra daedalea	*Cladocopium*	C3u	Chankong et.al., 2020	DGGE
Platygyra sinensis	*Cladocopium, Durusdinium*	D17*, D6, C118	Pootakham et.al., 2021	NGS
Pocillopora damicornis	*Cladocopium, Durusdinium*	D1-6, D17*, D6, C119	Chankong et.al., 2020 and Pootakham et.al., 2021	DGGE&NGS
Porites lutea	*Cladocopium*	C116, C15, C15f, others C	Chankong et.al., 2020 and Pootakham et.al., 2021	DGGE&NGS
Sinularia sp.	*Cladocopium, Durusdinium*	-	Panithanarak et.al., 2014	Sanger

(* Dominant types)

Moreover, the genetic diversity present within the Symbiodiniaceae genus may provide potential for dynamic ecological strategies of corals (Brener-Raffalli et al., 2018; Claar et al., 2020). Two potential adaptive mechanisms, namely, “ shuffling” (change in Symbiodiniaceae community composition) and “switching” (uptake of exogenous Symbiodiniaceae type from natural sources), were established in corals (Baker, 2003; Fautin & Buddemeier, 2004). Symbiont shuffling or switching consistently occurs when corals encounter bleaching phenomenon as described in the adaptive bleaching hypothesis (Kemp et al., 2014; Keshavmurthy et al., 2014). Symbiont shuffling is more frequently observed in wild coral colonies during and after bleaching than switching (Hume et al., 2015; Quigley et al., 2019). Bleached corals may become more resistant to bleaching by increasing abundance of thermally tolerant symbiont (Császár et al., 2010; Cunning, Yost, et al., 2015).

Several studies have shown that most of hermatypic corals do not change the Symbiodinium type but only the proportion of intraspecific types (Baker, 2003; Goulet, 2006). Studies conducted on several scleractinian corals at Dongsha island, Taiwan reported that corals in shallow waters exhibited a significantly higher proportion of *Durusdinium* (the most thermally resistant type known) compared with depth during thermal stress (Keshavmurthy et al., 2017). Similarly, *Orbicella faveolata* coral at high-latitude reefs was associated with different proportions of Symbiodiniaceae type before, during, and after recovery from bleaching. However, no new type of Symbiodiniaceae has been detected over the years (Kemp et al., 2014). In addition, the genetic diversity of Symbiodiniaceae genus/type present within a single coral colony may lead to a rapid selective mechanism of tolerant partnerships in response to environmental changes (Baker, 2003; Jones et al., 2008). Although, a miscellaneous number of Symbiodiniaceae types was found in environment sources, such as sediment and water column, several corals exhibited no uptake of new tolerant types during environmental stress condition (Goulet, 2006; Shinzato et al., 2018; Sweet, 2014). Several possible hypotheses can be used to explain this phenomenon, that is, the prevention of inter- and intraspecificity among Symbiodiniaceae types which

maintains the cellular mechanism activity and adaptation experience selected (Klepac et al., 2015; Putnam et al., 2012).

The ability of corals to recover from loss, bleaching, and/or recruitment is an important indicator of reef resilience. Several factors influence the success of this process; these factors include the water quality, outbreak of crown of thorn, coral species diversity, and local environment conditions (Bang et al., 2021; MacNeil et al., 2019). The use of the benthic model reef community to predict the potential of coral resilience revealed that ocean acidification and warming condition can cause the low calcification rate and mortality of corals (Anthony et al., 2011; McCulloch et al., 2012). Low coral reef resilience occurs when the reef consists of mono-dominant coral species (Rinkevich, 2019). Genetically diverse of corals and Symbiodiniaceae are and important keys for coral reef resilience. The differences in their genotypes or gene expressions provide varied individual tolerances to response to environmental stress (Sakai et al., 2019). *Acropora* spp. or staghorn coral is the most susceptible coral to bleaching. Both factors, including *Acropora* morphology and Symbiodiniaceae-type association, may drive the strong effect of coral bleaching and mortality (Cooper et al., 2011; Hoogenboom et al., 2017). However, the offspring of this coral obtain endosymbionts, several of which are associated to a variety of Symbiodiniaceae types, from the environmental pool (Yorifuji et al., 2017). Recently, Several studies indicated that *Acropora* corals showed high resistance to heat stress, suggesting that the benefit of coral association with genetically diverse Symbiodiniaceae may allow corals to be selected and survive environmental stress (Jones & Berkelmans, 2010; Putchim et al., 2017), or possibly, corals with historical experience to an environment stressor can manifest their adaptation (Sully et al., 2019). By contrast, several corals maintaining a specific Symbiodiniaceae organism may have a beneficial coral response to uncertain environment that is mostly found in brooder corals (Qin et al., 2019; Rouze et al., 2017). However, the lack of adaptive capacity of corals may result in reef ecological collapse in the near future (Coles & Riegl, 2013; Mora et al., 2016).

1.6 Rational and Objectives

Significant losses and changes in coral reef ecosystems have occurred due to anthropogenic activities and natural phenomena. The study of coral biology, especially coral reproductive and coral-Symbiodiniaceae association, is important to understand the future survival of corals in response to climate change. Although Thai waters consists of up to 380 species of scleractinian corals, little is known about the variability and dynamics between Symbiodiniaceae and their coral host. To fill this gap, studies should focus more on the coral species from different conditions. This research had four main objectives: 1) increasing the database of *Acropora* spp. spawning and Symbiodiniaceae diversity in the upper GoT 2) ontogenetic investigation of coral-Symbiodiniaceae association in *Acropora humilis* and *Pocillopora damicornis* under rearing hatchery condition (*ex situ*); 3) seasonal investigation of coral-Symbiodiniaceae community in *Acropora humilis* and *Pocillopora damicornis* (*in situ*) and 4) evaluation the potential of coral adaptation under environmental change (*in situ* and *ex situ*). The evidence from these studies are important to identify coral-Symbiodiniaceae associations and can be used to better understand localized coral reef adaptive potentials under different environmental conditions. In addition, understanding coral and Symbiodiniaceae relationship during coral development can improve our knowledge on coral cultivation technique. To access all of this information, we used conventional and developed methods together with statistical analysis to evaluate the data of each experiment.

CHAPTER 2

Sporadic asynchronous spawning in two *Acropora* species in the upper Gulf of Thailand

Introduction

It is estimated that the majority of known scleractinian corals are simultaneous hermaphrodite broadcasters, releasing both eggs and sperm into the water column for external fertilization (Baird & Guest, 2009; Harrison & Wallace, 1990; Harrison, 2011). Typically, this involves mass spawning events, which is thought to promote successful fertilization (Olive et al., 2000) and increased survival due to over-saturation of prey (Westneat & Resing, 1988). Mass Spawning events can involve over 100 species of corals in a given reef spawning in a single night (Babcock et al., 1986). It has been shown that most broadcasting coral species spawn once a year as opposed to the two (or more) spawning peaks seen in some corals (Baird et al., 2015; Harrison & Wallace, 1990). These events require synchrony across multiple colonies of a given species to be successful, however a wide array of conditions and cues have been linked to the synchrony of such events (Mendes & Woodley, 2002; Penland et al., 2004; Van Woesik, 2009; Van Woesik et al., 2006) most predominantly the role of the lunar cycle (Babcock et al., 1986; Gorbunov & Falkowski, 2002) and temperature (Keith et al., 2016; Kojis, 1986)

Temperature cues in combination with solar irradiance, are considered to contribute significantly to spawning timing over seasonal cycles (Keith et al., 2016; Penland et al., 2004). Temperature, specifically the relatively rapid increase from 27-30^{o}C, has been theorised to be the greatest influencing factor for Acroporid spawning synchrony in the Gulf of Mannar, India (Raj & Patterson, 2010). However, in recent decades, the significance of seasonal thermal changes in coral reefs has shifted focus towards monitoring the now regular occurrences of above average ocean temperature. Temperatures elevated beyond ambient conditions have been linked to reduced larval survival in numerous studies (Bassim & Sammarco, 2003; Nozawa & Harrison, 2007;

Wilson & Harrison, 1997). Elevated temperatures have also been shown to influence spawning time by causing earlier planula release in brooding *Pocillopora damicornis* corals (Crowder et al., 2014).

Studies on larval settlement have indicated that elevated temperatures initially facilitate higher settlement rates in larvae of *Acropora solitaryensis* and *Favites chinensis*, however this is quickly followed by higher post-larval mortality, resulting in a net reduction in survival in coral offspring (Nozawa & Harrison, 2007). A 1.5°C increase temperature has also been shown to result in a 5 folds decrease in larval survival in the Caribbean coral *Acropora palmata* (Randall & Szmant, 2009). Thermal stress due to elevated temperatures and excessive solar irradiance are known to be key contributing factors in mass coral bleaching events, which in turn have been linked to reduced gamete development prior to spawning (Fine et al., 2001; Lesser & Farrell, 2004; Szmant & Gassman, 1990).

While solar insolation cycles have been considered a key factor in determining spawning synchrony for both month of the year and time of the day (Brady et al., 2009; Penland et al., 2004), the lunar cycle is thought to be a major influence in synchrony for date (typically in the form of days after full moon/new moon) (Baird & Guest, 2009; Guest et al., 2005; Willis et al., 1985). Recently, Foster et al. (2018) documented periodic split spawning events at the Great Barrier Reef (GBR) during spawning seasons where the full moon fell upon the last week month prior, or the first week of the main 'spawning month'. When however, the full moon fell upon the middle of the month, a singular spawning period occurred within the typical month.

Though the lunar cycle determines spawning day in a given calendar month, the determination of spawning season does not follow the synodic lunar cycle precisely, due to the discrepancy between seasonal influences and number of lunar months in a given year. Over the years, multiple theories have been proposed regarding the relationship between lunar cycles and spawning events, such as the annual realignment theory as mentioned in Baird and Guest (2009) and the more recent coincidence of factors between synodic and sidereal lunar declination cycles

(Wolstenholme et al., 2018). Studies have revealed the dependency of illumination cycles on gene expression in the coral *Acropora millepora* (Brady et al., 2011; Vize, 2009). A recent study showing that replacing more typical lunar-like cycles of illumination with those of constant new moon or full moon cause almost complete disruption in expression of numerous genes in *A. millepora* (Brady et al., 2016)

As is the trend in the Scleractinia, the vast majority of the Acroporidae are known to be hermaphroditic broadcasters, with no known gonochoric species and only 6 brooding species (Baird & Guest, 2009). Acroporid species have been shown across the tropics to spawn with varying degrees of synchrony across the genus, such as with synchrony among multiple species in a given month to split spawning between particular species groups across multiple months (Baird et al., 2002; Bouwmeester et al., 2011; Carroll et al., 2006; Lin & Nozawa, 2017) Gametogenic development and synchronous spawning has been recorded for *A. humilis* and *A. millepora* in particular from multiple locations in the Indo-Pacific (Baird et al., 2002; Baird et al., 2010; Chelliah et al., 2015; Jamodiong et al., 2018).

Gilmour et al. (2016) however, documented asynchrony in seasonality between both species, with *A. millepora* spawning exclusively during the spring and *A. humilis* spawning exclusively in autumn. Spawning events over consecutive months (split spawning) events have also been documented for *A. humilis* (Foster et al., 2018), further supporting that variation in spawning synchrony is less influenced by species or genus, but rather variations in key environmental cues combined with lunar and solar cycles from year to year. Nozawa (2012) reported strong inter-annual variation in two *Acropora* species, ranging over 7 days in the optimal spawning period over the 4 year study. Reduced fertilization success has been shown for *Acropora* gametes after 7-8 hours post spawning (Omori, 2011; Willis et al., 1997) which highlights the importance of synchronous spawning events in maximising population growth.

Synchrony has been suggested to require large environmental fluctuations, the lack of which may cause reduced synchrony (Richmond & Hunter, 1990). However subsequent circumtropical observations have revealed extensive variability in patterns

of synchrony. Split spawning has been observed in multiple genera and at numerous locations (Foster et al., 2018) and has been known in Acroporid corals for over three decades (Wallace, 1985). Sequential spawning over periods of multiple months has also been documented from certain reefs where synchrony in diminished (Fogarty et al., 2012; Mangubhai & Harrison, 2008).

Lack of synchrony in spawning events has be shown to drastically reduce gene exchange between colonies (Levitan et al., 2011; Rosser, 2016) and therefore act as a driver for genetic divergence (Bird et al., 2011; Dai et al., 2000; Rosser, 2015; Rosser, 2016). Divergences by as little as 1-3 hours in spawning time has been shown to lead to independent genetic clades in congeners (Fukami et al., 2003).

Thailand is considered an under-represented region with regards to data on spawning, with sparse observations and studied carried out in the region (Kongjandtre et al., 2010; Piromvaragorn et al., 2006). A few studied of Acroporidae spawning in Thailand indicated that there is two seasons for *Acropora* spp. spawning depends on location. Putchim et al. (2006) found that Acropora corals around Ko Kram, upper Gulf of Thailand spawning occurred in February – March during neap tide while Acropora in Andaman sea matured in September and releasing the gamete around September – October each year (Piromvaragorn et al., 2006).

However, the lack information about coral spawning in Thailand and there is no study was done for long term monitoring of Acropora spp. spawning before. In this study, we investigated gamete development and spawning periods of two Acropora species (*Acropora humilis and Acropora millepora*) at Ko Tao Mo in the upper gulf of Thailand from 2006-2018.

Materials and Methods

Colonies of *Acropora humilis* and *Acropora millepora* were monitored at Ko Tao Mo at Sattahip, Chonburi Province, Thailand (Fig. 2.1). The dominant genus of coral Ko Tao Mo is *Acropora* (Fig. 2.2), which has long been documented to spawn between

the months of January and February annually. Date, time, and other spawning data was recorded for numerous monitored colonies at the site from the years 2006 – 2018 (except 2009 where no data was collected). All monitored colonies at the study site were at a depth of 4-5 meters. A total of 50 colonies were tagged and assessed for gamete development for the months leading up to the spawning season. The colony size ranged from 30 to 100 cm in diameter. Gamete development was monitored based on standard visual assessment of pigmentation in gametes with white (unpigmented) indicating early stages, pale-yellow indicating mid-stages and finally pink with sperm sacs indicating maturity and imminent release. Upon assessments revealing colonies with pink gametes, SCUBA surveys were undertaken daily from 5 PM – 9PM a day after the first new moon or full moon, until early February. Duration of spawning events were timed from the first signs of gametes prior to release till the majority gametes had been released in the last colony. All of spawning data were recorded such as time of setting, spawning and finishing time for each coral species. HOBO data loggers (UA-002 Onset HOBO) were installed at the same depth and location as the coral colonies to measure the variation of temperature during the coral spawning period between 2013-2018. The Fisher exact test was used to investigate the association on spawning of one species to the other. To investigate the significance of temperature on spawning date, the Kruskal-Wallis chi-squared test was used investigating the significance of spawning events as a function of temperature. The test was applied to spawning of both species together, as well as each species separately.

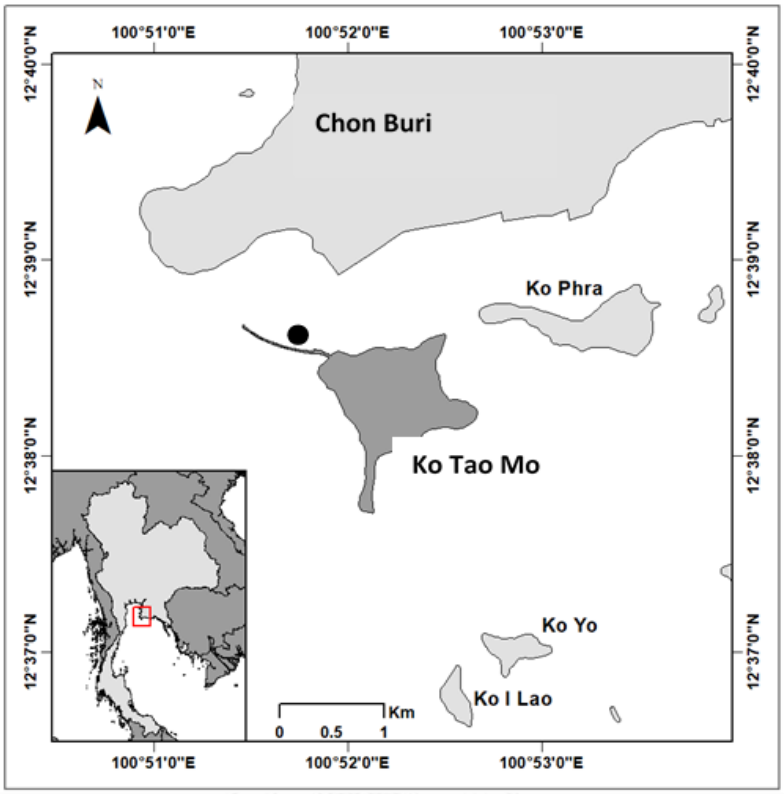

Figure 2.1 Study site at Ko Tao Mo, Sattahip, Chonburi Province ,where coral spawning was monitored from 2006 – 2018.

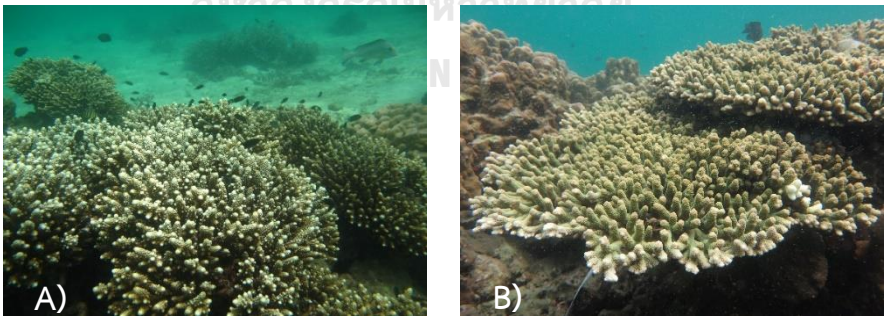

Figure 2.2 Two dominant species of *Acropora* coral around Ko Tao Mo, Upper Gulf of Thailand ;A) *Acropora humilis* B) *Acropora millepora.*

Results

Gamete development

Observation of coral gamete development showed that gametes for *Acropora humilis* and *Acropora millepora* take between 90 - 120 days from initiation of observed gametes till release. It was observed that *A. millepora* gametes proceeded towards maturity more quickly than *A. humilis*, with approximately 40% of colonies of *A. millepora* achieving pigmentation at both intermediate and mature stages (Fig. 2.3) between 14 - 28 days before *A. humilis* colonies. Coral oocytes were first observed around October in both species every year. The early stage of oocyte development was showed the small egg with no pigmented (white) while the intermediate stage contained of yellow eggs around November-December and the matured stage with pigmentation was exhibited around mid of January (Fig 2.4). When coral reached the matured stage, pink oocyte and sperm sac were appeared inside of the polyps. However, in this particular area contained of diverse species of *Acropora* corals including *A. hyacinthus*, *A. formosa*, *A. nusuta and A. digitifera* etc. but the gametogenic cycle was quite different from these two species.

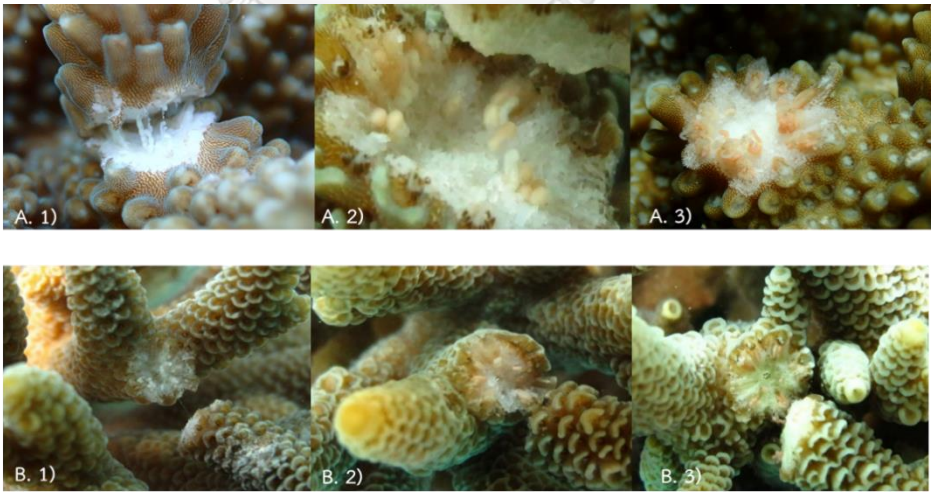

Figure 2.3 Gamete development of staghorn coral ;A) *Acropora humilis* and B) *Acropora millepora* at Ko Tao Mo. Number of 1-3 is represent of gamete development stage followed by early, intermediate and mature stage.

Lunar Asynchrony

Spawning occurred during both neap and spring tides across all lunar periods with no clear indication of lunar-associated cue. Typically, spawning was observed between 2-9 days after the full moon and 3-13 days after the new moon with peak spawning periods at 7 days after the full moon and 10 days after the new moon (Fig 2.5). It was sometimes observed that some colonies of both species (usually < 60 cm diameter in size) may spawn more than once, on account of irregular and asynchronous development of gametes across the colony. Setting of gametes of both species was usually observed around 7 PM. Mean duration between initial setting and first spawning over the years for both species was 69 minutes (60 mins and 78 mins for *A. humilis* and *A. millepora* respectively) (Fig 2.6).

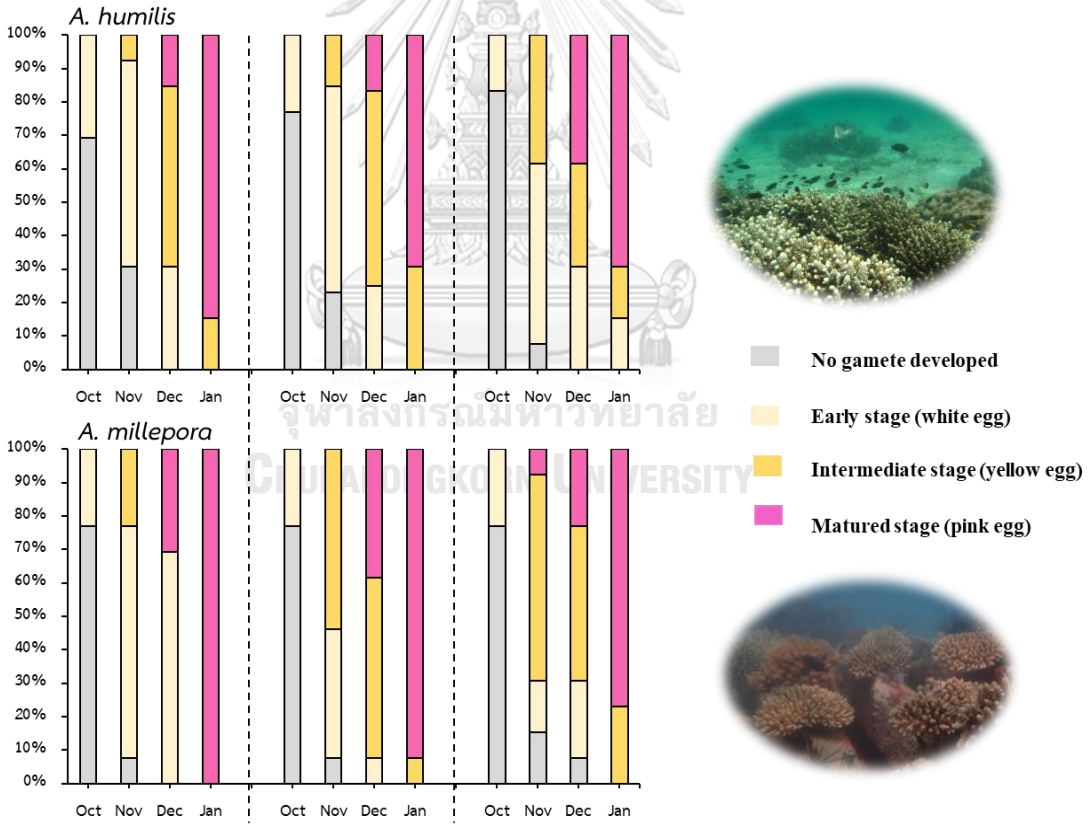

Figure 2.4 The proportional of gamete development in *Acropora humilis* and *Acropora millepora.*

Mean duration between first observed spawning colony and final release of gametes was 35 minutes for both species *(*36 mins and 33 mins for *A. humilis* and *A. millepora* respectively*).* Throughout the 12 year period, spawning was observed on a total of 81 days. The total spawning period across the years was an estimated 54 days, with the earliest spawning being recorded on 15th January *(A. millepora*, 2008*)* and the latest on 9th March *(A. humilis*, 2007*).* Fisher exact test revealed no significance between spawning date of one species on the other *(*p=0.15).

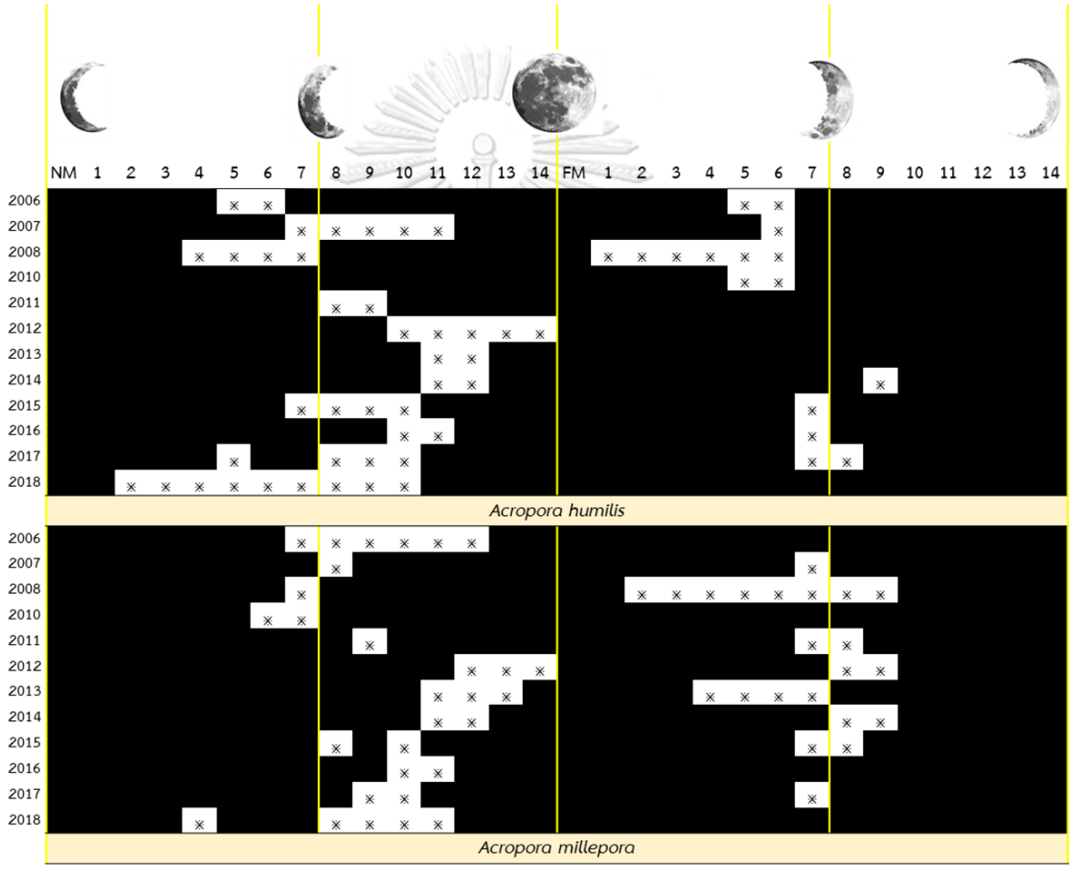

Figure 2.5 Spawning pattern according to phases of the moon at Ko Tao Mo from 2006 – 2018 (no data recorded in 2009).

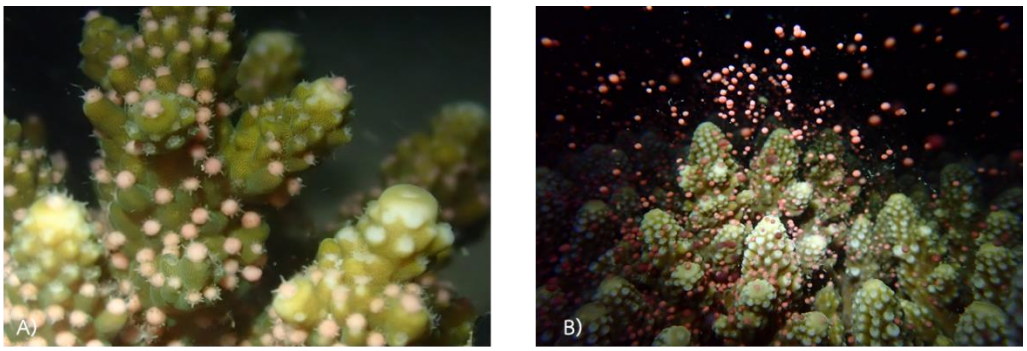

Figure 2.6 Night of coral spawning ;A) coral bundles protrude through the mouth of polyps (setting) and B) coral spawning in the water column.

Temperature

The mean water temperature at Ko Tao Mo during the spawning periods of 2013 - 2018 ranged from 24.8 – 31.1°C, with temperature on spawning days ranging from 26.2 – 29.0 °C. It was observed that the temperature recorded during the period for 2014 was considerably below the typical range of the 6 year period. Analysis using the Kruskal- Wallis chi- squared test yielded resulted in no significant association between spawning date of all spawning events and temperature ($p = 0.12$) (Fig 2.7). Similarly, no significance was observed with *Acropora millepora* and *Acropora humilis* tested separately (p=0.30 and p=0.08 respectively).

Discussion

This study provides the first *in situ* observation of *Acropora* coral spawning at Ko Tao Mo, upper GoT in over 12 years. *Acropora* spp. spawn approximately 2-3 h after sunset to avoid predators, high temperature, and irradiance (Fan et al., 2006; Forrest & Miller-Rushing, 2010). However, we observed that several fish species feed on coral bundles during coral spawning including *Abudefduf sexfasciatus, Chaetodon octofasciatus*, *Chromis* spp. and *Pomacentrus* spp. (Fig 2.8). A group of planktivorous fishes (*Abudefduf* spp.) feeds on coral gametes, especially switching the feeding diet to omnivorous during coral spawning which can cause the low fertilization rate of

corals (Westneat & Resing, 1988). Corals are most likely to spawn during neap tide to prevent gamete dilution and increase fertilization. (Mendes & Woodley, 2002; Monteiro et al., 2016). In contrast, the *Acropora* spp. observed in this study spawned during both neap and spring tides and may produce the larva supply of coral recruitment in this area and other nearby reef ecosystems (Hock et al., 2019; Keith et al., 2016; Mangubhai & Harrison, 2008).

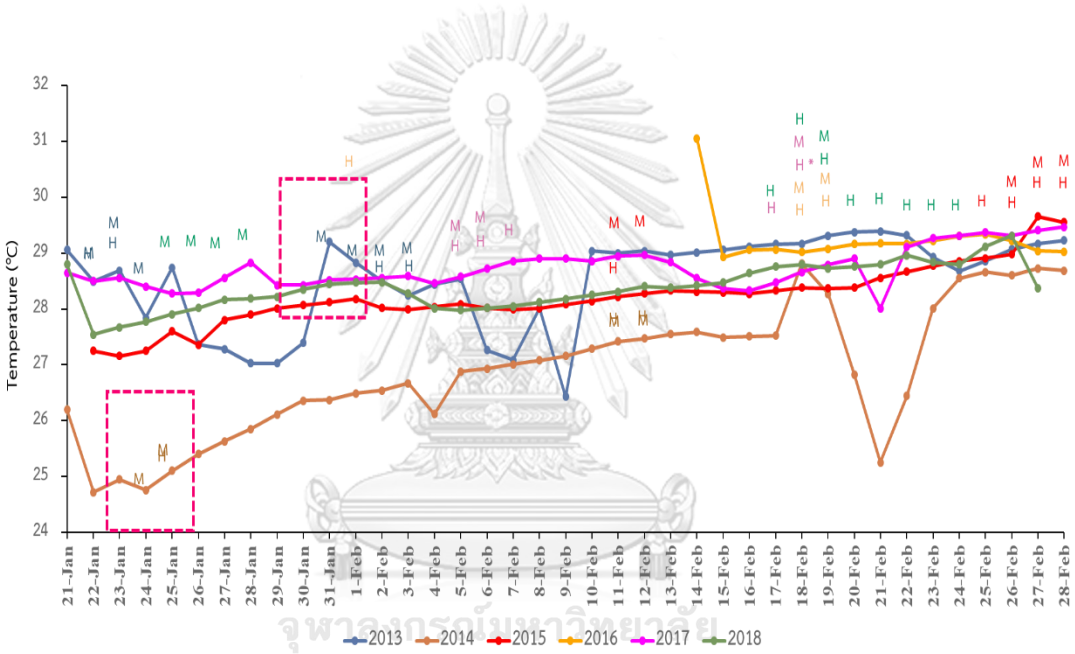

Figure 2.7 Mean water temperatures between 3-8 m at Ko Tao Mo during the spawning period of 2013 - 2018. Days of spawning for Acropora spp. overlayed (H for *Acropora humilis* and M for *Acropora millepora*) (* more spawning in March)

Several equatorial scleractinian corals, including those in this study, have been reported for asynchronous spawning (Mangubhai & Harrison, 2008; Wijayanti et al., 2019). Variable local environmental conditions may drive corals to extend their breeding season with different gametogenic cycles and spawn in more than one lunar period (Foster & Gilmour, 2020; Mangubhai & Harrison, 2008). *Acropora* spp. at Ko Tao Mo exhibited the split spawning in several years, when the gametes matured, and mass

spawning occurred in two consecutive months (Foster et al., 2018). This spawning pattern was also recorded at the Scott reef, west of the Great Barrier Reef, suggesting that this event may be driven by other factors, such as coral biological adaptation (Foster et al., 2018; Hock et al., 2019). However, new evidence showed that the larvae produced by split spawning may increase coral resilience in other reef because of the variable currents and/or conditions of each spawning period and may result in the larva dispersal area (Hock et al., 2019).

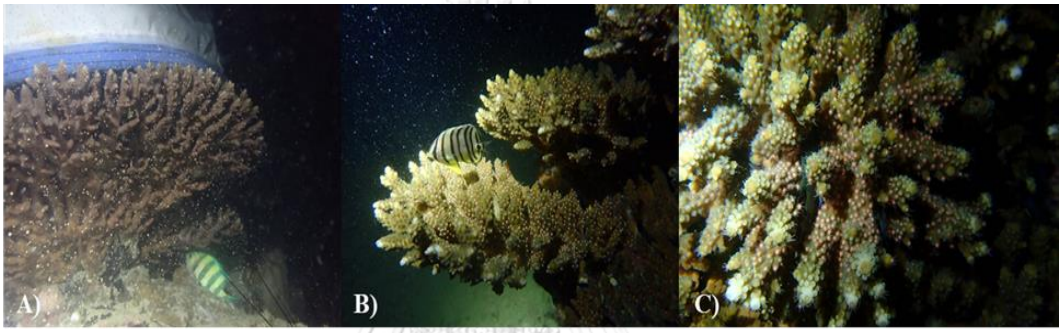

Figure 2.8 Reef fishes feeding on coral gamete ;A) *Abudefduf sexfasciatus* B) *Chaetodon octofasciatus* and C) *Pomacentrus* spp.

The spawning of *Acropora* spp. generally occurs around full moon or approximately 3-8 days after full moon (peaking at 5-7 after full moon) along latitudinal regions. For example, *A. hyacinthus* and *A. japonica* at Kochi island, Japan and *Acropora* spp. in Red sea Saudi Arabia spawn near the full moon (Bouwmeester et al., 2015; Nozawa, 2012), whereas a different pattern was observed in Kenya reefs and Ko Tao Mo, Thailand (Fig. 2.9). These locations showed the same rare pattern in which corals spawn across all lunar periods and during stress environmental conditions, such as temperature and tides (Mangubhai & Harrison, 2008). Several hypotheses can possibly explain this spawning event: (1) *Acropora* corals may rely on exogenous/environmental conditions than endogenous factors, and the trigger of stress condition may influence coral spawning in several coral species (biological rhythm); (2)

the different gametogenesis in each cycle may lead to the distinct lunar phase spawning; (3) corals may adapt to local environment conditions based on historical experience (Chelliah et al., 2015; Foster & Gilmour, 2020; Nozawa, 2012; Nozawa & Harrison, 2007); (4) differences in the observation protocol may result in varied output information.

Most healthy reefs exhibit multi-specific spawning within a single night (several coral species spawned together) (Babcock et al., 1986; Baird & Guest, 2009; Wolstenholme et al., 2018). Similarly, our study showed that at least four species of *Acropora* corals were spawned within the same night, but the time of spawning differed depending on the coral species. *Acropora millepora* spawned earlier, followed by *Acropora humilis* and two other unknown *Acropora* spp. According to the different duration times of spawning (1-3 h), these corals can be separated into two groups, namely, early (*A. millepora* and *A. humilis*) and late spawners (*Acropora* spp.) (Chan et al., 2018; Chan et al., 2019; van Oppen et al., 2001). Variation in the time of coral spawning occurs to prevent species-specific fertilization (hybridization) between coral species (Gilmour et al., 2016; Morita et al., 2019). However, the overlap of spawning times can cause coral hybridization in natural reefs (Chan et al., 2018). Hybrid corals have been reported mostly in *Acropora,* and different morphologies of *Acropora* colony were observed at the marginal reef in Caribbean Sea. Morphological and molecular evidence indicated that *Acropora prolifera* is a hybrid of *A. cervicornis* and *A. palmata* (Willis et al., 2006). Moreover, coral hybridization may occur more frequently than expected, especially in the massive reef containing the greatest diversity of coral species (Willis et al., 2006; Willis et al., 1985).

The asynchronicity of more than 7-8 h has been suggested as a possible contributing factor to the reduced fertilization success of the gametes of *Acropora* spp.(Omori et al., 2001; Willis et al., 1997). The consistent sporadic nature of spawning events at Ko Tao Mo may fundamentally reduce the population growth and recovery at the site. This finding coincides with the proposed factors in Wolstenholme et al. (2018) and annual realignment theory (Baird & Guest, 2009; Baird et al., 2002; Foster et al., 2018). Although the expression of genes pertaining to circadian rhythm and

entrained biological cycles has been investigated for *Acropora millepora*, most of the cyclic responses to environmental cues relating to spawning remain (Vize, 2009; Vize et al., 2012) suggesting the implications for genetic divergence between colonies and Sattahip and the rest of the gulf (with implication that more research is needed on spawning timings in the GoT).

Our findings suggest that unknown factors disrupt the natural cycling in spawning synchrony, as conceptualized by either the "hourglass" or "oscillator" models (Lin et al., 2013; Lin and Nozawa 2017). Additional research is required to investigate the influencing factors, as the percent of cloud cover, wind speed, and precipitation rate prior spawning season, of *Acropora* spp. spawning in this area such.

Conclusion

This study was monitored the spawning of two staghorn corals *Acropora humilis* and *Acropora millepora* which is the most abundant at Ko Tao Mo in the upper Gulf of Thailand. The spawning data was recorded from 2006-2018 (13 years). *A. humilis* and *A. millepora* spawned around January-February every year. The spawning of two species always occurred both neap and spring tides, and across all lunar periods. The spawning was observed between 2-9 days after full moon and 3-13 days after new moon. They are disconnected between temperature and spawning dates according the mean water temperature data. Our results suggest that there is currently unknown factors are disrupting the natural cycling in spawning and this asynchrony spawning is possible contributing factor to reduce fertilisation success in *A. humilis* and *A. millepora* in this area.

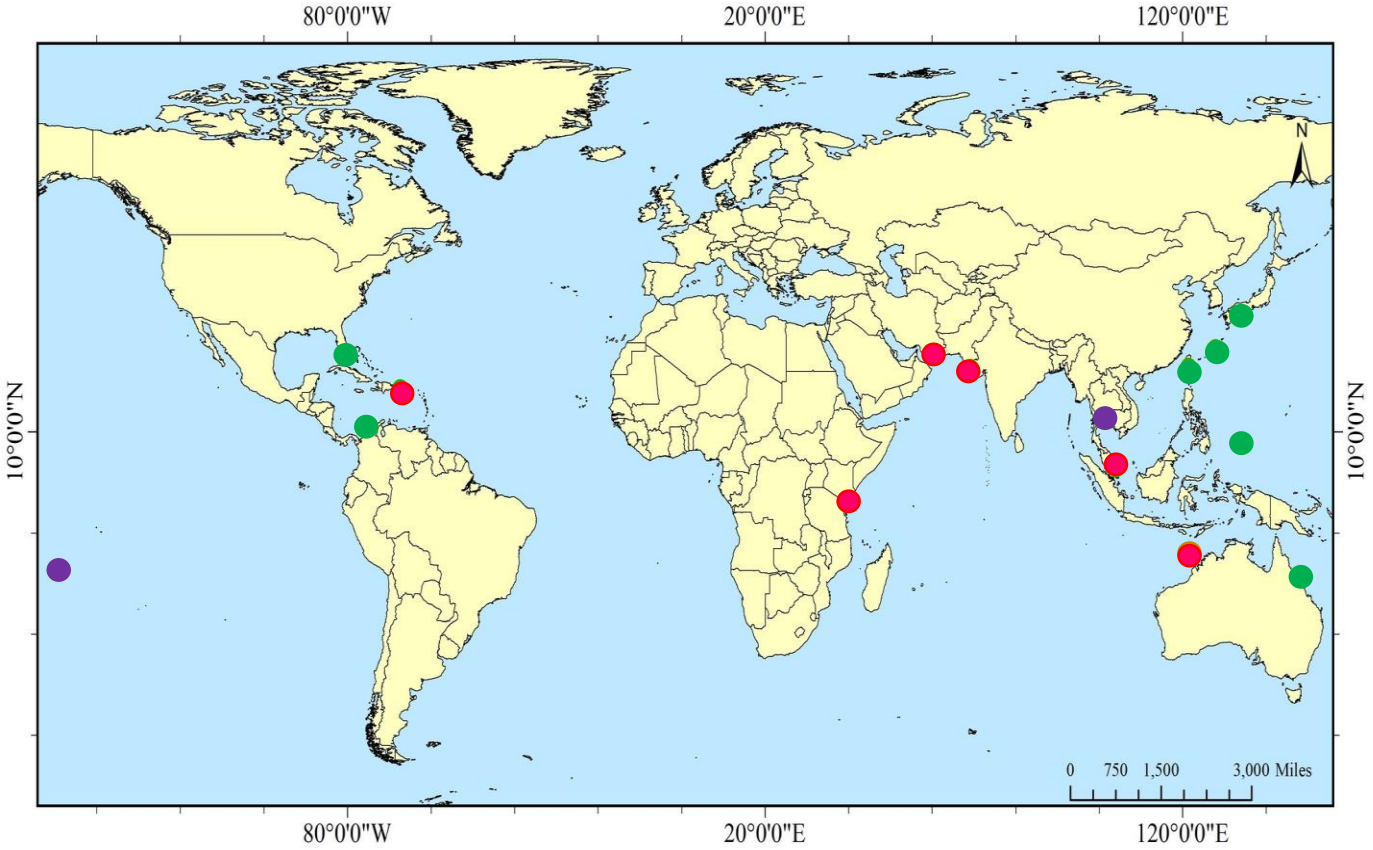

Figure 2.9 The locations of *Acropora* spp. spawning were recorded across biogeographic

;Red = during full moon, Green = days after full moon and Purple = both before and after full moon.

CHAPTER 3

Ontogeny change of Symbiodiniaceae community in *Acropora humilis* and *Pocillopora damicornis* under rearing hatchery

Introduction

The establishment of a symbiotic relationship between scleractinian coral and single–cell algae (known as zooxanthellae) in the family Symbiodiniaceae is essential for the development and survival of coral reefs (Decelle et al., 2018; LaJeunesse et al., 2018). Reef–building corals rely on their symbiotic association, especially among members of Symbiodiniaceae, which contribute up to 50%–95% of the nutrients needed by the coral host by supplying photosynthetic products (Fabricius & Klumpp, 1995; Muscatine, 1990). To date, seven new genera of zooxanthellae, namely, *Symbiodinium*, *Breviolum*, *Cladocopium*, *Durusdinium*, *Effrenium*, *Fugacium*, and *Greakladium*, have been described on the basis of morphological, physiological, ecological, and molecular evidence (LaJeunesse et al., 2018). The physiological response of corals to environmental stresses may be different among genera of Symbiodiniaceae (Berkelmans & van Oppen, 2006). For example, *Cladocopium* can produce and translocate large amounts of photosynthates to their coral hosts via photosynthesis, allowing the rapid growth of some *Acropora* spp (Cantin et al., 2009; Quigley et al., 2020). By contrast, corals that host *Cladocopium* are more susceptible to bleaching than other genera (Sampayo et al., 2008; Thinesh et al., 2019), with the exception of C15 and C15–related Symbiodiniaceae, which are thermally tolerant types (Gong et al., 2018; Putnam et al., 2012). *Durusdinium* is considered a stress–tolerant Symbiodiniaceae, most coral distributed in shallow water reef frequently exposed to high temperature, irradiance and turbidity were found associate with this genus (Claar et al., 2020; Rowan, 2004) including *Pocillopora*, *Platygyra* and *Pavona* corals in the GoT (Chankong et al., 2020; Pootakham et al., 2021). In comparison, *Pocillopora* colonies containing with *Cladocopium* (C1b-c) are bleached, whereas those harboring *Durusdinium* (D1) are not affected by environment variability (LaJeunesse et al., 2010).

The assemblage of Symbiodiniaceae in corals may be driven by several factors, including biological and physical factors (LaJeunesse et al., 2010; Stat et al., 2013). Differences in life–history strategies are correlated with the pattern of symbiont acquisition in corals (Nicholas S. Fabina et al., 2012; Stat et al., 2008). The offspring of corals exhibit the distinct modes of Symbiodiniaceae cell acquisition, namely, horizontal symbiont transmission (HT), in which the larvae and/or the primary polyps acquire symbionts from their environment, and vertical symbiont transmission (VT), in which symbiont communities are directly transferred from the parent to their offspring (Hirose et al., 2008; Reich et al., 2017). *Acropora*, *Fungia*, and *Galaxea* spp. belong to groups with HT. These corals may develop an association with several types of Symbiodiniaceae via uptake from their environment (Qin et al., 2019; Quigley et al., 2017). This characteristic implies that corals have a highly flexible relationship with members of Symbiodiniaceae. By contrast, corals that belong to groups with VT maintain a specific and stable Symbiodiniaceae community but with a low genetic diversity throughout biogeographic gradient. Examples of these corals are *Porites*, *Montipora*, and *Pocillopora* spp.(Douglas, 1998; LaJeunesse et al., 2004). *Porites lutea* is a specialist of Symbiodiniaceae (specialist), almost 100% of which is predominantly associated with *Cladocopium* type C15 (Qin et al., 2019; Tan et al., 2020). VT corals that maintain specific Symbiodiniaceae species that were inherited from their parents may rapidly adapt to warming period, whereas HT corals are timely required for adaptation (Császár et al., 2010; Rouze et al., 2019). However, a recent study found that a VT coral, *Stylophora hystrix*, exhibits both VT and HT strategies, suggesting that this species may become more resilient to environmental stresses (Quigley et al., 2018).

The diversity of members of Symbiodiniaceae supports the potential of coral flexibility under the changing climate through “switching” (uptake of exogenous Symbiodiniaceae from the environment) and “shuffling” (changes in endogenous Symbiodiniaceae community composition) mechanisms (Baker, 2003; Quigley et al., 2016). Changes in coral–Symbiodiniaceae community may occur during severe bleaching or coral development (Hirose et al., 2000; Toller et al., 2001). Corals that

establish a relationship with Symbiodiniaceae via the HT mode are allowed to uptake diverse Symbiodiniaceae genera from the environment, especially during the early settling stage (Adams et al., 2009; Yamashita et al., 2014). *Acropora gemmifera* larvae are associated with various *Cladocopium* species, whereas their settled colony changes to *Durusdinium* under *ex situ* experiment (Zhou et al., 2017). Similarly, gorgonian species newly recruited from the natural environment acquire three genera, namely, *Symbiodinium*, *Breviolum*, and *Cladocopium*, and almost all of these hosts change to *Breviolum* after 1 year, which similar to their mother colony (Coffroth et al., 2001). Although VT corals are maternally transmitted symbionts, but the juvenile colonies of these coral types reared in laboratory do not reflect the Symbiodiniaceae community of their parents (Nitschke et al., 2016). However, most of previous studies investigated coral–Symbiodiniaceae association within a short term only. Thus, long–term monitoring is needed to understand coral adaptation mechanisms.

Over the past years, dramatic losses and changes in coral reef ecosystems caused by both anthropogenic activities and natural phenomena have become urgent issues (Grottoli et al., 2014; Thinesh et al., 2019; Wilkinson, 2004). More importantly, coral bleaching is a global and natural concern. Abnormally high ocean temperatures have been frequently observed and had led to mass bleaching events (Baird et al., 2009; Chavanich et al., 2009). Various management tools and strategies can be employed to enhance coral reef resilience and reduce the decline in coral reefs (Omori, 2019; Omori & Iwao, 2014). An active coral restoration technique for increasing the abundance of corals is cultivation via sexual reproduction followed by transplantation of juvenile corals into degraded reefs. In Thailand, this method is initiated by the Reef Biology Research Group in collaboration with the Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, the Royal Thai Navy, and Akajima Marine Science Laboratory in Japan. However, limited information is known about *A. humilis* (HT) and *P. damicornis* (VT) in terms of their symbiotic relationship with Symbiodiniaceae. A firm knowledge of ontogenetic changes in the Symbiodiniaceae community of reared coral under

hatchery conditions and transplanted corals is an essential information for coral cultivation and improvement of coral conservation techniques.

Materials and Methods

Coral species and study site

This study investigated the ontogenetic change of Symbiodiniaceae community of two different coral reproductive background and symbiont transmission modes, *A. humilis* (broadcast spawner; HT) and *P. damicornis* (brooder; VT) (Fig 3.1). Study site consists of three different locations followed by Ko Tao Mo (KTM) (12° 38' 33.78" N 100° 51' 40.13" E) Samae San Island (SSI) (12° 35' 05.51" N 100° 57' 14.50" E) and Khao Ma Chao (KMC) (12° 35' 55" N 100° 56' 59" E) (Fig 3.2).

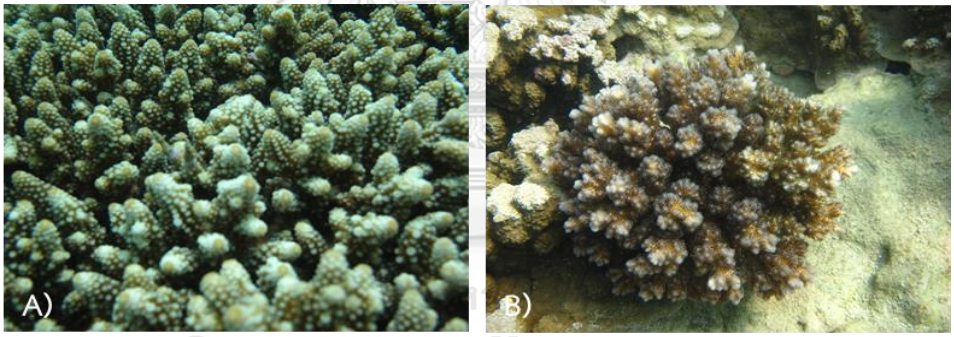

Figure 3.1 Coral colony of A) *Acropora humilis* and B) *Pocillopora damicornis*.

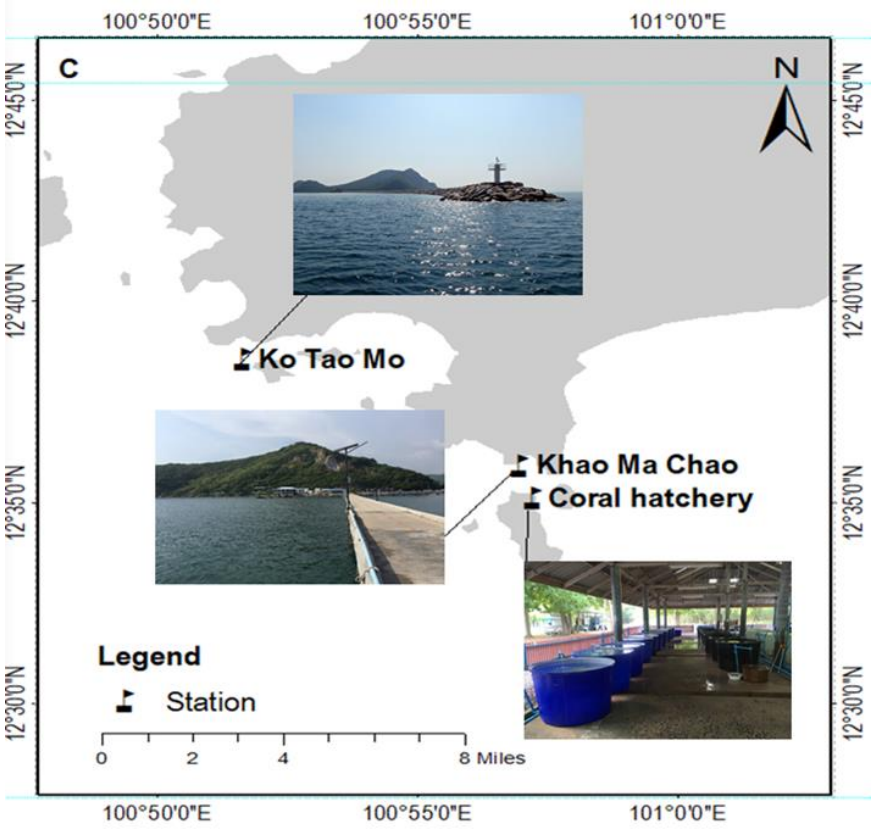

Figure 3.2 Study site and sampling locations.

(1) Coral gamete/larva collection and cultivation processes

Collection of *Acropora humilis* samples

A. humilis gametes at KTM were monitored during the gametogenic months. Twenty adult colonies of *A. humilis* approximately 30–100 cm in diameter were tagged. Their gamete development was monitored from September 2015 to February 2016. Coral fragments were carefully detached using a diving knife to evaluate their stage of gamete development. Gametes were categorized into four stages according to standard visual assessment of their pigmentation: N = no gamete has developed, W = white eggs have developed (unpigmented), Y = yellow eggs have developed, and P =

pink eggs with sperm sac have developed (mature stage). The stage of gamete that developed each month was recorded until they reached maturity (Fukami et al., 2003).

SCUBA surveys were conducted daily from 5:00–9:00 PM a day after imminent release of coral gamete was determined. On the night of coral spawn, a gamete collector was used to collect a partial gamete of spawning colonies (Fig 3.3). All gametes were kept in a separate container and directly transferred to the coral hatchery on SSI. Coral gametes were randomly sampled and preserved in 95% absolute ethanol for Symbiodiniaceae type analysis prior to fertilization. The remaining gametes of each container were pooled in one tank for coral gamete fertilization. Fertilized eggs were washed three times by using filtered seawater (200 μm mesh) to eliminate remnant sperm cells. The eggs were then transferred to a separate tank with filtered seawater. Five replications of fertilized (embryo with cell division) and unfertilized (no cell division) embryos in each experimental tank were counted after 8 h (Omori, 2019; Suzuki et al., 2013). Afterward, the percentage of fertilization was calculated. A terracotta tile with a dimension of 5 cm × 5 cm was prepared for coral larvae settlement. The rearing tanks were continuously aerated while raising the corals, except on the 4th day after fertilization (Fig 3.4). Ten replicates of the settled corals were sampled at 1-, 3-, 6-month-old, 1-, 1.5-, 2-year-old and five replications of 4- and 5-year-old (different batch of reared coral in hatchery) The corals were preserved in 95% absolute ethanol for Symbiodiniaceae community analysis. Algae that overgrew on the juvenile coral colonies were removed during the trials. Coral cultivation and maintenance during rearing were adapted from Omori (2005) and Omori and Iwao (2014). Various physical factors, including light intensity and temperature, in the rearing hatchery were recorded using HOBO data loggers (UA–002 Onset HOBO). The survival rate of juvenile corals was observed every 3 months until they reached 2 years old.

After 2 years in the coral hatchery, the reared corals that survived were translocated to another nearby natural reef on SSI. The terracotta tile encrusted with *A. humilis* colonies were attached to an artificial underwater structures at a depth of 2–4 m (Fig 3.5). 5 replications of transplanted coral at 9-month-old, 2-, 3-, 4- and 5-year-old were collected for Symbiodiniaceae community analysis.

(2) Collection of *Pocillopora damicronis* samples

In general, *P. damicronis* tends to release its larvae almost every month within 1–12 days after the new moon (Kuanui et al., 2008). SCUBA surveys were conducted to collect mature *P. damicronis* colonies at KMC, the area where the greatest number of *P. damicornis* colonies is distributed. Small fragments were detached from the coral colony (colony size < 20 cm in diameter) by using a chisel to determine their developmental stage. Once the corals reached maturity, small larvae appeared inside the fragments. Five mature colonies were brought to the coral hatchery on SSI and placed in separate tanks with aeration and filtered seawater for 24 h. Three small fragments and 10 larvae from each colony were collected and preserved in 95% absolute ethanol. The remaining larvae were transferred to another tank containing terracotta tiles for settlement (Fig. 3.6). The settled corals were raised under rearing and hatchery conditions similar to those in *A. humilis*. About three to five replications of corals at 1-, 3-, 6-, 12-, 18-, and -24-month-old were collected and preserved in 95% absolute ethanol for analysis of Symbiodiniaceae diversity.

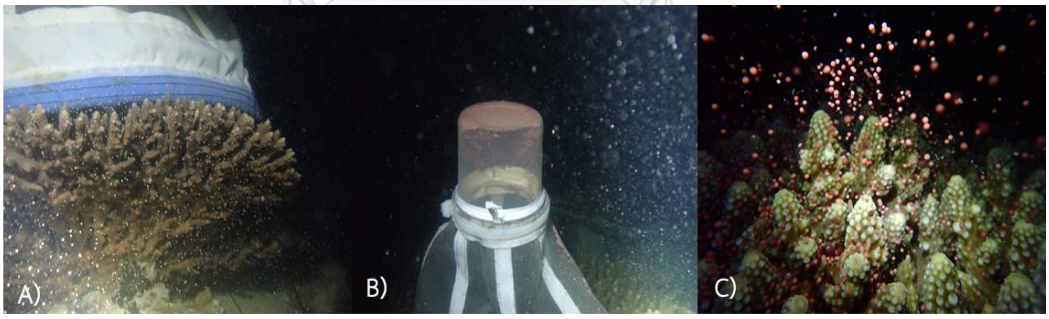

Figure 3.3 Gamete collection of *Acropora humilis* ;A) placed gamete collector on the setting colony B) coral bundles floating to the collector cup and C) coral spawning to the water column.

Figure 3.4 Coral cultivation process ;A) coral gamete from different colony B) artificial fertilization C) gamete fertilizing D) rearing of early stage coral and E) coral hatchery system.

Figure 3.5 The formation of underwater structure for coral transplantation ;A) table shape B) ladder or stair shape and C) stainless streel dome shape.

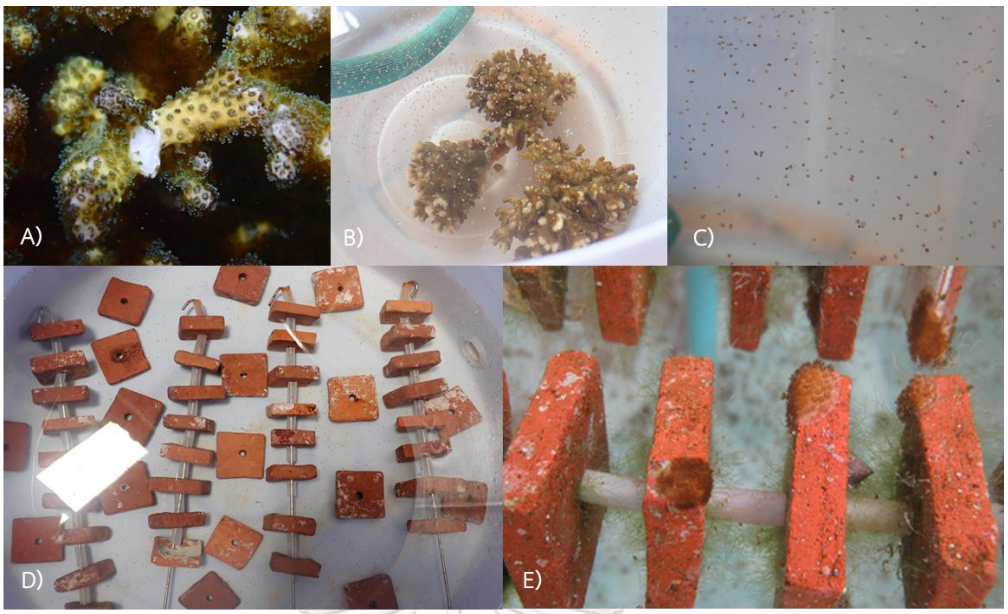

Figure 3.6 Sample collection and rearing of *Pocillopora damicornis* ;A) observation of larva development B) larva releasing C) larvae swimming in the water column D) terracotta tile for larva settle and E) settled colonies.

Transmission electron microscope (TEM) and Fluorescence microscope (FM) analysis

Five pre-fertilize and fertilized oocytes of *A. humilis* and planulae of *P. damicornis* were collected and preserved in 2.5% glutaraldehyde to observe their ultrastructure at the early stage via transmission electron microscopy (TEM). Moreover, zooxanthella cells in coral tissues were investigated via fluorescence microscopy (FM). All samples were processed and analyzed at the laboratory of Scientific and Technological Research Equipment Center and Department of Marine Science, Faculty of Science, Chulalongkorn University.

DNA extraction and PCR amplification

The preserved samples included parent colonies of *A. humilis* from KTM; coral gametes (prior to fertilization); fertilized eggs; 1-, 3-, 6-month-old, 1-, 1.5-, 2-,4-and 5-year-old corals collected from SSI; and colonies from transplanted area after 09-month-old, 2, 3, 4, and 5 years. Small coral fragments were ground using a mortar and pestle, whereas coral gametes were directly lysed in an incubator before proceeding to the next step. Total genomic DNA from each sample was extracted using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The quality and concentration of extracted DNA were examined by both gel electrophoresis and NanoDrop 2000c (ThermoFisher Scientific™). Symbiodiniaceae DNA was amplified using 10 µL reaction volumes of mixtures per sample containing 0.2 µL DNA Polymerase, 2 µL buffer, 0.8 µL dNTP (TaKaRa Bio Inc.), 4 µm ddH_2O (Sigma® Life Science), 1 µL each of forward and reverse primer, and 1 µL (~5 ng) of DNA template. Both the forward (r18Sf; 5'–CGCTCTTCCGATCTCTGGAAAGTTTCATGAACCTTAT3–') and reverse (Sym28Sr–1st; 5'–TGCTCTTCCGATCTGACCTTGTRTGACTTCATGCTA–3') sequences of the ITS1 and ITS2 regions were used to identify genera or types of Symbiodiniaceae in the corals. These genes were designed by Dr. Chuya Shinzato of Atmosphere and Ocean Research Institute (AORI) in Japan. The mixtures were amplified under the following conditions: 3 min at 94 °C, followed by 33 cycles of 98 °C for 10 s, 50 °C for 0.15 s, 68 °C for 60 s, and a final extension step at 68 °C for 3 min on an AllInOneCycler™ (Bioneer Inc., United States of America). The PCR products were cleaned using QIAquick PCR purification kit (Qiagen, Hilden, Germany). Each purified amplicon was diluted to ~5 ng and used as a template DNA for the second PCR (the unique molecular barcode was attached to the first PCR product). The final volume of the second PCR mixtures were adjusted to10 µL. The mixtures contained 0.24 µL DNA polymerase, 2.4 µL buffer, 0.96 µL dNTP, 1 µL (0.1 µM) of each unique short primer combination of forward and reverse primers (Eurofins Scientific), 3.4 µL ddH_2O, and 1 µL of purified amplicon. PCR was performed under the following conditions: 3 min at 94 °C, followed by 13 cycles of 98 °C for 10 s, and 68 °C for 60 s. The PCR products

were run in a 1.5% agarose gel with EcoDye nucleic acid staining solution (Biofact Co., Ltd.) to evaluate the targeted size of DNA (~820 bp).

Next generation sequencing and Bioinformatics analysis

Each purified amplicon was pooled in equimolar amounts in a single Eppendorf tube to assemble DNA libraries. The pooled samples were quantified, and their quality was examined via qPCR (Thermo Fisher Scientific). The final volume of the DNA libraries was adjusted to 8 pM and applied on an Illumina Mi–Seq platform (Illumina, San Diego, CA, United States of America) according to the standard protocol by the AORI, The University of Tokyo, Japan. However, the PCR products of 2–5–year–old and transplanted *A. humilis* along with most of the *P. damicornis* samples were sent to U2Bio Company, Korea, for second batch sample processing. Symbiodiniaceae diversity and community composition were analyzed by identifying sequence targets of Symbiodiniaceae and then trimming and chimera filtering these targets, High–quality filtered reads were aligned to the ITS2 database by using BLASTN. Non-Symbiodiniaceae and low-quality sequence reads were removed. Detailed parameter settings and pipeline development are described in Shinzato et al. (2018). The resulting counts of Symbiodiniaceae ITS 2 types were used to assess Symbiodiniaceae community composition. After normalized the quality data, low read below For the statistical analysis of difference in Symbiodiniaceae diversity, Shannon and Simson were calculated to access the level of alpha diversity across corals (Simpson, 1949). The Kruskal-Wallis test was performed to test the different of Symbiodiniaceae community of corals in different stages. Permutation multivariate analysis of variance (PERMANOVA) test were used to compare the Symbiodiniaceae community compositions among coral species. To visualize the beta diversity of Symbiodiniaceae community structure among coral host, non-metric multidimension scaling (nMDS) based on Bray-Curtis dissimilarity was plotted using Phyloseq and VEGAN packages in R software (McMurdie & Holmes, 2013; Oksanen et al., 2020).

Results

Physical factors in rearing hatchery system

Light intensity and temperature were recorded from March 2016 – November 2017. The average light and temperature approximately 18.21 µmole m^{-2} s^{-1} and 28.97 ^{o}C respectively. The highest temperature was detected at 33.13 ^{o}C around June 2016 whereas the minimum temperature was at 23.79 ^{o}C in December 2016. However, the average light intensity in hatchery is low compared to the ambient condition (~70 µmole m^{-2} s^{-1}) (Fig 3.7)

Gamete development and Coral spawning

The tagged colonies of *A. humilis* showed different stages of gamete development. Fourteen *A. humilis* colonies started to develop their gametes at the beginning of October 2015. During this time, white eggs were observed and another six colonies were detected, but no gamete was found at the beginning of the observation period. Most colonies continued to develop to maturation stage (pink eggs with sperm sac had developed) midway through January 2016. Fourteen mature colonies released their gametes at their mouths (polyps) at approximately 6:50 PM (setting time). The mean duration of coral spawning lasted for approximately 1 h after the setting time. Most corals tended to spawn together approximately 7 days after the full moon in January. Moreover, six other colonies spawned consecutively for 2 days, 9 and 10 days after the new moon in February. In addition, the *Acropora* spp. in this area spawned during both neap and spring tides. On the other hand, mature colonies of *P. damicornis* released their larvae during the night and the early morning at about 3–6 days after the new moon.

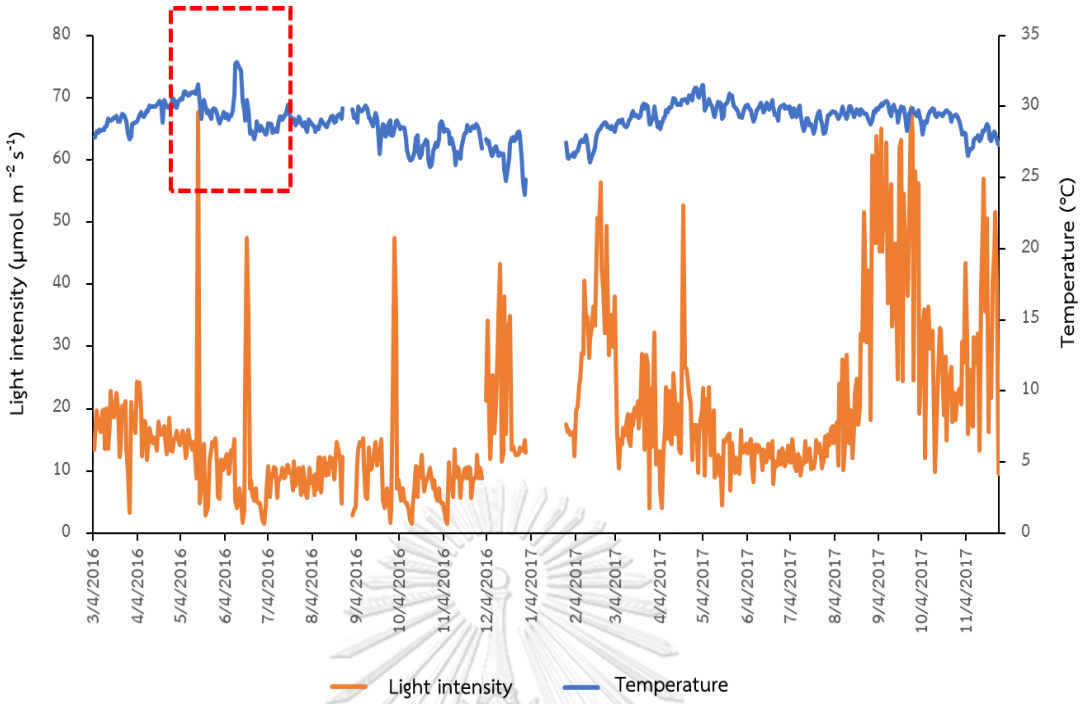

Figure 3.7 Average light intensity and temperature in rearing hatchery.

Fertilization and survival rate

The fertilization success of *A. humilis* in each tank ranged from 97.2%±1.72% to 99.8%±0.75% (Fig 3.8). The fertilized embryos underwent cell division 1 h after fertilization and became flat similar to a red blood cell after 8 h (Fig 3.9). However, both abnormally shaped and round embryos were considered unfertilized. The corals' embryos then developed to larvae (swimming stage) and settled on the substrate within 2–3 days. After 2 years, the juvenile corals grew and encrusted themselves on the substrate. The survival rate of 2–year–old *A. humilis* at the end of experiment was 45.93%±3.81% (Fig 3.10). However, all settled colonies of *P. damicornis* died after 3 months during the observation period. Thus, another batch of *P. damicornis* from the hatchery was used. *P. damicornis* colonies were selected by their average size to represent of each stage.

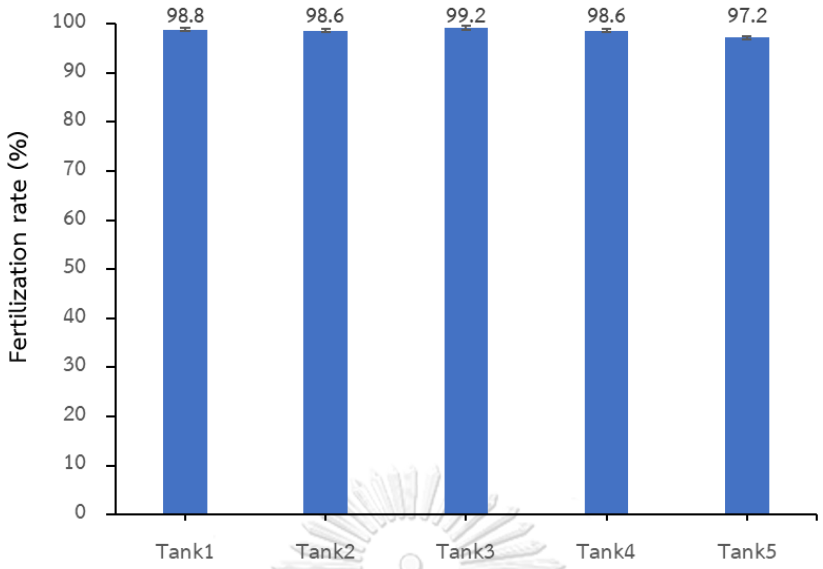

Figure 3.8 Fertilization rate of *Acropora humilis* gamete after 8 h.

Figure 3.9 Coral gamete and embryo ;A) before fertilization B) 1 h after fertilization and C) 8 h after fertilization.

Investigation of zooxanthellae cell in pre-settled coral stages: TEM and FM

No zooxanthella cells were detected in the eggs, fertilized gametes, and larvae of *A. humilis* samples. However, some organelles were observed during coral development via TEM. The distinct composition of the ultrastructure of the corals indicated that they were at different growth stages. The corals' eggs contained numerous yolk bodies and lipid granules, and the number of these organelles tended to decrease during coral development. Nematocyte cells were found in the larval stage of both *A. humilis* (Fig. 3.11) and *P. damicornis*. The larvae of *P. damicornis* had

numerous zooxanthella cells under a fluorescence microscope. The small red spot referring to zooxanthellae cells in the corals' tissues due to plant cell emitted red light under the fluorescence microscope. Small zooxanthella cells were observed in epidermal layer. Cell walls, chloroplasts, and nuclei were also observed via TEM (Fig. 3.12).

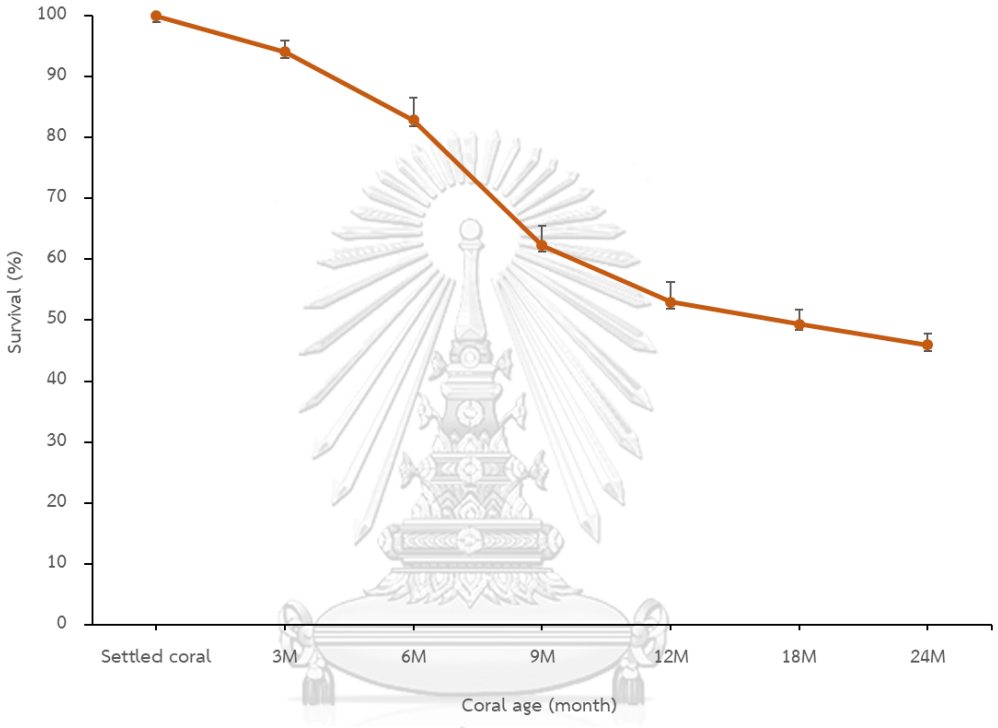

Figure 3.10 Survival rate of *Acropora humilis* under rearing hatchery system.

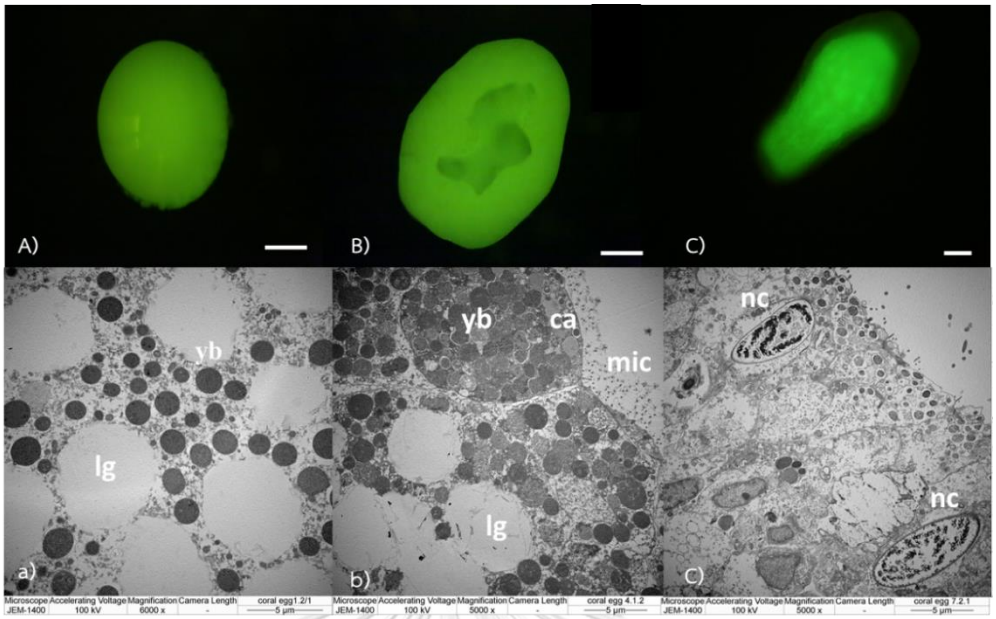

Figure 3.11 Early stage of *Acropora humilis* metamorphosis under fluorescence microscope ;(A-C) and transmission electron microscope (a-c); A and a) prior fertilization, B and b) 8 h after fertilization, and C and c) swimming larva stage (yb = yolk bodies, lg = lipid granule, mic = microvilli, ca = cortical alveoli and nc = nematocyst). Scale bars (A-C) = 100 µm.

Symbiodiniaceae diversity and community

The results of the analyses of Symbiodiniaceae diversity and community were divided into two groups because the output data were obtained from different places: the first set of sequencing data was from AORI, Japan, and the second set of sequencing data was from U2BIO Company, Korea. In the first set of sequencing data, approximately 1,429,599 high–quality sequences were obtained from 66 samples, including *A. humilis* (parent colonies and most of the corals in the hatchery) and *P. damicornis* (parent colonies, larvae, and 1 sample of a 3–month–old coral). Three genera and 18 types of Symbiodiniaceae were detected (C13, C15.6, C1p_C1.8, C3w, C42type1_C42a, C42type2, C70, C74, C89, C93type1, Cspc_C3, Cspf) and *Durusdinium*

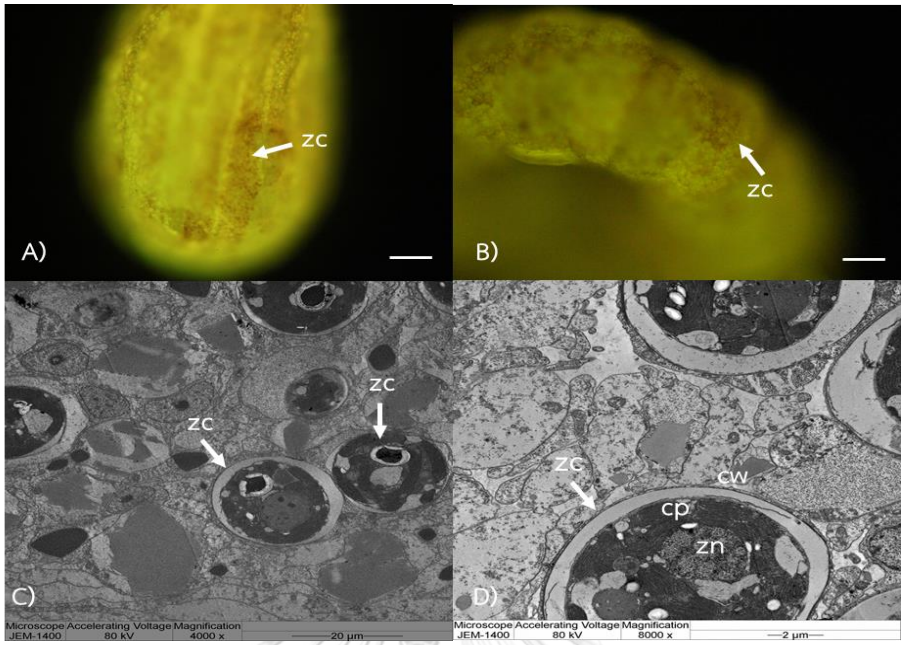

Figure 3.12 Larva of *Pocillopora damicornis* under fluorescence microscope ;(A-B) and transmission electron microscope (C-D); A) swimming stage, B) primary settled polyp, C) zooxanthellae cells, and D) microstructure of zooxanthellae (zc = zooxanthellae cell, cw = cell wall cp = chloroplast and zn = zooxanthellae nucleus). Scale bars (A-B) = 100 µm.

(D1, D4, D5, D8, D10). The second set of sequencing data, approximately 2,068,379 high–quality sequences were obtained from 32 samples, including 2–year–old *A. humilis* colonies reared in the tanks, 2–5–year–old transplanted colonies of both *A. humilis* and *P. damicornis*, and 3–24–month–old colonies from the coral hatchery. Two genera and 21 types of Symbiodiniaceae were found (C#, C1, C1.5, C1.6, C1.v1b, C115a, C3w, C39, C50, C59, C71, C71a, C72, C78a, C89, C91, C93type1, Cspc_C3) and *Durusdinium* (D1, D1a, D6).

However, the Symbiodiniaceae composition of *A. humilis* eggs and larvae was not amplified because this coral species is not yet associated with these symbionts at these stages. The diversity of Symbiodiniaceae types from both the first and second

sets of sequencing data was analyzed by calculating their alpha–diversity indices (Shannon and Simson). Results showed that *A. humilis* was associated with a higher diversity of Symbiodiniaceae types than *P. damicornis* (Fig 3.13). The relative abundance of Symbiodiniaceae types in *A. humilis* was remarkably different between the parent (wild coral) and the cultured colonies (corals raised in the hatchery). The parent colony of *A. humilis* was associated with the dominant *Cladocopium* types Cspc_C3 (59.06%) and C3W (31.02%), as well as with *Durusdinium* (1.45%), whereas the cultured colonies predominantly harbored *Durusdinium* during the growth period. Moreover, 1–month–old colonies were associated with *Durusdinium* type D1 (44.03%), *Cladocopium* types Csps_C3 (33.31%) and C3w (16.55%), and 10 minor types. Furthermore, although 3–month–old corals were associated with genetically diverse types of Symbiodiniaceae, they were predominantly associated with type D1 (80.18%). However, 6–18–month–old corals were found to be consistently associated with *Durusdinium* type D1 (almost 100%). By contrast, the 2–5–year–old corals from the second set of sequencing data contained other types of *Durusdinium* (D1a). Interestingly, the Symbiodiniaceae diversity and community of the transplanted corals gradually changed. These corals acquired novel types of Symbiodiniaceae from their environment, and they seemed to harbor Symbiodiniaceae community similar to that of the original parent colonies (Fig 3.14). The first set of sequencing data of *P. damicornis* consisted of parent colonies, larvae, and a colony of 3–month–old corals. *Durusdium* type D1 was found to be associated with the parent colonies (95.64%) and their larvae (94.21%). However, the Symbiodiniaceae community of 3–month–old colony changed from D1 to *Cladocopium* type Cspc_c3 (65.07%) and C3w (29.01%). From the second set of sequencing data, most of the 3–24–month–old *P. damicornis* colonies selected from the coral hatchery to replace the dead colonies had a similar community. Two dominant *Cladocopium* types, namely, C1 and C78a, were detected at all ages of the corals (Fig 3.15). Dissimilarities in the Symbiodiniaceae types of the coral samples were plotted via non–metric multidimensional scaling. The 3–18–month–old *A. humilis* colonies from the hatchery appeared to group together, whereas the 1–month–old colonies, parent colonies, and transplanted corals were individually

separated (Fig 3.16). However, a small number of *P. damicornis* samples could not be statistically analyzed via PERMANOVA as well as the nMDS calculation and visualization.

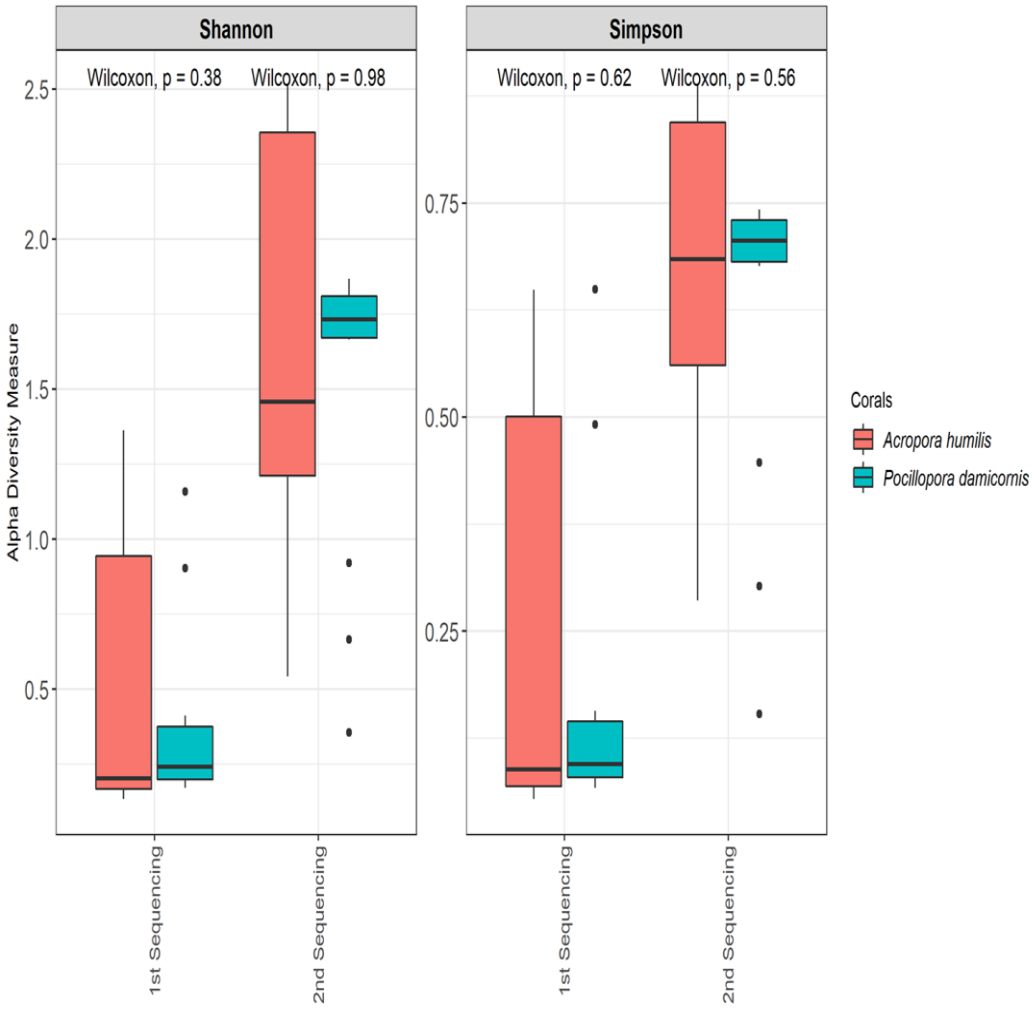

Figure 3.13 Alpha diversity indices (Shannon and Simpson) of Symbiodiniaceae community among two coral species in different time of sequencing.

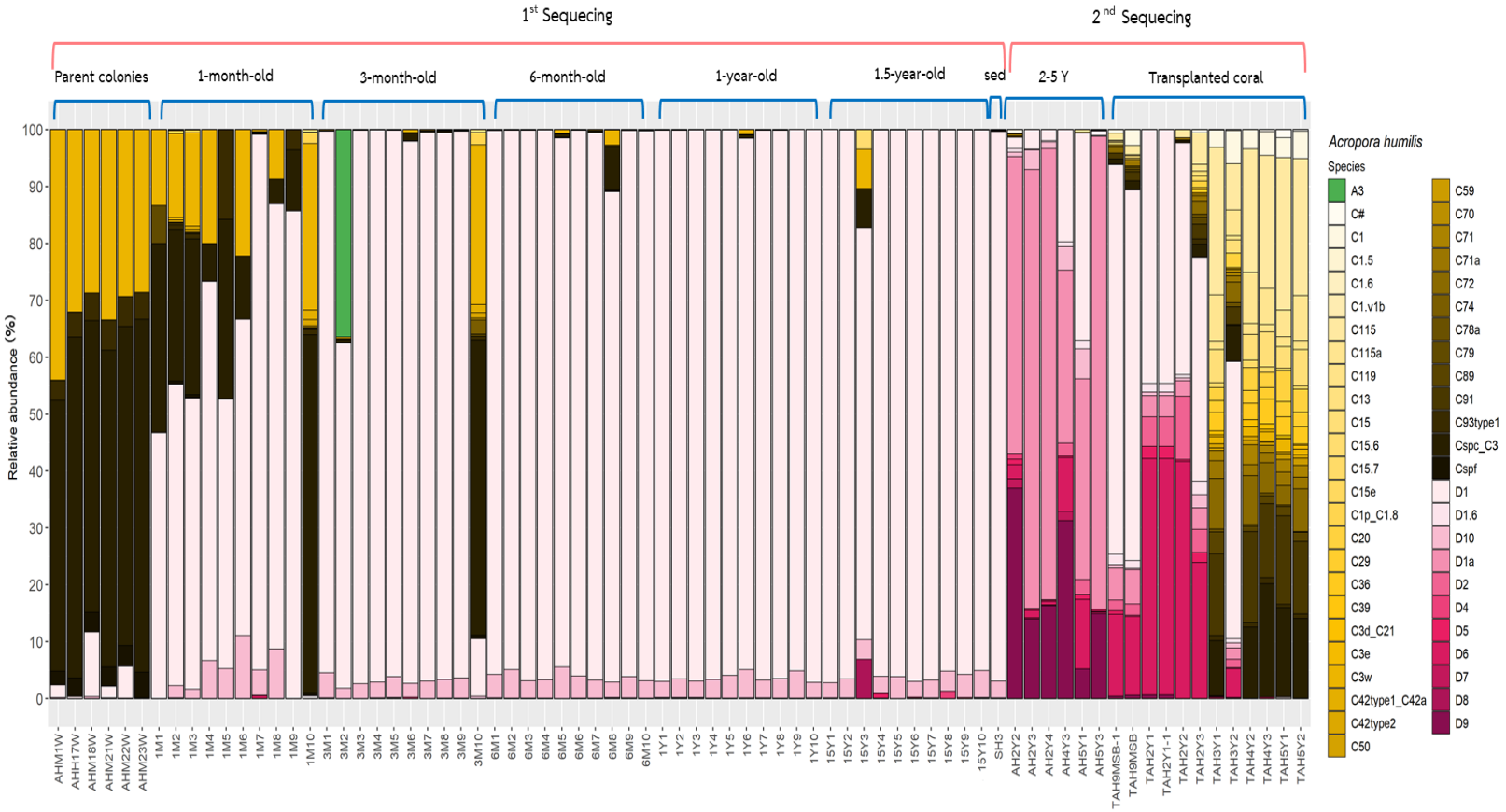

Figure 3.14 Relative abundant of Symbiodiniaceae communities *in Acropora humilis* from parent colony (wild) and coral cultivation using sexual propagation under rearing hatchery.

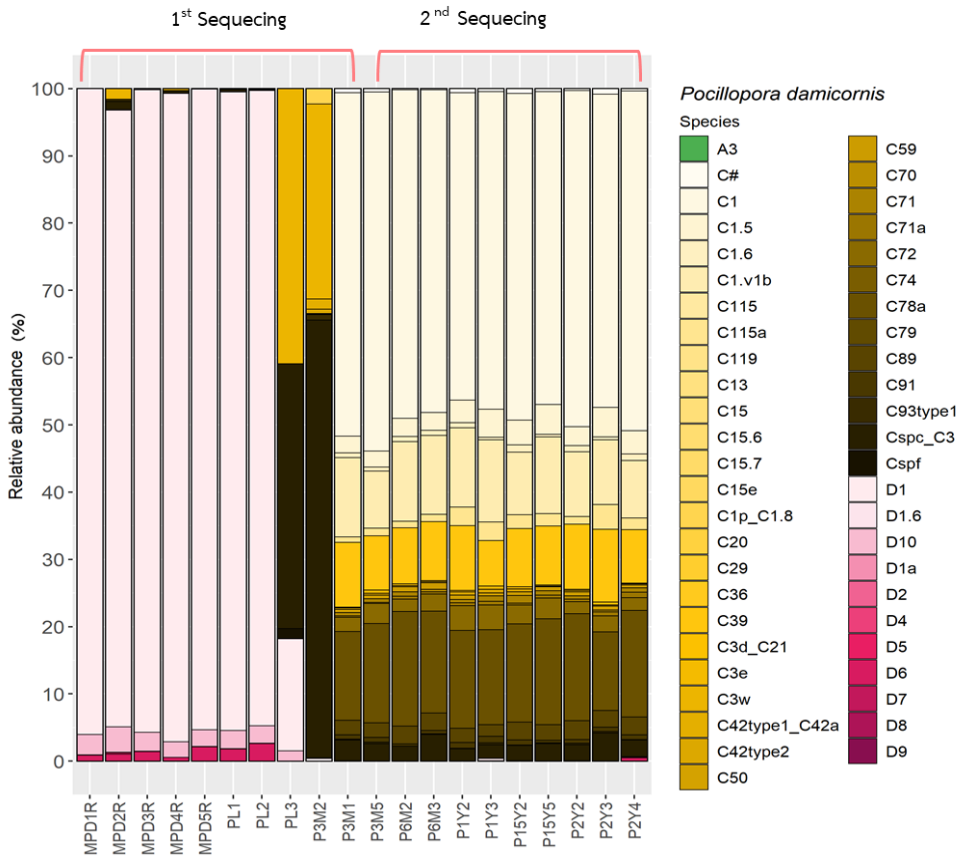

Figure 3.15 Relative abundant of Symbiodiniaceae communities in *Pocillopora damicornis* from parent (wild) and reared colonies (hatchery).

Discussion

Coral gamete development and spawning / larva releasing

The gametogenesis of adult *Acropora* colony was established within 4–7 months (Gouezo et al., 2020; Ibrahim et al., 2021; Mangubhai & Harrison, 2008). The duration of gametogenesis may differ depending on the local environmental conditions (Bouwmeester et al., 2015; Bouwmeester et al., 2011; Chelliah et al., 2015). In this study, *A. humilis* underwent a single gametogenic cycle. The corals started to produce gametes in October and became fully mature in 4–5 months in January and February.

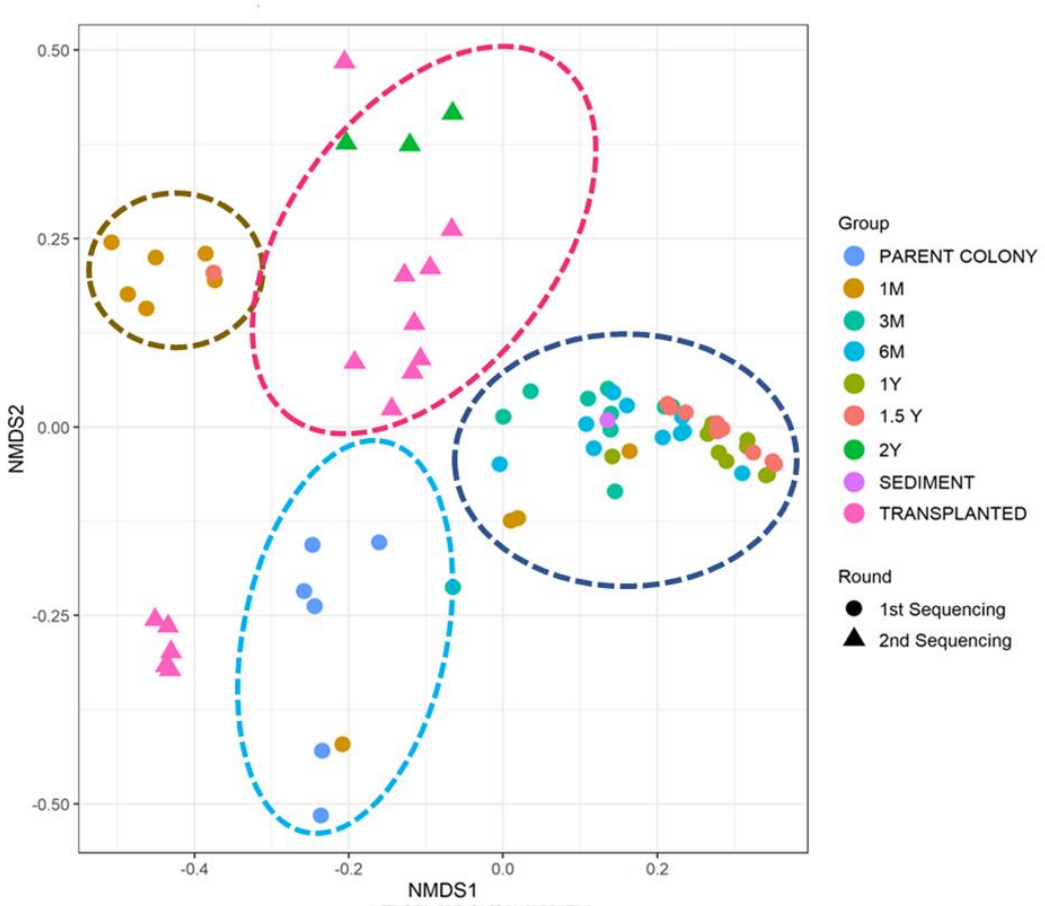

Figure 3.16 Non-metric multidimensional scaling (nMDS) plotting of the Symbiodiniaceae type composition of *Acropora humilis* from different stage based on of Bray-Curtis dissimilarity indices.

In general, oogenesis occurs before spermatogenesis, similar to that observed in this study as the sperm sac was observed approximately a month before coral spawning. However, spermatogenesis may develop before but it is required on the evidence of histological study (Ibrahim et al., 2021). Large colonies of *A. humilis* displayed spit spawning in consecutive months probably because corals reproduce gametes at different times (Foster & Gilmour, 2020; Foster et al., 2018) or corals have the ability to adapt physiologically in response to environmental variations to ensure larval survival (Sakai et al., 2020). Most *Acropora* corals reportedly spawn around the full

moon or several days after the full moon in both the Pacific and Atlantic Oceans, excluding in Kenya and the upper GoT (this study). (Bouwmeester et al., 2015; Kaniewska et al., 2015; Nozawa, 2012; Wolstenholme et al., 2018). In this study, *Acropora* corals were observed to spawn across the lunar cycle. Several factors, such as biological, chemical, and physical factors, may account for the varying patterns noted in these particular locations (Baird & Guest, 2009; Lin & Nozawa, 2017; Nozawa, 2012). Most corals tend to spawn at night to avoid predation and high irradiance (Fan et al., 2006). To ensure successful fertilization, corals tend to spawn during the neap tide to minimize the effects of tides on gamete concentration (Babcock et al., 1986; Mendes & Woodley, 2002). However, in this study, coral spawning was recorded in both neap and spring tides. The fact that some corals selected unfavorable conditions for spawning may provide evidence of genetic variation among coral populations (Babcock et al., 1994). Pocilloporidae species in different locations exhibit mixed reproduction modes. *P. damicornis* in GoT is a hermaphroditic brooder that can produce offspring throughout the year. *Ex situ* experiments revealed that *P. damicornis* released its larva during nighttime and the early morning 3–5 days after the new moon (Kuanui et al., 2008). By contrast, a lower GoT was recorded of day time spawning of *Pocillopora* spp., suggesting that corals have a different spawning behavior along biogeological gradients. This behavior may be influenced by the local environment or genetic differences among species (Fogarty & Marhaver, 2019; Plathong et al., 2005).

Fertilization and survival rate

Ex situ experiments showed that *Acropora* corals may have a high rate of successful fertilization in natural reefs. The contact time (10 min) of gamete fertilization was reduced in merulinid fertilization, but the short contact time had no effect on *Acropora* spp. (Nozawa et al., 2015). A high fertilization rate of *A. humilis* (99.8%) was achieved in this study likely because of the stable laboratory conditions. Under this setting, current was lacking, which could disturb coral gametes during fertilization and reduce sperm concentration (Babcock et al., 1986; Mendes & Woodley, 2002). Another plausible explanation for the high fertilization rate was the provision of a multiple donor colony for fertilization. The number of gametes from different mother colonies

were also the key to successful fertilization and provided the coral genetic diversity (Omori & Iwao, 2014). After 8 h of fertilization, the embryos changed from circle to flat (like a red blood cell), and they became swimming larvae within 3 days under optimum conditions (Omori, 2005; Omori & Iwao, 2014). The survival rate of juvenile corals under hatchery conditions for rearing rapidly decreased after 3–9 months. Several limiting factors, such as water movement/water flow, in the hatchery possibly affected the corals' survival rate. Moreover, *Acropora* spp. preferably grow in environments with a high water flow rate (Boch & Morse, 2012). By contrast, in this study, the rate of water flow in the hatchery was low because of limited power supply (in the island, only a power generator was used to supply electricity). Moreover, the hatchery had a low light level, and it might have lowered the photosynthetic rate of zooxanthella cells (Bessell-Browne et al., 2017; Kuanui et al., 2020). Thus, the rate of coral growth and survival was low. The unrestricted growth of macroalgae, sponges, or tunicates on the coral colony may also account for the low survival rate of the corals in the hatchery (Henry et al., 2019; Ligson et al., 2020; Omori, 2005). However, after 3 months, *P. damicornis* colonies died likely because of the high temperature in summer to which the corals could not adapt.

Zooxanthellae cell observation by TEM and FM

Zooxanthella cells were not detected in both the oocytes and planulae of *A. humilis* via TEM and FM. The results offered evidence that the *A. humilis* in upper GoT belongs to a group with HT as it spawns zooxanthella–free eggs. Most *Acropora* corals acquire free–living zooxanthellae from their environment, including seawater and sediments (Byler et al., 2013; Nitschke et al., 2016). Three stages (oocytes, fertilized eggs, and larvae) of *A. humilis* were observed, and the composition of organelles in each stage was different. The oocytes and embryos contained numerous yolk bodies and lipid granules for energy storage and buoyancy, respectively. *Acropora* eggs contained no zooxanthella cells (Tsai et al., 2016). Therefore, these organelles are an essential nutrient source for coral embryogenesis during this stage (Shikina et al., 2013). Nematocyst cells are a common feature of cnidarians (Ben-Ari et al., 2018; Kitahara et al., 2020). In this study, nematocysts developed in the larval stage of both *A. humilis*

and *P. damicornis.* Although nematocysts are used for defending against invasion and capturing prey, several studies suggested that this type of cell is predominantly used for locomotion of coral larvae (Beckmann & Özbek, 2012; Kass-Simon, 2002; Larsson et al., 2014). *P. damicornis* is a brooder coral that produces larvae containing zooxanthella cells (Combosch & Vollmer, 2013; Crowder et al., 2014). Zooxanthella cells were observed in coral tissues via TEM and FM. Like adult colonies, larvae contain zooxanthellae for photosynthesis and cell activity (Gaither & Rowan, 2010; Harii et al., 2002). Moreover, brooded larvae containing zooxanthella cells are important for coral reef recruitment by supplying coral populations in local reefs owing to the competency period of settlement (Harii et al., 2002).

Symbiodiniaceae diversity of two coral species

In the past, corals were believed to be associated with a genus or a single type of Symbiodiniaceae owing to the sensitivity and/or limitation of molecular technology (Ng & Ang, 2016). Sanger sequencing revealed that the *A. millepora* in Sattahip, GoT, harbors *Cladocopium* type C1 both before and after bleaching (unpublished data). New molecular technologies have been developed to determine the DNA or genome of various organisms. Next–generation sequencing (NGS) is a molecular tool that rapidly produces multiple sequencing reads (Arif et al., 2014). Many studies that employed NGS to analyze Symbiodiniaceae diversity/community found a high diversity level of Symbiodiniaceae associated within coral colonies (Pootakham et al., 2021; Qin et al., 2019; Shinzato et al., 2018). In the present study, both *A. humilis* and *P. damicornis* were found to harbor several types of Symbiodiniaceae in a single colony.

Cladocopium is considered the most–species rich and broadly distributed dinoflagellates in the Indo–Pacific reef (LaJeunesse et al., 2004; LaJeunesse et al., 2018). Herein, at least 26 types of *Cladocopium* were recorded. Wild colonies of *A. humilis* mostly contained *Cladocopium* types Cspc_C3 and C3W, similar to the *Acropora* spp. in the South China Sea (Gong et al., 2018; Qin et al., 2019). However, the offspring of *A. humilis* had a different Symbiodiniaceae community under the rearing conditions in the hatchery. Aposymbiotic recruits colony frequently exhibited

the distinct Symbiodiniaceae community compared with the parent clone because of their mode of symbiont acquisition (Ali et al., 2019). Free–living Symbiodiniaceae in sea water and sediments are an important reservoir for newly recruited HT corals (Hirose & Hidaka, 2006; Hirose et al., 2008; Sweet, 2014). The 1–3–month–old corals in the hatchery were found to harbor multiple types of Symbiodiniaceae that were probably randomly uptaken from sea water in the hatchery system (Yamashita et al., 2014). However, after 3 months, the corals likely became an attractive host for the Symbiodiniaceae type *Durusdinium* D1 under stressful conditions. The presence of Symbiodiniaceae types may change because of the differential metabolic needs of juvenile corals during their development (Gordon & Leggat, 2010; Hillyer et al., 2017). *Durusdinium* is one of the most tolerant to uncertain environments, and they are mostly found to be associated with hosts living at shallow reefs where they are exposed to high temperatures, intense light, and turbidity (LaJeunesse et al., 2010; Wham et al., 2017). Reef–building corals associated with *Durusdinium* experience a lower mortality than *Cladocopium* at high temperatures and intense irradiance (Yorifuji et al., 2017; Yuyama et al., 2016). In this study, the potential of corals to adapt and associate with the stress resistance genera of Symbiodiniaceae was explored. Both shuffling and switching occurred, especially in 1–3–month–old corals reared in the hatchery and transplanted colonies. Interestingly, the proportion of *Durusdinium* gradually decreased after 0.75, 2, and 3 years of transplantation and switched to several types of *Cladocopium* 4 and 5 years after transplantation, similar to that observed in the parent colony.

The Symbiodiniaceae composition of *A. humilis* changed from *Durusdinium* to *Cladocopium* likely because of differences in the environment where the juveniles were reared. In the hatchery, the corals were exposed to low light and high temperature with limited food compared with corals in natural reefs (Reich et al., 2017). Corals that host *Cladocopium* reportedly have a higher growth rate than those associated with *Durusdinium* (K. M. Quigley et al., 2020). *Cladocopium* translocates large amounts of the photosynthates produced by photosynthesis to its coral host (Cantin et al., 2009), suggesting that transplanting *A. humilis* harboring *Cladocopium*

may enhance the benefit to its coral hosts under certain natural conditions (Fisher et al., 2012; Thinesh et al., 2019). However, many scleractinian corals associated with *Cladocopium*, especially *Acropora* spp., are susceptible to bleaching (Thinesh et al., 2019).

Previous studies reported that *P. damicornis* in both the upper and lower GoT hosts *Durusdinium* types D1–6 and D17 (Chankong et al., 2020; Pootakham et al., 2021). In the present study, *P. damicornis* had the similar Symbiodiniaceae community, in which the parent colony contained <95% of *Durusdinium* D1 similar to that reported by previous studies. However, all colonies switched to *Cladocopium* after 3 months under hatchery conditions, which is an unexpected result because most VT corals possibly maintain the Symbiodiniaceae community they have inherited from the parent colony (Baker, 2003; Douglas, 1998). For example, the offspring of *Porites* spp. contain *Cladocopium* C15, which is transmitted from the parent and does not change even under thermal stress throughout their biogeographic distribution (Gong et al., 2019; Pootakham et al., 2018). In brooded corals, the types of Symbiodiniaceae may be selected from the parent to confer their offspring with high fitness and survival rate (Kenkel & Bay, 2016; Quigley et al., 2016). However, some studies on the brooded larvae of *Seriatopora hystrix* showed that this species exhibits a mixed mode of symbiont acquisition as an adaptation mechanism to be more resilient to stressful environmental conditions (Byler et al., 2013; Quigley et al., 2018).

This study found that the relative abundance of Symbiodiniaceae genera and types in both species corals substantially changed after 3 months, and this phenomenon was correlated with the dramatic decreased in the survival rate of the corals. Therefore, during this particular stage, *A. humilis* colonies associated with other genera of Symbiodiniaceae, including *Symbiodinium* and *Clodacopium*, cannot adapted under hatchery conditions. However, those associated with *Durusdinium* survived and tended to grow. Conversely, *P. damicornis* colonies associated with *Cladocopium* survived, but the remaining colonies associated with the other genera died. The preference of corals in hosting different genera or types of Symbiodiniaceae in the hatchery may be attributed to microhabitat differences (Leveque et al., 2019;

Quigley et al., 2018). Moreover, the energetic requirements associated with these corals implied that different coral species have different metabolic rates and nutritional requirements (Obura, 2009).

However, the variations observed in Symbiodiniaceae types at different times might be attributed to the different sequencing methods employed. The laboratory in AORI provided kits with 600 cycles, whereas the U2BIO Company used 300 cycles. The kit with 300 cycles generated a shorter sequencing read than the kit with 600 cycles, and this discrepancy might have resulted in a higher number of top hit sequences in the database. Thus, most of the sequences were removed, and the sample that revealed the highest BLAST score was excluded.

Conclusion

To the best of our knowledge, this study was the first to examine coral–Symbiodiniaceae association both *ex situ* and *in situ*. We documented a greater diversity of Symbiodiniaceae associated with *A. humilis* and *P. damicornis* reared in a hatchery and adult colonies from natural reefs in upper GoT. These coral species exhibited adaptation mechanisms, shuffling, and switching, indicating that they may have more resilience to uncertain environmental conditions than previously thought. However, more extensive collections of corals and free–living Symbiodiniaceae from the environment will help us in understanding better the specificity of coral–Symbiodiniaceae relationship and their adaptation potential.

CHAPTER 4

The seasonal investigation of Symbiodiniaceae in broadcasting, *Acropora humilis* and brooding, *Pocillopora damicornis* corals

Introduction

Coral reefs are well established as habitats of high ecological complexity and biodiversity in the marine environment (Blackall et al., 2015; Brown et al., 1999a). Most reef-building scleractinian corals are known to sustain a symbiotic relationship with dinoflagellate algae belonging to family Symbiodiniaceae (also known as zooxanthallae) (Eckert et al., 2020; LaJeunesse, 2002). This relationship has been shown to contribute up to 50-95% of the metabolic needs of the coral host by the symbiont supplying photosynthetic products (Fabricius & Klumpp, 1995; Muscatine, 1990). Therefore, the symbiosis between corals and zooxanthellae is essential for the development and survival of coral reefs (Baker et al., 2013; Wilkinson et al., 2015). The diversity and community of zooxanthellae associated with corals is influenced by different factors including ecological and physical conditions (Terraneo et al., 2019; Thornhill et al., 2009). Seasonal variation is known to effect coral health indices such as Chlorophyll *a* and zooxanthellae density, due to both physical and chemical factors of the surrounding environment (Hinrichs et al., 2013; Scheufen, Iglesias-Prieto, et al., 2017). The density of zooxanthellae in several scleractinian corals around the world has been shown to be variable throughout the year, in response to seasonality (Li et al., 2008; Stimson, 1997; Zhou et al., 2017). Higher irradiance levels and temperatures may have severe impacts on zooxanthellae density (Fisher et al., 2012; Glynn, 1993; Pillay et al., 2005). Most tropical reef-building corals possess their highest zooxanthellae densities during winter months, declining in the summer (Brown et al., 1999a; Fitt et al., 2001; Stimson, 1997). This has been shown to correlate with minimum coral growth rates, which occur simultaneously with low zooxanthellae density during summer season as well (Al-Hammady, 2013; Pillay et al., 2005).

Reef-building corals possess symbiotic relationships with a genetically diverse (clade/types) range of zooxanthellae, which have been attributed to coping with different environmental stressors (Rouze et al., 2019; Silverstein et al., 2011; Silverstein et al., 2012). One genus (*Symbiodinium*) and nine clades (A-I) of zooxanthellae were identified based on nuclear ribosomal DNA (rDNA) and internal transcribed spacer (ITS) region (Baker, 2003; Barbrook et al., 2006; Coffroth & Santos, 2005; Pochon et al., 2007; Stat et al., 2011). However, the revised systematics of zooxanthellae were recently defined based on genetic variation (LaJeunesse et al., 2018). Seven genera of zooxanthellae were described including *Symbiodinium*, *Breviolum*, *Cladocopium*, *Durusdinium*, *Effrenium*, *Fugacium* and *Greakladium* (formerly clade A, B, C, D, E, F and G, respectively). The physiological responses of corals to environmental stressors may differ among different genera/type of zooxanthellae (DeSalvo et al., 2010; Rowan, 2004; Sampayo et al., 2008). The selective specific uptake of symbiont types could lead to differentiation of coral fitness and survival rates (Quigley et al., 2016; Tan et al., 2020). However, the relative abundance of genetically different types of zooxanthellae in corals may change over time (month or season) (Baker & Romanski, 2007; Berkelmans & van Oppen, 2006). For example, abnormally high ocean temperatures have been frequently observed and may lead mass bleaching events especially during summer season (Al-Sofyani & Floos, 2013; Brown, 1997; Chavanich et al., 2009).

Several studies have corroborated that the genus *Durusdinium* (clade D) may be found at significantly higher proportions during thermal stress than normal environmental condition (Chen et al., 2020; Cunning, Silverstein, et al., 2015; Dilworth et al., 2021; Poquita-Du et al., 2020; Thinesh et al., 2019). Under thermal stress, corals associated with *Durusdinium* exhibit less damage of photosystem II (PSII) compared to those corals associated with *Cladocopium* (Jones & Berkelmans, 2011; Little et al., 2004; Suwa et al., 2008). suggesting that corals harboring *Durusdinium* may display the most tolerance to heat stress (Fabricius et al., 2004; Ghavam Mostafavi et al., 2007). Meanwhile, corals containing the genus *Cladocopium* translocated large amounts of carbon to the host compared with other zooxanthellae type (Cantin et al., 2009).

Corals are flexible in acquiring new symbionts from the environment when physical conditions change (Baker, 2003; Claar et al., 2020; Putnam et al., 2012). The capacity of coral hosts to differentiate between symbionts type are related to two potential adaptive mechanisms, namely, "shuffling" (change in relative abundance of zooxanthellae type within a colony) and "switching" (uptake of new exogenous zooxanthellae types from the environment) (Berkelmans & van Oppen, 2006; Fautin & Buddemeier, 2004; Rouze et al., 2016; Sampayo et al., 2008). Symbiont shuffling of corals can enhance coral fitness, growth, and survival (Abrego et al., 2009; Stat & Gates, 2011). The flexibility of zooxanthellae type is important in understanding their future survival in response environmental stressors (Baskett et al., 2009; Huang et al., 2019; Sampayo et al., 2016). The thermal sensitivity of corals are often related to the physiological properties of symbiont type (Reed & Frankham, 2003). Bleached corals may become more resistant to higher temperature by symbiont shuffling and switching (Császár et al., 2010; Davies et al., 2018).

The different reproductive mode of corals can affect establishment of coral-endosymbiosis. Broadcasting corals (horizontal zooxanthellae transmission) acquire their zooxanthellae from their environment, whereas in contrast, brooding corals (vertical zooxanthellae transmission), acquire initial zooxanthellae cells from their parents (Baird et al., 2009; Sweet, 2014). Almost all Acroporidae corals are believed to be broadcast spawning corals, which acquire zooxanthellae from surrounding environment during development to planula or primary polyp stage (Hirose et al., 2008; Kayanne, 2016). Conversely, the Pocilloporidae have species that are considered brooding corals such as *Pocillopra* spp., *Seriatopora* spp and *Stylophora* spp. (Hirose & Hidaka, 2006; Hirose et al., 2000; Nishikawa et al., 2003; Prasetia et al., 2017). However, the higher opportunity of coral associated with genetically diverse of symbiont could be occurred more on a group of horizontal symbiont transmission by selecting a partner from their various environment sources (Byler et al., 2013; Padilla-Gamiño et al., 2012; Rouze et al., 2019; Sweet, 2014).

Little is known about the variability and dynamics between Symbiodiniaceae and their coral host in Thai waters. This study provides the first annual investigation of

Symbiodiniaceae communities and diversity associated with corals with different reproductive modes, *A. humilis* (horizontal, gonochroric broadcaster) and *P. damicornis* (vertical, hermaphroditic brooder) from the upper Gulf of Thailand. The evidence from this study is important to identify the coral-Symbiodiniaceae associations and can be used to better understand localized coral reef adaptive potentials under different environmental conditions.

Materials and Methods

Sampling site

Acropora humilis and *Pocillopora damicornis* corals were collected from Ko Tao Mo, Sattahip, Upper Gulf of Thailand (12° 38' 33.78" N 100° 51' 40.13" E) (Fig 4.1) in 2018. These corals were distributed in shallow water at 5-7 m depth. Coral colonies were tagged numerically (n=15) for each species. The size of tagged colonies ranged from 30-100 cm in diameter. We collected coral samples following different seasons, referring to the local information of the Thailand Meteorological Department. These were divided into three seasons including summer (March-June), rainy (July-October) and winter (November-February). Fragments of approximately 2-3 cm from tagged colonies of each coral species and season within a year were collected by SCUBA diving (Fig 4.2) and preserved in 95% ethanol. In addition, ambient sea water temperature and light intensity data were recorded using underwater temperature data loggers (Hobo, Onset Corporation LtD). The average light intensity and temperature of each season were calculated based on logged data. The unit of light intensity (lux) was converted to PAR, µmol quanta m^{-2} s^{-1} (approximate 1 µmol m^{-2} s^{-1} = 52 lux) following the calculation method according to Bednarz et al. (2015).

Determination of Zooxanthellae Density

Fresh coral fragments were seasonally collected (January, May and October respectively) from each of the 15 tagged colonies of *A. humilis* and *P. damicornis* corals using hammer and chisel. Coral fragments were then transferred and preserved in 10% buffered formalin. For zooxanthellae density determination, we adapted the decalcified method from McCowan et al. (2011). In brief, preserved coral branches were placed in a glass container with 5% HCL solution until the coral skeleton was completely dissolved (~ 4-7 days depend on size and coral species). The remaining tissues were rinsed and preserved in 70% ethanol. Three pieces of fixed tissue were cut into 1x1cm sections and then preserved with 70% ethanol. Prepared samples were homogenized using Nissei ACE homogenizer (Nihonseiki Kaisha LTD.) for 5 min until the tissue was well-mixed. Subsamples from homogenate of 2.5 µl coral tissue were immediately placed on each chamber of a Neubauer-improved bright line hemacytometer (Marien Feld Germany) to count the number of zooxanthellae cells under 40x magnification (Fig 4.3). Four corner squares of each chamber were counted as one replication (6 times counted/ one sample). To calculate the mean zooxanthellae density per cm^2, the average total of zooxanthellae cell counted on each square was multiplied by 10^4 ml (the volume of chambers) and the dilution then divided by the coral tissue area (cm^2). The average zooxanthellae density of two coral species from three different seasons were test a mean of significant differences density using two-way ANOVA and Post Hoc.

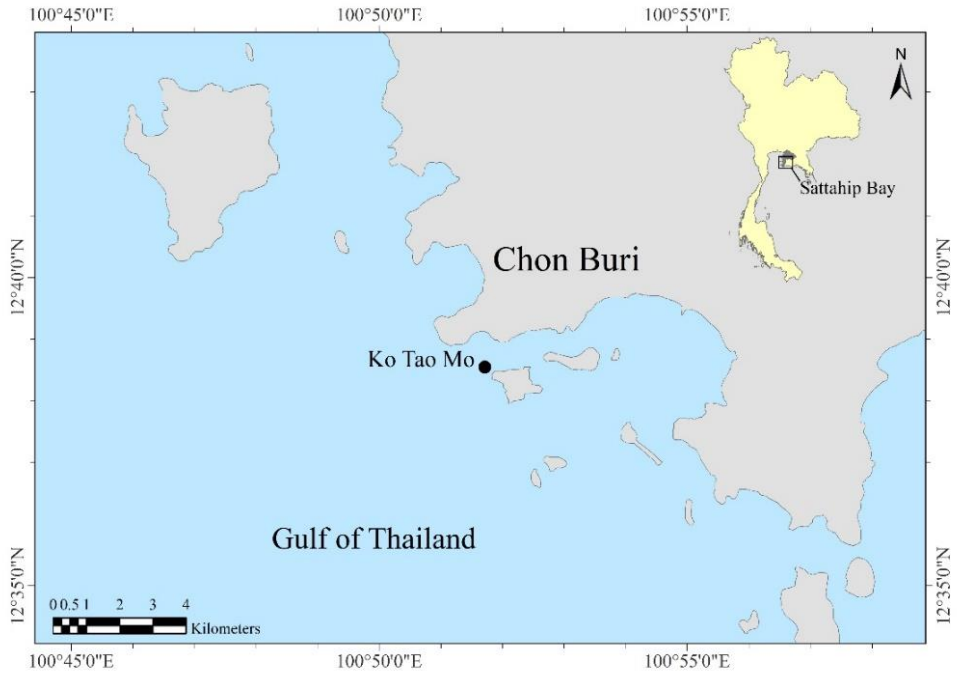

Figure 4.1 Study area and sampling site. Ko Tao Mo, Sattahip, Upper Gulf of Thailand.

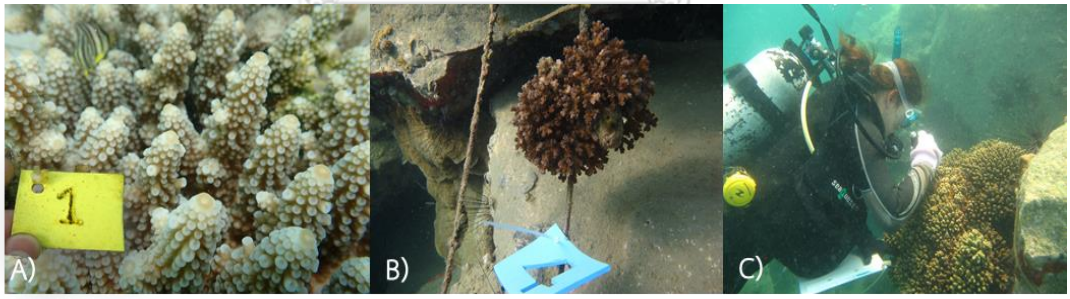

Figure 4.2 Tagged colony of two corals species ;A) *Acropora humilis* B) *Pocillopora damicornis* and C) Sample collection by SCUBA diving.

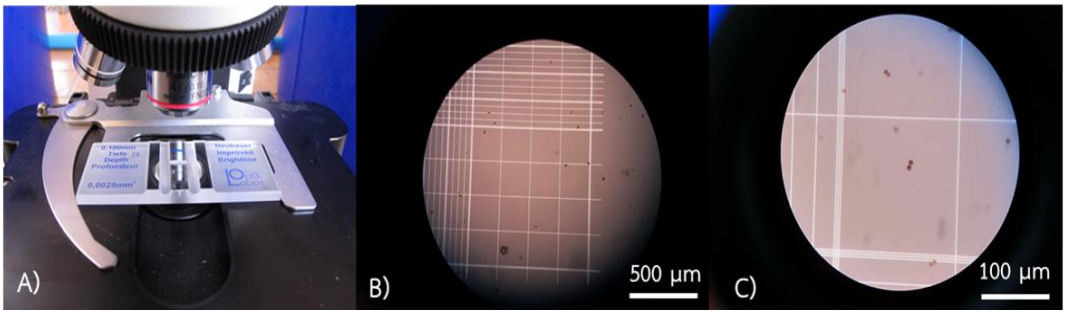

Figure 4.3 Zooxanthellae density count ;A) hemacytometer B) zooxanthellae cell under 10X and C) zooxanthellae cell under 40X magnification.

DNA extraction and PCR amplification

Small pieces of coral preserved in 95% ethanol were dried and ground using a pestle and mortar to create a coarse powder. Each powdered coral was used to extract the genomic DNA of zooxanthellae using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions with minor modifications. Crude DNA was analyzed using a NanoDrop 2000c (ThermoFisher Scientific™) to examine the quality and concentration of DNA. Extracted DNA was used as a PCR template to amplify the DNA of zooxanthellae. The PCR amplification was performed using primers for the internal transcribed spacers 1and 2 (ITS-1 and ITS-2) genes. The targeted ITS-1 and ITS-2 regions were amplified using the specific primers for zooxanthellae type identification including forward: r18Sf (5'- CGCTCTTCCGATCTCTGGAAAGTTTCATGAACCTTAT3-') and reverse: Sym28Sr-1st (5'-TGCTCTTCCGATCTGACCTTGTRTGACTTCATGCTA -3'). PCR was carried out using AllInOneCycler™ (BIONEER, Inc. USA) with a reaction volume of 10 µl mixture for each sample which consisted of 0.2 µl PrimeSTAR GXL DNA Polymerase, 2 µl Taq buffer, 0.8 µl dNTP of Takara (TAKARA Bio Inc.), 1 µl of each forward and reverse primer, 4 µl of 0.1 µm PCR grade water (Sigma® Life Science) and 1 µl of DNA template. The PCR conditions were 3 min at 94 °C, followed by 33 cycles of 98 °C for 10 s, 50 °C for 0.15 s, 68 for 60 s, and a final extension step of 3 m at 68 °C. PCR product were

cleaned using the FavorPrep® gel/PCR Purification Kit (FAVOGEN Biotech Corp.) following the manufacturer's protocol. Each purified sample was then used for the second PCR. The unique short nucleotides barcode (Eurofins Sciencetific) including forward and reward were performed. Here 90 pairs of different primers (90 coral samples) were used to include in the second PCR mixture. Each 10 µl PCR sample included the following components: 0.24 µl PrimeSTAR GXL DNA Polymerase, 2.4 µl Taq buffer, 0.96 µl dNTP of Takara (TAKARA Bio Inc.), 1 µl (0.1 µM) of each unique primer combination of forward and reverse primer, 3.4 µl Sigma water and 1 µl of purified PCR product. The PCR conditions were 3 min at 94 °C, followed by 13 cycles of 98 °C 10 s, 68 °C 60 s. Second PCR products were run in a 1.5% agarose gel with EcoDye Nucleic Acid Staining Solution (BIOFACT Co.,Ltd.) to check the targeted size of DNA.

Next-generation sequencing and data analysis

Each success PCR amplicon was pooled together in equimolar concentration for sequencing. Pooled samples were cleaned using SPRI Based Size Selection (Beckman Coulter, Inc, USA). DNA libraries then were quantified on Agilent Bioanalyzer 2100 (©Agilent Technologies, Polo Alto, CA, United States) and qPCR (Thermo Fisher Scientific) to examine the concentration and average DNA fragment size (bp). We used 4nM of DNA libraries and make a dilution to 8 pM followed by sequencing on an Illumina Mi-Seq platform (Illumina, San Diego, CA, United States) by the Atmosphere and Ocean Research Institute, The University of Tokyo, Japan. The analysis pipeline and analytical procedures were carried out following Shinzato et al. (2018). The raw data were submitted to the NCBI Sequence Read Archive (BioProject ID: PRJNA747703). For statistical analysis see in chapter 3.

Results

Local Environment Condition

The local condition at Ko Tao Mo reef were continuously recorded during each season over a year. The seasonal minimum and maximum temperature were recorded in January (26.90 °C) and May (31.98 °C) contrasting with light intensity which was lowest in August and September (1 μmol m^{-2} s^{-1}) and highest in April (342 μmol m^{-2} s^{-1}), respectively. An average local light intensity and temperature in each season was found to be 121.56 ± 8.44, 55.60 ± 3.88, 61.67 ± 2.09 μmol m^{-2} s^{-1} and 31.06 ± 0.06, 30.66 ± 0.05 and 29.26 ± 0.12 °C following summer (March-June), rainy (July-October) and winter (November-February), respectively (Fig 4.4). The summer season exhibited the highest average of both light intensity and temperature across all seasons ($p=0.001$) while rainy and winter season differed with the temperature only ($p=0.001$). However, there was no significant difference in the average light intensity between rainy and winter seasons ($p>0.05$). While both light intensity and temperature were highest in the summer, field observations found no sign of coral bleaching (though a slightly paler colour was recorded in both *A. humilis* and *P. damicornis* colonies) during 2018.

Zooxanthellae Density

Zooxanthellae densities (ZDs) of the two coral species were measured across the three different seasons. Average ZDs in *A. humilis* and *P. damicornis* across all seasons ranged from 0.66 $x10^6$ to 3.69 $x10^6$ cells/cm^2. One-way ANOVA showed significant differences in mean ZDs between the coral species ($p<0.005$). The mean ZDs of *P. damicornis* were twice as high (3.45 ± 0.62 x 10^6 cells/cm^2) as *A. humilis* (1.75 ± 0.42 x 10^6 cells/cm^2). Two-way ANOVA indicated that ZDs in different seasons were significantly different ($p<0.001$) (Table 4.1). The mean ZDs in both species showed the lowest number during the summer with *A. humilis* having 0.8 ± 0.18 x 10^6 cells/cm^2 and *P. damicornis* having 3.66 ± 0.42 x 10^6 cells/cm^2. (Fig 4.5). ZDs were found to be significantly different between different light intensity especially in *A. humilis* ($p<0.001$). The highest ZDs in *P. damicornis* were found to occur in winter (3.69 $x10^6$ cells/cm^2) while the highest in *A. humilis* was in the rainy season (2.21 ± 0.03 x 10^6

cells/cm^2). However, the comparison of ZDs between winter and rainy season among coral species was not found to be significantly different ($p>0.05$). Although, higher light intensity was found to correlate with lower zooxanthellae density in both corals, most colonies in the area remained healthy with no sign of coral bleaching during 2018 (sample collection year).

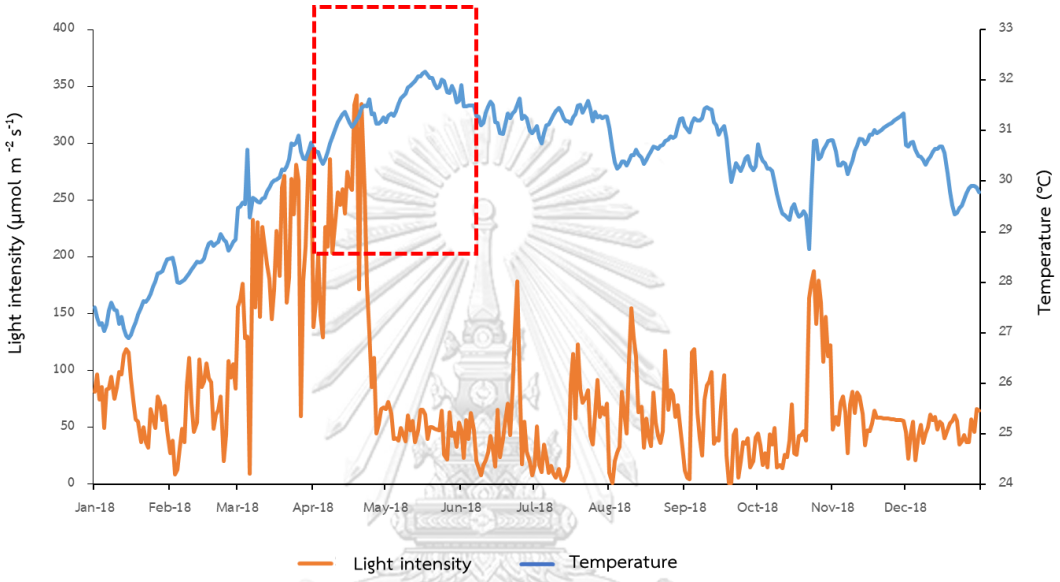

Figure 4.4 Average light intensity and temperature from winter to rainy seasons at Ko Tao Mo, Chon Buri, Upper Gulf of Thailand during 2018.

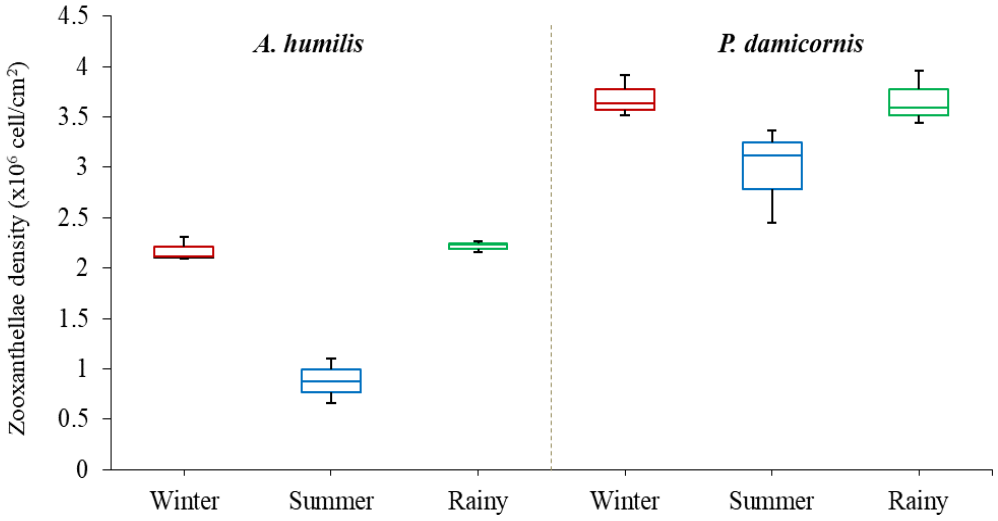

Figure 4.5 Box plots describing average of zooxanthellae densities of *Acropora humilis* and *Pocillopora damicornis* from winter, summer and rainy seasons. Boxes encompass the 25 and 75% quartile of all data. The central line corresponds to the median and bar extended to 95% and 5% of the confidence limits. Turkey Post-Hoc test ($p<0.001$) between two corals in different seasons.

Diversity and composition of Symbiodiniaceae of *Acropora humilis* and *Pocillopora damicornis* corals

We obtained genomic DNA from 80 colonies of two coral species from three different seasons. Sequencing of the ITS-1 and ITS-2 amplicons yielded 3,450,698 raw demultiplexed reads. Adaptor and quality trimmed reads resulted in 1,488,632 raw contigs and yielding 1,137,320 contigs after chimera removed reads from 80 individuals. An average 14,217 contigs/sample. Based on GeoSymbio database, two genera of Symbiodiniaceae were detected, *Cladocopium* (14 types) and *Durusdinium* (6 types) were identified in this study. The alpha diversity indices (Shannon and Simson) of coral were found *A. humilis* appeared to associate Symbiodiniaceae community with higher level of diversity than *P. damicornis* (Fig. 4.6).

Table 4.1 Summary of two-way ANOVAs analyses on Symbiodiniaceae density in *Acropora humilis* and *Pocillopora damicornis* at different local light intensities and temperature.

	Light intensity ($\mu mol\ m^{-2}\ s^{-1}$)	Temperature (°C)	*A. humilis* ($x10^6$ cells/cm^2)	*P. damicornis*
Light intensity	1			
Temperature	0.61	1		
ZDs of *A. humilis*	-0.98 **	-0.64	1	
ZDs of *P.damicornis*	-0.76*	-0.53	0.71	1

A total of 14 clades/types were identified in abundance in the two coral species, with *A. humilis* consisting of two dominant subclades including Cspc_C3 (56.39%), C3w (33.62%) and other minority subclades C93type1 (4.42%), Cspf (3.59 %) D1 (1.03%) and other types (0.95%). In the other hand, the majority of *Durusdinium* was found in *P. damicornis* following D1 (95.58%), D6 (1.01 %) D10 (2.70 %) and other (0.71%) subclades (Fig 4.7). A Bray-Curtis based nMDS plot illustrated a significantly different between Symbiodiniaceae community between *A. humilis* and *P. damicornis* (PERMANOVA, $p<0.001$) (Fig 4.8). The number of valid alignments of Symbiodiniaceae subclades across the different seasons remained relatively consistent in *A. humilis* and *P. damicornis* throughout the year but a greater dominant of *Durusdinium* D1 were found in one particular colony of *A. humilis* during the rainy season (Fig 4.9).

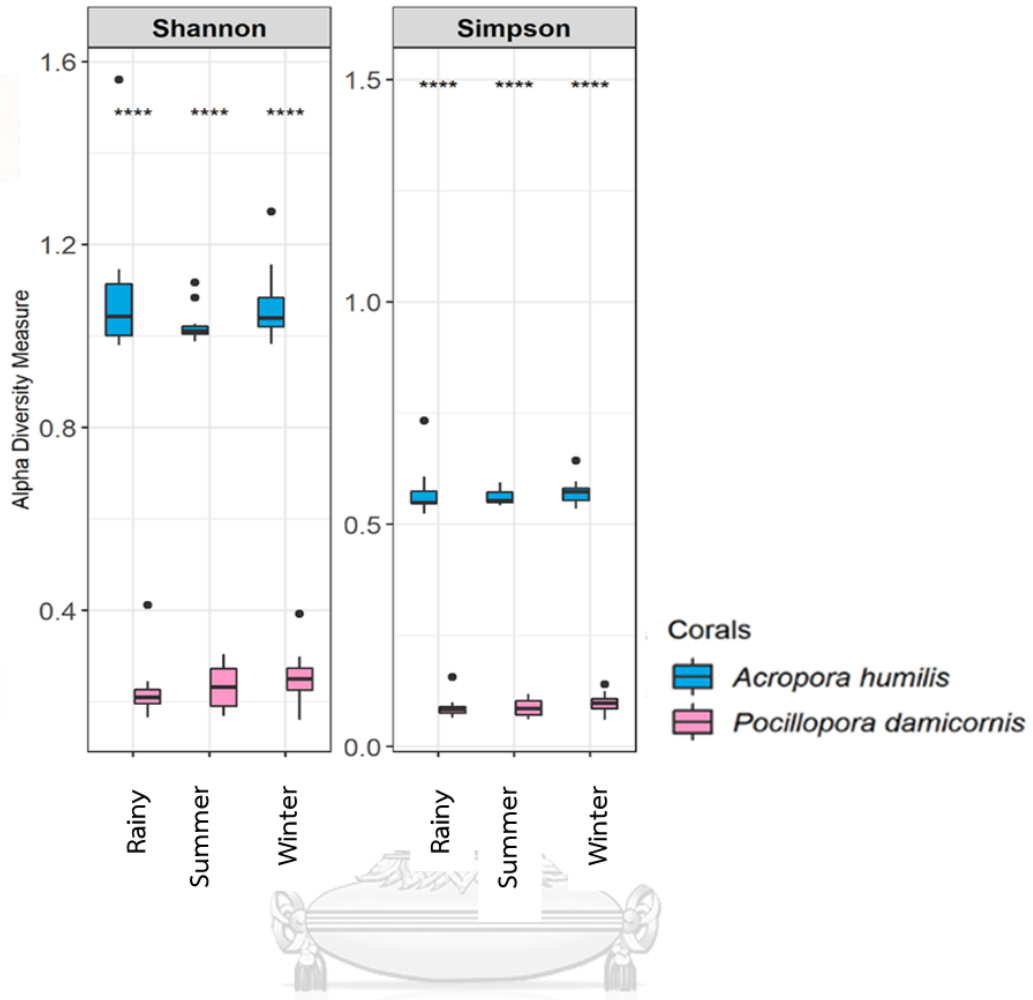

Figure 4.6 Alpha diversity indices (Shannon and Simpson) of Symbiodiniaceae community among samples from two coral species in different seasons.

Discussion

The average densities of zooxanthellae in both *A. humilis* and *P. damicornis* (1.75 and 3.44 x 10^6 cells/cm^2) in this study were within a range shown in other studies of a similar coral genera in the South China sea, Bahamas and Great Barrier Reef with approximately 1-3 x 10^6 cells/cm^2 (Fitt et al., 2001; Li et al., 2008; Pillay et al., 2005; Qin et al., 2019; Stimson, 1997; Xu et al., 2017b; Zhou et al., 2017). However, lower densities have also been recorded in *Pocillopora* spp for example in Wuzhizhou island,

SCS and Kenya's reefs were found to express a lower average (~0.6 to 1 x 10^6 cells/cm^2) (Mwaura et al., 2010; Xu et al., 2020). Observations that *P. damicornis* contained zooxanthellae cells at a higher density than *A. humilis* may be due to the capacity of light absorption (A_{max}) of each coral species being different (Scheufen, Iglesias-Prieto, et al., 2017). In addition, most branching corals contained lower numbers of zooxanthellae than other coral forms such as *Oculina patagonica, Porites* spp., *Montipora* and *Galaxea* spp. These corals displayed higher ZDs range from ~2 to 12 x10^6 cells/cm^2 (Ben-Zvi et al., 2015; Li et al., 2008; Mwaura et al., 2010; Rodolfo-Metalpa et al., 2006). It is however important to state that the variation of ZDs depends on sampling depth, coral species, local environmental factors (Al-Hammady, 2013; Brown et al., 1999b; Ladrière et al., 2014; Stimson, 1997; Xu et al., 2017b) zooxanthellae count preparation method (McCowan et al., 2011) and interval time of sample collection.

Seasonal changes have been shown to strongly influence zooxanthellae density variation (Pillay et al., 2005). High light intensity (342 µmol m^{-2} s^{-1}) during the summer in this study is significantly correlated with ZDs, particularly in *A. humulis*. Assessments of zooxanthellae function have also been carried out *ex-situ* based-on colonies reared from reef near the present study site of Ko Tao Mo, The corals *A. millepora* and *Platygyra sinensis* were shown to have a statistically significant reduction of photosynthetic rate (*Fv/Fm*) under higher light intensity (Kuanui et al., 2020). Similarly, data has shown a reduction of ZDs and PSII efficiency in *P. damicornis* during elevated temperature and intense light level (Hill & Ralph, 2007). The lowest zooxanthellae density was found during summer in both *A. humilis* and *P. damicornis*, similar to several other studies on scleractinian corals. Fitt et al. (2000) indicated that the lowest density of zooxanthellae and coral tissue biomass occurred during the summer, caused by bleaching of five hermatypic coral species. Rising temperature and light intensity were found to cause zooxanthellae cell reduction of 53% in the end of summer season in several shallow water corals in Thailand (Andaman sea) (Brown et al., 1999b). This suggests that corals have been shown to regulate their intracellular nutrient optimization by reducing the number of zooxanthellae, especially during

summer, due to nutritional limitations (Houlbrèque & Ferrier-Pagès, 2009; Kemp et al., 2014). Conversely, corals distributed along the Red Sea and high latitudes have been shown to have the opposite trend with the highest ZDs present around northern monsoon, which is associated with higher temperatures and light intensities (Al-Hammady, 2013; Mwaura et al., 2010). However, the maximum average temperature (25-29 ^{o}C) at higher latitude during summer months is still an optimum range for coral photosynthetic activity (Vaughan, 2014). Generally, corals were established higher ZDs during winter including this study (Costa et al., 2004; Ferrier-Pagès et al., 2011; Fitt et al., 2000; Pillay et al., 2005; Stimson, 1997) may be due to physiological seasonal adaptations of corals during low light conditions for harvesting capacity (Pillay et al., 2005). In contrast, three dominant species of *Acropora* corals in northern South China Sea and four Caribbean reef corals were observed to have the lowest density of zooxanthellae during winter (Scheufen, Krämer, et al., 2017; Xu et al., 2017a). This might have occurred after corals experience heat-stress previously (summer) and attempting to acclimatize during winter (Scheufen, Krämer, et al., 2017).

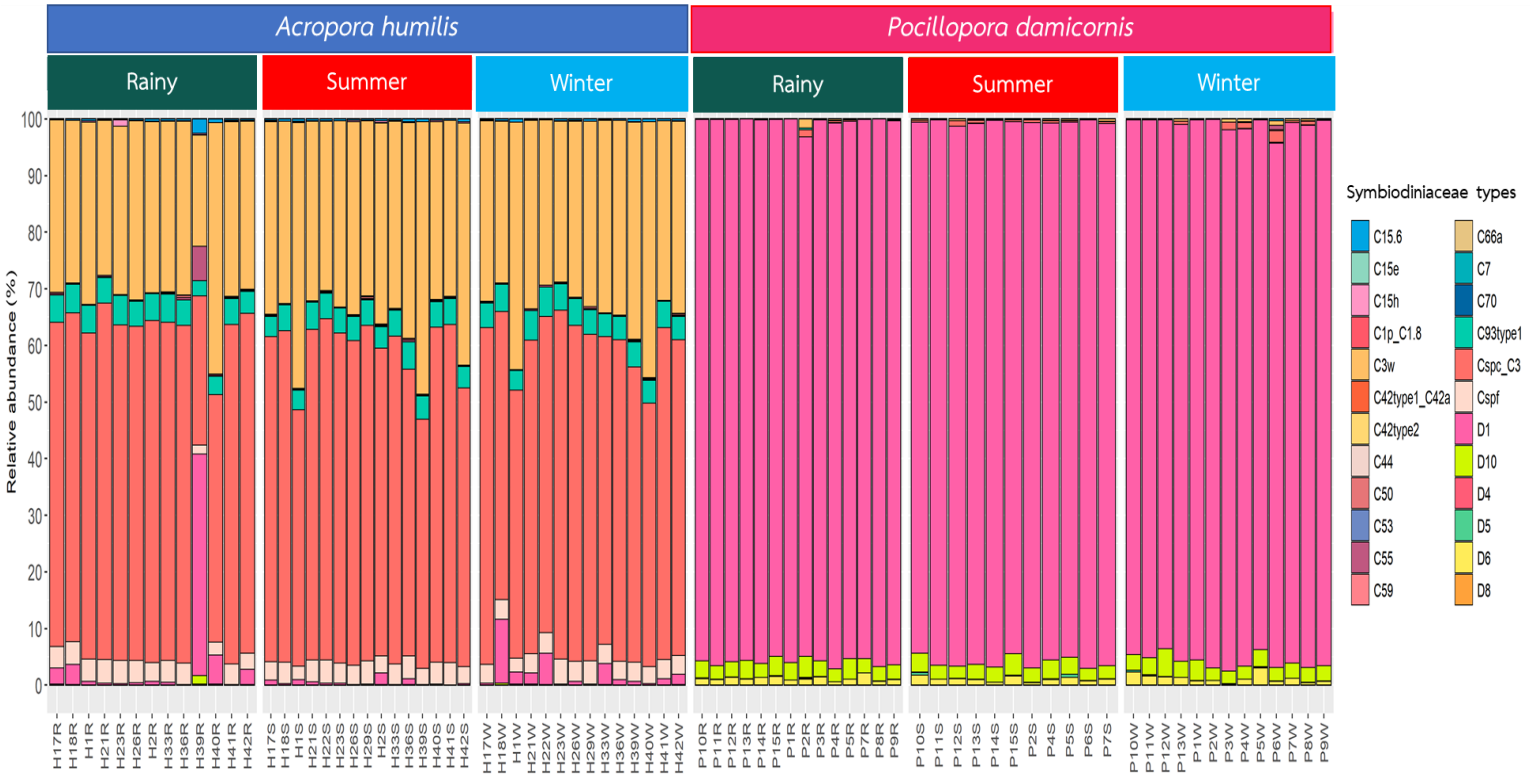

Figure 4.7 Relative abundance of Symbiodiniaceae communities in *Acropora humilis* and *Pocillopora damicornis* across different seasons in 2018.

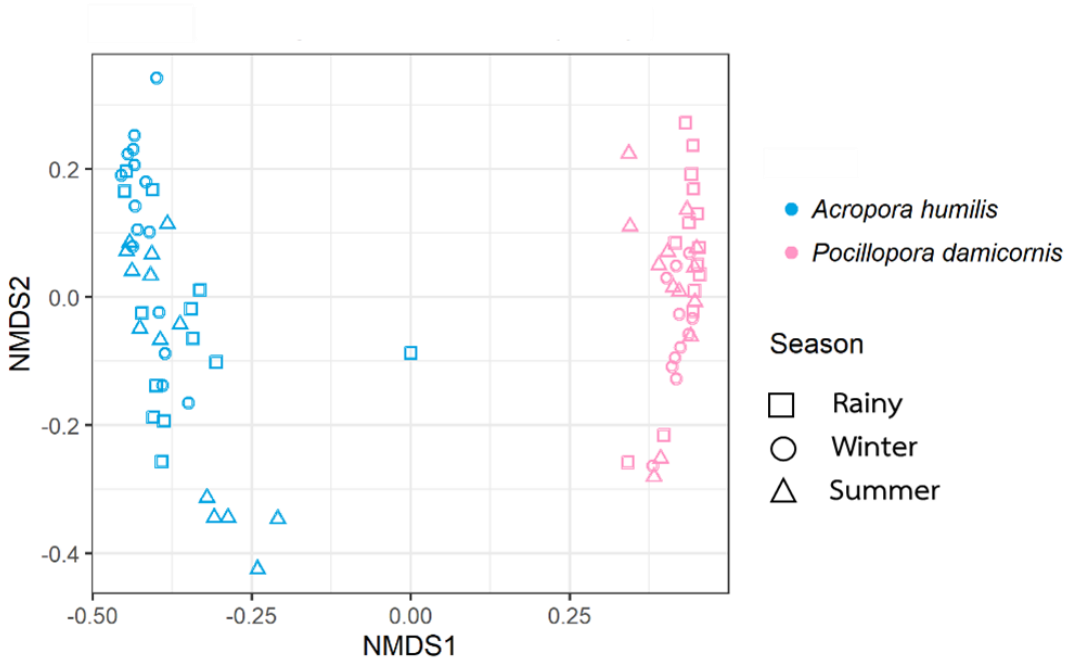

Figure 4.8 Non-metric multidimensional scaling (nMDS) plotting of the Symbiodiniaceae type composition of two coral species based on of Bray-Curtis dissimilarity indices.

Coral-Symbiodiniaceae association is influenced by several factor including life history of the host and environmental variability (LaJeunesse et al., 2004; LaJeunesse et al., 2010). This study, two distinct genera of Symbiodiniaceae, *Cladocopium* and *Durusdinium* were detected among *A. humilis* and *P. damicornis*. Our results indicate that corals were associated with a strong specificity to a particular Symbiodiniaceae genus/type in this location. Specifically, *A. humilis* was comprised with two genera of Symbiodiniaceae and five types (*Cladocopium;* Cspc_C3, C3w, C93type, Cspf and > 2% of *Durusdinium*: D1) while *P. damicornis* exhibited only one genus, *Durusdinium* (D1, D6 and D10). Symbiodiniaceae specificity is correlated with the pattern of symbiont acquisition of corals (N. S. Fabina et al., 2012; LaJeunesse et al., 2004; Stat et al., 2008; Thornhill et al., 2006).

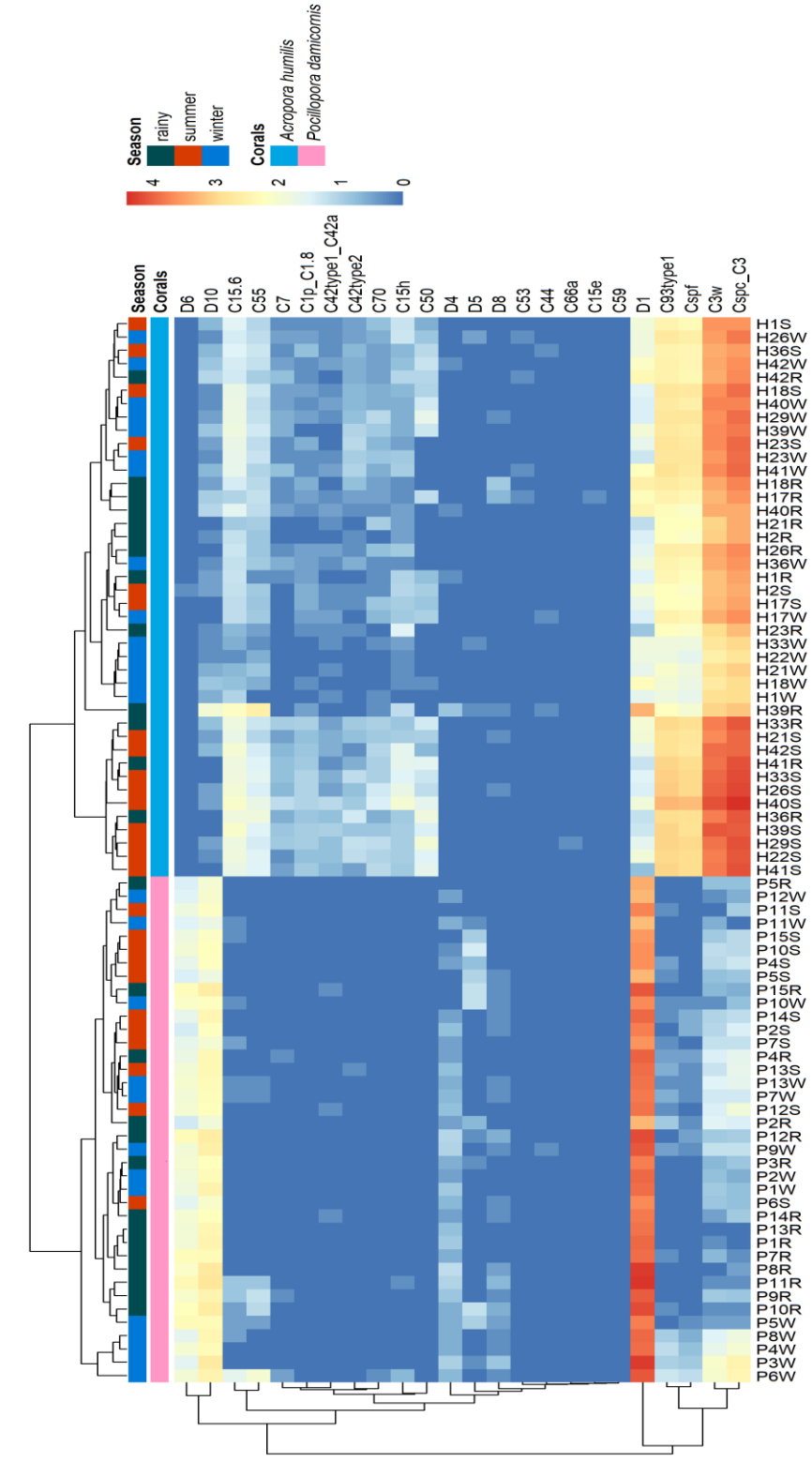

Figure 4.9 Number of valid alignments heatmap of the dominant and sub-dominant of Symbiodiniaceae types among *Acropora humilis* and *Pocillopora damicornis*.

Approximately 80-90% of reef building corals in the Indo-Pacific region are broadcast spawners which produce larvae free-zooxanthellae cells (Richmond & Hunter, 1990). Generally, *Acropora* spp. were considered to be a horizontal transmission coral harboring several types of Symbiodiniaceae to cope with environment stress including *A. humilis* in this study (Gong et al., 2019; LaJeunesse et al., 2010; Putnam et al., 2012; Quigley et al., 2017). Offspring of these hosts probably reflected local environmental conditions during early life stages when symbiosis with Symbiodiniacea was initially developed (LaJeunesse et al., 2004). Free-living Symbiodiniaceae cells, both in the water column and sediment, are an important natural pool for corals during larval or primary polyp stage and/or under critical conditions (Adams et al., 2009; Hoegh-Guldberg et al., 2008). Work by Yamashita et al. (2014) found that *Cladocopium* were detected mostly in the sediment while *Symbiodinium* and *Durusdinium* were found in the water column. This is could explain how Symbiodiniaceae cells in the sediment play a significant role with *A. humilis.* On the other hand, vertical symbiont transmission mode corals such as *Porites*, *Montipora* and *Pocillopora* have been shown to possess greater stability with fewer Symbiodiniaceae types across biogeographic gradients (Muller-Parker & Davy, 2001; Thornhill et al., 2006) due to the offspring of these corals being hypothesized to be more similar to their parent colony (Quigley et al., 2018). Similar results were shown in *P. damicornis* in this study and other investigations in Thailand with almost 100% associated with *Durusdinium* type D1 (Chankong et al., 2020; Pootakham et al., 2021) that the present study supports the idea that mode of symbiont acquisition may play an important role in Symbiodiniaceae structure in *A. humilis* and *P. damicornis* corals.

Distinct genera/types of Symbiodiniaceae have been shown to perform differently under changing environments. For example, *Durusdinium* and *Symbiodinium* are considered to be stress-tolerant or opportunistic (ie., warm, high turbidity, high irradiance) genera/types (LaJeunesse, 2002; Levas et al., 2013; Rowan, 2004; Wham et al., 2017) whereas corals associated with *Cladocopium* exhibited more susceptibility during stress conditions (Suwa et al., 2008; Thinesh et al., 2019). Corals

in the Indo-Pacific and Indian Ocean have been found associated with high abundance of *Cladocopium* and *Durusdinium* (Baker, 2003; LaJeunesse, 2005; LaJeunesse et al., 2010) whereas in the Caribbean, corals were associated with *Symbiodinium* and *Breviolum* (Cunning, Silverstein, et al., 2015; LaJeunesse, 2002; Thornhill et al., 2006). The Symbiodiniaceae community in the present study is similar to previous studies in Thailand, with only two genera, *Cladocopium* and *Durusdinium,* being found both in the Gulf of Thailand and Andaman sea (Chankong et al., 2020; Pootakham et al., 2018) but different types were identified regarding to the specific of coral genera. For example, *A. humilis* in this study hosted *Cladocopium* types and were found to be more susceptible to bleaching when higher light intensity was presented. Similarly, several studies have been affirmed that *Acropora* spp. tend to be more susceptible to bleaching which may be linked to the type of Symbiodiniaceae association (Hoogenboom et al., 2017; Putchim et al., 2017; Sakai et al., 2019) whereas *P. damicornis* contained *Durusdinium* and showed no significant effect on coral throughout seasons. Comparing this different pattern of Symbiodiniaceae community among *A. humilis* and *P. damicornis* may suggest that *A. humilis* is more vulnerable under stress conditions.

The community structure of Symbiodiniaceae association in the particular corals in this study showed a similar pattern to those in Xinyi and Palau reefs, in which, *Acropora* spp. hosted *Cladocopium* types while *Pocillopora* spp. contained almost exclusively D1 (Qin et al., 2019; Wham et al., 2017). Nevertheless, the different patterns of the same coral genus association with Symbiodiniaceae genus/type is known to occur across both local scales and wide geographic zones as well (Rodriguez-Lanetty et al., 2001; Ulstrup & Van Oppen, 2003; van Oppen et al., 2005). Several studies in the Great Barrier reef were found to show the opposite trend in Acroporidae and Pocilloporidae, at Lizard island with *Acropora* spp. being associated with a greater abundance of Symbiodiniaceae types than *Pociillopora* which were associated with almost 100% of *Cladocopium* (Ziegler et al., 2018). This suggests that the variety of Symbiodiniaceae genus/type diversity and pattern along the region may be driven by several factors including local environmental variability, coral species background,

Symbiodiniaceae supply sources such as seawater, sediment etc. and investigation techniques (Baird et al., 2009; LaJeunesse et al., 2010; Ng & Ang, 2016; Sweet, 2014; Thornhill et al., 2014; van Oppen et al., 2005). Most corals have not been shown to change their type of Symbiodiniaceae, despite continued changes in environmental conditions (Coffroth et al., 2010; Goulet, 2006; Lewis & Coffroth, 2004). Our results revealed that the seasonal variation does not affect the dominant genus/type of *Symbiodiniacea* in *A. humulis* and *P. damicornis* corals across all seasons. No coral bleaching was observed at Ko Tao Mo and the average sea water temperature was not higher than annually expected. This could influence the finding of no variation of Symbiodiniaceae type within the coral host during the study period. However, we detected a greater relative abundance of *Durusdinium* type D1 in certain colonies of *A. humilis* during rainy season without an occurrence of bleaching in summer. There are several studies that have shown a similar trend along tropical to temperate regions (Berkelmans & van Oppen, 2006; Cunning et al., 2018; Rouze et al., 2017). Ng and Ang (2016) who investigated labeled colonies of *Platygyra acuta* and *Porites* spp. from Hong Kong reef in different seasons found that there was no change in the Symbiodiniaceae type in all corals. Similarly, *Porites lutea* in Andaman Sea, Thailand retain a stable Symbiodiniaceae community structure (C116, C15f, C15g, C15h, C15.2) compared to healthy and bleached coral (Pootakham et al., 2018). No variation of Symbiodiniaceae (C1, C3-related) were showed in *Zoanthus sensibaricus* (zoanthids) across twelve months of the experiment at Kagoshima Bay, Japan despite this site showing a high variability of temperature (Reimer, 2007).

Several possible hypotheses could explain why corals maintain a stable community of Symbiodiniaceae during environmental condition stress; 1) avoiding intraspecific competition among Symbiodiniaceae types that could lead to disequilibrium of nutrients within the coral host and effect coral growth 2) coral response to historical environment stressors may exhibit higher potential of stable Symbiodiniaceae association 3) higher composition of some Symbiodiniaceae types may effect coral-cell metabolism 4) no severe effect on corals after experiencing stress conditions (Jones & Berkelmans, 2011; Klepac et al., 2015; LaJeunesse et al.,

2010; Putnam et al., 2012) or 5) limitation of high-stress tolerant Symbiodiniaceae genotypes within the host coral and/or environment. Nevertheless, some corals were able to acclimatize to environmental stresses by shuffling the relative abundance of their symbiont type or switching by uptake of exogenous Symbiodiniaceae types from the environment (Baker, 2003; Berkelmans & van Oppen, 2006; Cunning, Silverstein, et al., 2015; Quigley et al., 2016; Rouze et al., 2017; Sweet, 2014). Although corals-Symbiodiniaceae associations in upper Gulf of Thailand were found to be stable during the presently studied year, we predict that increasing severe coral bleaching in the future may result in acclimatization or adaptation of some corals by Symbiodiniaceae shuffling due to the diverse of Symbiodiniaceae types within hosts.

Conclusion

Both *A. humilis* and *P. damicornis* in this study exhibited the lowest zooxanthellae density during summer, which we infer is likely caused by light intensity. Our corals had a strong relationship with particular Symbiodiniaceae types, specific to each species, and showed no variation of Symbiodiniaceae type throughout the year. This may suggest that corals in upper Gulf of Thailand may select the appropriate genus/type of Symbiodiniaceae in response to local environmental stressors. This study is useful in understanding the coral-Symbiodiniaceae relationship and may be applied to predicting the potential adaptation of coral in localised reefs. However, more coral species and study sites are needed to further study the examined seasonal variation on coral-Symbiodiniaceae in Thailand.

CHAPTER 5

SUMARY AND CONCLUSIONS

5.1 Coral spawning study

Coral reefs in Thailand span more than 238.44 square kilometers. The deterioration of coral reef habitats and structural complexity in Andaman Sea and Gulf of Thailand are frequently influenced by anthropogenic activities and natural phenomena. Therefore, several national organizations have raised awareness regarding the conservation of coral populations. However, information on coral biology, including coral reproductive strategies, larva dispersal ability, and adaptation capacity, is required to improve coral reef restoration techniques and management. The corals of the genus *Acropora* are widely accepted to be among the most threatened reef building scleractinia, highlighting the importance of monitoring their reproductive strategies and development under the changing climatic conditions seen in recent decades. Gametogenesis of *Acropora* spp. develop approximately 4-5 months to achieve fully maturity. Coral spawning occurs annually during January- February. Most *Acropora* coral on a global scale tend to spawn around the full moon or days after full moon, but *A. humilis* and *A. millepora* in this study spawned across lunar cycles and tidal ranges. We observed the different time (5-40 min) of coral spawning among *Acropora* species in the upper GoT, including *A. hyacinthus*, *A. humilis*, *A. millepora*, and two unknown species. Coral spawning within a wide temperature range suggests that other physical or chemical factors may be driving the coral spawning mechanism in this particular area. However, coral spawning in consecutive days with low amount of gamete is a critical concern of less successful fertilization and coral recruitment. In addition, *Acropora* spp. in Thailand exhibited different times for gametogenesis and spawning. *Acropora* in Andaman Sea develop around June and tend to spawn in November or December every year (unpublish data). The different gametogenesis patterns along biogeographic gradients occurred possibly because of genetic differences among coral populations.

5.2 Coral-Symbiodiniaceae diversity and community

Endosymbiotic dinoflagellates in the family Symbiodinaceae are genetically and functionally diverse. More than 7 genera were identified based on ITS regions, but the total number of defined genera may reach 15 or more across regions. The community and diversity of Symbiodiniaceae associated with corals are related to several factors, including biological, ecological and physical conditions. This study provided a greater number of Symbiodiniaceae in Thai waters detected from 2 coral species, namely, *A. humilis* and *P. damicronis*. In comparison, 2 genera, *Cladocopium* and *Durusdinium* along with 13 types of Symbiodiniaceae were found from 6 coral species in previous studies, while one more genus, *Symbiodinium* and 21 distinct types were recorded in this study (1^{st} sequencing result only) (Fig 5.1). The first-record Symbiodiniaceae genus *Symbiodinium* was detected in 3-month-old of reared *A. humilis* under hatchery conditions. *Cladocopium* has the highest types number (18 types) followed by *Durusdinium* (7 types) and *Symbiodinium* (1 type), detected in this study. *A. humilis* is associated with a greater number of Symbiodiniaceae types compared with *P. damicornis*. Wild and transplanted *A. humilis* and ≤ three-month-old reared colonies of *P. damicornis* were associated with predominantly *Cladocopium*, whereas wild colonies of *P. damicornis* and ≤ three-month-old reared colony of *A. humilis* were associated with *Durusdinium*. The number of Symbiodiniaceae in this study may be lower than what was originally estimated because limited numbers of coral samples were studied. In addition, different investigation techniques applied in studies on Symbiodiniaceae diversity possibly affected the types of detected variations.

5.3 Coral adaptation/ Resilience

The adaptability of coral under stress condition is well known to rely on their genetically diverse Symbiodinaceae. Corals in this study hosted at least 3 types of Symbiodiniaceae within a single colony. Each Symbiodiniaceae type/genus exhibits a different response to environmental change; thus, hosting with a higher diversity may provide coral with an option for rapid selection during unsuitable conditions. Horizontal symbiont transmission (HT) corals are believed to acquire a greater novel

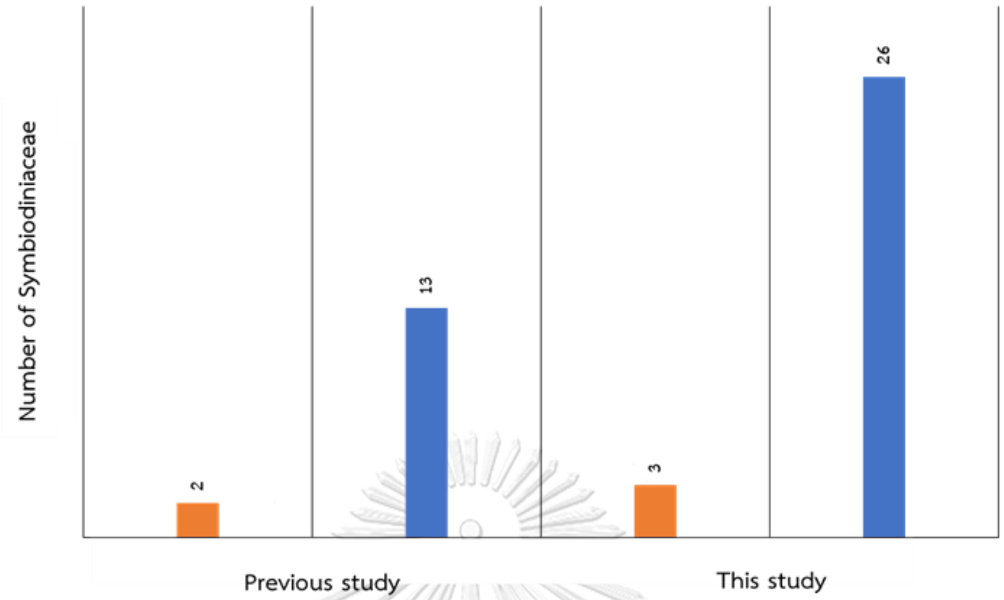

Figure 5.1 Number of Symbiodiniaceae recorded in Thai waters.

or change in Symbiodiniaceae composition than vertical symbiont transmission (VT). However, HT and VT corals exhibited both shuffling and switching mechanisms, especially during *ex-situ* experiments. Both corals exhibited the opposite community of Symbiodiniaceae compared with the wild parent colony, thereby suggesting that corals adjust their symbiont to survive under stress conditions. However, after four to five years of transplantation, reared *A. humilis* showed resemblance to the parent Symbiodiniaceae community, indicating that corals living in a natural reef may have more benefits to harbors with *Cladocopium*, and such benefits may be correlated with the amount of nutrients produced by their symbiont. Tagged colonies of wild corals had a strong relationship with particular Symbiodiniaceae types and were unable to change their symbiont throughout the year. A possible explanation for this situation is that no coral bleaching was observed within the year of sample collection. However, corals exhibit a simple adaptation through reduced Symbiodiniaceae cells during summer as a result of higher temperature and light intensity. This study reveals that

corals develop adaptation mechanisms to select the appropriate genus/type or adjust the number of Symbiodiniaceae in response to local environmental stressors.

5.4 Recommendations of further study

Coral spawning in the upper GoT, especially in Ko Tao Mo in Chon Buri province, exhibited no clear patterns according to the lunar period and tide range. Several physical factors have to be considered for additional data collection, such as wind speed, percentage of cloud cover, light intensity, and precipitation rate for spawning pattern analysis. Thailand is considered an underrepresented region with regard to data on spawning. Therefore, more coral species and study sites are needed to monitor reproductive strategies. Coral is vulnerable to environment changes. An *ex-situ* experiment requires careful attention to coral raising in the aquarium system. Slight physical factor variability, including changes in salinity, temperature, and light intensity, can cause coral mortality. Biological disturbance, such as the growth of algae, cyanobacteria, or sponge, also affects coral growth rate and survival. These organisms have to be removed to prevent competition between corals during experiments. DNA extraction and amplification should be completed as soon as possible after sample collection. Otherwise, some DNA would degrade and may result in amplification difficulty. The important factor that influences coral-Symbiodiniaceae establishment is zooxanthellae cell in the environment pool. Therefore, more free-living Symbiodiniaceae need to be collected from seawater and sediment for DNA analysis comparison. Single next-generation sequencing is recommended for DNA amplicon analysis; otherwise, the results may vary between sequencing batches.

APPENDICIES

Table A-1 Number of sequencing reads of two coral species, *Acropora humilis* and *Pocillopora damicornis* from 1st sequencing result: CHAPTER 3.

	Raw sequencing reads		Adaptor and quality (QV>20) trimmed reads			Chimera removed reads	
	Sample name	Number of reads	Basepair	Number of reads	Basepair	Number of reads	Basepair
1	1M1	748	225148	334	70167	179	35159
2	1M2	265292	79852892	113703	18541047	85823	14103829
3	1M3	290823	87537723	128936	20934232	97193	15883054
4	1M4	647	194747	302	69384	125	26949
5	1M5	789	237489	399	87099	185	38008
6	1M6	742	223342	314	61977	187	35061
7	1M7	181699	54691399	38336	6978240	27884	5170763
8	1M8	174670	52575670	114536	19108868	96504	16289588
9	1M9	768	231168	378	79686	221	45209
10	1M10	79905	24051405	40021	7816143	30661	5830794
11	3M1	67277	20250377	43611	7787215	34684	6002821
12	3M2	50420	15176420	26035	4981619	22497	4411316
13	3M3	62067	18682167	41601	6881096	36242	6054693
14	3M4	45607	13727707	37772	7029597	32313	6160477
15	3M5	43291	13030591	27094	5394766	23821	4830243
16	3M6	44033	13253933	26813	4189946	22367	3544748
17	3M7	60543	18223443	38077	6548935	32424	5658991
18	3M8	199451	60034751	112807	16967287	95110	14300511
19	3M9	121336	36522136	32150	5345706	21182	3821152
20	3M10	147090	44274090	48496	7379887	29275	4596280
21	6M1	26219	7891919	15674	4185733	14255	3851082
22	6M2	26867	8086967	16630	4332039	14746	3897232
23	6M3	44162	13292762	32333	8811543	29627	8150888
24	6M4	26326	7924126	14555	3937154	13343	3654091
25	6M5	20119	6055819	7698	1999041	6622	1745304
26	6M6	19198	5778598	8188	2204647	7460	2032456
27	6M7	46729	14065429	6891	1798263	6000	1600962
28	6M8	35904	10807104	4192	1024158	3399	855974
29	6M9	16795	5055295	8142	1981534	6845	1744551
30	6M10	22849	6877549	14081	3732427	12683	3428098
31	1Y1	43297	13032397	22573	6004925	20568	5567037
32	1Y2	42033	12651933	22792	5992028	20538	5504044
33	1Y3	53985	16249485	22812	5764154	19894	5199555

Table A-1 Number of sequencing reads of two coral species, *Acropora humilis* and *Pocillopora damicornis* from 1st sequencing result: CHAPTER 3 (cont.).

	Raw sequencing reads		Adaptor and quality (QV>20) trimmed reads			Chimera removed reads	
	Sample name	Number of reads	Basepair	Number of reads	Basepair	Number of reads	Basepair
34	1Y4	64250	19339250	41020	11166387	37398	10289199
35	1Y5	72717	21887817	46499	11863475	40440	10593953
36	1Y6	27522	8284122	10116	2602977	8748	2282372
37	1Y7	63866	19223666	33031	8355146	29111	7618308
38	1Y8	48166	14497966	23763	6460706	21624	5939453
39	1Y9	65737	19786837	35150	8837767	28961	7458251
40	1Y10	60759	18288459	36478	9286293	32243	8472906
41	15Y1	83230	25052230	47433	12059768	41957	11028823
42	15Y2	48712	14662312	22817	4882472	17780	4068086
43	15Y3	276	83076	148	34089	83	19432
44	15Y4	44830	13493830	24580	6658215	22456	6147438
45	15Y5	38359	11546059	14778	3948694	13317	3609883
46	15Y6	74832	22524432	42402	10663527	35993	9349236
47	15Y7	66013	19869913	43683	11830354	39345	10784445
48	15Y8	31738	9553138	13864	3670966	12809	3430224
49	15Y9	69775	21002275	34483	7779875	28077	6703118
50	15Y10	44163	13293063	26808	6964814	22820	6002811
51	SH3	59758	17987158	8658	2212589	7542	1972342
52	AH1PA	47441	14279741	11478	1947181	7428	1307363
53	AH17PA	14248	4288648	11073	2948691	8285	2190609
54	AH1PA	23794	7161994	3237	853682	2021	514935
55	AH2PA	61009	18363709	14452	2402757	9405	1605057
56	AH22PA	53691	16160991	3367	683557	2141	454210
57	AH23PA	28624	8615824	17192	4703638	12708	3423383
58	PDL1	32702	9843302	8772	2320813	7063	1893854
59	PDL2	36755	11063255	13514	3593486	10820	2912480
60	PDL3	471	141771	242	56292	123	28479
61	P3M2	36489	10983189	32075	8795064	18271	5136744
62	PD1PA	22528	6780928	17663	4700763	15043	4047777
63	PD2PA	8447	2542547	4903	1257045	2988	787641
64	PD3PA	37277	11220377	11556	3017479	9424	2508691
65	PD4PA	42754	12868954	17193	4649094	14181	3866757
66	PD5PA	39993	12037893	6841	1804964	4137	1075173

Table A-2 Number of sequencing reads of two coral species, *Acropora humilis* and *Pocillopora damicornis* from 2nd sequencing result: CHAPTER 3.

	Raw sequencing reads		Adaptor and quality (QV>20) trimmed reads			Chimera removed reads	
	Sample name	Number of reads	Basepair	Number of reads	Basepair	Number of reads	Basepair
1	AH2Y2	101366	9998669	78772	7952176	62841	6343947
2	AH2Y3-1	47748	4808698	44699	4513447	35617	3596419
3	AH2Y4	103799	10221254	80180	8093354	63363	6395870
4	AH4Y3	105077	10347065	80571	8133492	63509	6411192
5	AH5Y1	106447	10516582	83904	8470493	66878	6751708
6	AH5Y3	109885	10792763	83158	8395073	66112	6674234
7	TAH2Y1	43885	4421245	40768	4116191	32053	3236281
8	TAH2Y1-1	43885	4421245	40768	4116191	32053	3236281
9	TAH2Y2	103644	10232154	81108	8188384	64551	6516962
10	TAH2Y3	105257	10356396	79951	8071270	64895	6551352
11	TAH3Y1	105807	10354590	77898	7863739	67542	6818446
12	TAH3Y2	102377	10126880	81112	8187534	65965	6658649
13	TAH4Y2	100679	9926913	78471	7921952	68243	6889540
14	TAH4Y3	104180	10223493	79329	8008346	69267	6992692
15	TAH5Y1	100681	9895516	77439	7818102	67707	6835702
16	TAH5Y2	105670	10370329	80249	8101135	69980	7064578
17	TAH9MSB	107316	10585564	82831	8362115	65827	6645509
18	TAH9MSB-1	78939	7951329	73550	7426964	58538	5911122
19	MOPL2	102845	10175765	82224	8300777	64702	6531925
20	MOPL4	105049	10384763	82835	8362216	64323	6493471
21	P1M2	108930	10797887	87478	8830310	68874	6952367
22	P3M1	105350	10450242	86543	8737052	74046	7475518
23	P3M5	110018	10917264	91040	9191539	77811	7856015
24	P6M2	100812	9980884	81397	8216864	70012	7067588
25	P6M3	111255	11050334	91148	9201209	78104	7884511
26	P1Y2	106017	10426718	81286	8205584	70173	7083803
27	P1Y3	112820	11102446	86923	8774877	74796	7550725
28	P15Y2	108034	10633654	83398	8419113	71557	7223824
29	P15Y5	112773	11120887	88658	8950009	76152	7687566
30	P2Y2	107551	10588410	83312	8410135	71296	7197211
31	P2Y3	63928	6441860	59875	6045943	51524	5202710
32	P2Y4	101890	10073438	81758	8253720	70068	7073692

Table A-3 Number of sequencing reads of two coral species, *Acropora humilis* from 1st sequencing result: CHAPTER 4.

	Raw sequencing reads		Adaptor and quality (QV>20) trimmed reads			Chimera removed reads	
	Sample name	Number of reads	Basepair	Number of reads	Basepair	Number of reads	Basepair
1	H1W	47441	14279741	11478	1947181	7428	1307363
2	H17W	14248	4288648	11073	2948691	8285	2190609
3	H18W	23794	7161994	3237	853682	2021	514935
4	H21W	61009	18363709	14452	2402757	9405	1605057
5	H22W	53691	16160991	3367	683557	2141	454210
6	H23W	28624	8615824	17192	4703638	12708	3423383
7	H26W	18731	5638031	14829	4046182	11081	2977284
8	H29W	23884	7189084	19468	5276333	14589	3899601
9	H33W	22335	6722835	7967	1495170	5305	1029367
10	H36W	25699	7735399	14329	3310923	10254	2406651
11	H39W	32145	9675645	19413	5030232	14438	3720675
12	H40W	24602	7405202	17768	4697091	13385	3482496
13	H41W	50223	15117123	26459	6545697	19119	4775202
14	H42W	42872	12904472	15372	3553919	10892	2568500
15	H1S	55128	16593528	21564	4726288	15298	3425330
16	H2S	66063	19884963	15823	3004212	10616	2090239
17	H17S	33759	10161459	13962	2933853	9358	2048810
18	H18S	21733	6541633	18297	4951440	13638	3641961
19	H21S	25773	7757673	19961	5407397	15010	4013735
20	H23S	30221	9096521	21856	5784323	16397	4306406
21	H26S	60105	18091605	40887	10964942	31092	8212507
22	H22S	36620	11022620	28299	7677471	21352	5716847
23	H29S	48182	14502782	33556	9034691	25199	6691789
24	H33S	45050	13560050	37562	10044629	28256	7483939
25	H36S	26938	8108338	9871	2530301	7336	1852651
26	H39S	58223	17525123	39005	10200276	29811	7655321
27	H40S	80945	24364445	59643	16296323	44658	12032921
28	H41S	57126	17194926	30941	8275268	23242	6132599
29	H42S	45400	13665400	27378	7242234	20739	5419740
30	H1R	78174	23530374	13575	2726321	8875	1845620
31	H2R	100368	30210768	14402	2626610	9036	1726772
32	H17R	59692	17967292	13684	3100771	9535	2216944
33	H18R	40252	12115852	12826	3496450	9523	2553336

Table A-3 Number of sequencing reads of two coral species, *Acropora humilis* and *Pocillopora damicornis* from 1st sequencing result: CHAPTER 4 (cont.).

	Raw sequencing reads		Adaptor and quality (QV>20) trimmed reads		Chimera removed reads	
	Sample name	Number of reads	Basepair	Number of reads	Basepair	Number of reads	Basepair
34	H21R	54700	16464700	6061	1584133	4396	1147131
35	H23R	62455	18798955	5427	1305026	3800	909284
36	H26R	50667	15250767	11872	3205460	8842	2358137
37	H33R	51292	15438892	26255	7155896	19771	5314779
38	H36R	42123	12679023	26764	7306123	20226	5447614
39	H39R	36107	10868207	9599	2513613	7934	2077974
40	H40R	32083	9656983	11601	2719234	8394	1992177
41	H41R	39178	11792578	30130	8277499	22354	6059870
42	H42R	25988	7822388	12041	3248994	9179	2465329
43	P1W	21202	6381802	16561	4350560	13221	3503659
44	P2W	25747	7749847	16174	4407559	13312	3654994
45	P3W	44676	13447476	32650	8930007	28697	7914975
46	P4W	24115	7258615	14790	4004256	12471	3411344
47	P5W	42978	12936378	23903	5127479	16141	3617469
48	P6W	32277	9715377	20753	5659519	17635	4850806
49	P7W	28471	8569771	13499	3619107	11003	2980971
50	P8W	40335	12140835	17437	4597553	14282	3817475
51	P9W	37365	11246865	22057	5982091	18311	5010525
52	P10W	31417	9456517	13188	3061935	10418	2546145
53	P11W	150236	45221036	38044	5833403	23646	3786854
54	P12W	75223	22642123	19014	3185108	12190	2166872
55	P13W	28399	8548099	13512	3611335	11174	3020831
56	P2S	23859	7181559	10622	2879405	8782	2402841
57	P4S	24518	7379918	9590	2471323	6380	1684756
58	P5S	61910	18634910	5518	1195594	4127	966166
59	P6S	40462	12179062	9429	2567281	7357	2013112
60	P7S	17374	5229574	7227	1957527	5945	1624573
61	P10S	23918	7199318	10891	2650057	8932	2277031
62	P11S	28292	8515892	14630	3466353	11696	2901682
63	P12S	43872	13205472	13511	3661541	11077	3022632
64	P13S	17093	5144993	15038	4086504	12746	3485571
65	P14S	26855	8083355	15583	4257072	13110	3604201
66	P15S	26136	7866936	11492	2534580	8968	2095410

Table A-3 Number of sequencing reads of two coral species, *Pocillopora damicornis* from 1st sequencing result: CHAPTER 4 (cont.).

	Raw sequencing reads		Adaptor and quality (QV>20) trimmed reads		Chimera removed reads	
	Sample name	Number of reads	Basepair	Number of reads	Basepair	Number of reads	Basepair
67	P1R	22528	6780928	17663	4700763	15043	4047777
68	P2R	8447	2542547	4903	1257045	2988	787641
69	P3R	37277	11220377	11556	3017479	9424	2508691
70	P4R	42754	12868954	17193	4649094	14181	3866757
71	P5R	39993	12037893	6841	1804964	4137	1075173
72	P7R	39228	11807628	15848	4201533	13385	3602215
73	P8R	83470	25124470	33943	9248546	28214	7733221
74	P9R	31806	9573606	21784	5988784	16741	4605267
75	P10R	50832	15300432	25269	6771414	22179	6054805
76	P11R	60605	18242105	40764	11108890	35069	9648551
77	P12R	58542	17621142	32784	8507673	27527	7275830
78	P13R	73086	21998886	25768	5522079	19434	4414419
79	P14R	69393	20887293	13214	3584973	10304	2810021
80	P15R	82364	24791564	26743	6520973	22155	5643216

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AWARD RECEIVED

April 2017 The winner of the "WESTPAC Young Scientists Travel Grant for the 10th WESTPAC International Scientific Conference, China

July 2017 The Best student presentation award from 1st Zhejiang University International Ocean Youth Camp, China

November 2018 1st Prize of the outstanding poster presentation award from International Conference on the Environmental Management of the Enclosed Coastal Seas (EMECS12), Thailand