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Master Thesis

持久熱逆境決定共生藻洗牌程度與維氏腦紋珊瑚白化後之生存率

Prolonged thermal stress determines the degree of symbiont shuffling and *Platygyra verweyi* survival after bleaching

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誌謝

　　能完成這本論文、走完碩士生涯，我對於一路上引導、協助、支持我的所有人，在這邊致上由衷的感謝。對於原本對海洋生態研究一無所知的我而言，指導教授 Allen 的引導至關重要；從實驗設計發想、資料蒐集及解讀，到報告與論文的呈現，讓我逐步建立起對生態研究的認知。論文裡所收集的每一個珍貴實驗樣本，皆是在實驗室成員齊心協力幫助下採取完成。小曹於整段實驗時期在移植板的設立與潛水採樣上的大力協助，是實驗能完成的最大支柱。Christine、Sophia、Rod、Sophie、Lauriane、佳妍、山豬等實驗室夥伴也義不容辭地幫我分擔了繁重地採樣工作，謝謝有你們在墾丁一起跟我奮鬥，讓看似再不可能的任務都能逐一達成。在收集完實驗數據後，資料的解釋與統計方法的應用，也多虧有 Garmin、Vianney 時常給我建議，讓我減少許多摸索的時間。在實驗室分析樣本的過程中，綵葭、Shanky 的示範，讓我對各項實驗方法，有了更深一層的了解。也感謝其他的實驗室成員，若譯、Stéphane、警衛沈大哥的加油打氣，讓我充飽電力繼續往前。當然還有雁文、文華在行政事務與經費上的管理，讓我在實驗上無後顧之憂。特別感謝 Shanky 在協助我審閱論文草稿時，在圖表的編排、文章段落的調整上給我許多寶貴的建議。除了與實驗室夥伴的合作之外，在墾丁採樣時能順利完成，也要謝謝魏杰教練在實驗初期的幫忙及水下工作的經驗分享，蔡教練提供南北舒適的住宿環境與車輛使用上的支援。海生館孟培傑老師、家銘學長、宋秉鈞老師、王立雪老師在水缸實驗、樣本保存等器材使用上的協助，以及核三廠李先生在水下作業安全部分的叮嚀。實驗後期也謝謝生多中心的李文雄老師、王子元老師、呂美曄老師提供 qPCR 儀器相關的協助。口試前後，也感謝口試委員們對於論文編排、圖表呈現，以及文章細節上的提醒，讓我的論文更加完整。最後，最感謝的就是我的家人，支持我轉換領域且三年來不斷地給我鼓勵，還有女友佳瑜，陪伴及勉勵我，度過三年來難忘的碩班生活。
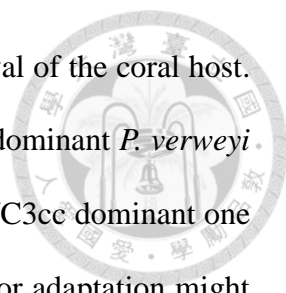
中文摘要

由全球氣候變遷造成之海水暖化已然威脅造礁珊瑚的生存。共生藻洗牌為珊瑚宿主對抗熱逆境的數種機制之一，藉由改變體內既有不同抗熱性之共生藻相對豐度，來度過熱逆境所帶來之生存壓力。目前已知有少數幾種珊瑚具有執行共生藻洗牌能力，然而，熱逆境之歷程對於珊瑚執行共生藻洗牌之過程以及洗牌過後珊瑚宿主生存率之影響，至今還不清。本篇實驗採取位於墾丁核三廠出水口、入水口以及萬里桐之維氏腦紋珊瑚片段，於 2014 年與 2015 年分次進行野外三個地點間之交互移植實驗。由出水口採取之珊瑚片段，其體內共生藻族群以抗壓性強之 D1/D1a 型共生藻為主，而其餘兩地採取之珊瑚片段則以對熱敏感之 C3/C3cc 型共生藻為主。在 2014 年進行之實驗中，所有由入水口與萬里桐移植至出水口之珊瑚片段皆於七月產生白化現象（熱周度指標＝10.43），對比之下由出水口移植至其餘兩地之珊瑚片段則無此現象。所有白化之珊瑚片段中，共有 40%完成共生藻洗牌，其體內共生藻族群轉為以 D1/D1a 型為主，然而在已完成洗牌之珊瑚片段中，有 75%在熱逆境消散後死亡。在 2015 年進行之實驗中，由萬里桐移植至出水口之珊瑚片段共有 73%產生白化現象（熱周度指標＝5.7），而其中又有 73%完成共生藻洗牌，其體內共生藻族群轉為以 D1/D1a 為主並且全數存活。本篇研究結果證明了在面對氣候變遷影響下，維氏腦紋珊瑚具有進行共生藻洗牌以對抗海水暖化之能力，然而持久熱逆境可能扮演著決定最終共生藻洗牌成功率以及珊瑚宿主最終存活率的關鍵角色。此外，本篇研究亦在野外的條件下證明了體內以 D1/D1a 型共生藻為主之維氏腦紋珊瑚可有著與體內以 C3/C3cc 型共生藻為主之維氏腦紋珊瑚相等甚至更高的生長率，此結果暗示著位於出水口之維氏腦紋珊瑚族群可能已在長期熱逆境下藉由調適（acclimatization）或適應（adaptation）或兩者，發展出能更有效率地與 D1/D1a 型共生藻共生之機制。

關鍵字：熱周度、共生藻洗牌、生存率、珊瑚生長、維氏腦紋珊瑚

Abstract

Rising seawater temperature caused by climate change is threatening the survival of reef-building corals. One of the mechanisms for corals to overcome thermal stress is to shuffle *in hospite,* the relative dominance of *Symbiodinium* from thermally susceptible types to tolerant ones. Although the shuffling of *Symbiodinium* has been observed in a several of coral species, the influence of thermal history on the process of symbiont shuffling and subsequent survival of coral hosts remains unclear. In this study, we conducted *in situ* reciprocal transplantation experiments (RTE) on nubbins of brain coral, *Platygyra verweyi*, collecting from a nuclear power plant outlet (NPP-OL), inlet (NPP-IL) and Wanlitung (WLT) of Kenting National Park in 2014 and 2015. While the symbiont community of *P. verweyi* in NPP-OL was dominated by stress-tolerant types D1/D1a, the ones in NPP-IL and WLT were dominated by the thermal- susceptible types C3/C3cc. In 2014, all the nubbins transplanted from NPP-IL and WLT to NPP-OL bleached in July after the prolonged high seawater temperature (Degree Heating Week, DHW=10.43) in NPP-OL. In contrast, those ones transplanted from NPP-OL to the other two sites remained unbleached. Forty percent of bleached nubbins showed sign of shuffling from C3/C3cc to D1/D1a, although 75% of them died in the subsequent fall. In 2015, 73% of the RTE nubbins from WLT to NPP-OL bleached under less prolonged high seawater temperature (DHW=5.7) with 73% shuffling their symbiont community from C3/C3cc to D1/D1a dominant and surviving after a reduction of the thermal stress. Our results show that in the facing of climate change impact, *P. verweyi* is capable of shuffling symbiont community towards the stress-tolerant type in order to respond to the rising seawater temperature. However, the degree of prolonged thermal stress may play

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a crucial role in determining the success of shuffling and final survival of the coral host. In addition, our study also indicates that the *Symbiodinium* D1/D1a dominant *P. verweyi* can have a comparable or higher growth rate than *Symbiodinium* C3/C3cc dominant one under field condition. This suggests a potential acclimatization and/or adaptation might occur in the *P. verweyi* population at NPP-OL after long-term association with *Symbiodinium* D1/D1a.

Keywords: Degree Heating Week, Shuffling, Survival rate, Coral growth, *Platygyra verweyi*

Contents

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Abbreviations

NPP-OL: Nuclear Power Plant Outlet

WLT: Wanlitung

NPP-IL: Nuclear Power Plant Inlet

NOO: Native group from NPP-OL to NPP-OL

TOW: Transplant group from NPP-OL to WLT

TOI: Transplant group from NPP-OL to NPP-IL

NWW: Native group from WLT to WLT

TWO: Transplant group from WLT to NPP-OL

NII: Native group from NPP-IL to NPP-IL

TIO: Transplant group from NPP-IL to NPP-OL
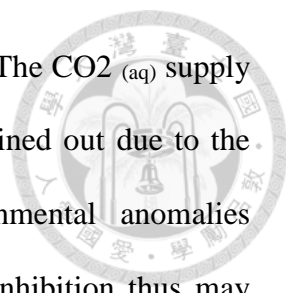
Introduction

Rising sea surface temperature caused by global climate change is threatening the survival of coral reefs. Due to the current pace of CO_2 emissions[1], the mean global sea surface temperature (SST) were predicted to rise by 1.0 to 3.7 °C by 2100[1], and up to 90% of coral reefs may suffer from annual bleaching by 2055 when severe and prolonged thermal stress events occur every year[2]. Adding up the human impact, global coral reefs are likely to encounter serious degradation in the near future[2, 3, 4].
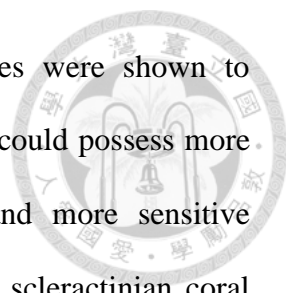
Corals form symbioses with the single-celled dinoflagellate (genus *Symbiodinium*) as part of the holobiont[5, 6, 7, 8]. The tight linkage between coral host and *Symbiodinium* may breakdown when environmental conditions derail from an equilibrium state which results in significant decrease in the number of *Symbiodinium* cells associated with a coral host[8, 9, 10, 11] and/or the chlorophyll content per *Symbiodinium*[12, 13] (a phenomenon called coral bleaching). High seawater temperature has been widely reported as one of the factors for causing coral bleaching. Generally, a deviation of 1.0 ℃ of seawater temperature above the local summer average over a couple of weeks, such phenomenon would cause negative effects on coral hosts such as reduction in energy stores, coral growth rates, and reproduction performances[9, 10]. Coral with mild bleaching could recover quickly if the stressors disappear[11, 12, 13, 14, 15, 16] while in severe cases could lead to host mortality[16, 17].

The onset of coral bleaching has been proposed to involve the disruption in symbiont photosynthesis[18, 19] and/or in the host CO2 concentration mechanisms (CCMs)[18]. One model proposed by Wooldridge 2009a integrated components of proposed cellular process lie behind coral bleaching which pointed out that the
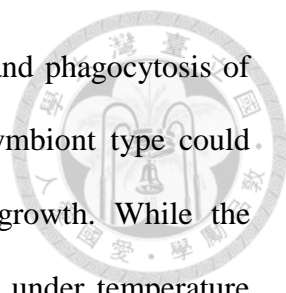
dysfunction of host CCMs may be the trigger of the whole process. The $CO2_{(aq)}$ supply to the symbionts from the coral host for the dark reaction was drained out due to the increasing demand from fast-growing symbiont under environmental anomalies (temperature anomalies or excess nutrition). The following photoinhibition thus may damage the photosystems[20] and generate the damaging reactive oxygen species (ROS) accumulated in the coral membrane[21] which ultimately lead to the mass expulsion of symbiont as a defending strategy of coral host[22, 23]. The theory was further supported by Cunning and Baker 2013, showing that *Pocillopora damicornis* with higher symbiont density was more susceptible to bleaching[24].

    Symbiodinium is genetically diverse and has been classified into 9 clades (clade A-I)[25] and hundreds of subclade or types based on nuclear internal transcribed spacer (ITS) regions[26, 27, 28, 29, 30, 31, 32]. It was regarded that *Symbiodinium* generally possesses distinct physiology traits between different clades. Members of the same clades share similar traits, such as clade C is thermal-susceptible while clade D is stress-tolerant[33, 34, 35, 36, 37]. However, recent studies have found that the physiological performances of *Symbiodinium* can also vary among types[38, 39, 40, 41, 42, 43] or populations within single type[44] under different environment. The various physiological traits of symbiont can also affect responses of the coral host against changing environment when different coral-algal combinations establish. For instance, thermal tolerance of coral hosting symbiont type D1a (*Symbiodinium trenchii*) are better than conspecific hosting type C3[45, 46, 47, 48]. However, one recent study described a new thermally tolerant symbiont species "*Symbiodinium thermophilum*" was described to prevail in Persian/Arabian Gulf that is genetically within but distinct from ITS2 type C3 which suggests high phenotypic plasticity in type C3 than common thought[49]. Various combinations of coral-algal relationships thus can provide different functionality against environmental

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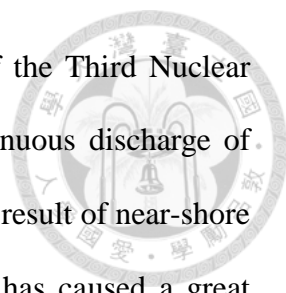
fluctuations. Before 2007, most of the scleractinian coral species were shown to associate with only one single type of symbiont while few of them could possess more than one type simultaneously[50]. However, utilizing advanced and more sensitive techniques like real-time quantitative PCR assay[51, 52], most of the scleractinian coral species that were only found to host a single type of symbiont by conventional PCR assay was found to also harbor more than one type of symbiont in "background" level. The role of these background types of symbiont was suggested to provide functional redundancy that corals could maintain fitness while facing environmental changes[45, 53], however, the functional significance may only limit to certain types of symbiont[54].

To overcome thermal stress, the coral host itself can activate numerous protective mechanisms to prevent or mitigate the detrimental effect during bleaching. For example, the coral host can generate fluorescence pigment (FP)[55] and Mycosporine-like amino acid (MAAs)[56] to reduce UV damage, or to scavenge damaging ROS, which is generated by the dysfunctional symbionts, through antioxidant systems[57, 58]. The association with thermally tolerant symbiont may also mitigate thermal stress on the coral host. The adaptive bleaching hypothesis (ABH) proposed that bleaching may help corals to re-establish coral-algal combination against environmental changes and thus could increase its overall fitness[59, 60]. The proliferation of new symbiont might come from the uptake of the coral host from the environment (switching), or from the pre-existing symbiont in coral host tissue (shuffling)[28]. The cellular process of switching generally involve three stages. First, the recognition when the heterologous *Symbiodinium* makes contact with the host gastrodermal cell. Several pairs of protein patterns and receptors on both side of cell membrane act to distinguish symbiont from pathogen[61, 62, 63]. Second, the engulfment of symbiont through phagocytosis[64, 65]. Third, the regulation of symbiont and host cell proliferation to finally form a stable symbiosis[66].

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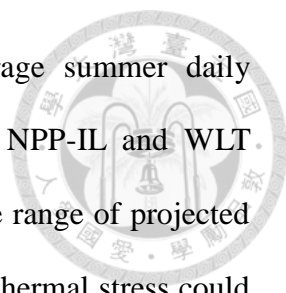In contrast, the shuffling process does not require the recognition and phagocytosis of *Symbiodinium* at the beginning that the pre-existing alternative symbiont type could proliferate directly when the environment condition favors its growth. While the evidence of switching is still limited[67], evidence of coral shuffling under temperature stress has been shown in numerous studies[13, 37, 45, 68, 69, 70, 71, 72]. For instance, in the case of *Acropora millepora*, it was shown that this coral may increase its thermal tolerance by 1.0-1.5°C as a result of shuffling to a stress-tolerant symbiont clade D[37]. However, symbiont clade D were shown to translocate fewer photosynthates compare to type C1 in *Acropora millepora* juveniles[73] and several studies also have shown that coral dominated by clade D could have lower growth rate than conspecific corals dominated by other types (C1, C1b-c, and C2)[10, 74, 75, 76]. In contrast, one study showed a reverse pattern in *Acropora tenuis* juveniles[77]. These physiological defects associated clade D also drew concerns on the trade-offs that coral may face in the long-term[78]. Although symbiont community of some corals could revert back to thermal-susceptible types to regain productivity when the thermal stress dissipate[46, 79], however, under current pace of rapid global warming and more frequent predicted bleaching events[1, 2], shuffling to a stress-tolerant symbiont type thus may become one of the fewer options, if any, for corals to overcome thermal stress in the future[3, 4, 80].

In order to assess how shuffling would affect future survival of corals, it is important to have a better understanding of how thermal history would play a role in determining the success of shuffling and subsequent host survival. Cunning et al 2015 suggested that the shuffling process would be affected by the disturbance severity and the recovery temperature from a short-term tank experiment[68]. However, the effect of prolonged thermal stress, a situation that coral reef actually facing under rapid climate change, on shuffling process and subsequent survival is still unclear. In Nanwan ,

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Kenting National Park, Taiwan, a reef site located at southwest of the Third Nuclear Power Plant Outlet (NPP-OL), and has been affected by the continuous discharge of thermal effluent flowing right into the existing coral community as a result of near-shore current[81] since the operation began in 1984. The thermal effluent has caused a great impact on the benthic invertebrate and fish communities at NPP-OL, indicated by significant different community compositions and settlement patterns compared to other sites such as the nuclear power plant inlet (NPP-IL) where temperature regimes are suggested to be a suitable reference for Nanwan[82, 83]. Corals present at NPP-OL have also experienced several bleaching events over time. Mass coral bleaching event (over 90% of coral cover) was observed in shallow water (<3m) of NPP-OL between 1987-1989 due to 4°C rise in the seawater temperature compared to other sites in Nanwan by the thermal effluent after the nuclear power plant started to operate in its full power.[84, 85, 86] The average monthly mean temperature of shallow water (<3m) of NPP-OL was 2.7°C to 1.6°C higher than other sites in Nanwan during 1986-2000[83]. Although the summer monthly maxima of surface water at NPP-OL had reduced from 32°C (1986-1991) to 30°C (1991-2000) after several improvements in the engineering and management of the NPP, the coral communities in the shallow water (3m) still remain dominant with thermally tolerant symbiont types[46, 47, 48]. The increasing prevalence of stress-tolerant symbiont type D1a at reef sites closer to NPP-OL reflects the consequences of long-term thermal effect, which led NPP-OL to become an ideal site for study of holobiont dynamics under thermal stress although other physical differences (temperature fluctuations, upwelling, and internal waves) between NPP-OL and other sites could also be involved[46, 47, 48, 87, 88].

In this study, we conducted *in situ* reciprocal transplant experiments (RTE) on nubbins of *Platygyra verweyi* collected from NPP-OL, NPP-IL, and Wanlitung (WLT)

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in Kenting National Park (KNP) in 2014 and 2015. The average summer daily temperature at NPP-OL (1-3m) is 1.2°C and 0.3°C higher than NPP-IL and WLT respectively, which temperature rises were approximately within the range of projected global SST in 2030-2050[89]. The aim was to test whether prolonged thermal stress could affect the process of shuffling and subsequent host survival, by monitoring the symbiont community dynamics between thermal-susceptible symbiont type C3/C3cc and stress-tolerant type D1/D1a in *P. verweyi*, coral host mortality, and Degree Heating Week (DHW)[90] for transplanted *P. verweyi* nubbins. We show that the prolonged thermal stress, in terms of maximum DHW (MDH), could affect the process of shuffling and the subsequent survival of *P. verweyi*. Symbiont shuffling could aid *P. verweyi* survival under moderate MDH, however, it cannot guarantee its survival when MDH rise beyond a potential threshold.

Materials and Methods

Study area and coral species

Three sites were included in this study, Nuclear Power Plant Outlet (NPP-OL), Nuclear Power Plant inlet (NPP-IL), and Wanlitung (WLT) in Nanwan, south Taiwan (Figure 1a). Among the three sites, NPP-OL (21°55'54.4"N, 120°44'42.7"E) and NPP-IL (21°57'20.3"N, 120°45'14.2"E) are located within Nanwan in Kenting National Park (KNP), Taiwan. A recent long-term (2007 to 2010, and 2013) seawater temperature data set obtaining from the deposited underwater data loggers shows that the average summer (June to August) daily seawater temperature at NPP-OL (29.31 ± 1.36ºC) is approximately 1ºC higher than at NPP-IL (28.14 ± 1.18ºC). Due to the tidally induced upwelling in Nanwan[87], the maximum daily seawater temperature fluctuation at NPP-OL and NPP-IL can be more than 8ºC in summer (Figure 1b). The third site, WLT (21°59'41.0"N 120°42'19.6"E), is located on the west coast of KNP with average summer (June to August) seawater temperature (28.99 ± 0.74ºC) similar to NPP-OL while the intervals of extreme temperature events ($\geq$ 30ºC) and the daily seawater temperature fluctuations are shorter and less extreme than NPP-OL (Figure 1b, c). In this study, NPP-IL and WLT were both include to assess the potential role of thermal variability on *P. verweyi* facing prolonged thermal stress. Massive coral species, *Platygyra verweyi*[91] presents in shallow water (2-4 m) was used for this study which was reported to associate with either or both ITS2 type C3 and D1a in the single colony in KNP[47, 92]. Symbiont type D1a has a very high prevalence of coral species in shallow water (< 3m depth) of NPP-OL and adjacent reef sites that are also affected by thermal effluent[47, 48].

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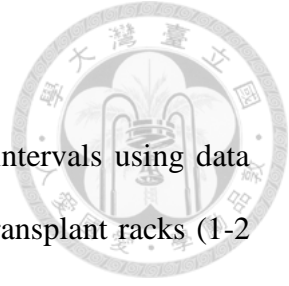
Seawater temperature

The seawater temperature was recorded *in situ* at 30-minute intervals using data loggers (HOBO; Pendant[TM], USA) deployed underwater near the transplant racks (1-2 m) at each study site. The raw temperature data were transformed into Degree Heating Week (DHW) [90, 93] to assess both the intensity and duration of the thermal stress for each experiment group. Although this indicator is typically used for reflecting large-scale bleaching monitoring, its application to experimental manipulation similar to our study had also been used in another study to assess the cumulative thermal stress on heat-treated corals but in daily scale[94]. The calculation of DHW was described in the following text. First, the weekly mean temperature for each study site was calculated from raw temperature data. Second, the maximum of the monthly mean temperature (MMM) were obtained from the recent long-term data set (NPP-OL = 29.63ºC; WLT = 29.28 ºC; NPP-IL = 28.37 ºC). Finally, the weekly mean temperature was subtracted to MMM to get the temperature anomalies; only those anomalies that were at least 1.0ºC above MMM were summed, within past 12-week windows, to obtain DHWs. The conceptual calculation equation was listed below. Using DHW for TWO, for example, $T_{NPP\text{-}OL}$ is the weekly mean temperature at NPP-OL and MMM_{WLT} is the MMM of WLT.

$$DHW_{TWO} = \sum [(T_{NPP-OL} - MMM_{WLT}) \geq 1°C]$$

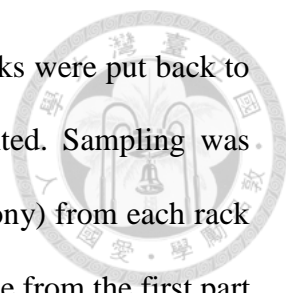The projections of DHW on Nanwan, which is included in one of the $1° \times 1°$ resolution grid reef cells locating at southern Taiwan (Appendix A1), were obtained from van Hooidonk et al. 2014[2].

Reciprocal transplant experiment design

Reciprocal transplant experiment - 2014

In the first reciprocal transplant experiment conducted in 2014 (from hereafter referred to as "2014RTE", Figure 1a), two sets of reciprocal transplant experiments (RTE) were carried with one set between Nuclear Power Plant Outlet (NPP-OL) and Wanlitung (WLT), and the other set between NPP-OL and Nuclear Power Plant Inlet (NPP-IL) in Kenting National Park (KNP), Taiwan in 2014. On March 2014, 25mm-dimeter cores from 5 colonies of *P. verweyi* from each study site (OL: 21 cores/colony, WLT: 16 cores/colony, IL: 10 cores/colony) at a depth of 1 to 2 m were sampled underwater using a pneumatic drill and placed in Ziploc bags underwater before being transferred to a wet lab at the National Museum of Marine Biology & Aquarium (NMMBA). Coral cores were maintained in indoor seawater tanks with constantly filtered seawater input. For RTE set between NPP-OL and WLT, nubbins from each site were randomly assigned to racks (a native group and a transplant group). Each rack thus contained 40 nubbins (5 colonies x 8 replicates). For another set between NPP-OL and NPP-IL, each rack contained 25 nubbins (5 colonies x 5 replicates). In the case of the native group of NPP-OL, the same rack was used for both sets of the experiments. Fewer replicates were used in the RTE set between NPP-OL and NPP-IL due to the small size of *P. verweyi* colonies at NPP-IL. Coral nubbins were made by attaching coral cores onto PVC adapters with underwater epoxy to be fixed onto the acrylic rack and were transferred back to their original sampling sites respectively with similar depths (1-2 m) as sampled colonies for 1.5-month recovery to ensure coral health before being transplanted. On April 2014, all the racks were retrieved and transported to NMMBA and stained with Alizarin Red S (Sigma-Aldrich, USA) 20mg/L,

24hrs[95] for the analysis of skeleton growth. Subsequently, all the racks were put back to the study sites with treatment groups being reciprocally transplanted. Sampling was conducted every 4 months, with 5 nubbins (1 nubbin from each colony) from each rack being retrieved. The sampled nubbins were cut into three parts. Tissue from the first part was removed by scraping the surface and stored in 95% Ethanol for DNA extraction. The second part was wrapped in aluminum foil and stored at -20ºC for chlorophyll concentration measurement and total symbiont density count, and the last part was snap-frozen in liquid nitrogen and stored for protein analysis. Monthly maintenance of the racks underwater was performed by cleaning the macro algae attached on the nubbins as well as the components of the racks to minimize any competition effect.

Reciprocal transplant experiment - 2015

Second reciprocal transplant experiment of *Platygyra verweyi* was conducted in 2015 (from hereafter referred to as "2015RTE", Figure 1a) between NPP-OL and WLT with a higher sample size (n=30 colonies from each site). NPP-IL was not included as one of the study sites in 2015RTE due to not enough *P. verweyi* colonies could be found to match a balanced design. Following identical procedures as 2014RTE, thirty colonies were sampled from NPP-OL and WLT in March 2015 and were reciprocally transplanted in April 2015 after 1 month of recovery.

Symbiont community dynamics

DNA extraction

Reciprocal transplant experiment - 2014

DNA extraction was carried out using salting-out method modified from Ferrara et al. 2006[96]. Coral tissue (30 mg) was cut and incubated overnight at 56ºC with 200 μL lysis buffer (1M Tris 25 mL, 0.5M EDTA pH8 10 mL, 20% SDS 10 mL, 5M NaCl 2 mL,

ddH$_2$O 53 mL) and 10 μL proteinase E (10 mg/mL). 7M NaCl (210 μL) was added to the tissue, centrifuged (6000 g, 30 sec), and transferred into B/T Genomic DNA Mini Column (Viogene, Taiwan). After a series washing with cold (-20ºC) 70% ETOH and centrifugation, the column was dried at 37ºC for 15 mins and finally the DNA was eluted with 50 μL of preheated (65ºC) 1X TE buffer and was isolated from the column after centrifugation (15000 g, 3 min). The concentrations of genomic DNA were determined by using NanoDrop 2000 (Thermal Scientific, USA).

Reciprocal transplant experiment - 2015

Approximately 30mg of coral tissue on each nubbin were cut *in situ* by using a bone-cutter per sampling time and placed in each Eppendorf tubes without retrieving the nubbins on a bimonthly basis. The seawater in each Eppendorf tubes was replaced by 95% ethanol for further DNA extraction. The DNA extraction was conducted following the same protocol as 2014RTE.

Denaturing gradient gel electrophoresis (DGGE)

The initial and final subclades (types) of the symbionts in the native and transplant groups in 2014RTE and 2015RTE were randomly selected and identified by amplifying internal transcribed spacer 2 (ITS2) region of DNA samples with primer sets ITSintfor2 5′GAATTGCAGA ACTCCGTG-3′ and ITS2clamp 5′CGCCCGCCGC GCCCCGCGC CCGTCCCGCCG CCCCCGCCC GGGATCCATA TGCTTAAGTT CAGCGGGT-3′. A touch-down PCR[97] program (92°C for 3 min, followed by 20 cycles of 30 s at 92°C. Annealing temperature starts from 62°C and then decrease 0.5°C in each following cycle to final temperature of 52°C, 30 sec at 72°C) were performed to ensure specificity. Denature gradient gel from 45% to 80% are used for electrophoresis under 115v for 15 hours using a CBS Scientific system (Del Mar, CA, USA). The gel was stained with SYBR Gold (Invitrogen, USA). Prominent bands are excised and amplified for
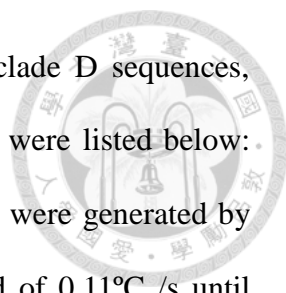
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sequencing. Samples used in Keshavmurthy et al 2012[47] and Lan 2011[92] were randomly selected and identified following the same process to examine any symbiont community shifting of natural *P. verweyi* colony through time.

Real-time quantitative PCR

Reciprocal transplant experiment - 2014

The copy numbers of symbiont clade C and clade D in *P. verweyi* samples were detected under LightCycler® 480 Instrument II (Roche, Switzerland) with the protocol modified from Mieog et al 2007[52]. Each 10 μL qPCR reaction consisted 5 μL of 1x SYBR Fast Master Mix, 0.5 μL of UF primer (2 nM/μL), 0.5μL of CR or DR primer (2 nM/μL), 7.5 μL of ddH$_2$O, and 2.5 μL of DNA templates (equal to 1 ng of genomic DNA) which primer sets, ITS1 clade C-specific reverse primer (CR) 5-AAGCATCCCTCACAGCCAAA-3, clade D-specific reverse primer (DR) 5-CACCGTAGTGGTTCACGTGTAATAG-3, and universal forward primer (UF) 5-AAGGAGAAGTCGTAACAAGGTTTCC-3[98] were used in this study. In each run, each sample was run in triplicate (technical replicates), and no-template control (NTC) was also run in triplicate with ddH$_2$O to inspect any contamination in the reagent. Plasmid standard curves were run in duplicate with *P. verweyi* samples to quantify the copy numbers of each symbiont. Plasmid standard curves were generated by using PCR product from symbiont clade C and clade D, which were ligated into pGem®-T Easy vector (Promega, USA), transformed, and amplified using *E. coli*. Copy numbers of final products were calculated by quantifying the concentration of plasmid DNA through NanoDrop 2000 (Thermal Scientific, USA) first, then divided by the mass of the plasmid (mass of each plasmid copy = 3015 bp (vector) + 100 bp (inserted PCR product) x 1.096e^{-21} g/bp = 3.4x10^{-18} g), finally multiplied by the plasmid DNA template volume (2.5 μL) in each reaction, and the serial dilutions of 1:10 from 3x10^6 copies to

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30 copies of plasmid standard containing symbiont clade C and clade D sequences, respectively, were generated at the end. The qPCR cycling settings were listed below: 40 two-step cycles of 15 s at 95ºC and 1 min at 60ºC. Melt curves were generated by starting at 60ºC and increasing the temperature with a ramp speed of 0.11ºC /s until 95ºC. Fluorescence data were collected when each annealing step was finished, and 5 data were collected every second during melt curve analysis. Crossing points (Cp) were determined by Light Cycler 480 software version 1.5 (Roche, Switzerland) using the second derivative method which represents the cycles when the maximum accretion of fluorescence signals of each sample occur[99]. Any Cp values from the samples that varied by 1 from other two technical replicates were excluded from analysis. If all Cp values of technical replicates varied by 1 from each other, sample were re-run. Since great variation occurred in Cp values (varied more than 1) within technical replicates of each sample when Cp>34, the cut-off cycle was set at 34 to avoid the inclusion of false positives causing by the formation of non-specific fluorescence. Average copy numbers of symbiont clade C and clade D were obtained respectively, and the formula for determining relative symbiont abundance in this study were listed in below following the correction suggested by Mieog et al. 2007[52]:

(Clade D copy numbers / 3) / [(Clade D copy numbers / 3) + Clade C copy numbers]
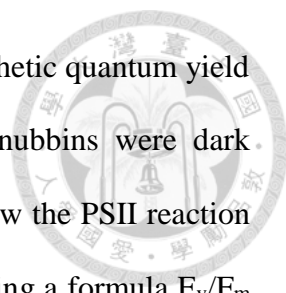
Reciprocal transplant experiment – 2015

The qPCR assay was conducted following the same protocol as 2014RTE.

Physiological parameters

Photochemical efficiency

Reciprocal transplant experiment - 2014

Photochemical efficiency for the nubbins was determined *in situ* by using a

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Diving- PAM (Waltz, Germany) to estimate the maximum photosynthetic quantum yield (F_v/F_m) of photosystem II (PSII) of the *Symbiodinium.* All the nubbins were dark adapted for 1 h by covering the racks with custom made covers allow the PSII reaction centers fully open. The maximum quantum yield is calculated by using a formula $F_v/F_m = (F_m-F_0)/F_m$, which F_0 is the background fluorescence yield that is obtained when giving the weak red light (< 1 µmol photons m^{-2} s^{-1}) with the fully-opened PSII, and F_m is the maximum fluorescence yield that is induced when giving a saturation light pulse to briefly suppresses photochemical yield to zero. The nubbins at each site were measured on each day with the similar time period in the morning, and with a fixing distance (1 cm) between the fiber and the coral tissue. Parameter settings using in this study are listed below and the photochemical efficiency of each colony was determined by averaging the photochemical efficiency of each replicate nubbins of each colony. (MI=8;SI=8;SW=0.8;AI=5;AW=0:30;AF=1.00;G=4;D=2;EF=0.84;FO=48;CT=40:00;CI=1;LW=0:10;LI=5;ID=0:40;IW=0:20;TO=11.2;TG=5.00;LO=0;LG=3.68)

Reciprocal transplant experiment – 2015

Photochemical efficiency of nubbins in 2015RTE was determined following the same procedure as 2014RTE which were described above.

General procedure for sample preparation for other parameters

Reciprocal transplant experiment - 2014

The general procedure for determining other physiological parameters (total symbiont density, chlorophyll pigments, and total soluble protein) is described below. The surface area of the subsamples was measured by using the aluminum foil. The tissue from the skeleton of each subsample was removed completely using airbrush filled with filtered seawater. The total volume of the tissue was adjusted to a final volume of 10mL. The slurry was shaken vigorously and vortexed for 10 seconds before

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being distributed into small aliquots (1 mL) for further analysis.

Reciprocal transplant experiment - 2015

The aim of 2015RTE was to further verify the results in 2014RTE by increasing sample size thus, considering the limiting quantity of big *P. verweyi* colony (which is needed for sampling enough pseudo-replicates for physiological analysis while not to put permanent stress on mother colony) in both study sites, no other physiological were measured in 2015RTE.

Total symbiont density

Symbiont pellet was obtained by centrifuging (1000 g, 10 min) 1 ml tissue aliquots. After discarding the supernatant, the pellet was homogenized by using Pellet pestle, and then fixed with 1 mL of 1% seawater-formalin. Symbiont cells were counted using a Neubauer haemacytometer (Assistant, Germany) under the microscope (BX40 Olympus, Janan), and the total symbiont density for each nubbin was determined by averaging 6 replicate counts.

Chlorophyll a concentration

Airbrushed tissue solution was centrifuged (1000 g, 10 min) to obtain the symbiont pellet. After discarding the supernatant, the pellet was homogenized by using a pestle. The resulting homogenate was then transferred to 1 ml Eppendorf tube containing 1 mL of 90% acetone and incubated at -20ºC for 24 h to extract the pigments. Absorbances of the supernatant (after centrifugation at 9000 g for 10 min) from triplicate aliquots (200 µL for each) were read at 630, 647, 664, and 750 nm wavelength using spectrometer (SPECTROstar[Nano] BMG LABTECH, Germany) with three wells containing 200 µL of 90% acetone as the blank. The chlorophyll concentration was then calculated using equations from Jeffrey and Humphrey (1975)[100] which is listed below and the

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chlorophyll a concentration of each nubbin was determined by averaging calculated concentrations from triplicate aliquots.

$$Chl\ a = 11.85\ A_{664} - 1.54\ A_{647} - 0.08\ A_{630}$$

Total soluble protein

Tissue solution obtained by airbrush was used for protein analysis. All the steps were performed inside 4ºC cold room to minimize protein degradation. Tissue aliquots were centrifuged (4000 g, 5 min)[101] before taking 25 µL of supernatant for analysis using BCA protein assay kit (Thermal scientific, USA) with bovine serum albumin (BSA) as standard to determine the total protein concentration using a spectrometer. The analysis was performed following the manufacturers' protocol of microplate protein analysis. Water (DEPC-treated) was added into three wells containing reagents as the blanks. Total protein concentration for each nubbin was determined by averaging the concentrations from triplicate.

Coral growth

Tissue coverage growth

Reciprocal transplant experiment - 2014

Top-view photos of all the nubbins on the rack were photographed *in situ* with a scale, and the surface area covered by the coral tissue of each nubbin were estimated by using ImageJ software (1.48v, USA). Data were transformed into the relative percentage change of tissue coverage from the initial sampling (April 2014).

Reciprocal transplant experiment - 2015

The tissue coverage growth was measured following the same procedure as 2014RTE.
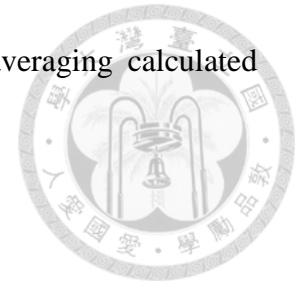
Skeleton growth

Reciprocal transplant experiment - 2014

At the end of the experiment, all the retrieving nubbins were sliced and airbrushed to remove the remaining tissue, and the length of the newly accreted skeletons perpendicularly above the Alizarin Red S stain mark from 6-10 septa were measured under the microscope (SZ61 Olympus, Japan), the results were averaged to determine the skeleton growth of each nubbin.

Reciprocal transplant experiment - 2015

Since none of the nubbins were retrieved, the skeleton growth was not measured in 2015RTE.

Statistical analysis

All the statistical analysis in this study were performed in R version 3.1.1.[102] Differences in daily mean seawater temperatures and daily seawater temperature fluctuations between sites were tested by using Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni adjusted p-value. Data were presented as the mean ± standard deviation (S.D.). Relative symbiont abundances of each sample were assigned into categories, C3/C3cc or D1/D1a dominant (either C3/C3cc or D1/D1a ≥ 90% relative abundance) and C3/C3cc+D1/D1a (10% < both C3/C3cc and D1/D1a < 90% relative abundance) and differences in symbiont community distributions were tested by using Fisher's exact test. For Photochemical efficiency and each physiological parameter, differences between each transplant groups and its native groups were tested by using Student t-test. Data were box-cox transformed[103] if data failed to meet normality and/or homogeneity of variance assumptions. Wilcoxon rank sum test was performed on raw data if statistical assumptions were violated even after box-cox

transformation. All data were presented as the mean ± standard deviation (S.D.). In 2014RTE, for tissue coverage growth and skeleton growth, differences between groups for each transplantation sets (WLT↔NPP-OL or NPP-OL↔NPP-IL) were tested by using one-way ANOVA followed by Tukey's post hoc test with Bonferroni adjusted p-value. In 2015RTE, for tissue coverage growth, differences between groups were tested by using one-way ANOVA followed by Tukey's post hoc test with Bonferroni adjusted p-value while the origin versus location effect was tested by using two-way ANOVA.

Results

Seawater temperature

Reciprocal transplant experiment - 2014

The average summer (June to August) daily seawater temperature at NPP-OL (30.11 ± 1.07ºC) was different from the temperature at WLT (29.59 ± 0.67°C; Dunn's post hoc test, p<0.001) and at NPP-IL (28.61 ± 0.96°C; p<0.001). The daily seawater temperature fluctuation at NPP-OL (2.23 ± 1.00°C; Figure 1b) was different from WLT (1.53 ± 0.58°C; p<0.001) and NPP-IL (1.80 ± 1.30°C; p<0.001) while there was no difference between WLT and NPP-IL (p=1.000). During summer, however, the daily seawater temperature fluctuation at both NPP-OL and NPP-IL could be more than 7°C (maximum 9.12°C at NPP-OL, and 7.19°C at NPP-IL; Figure 1b). The longest interval of the heating event (≥30°C) in each day also occurred at NPP-OL which was different from WLT an NPP-IL Figure 1c). The repeated seawater temperature anomalies (the weekly mean seawater temperature exceeding a bleaching threshold) occurring at NPP-OL during summer had caused the DHWs for both TWO and TIO piling up drastically (Figure 2a; Figure 3a). The DHWs for TWO had reached 4.41 in early July (>4.0: NOAA Alert level 1, bleaching likely[93]) and kept rising to a maximum value of 10.43 by early September (>8.0: NOAA Alert level 2, widespread bleaching likely and some mortality could be expected[93]). The DHWs then started to decrease gradually in fall while a relatively high value of DHW (DHW=4.6) still occurred in early October (Figure 3a). For TIO, the DHWs accumulated faster than TWO that had already exceeded 8.0 in July, and further escalated to a maximum value of 21.3 in early September. The DHW value for TIO started to decrease from fall but still appeared high (DHW=13.5) in early October (Figure 3a). In contrast to TWO and TIO, the DHWs for

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NWW and NII were all below 4.0 throughout experiment period. For NOO, high maximum DHW (DHW = 6.4) occurred that exceeded the bleaching alert level 1 while the DHWs for TOW and TOI remained 0 throughout the 9-month transplant experiment (Figure 3a).
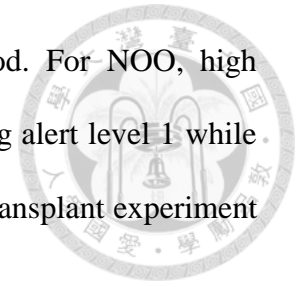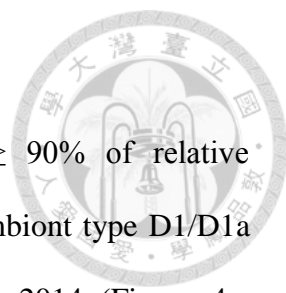
Reciprocal transplant experiment - 2015

The second reciprocal transplant experiment of our study carried out in 2015 (from hereafter referred to as "2015RTE), the average summer daily seawater temperature at NPP-OL (29.77 ± 1.12°C) was different from the temperature at WLT (29.52 ± 0.52°C; Wilcoxon rank sum test, W=5097, p<0.05). The daily seawater temperature fluctuation at NPP-OL (2.38 ± 1.04°C) was different from the fluctuation at WLT 1.57 ± 0.70°C (Wilcoxon rank sum test, W=655557, p<0.001; Figure 1b). The longer interval of the heating event (≥30°C) in each day also occurred at NPP-OL which was different from WLT (Figure 1c). The DHWs for TWO accumulated to a maximum of 5.7 in July 2015 (Figure 3b). However, because of several typhoon events during summer, which lowered the weekly mean seawater temperature in NPP-OL (Figure 2b), the DHWs did not increase further that it never reached the bleaching alert level 2 before decreasing in fall (Figure 3b). The DHWs for all the other groups were all lower than 4.0 that were below the bleaching alert level 1 (Figure 3b).
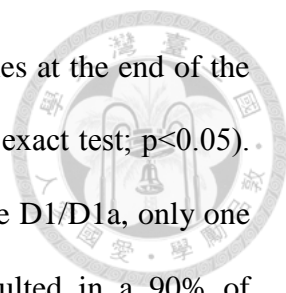
Symbiont community dynamics

Denaturing gradient gel electrophoresis (DGGE)

Sequences of *Symbiodinium* type C3 and C3cc[104] were recovered simultaneously in nubbins harboring symbiont clade C. For nubbins containing symbiont clade D, type D1, and D1/D1a existed simultaneously. Same patterns as this study were shown in those samples used in Keshavmurthy et al 2012 and Lan 2011 after reexamination.

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Reciprocal transplant experiment - 2014

Symbiont community of TWO was initially dominated ($\geq 90\%$ of relative abundance) completely by symbiont type C3/C3cc (n=5) while symbiont type D1/D1a had cell densities ranging from 0 to 4000 (cells cm^{-2}) in April 2014 (Figure 4a; Appendix A2, A4). After bleaching event occurred in summer, the symbiont type D1/D1a became dominant in 40% (n=2) of TWO colonies and almost all colonies had increased in symbiont type D1/D1a cell densities ranging from 0 to 1.8×10^5 (cell cm^{-2}) in September (Figure 4a; Appendix A2, A4) although all nubbins still remained bleached. The only surviving TWO colony in January 2015, which was still dominated by symbiont type C3/C3cc in September, was dominated by symbiont type D1/D1a with a recovered cell density of 6.3×10^5 (cell cm^{-2}) (Figure 4a; Appendix A2, A4). Symbiont community of TIO was initially dominated completely by symbiont type C3/C3cc (n=5) with no detectable symbiont type D1/D1a in April 2014 (Figure 4a; Appendix A2, A4). In September 2014, while all nubbins of TIO still remained visually bleached after the first observation of bleaching event in July 2014, the symbiont type D1/D1a became dominant in 20% of TIO colonies (n=1), and 60% of TIO colonies (n=3) had a mix symbiont type C3/C3cc+D1/D1a (each symbiont type showed a relative abundance falling between 10% ~ 90%) with symbiont type D1/D1a cell densities ranging from 3.4×10^4 to 1.3×10^5 (cells cm^{-2}) (Figure 4a; Appendix A2, A4). Both symbiont type C3/C3cc and D1/D1a were undetected in one colony of TIO in September (Figure 4a; Appendix A2, A4). All nubbins of TIO died in October 2014 thus no symbiont community data were collected in January 2015 (Figure 4a). Combining the symbiont community dynamic results from TWO and TIO, a total of 40% of the symbiont type C3/C3cc predominant *P. verweyi* nubbins shifted to symbiont type D1/D1a dominant (n=4), and another 30% were associated with mixed type C3/C3cc+D1/D1a (n=3)

during the 9-month transplant experiment. The symbiont communities at the end of the experiment had a different distribution compared to initial (Fisher's exact test; $p<0.05$). Despite a shifted dominance toward the stress-tolerant symbiont type D1/D1a, only one shifted nubbins survive from summer bleaching event which resulted in a 90% of overall mortality. Contrast to TWO and TIO, no change in symbiont community dominance for both NWW and NII happened, and both maintained symbiont type C3/C3cc dominance throughout the experiment period (Figure 4a; Appendix A4). Stable symbiont dominance with type D1/D1a appeared in both transplant groups of NPP-OL (TOW and TOI) and the native group of NPP-OL (NOO) throughout the experiment period (Figure 4a; Appendix A4).

Reciprocal transplant experiment - 2015

In 2015RTE, the symbiont community of TWO was dominated by symbiont type C3/C3cc (n=26), with few colonies possess a mix of symbiont type C3/C3cc+D1/D1a (n=3) or dominated by symbiont type D1/D1a (n=1) in April 2015 (Figure 4b; Appendix A3d). However, in July 2015, almost half (47%, n=14) of TWO colonies had a shifted in symbiont dominance from type C3/C3cc to type D1/D1a, seven percent (n=2) had shifted from a mix of type C3/C3cc+D1/D1a to type D1/D1a dominant, and 13% (n=4) from type C3/C3cc dominant to C3/C3cc+D1/D1a (Figure 4b; Appendix A3d) during summer bleaching event. The prevalence of symbiont type D1/D1a kept increasing in September, resulting in 73% of TWO colonies shifting from type C3/C3cc to type D1/D1a dominant (n=22), seven percent shifting from a mix of type C3/C3cc+D1/D1a to type D1/D1a dominant (n=2), and 7% from type C3/C3cc dominant to C3/C3cc+D1/D1a (n=2). As a result, the symbiont community was different from initial composition (Fisher's exact test; $p<0.001$; Figure 4b). Seventeen percent of overall mortality occurred for TWO while no mortality occurred for those shifting its symbiont

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community from type C3/C3cc dominant to type D1/D1a dominant (Figure 4b; Appendix A3d). Contrast to the dynamic symbiont community of TWO, the symbiont community of both native and transplant group of NPP-OL appeared to be stable throughout experiment period in 2015RTE (Figure 4b; Appendix A3a).
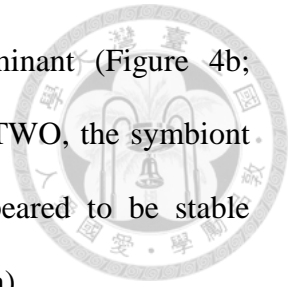
Physiological parameters

Reciprocal transplant experiment - 2014

The photochemical efficiency of TWO decreased 59% from initial ($p<0.001$; Figure 5a, Appendix A5) in July 2014, and was different from NOO ($p<0.001$; Figure 5a; Appendix A6) and NWW ($p<0.001$; Appendix A6, A7) when the sign of bleaching first occurred. A trend of recovery in photochemical efficiency was shown in September ($p<0.01$; Figure 5a; Appendix A5) and was no difference from NOO ($p=0.191$; Figure 5a; Appendix A6) and NWW ($p=0.151$; Appendix A6, A7). A lower photochemical efficiency of TWO was shown in October when only one colony survive (Figure 5a), but it recovered in January 2015 (Figure 5a). The photochemical efficiency of TIO in July decreased 68% from initial ($p<0.001$; Figure 5a, Appendix A5), and was different from NOO ($p<0.001$; Figure 5a; Appendix A6) and NII ($p<0.001$; Appendix A6, A7). A trend of recovery in photochemical efficiency was shown in September ($p<0.01$; Figure 5a; Appendix A5) but was still different from NOO ($p<0.001$; Figure 5a; Appendix A6) and NWW ($p<0.001$; Appendix A6, A7). No data were collected after October because of mortality.

The total symbiont density of TWO decreased 88% from initial ($p<0.001$; Figure 5b, Appendix A5) in September, and was different from NOO ($p<0.001$; Figure 5b; Appendix A7, A8) and NWW ($p<0.05$; Appendix A7, A8). The only survived colony of TWO showed a trend of recovery in total symbiont density in January 2015 (Figure 5b).

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The total symbiont density of TIO decreased 72% from initial ($p < 0.001$; Figure 5b, Appendix A5) in September, and was different from NOO ($p < 0.001$; Figure 5b; Appendix A7, A8) and NII ($p < 0.001$; Appendix A7, A8). No data were collected after October because of mortality.

The chlorophyll a content per unit area of TWO decreased 86% from initial ($p < 0.01$; Figure 5c, Appendix A5) in September, and was different from NOO ($p < 0.001$; Figure 5c; Appendix A7, A8) and NWW ($p < 0.005$; Appendix A7, A8). The only survived colony of TWO showed a trend of recovery in chlorophyll a content per unit area in January 2015 (Figure 5c). The chlorophyll a content per unit area of TIO showed a trend of decreasing from initial but was not significant ($p = 0.06$; Figure 5c, Appendix A5) in September, and was different from NOO ($p < 0.001$; Figure 5c; Appendix A7, A8) and NII ($p < 0.001$; Appendix A7, A8). No data were collected after October because of mortality.

The chlorophyll a content per symbiont cell of TWO showed no difference from initial ($p = 0.502$; Figure 5d, Appendix A5) in September, NOO ($p = 0.470$; Figure 5d; Appendix A7, A8) and NWW ($p = 0.418$; Appendix A7, A8). The only survived colony of TWO showed a trend of recovery in chlorophyll a content per symbiont cell in January 2015 (Figure 5d). The chlorophyll a content per symbiont cell of TIO showed no difference from initial ($p = 0.505$; Figure 5d, Appendix A5) in September, NOO ($p = 0.992$; Figure 5d; Appendix A7, A8) and NII ($p = 0.124$; Appendix A7, A8). No data were collected after October because of mortality.

The total soluble protein content of TWO decreased 77% from initial ($p < 0.01$; Figure 5e, Appendix A5) in September, and was different from NOO ($p < 0.01$; Figure 5e; Appendix A7, A8) and NWW ($p < 0.01$; Appendix A7, A8). The only survived colony of TWO showed a trend of recovery in total soluble protein content in January 2015

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(Figure 5e). The total soluble protein content of TIO decreased 85% from initial (p<0.001; Figure 5e, Appendix A5) in September, and was different from NOO (p<0.01; Figure 5e; Appendix A7, A8) and NII (p<0.001; Appendix A7, A8). No data were collected after October because of mortality.

Reciprocal transplant experiment - 2015

The photochemical efficiency of TWO in July decreased 20% (n=17; 5 nubbins were undetermined due to low signals) (p<0.001; Figure 5f; Appendix A9) and was different from NOO (p<0.05; Figure 5f; Appendix A10) when bleaching occurred in 73% (n=22) of nubbins. The photochemical efficiency of TWO showed a trend of recovery yet was still different from NOO (Figure 5f; Appendix A10)

Coral growth

Reciprocal transplant experiment - 2014

In 2014RTE, the tissue coverage growth of *P. verweyi* nubbins was measured in January 2015 after a 9-month period of *in situ* transplantation. For the RTE between NPP-OL and WLT, the tissue coverage growth in TOW nubbins had a highest mean (233 ± 68%) which was different from other groups (vs. NOO: 105 ± 23%, p<0.01; vs. NWW: 100 ± 42%, n=4, p<0.01; Figure 7a; Appendix A11) while no difference between TOW and NWW (p=1.000, Figure 7a; Appendix A11). No statistical analysis was performed with TWO due to insufficient sample size after high mortality (-56%, n=1). The negative value of tissue coverage growth for TWO was due to partial mortality on the nubbins from the only survive colony (Figure 7a). For the RTE between NPP-OL and NPP-IL, the tissue coverage growth of NII (187 ± 47%) had the highest mean than other groups (vs. TOI: 89 ± 26%, p<0.01; vs. NOO: 105 ± 23%, p<0.01; Figure 7a; Appendix A11) while no difference between NOO and TOI (p=1.000, Figure 7a;

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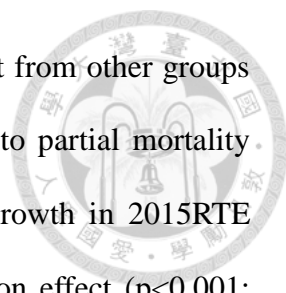
Appendix A11). No data were collected for TIO due to whole-group mortality.

The skeleton growth of *P. verweyi* nubbins was also measured in January 2015 after a 9-month period of *in situ* transplantation. For the RTE between NPP-OL and WLT, no difference in the skeleton growth between groups (one-way ANOVA, $F_{2, 11}$=1.89, p=0.198) although TOW (3.3 ± 0.8 mm) also tend to have the highest tissue coverage growth than other groups (NOO: 2.8 ± 0.9 mm; NWW: 2.4 ± 0.6 mm, n=3; Figure 7a; Appendix A12). No statistical analysis was performed with TWO due to insufficient sample size after high mortality (TWO: 1.79 mm; n=1). For the RTE between NPP-OL and NPP-IL, the skeleton growth of NII (4.3 ± 0.6 mm) had a highest mean than other groups (vs. TOI: 2.7 ± 0.4 mm, p<0.05; vs. NOO: 2.8 ± 0.9 mm, p<0.05; Figure 7a; Appendix A12) while no difference between NOO and TOI (p=1.000, Figure 7a; Appendix A12). No data were collected for TIO due to whole-group mortality.

Although due to insufficient sample sizes of TWO and TIO, no origin versus location effect can be tested for both tissue coverage growth and skeleton growth in 2014RTE.

Reciprocal transplant experiment - 2015

In order to verify the aforementioned patterns, tissue coverage growth of *P. verweyi* was measured with larger sample sizes (n=30 colonies for each group). In 2015RTE, the tissue coverage growth of *P. verweyi* nubbins was measured in November 2015 after a 7-month period of *in situ* transplantation. For the RTE between NPP-OL and WLT, the tissue coverage growth of TOW also showed a highest mean (158 ± 58%) and was different from other groups (vs. NOO: 81 ± 39%, p<0.001; vs. NWW: 82 ± 55%, n=28, p<0.001; vs. TWO: - 11± 66%, n=25, p<0.001; Figure 7b; Appendix A13) while no difference between NOO and NWW (p=1.000, Figure 7b; Appendix A13). The lowest
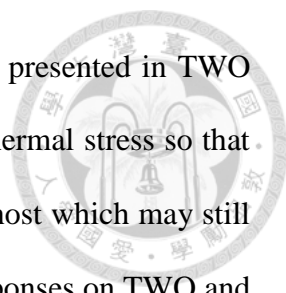
mean (-11 ± 61%) appeared in TWO and was significantly different from other groups (Figure 7b; Appendix A13), the negative value of TWO was due to partial mortality after bleaching event in summer. The results of tissue coverage growth in 2015RTE showed an origin ($p<0.001$; Figure 7b; Appendix A14) and location effect ($p<0.001$; Figure 7b; Appendix A14) respectively, however, there was no interaction between origin and location effect ($p=0.406$; Figure 7b; Appendix A14). *Platygyra verweyi* nubbins located in WLT (native in or been transplanted into WLT) tend to have higher tissue coverage growth rate than the ones in NPP-OL (native in or transplanted into NPP-OL).

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Discussion
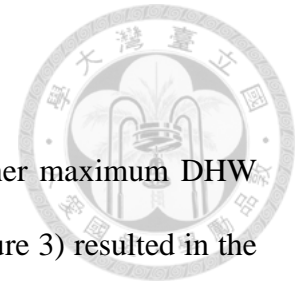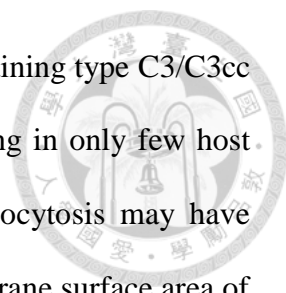
Comparing the results from 2014RTE and 2015RTE, the higher maximum DHW (MDH) in 2014RTE (TWO: 10.43& TIO: 24.3 vs. TWO: 5.68; Figure 3) resulted in the less proportion of colonies to complete shuffling process toward dominance of stress-tolerant symbiont type D1/D1a (2014RTE: 40% vs. 2015RTE: 73%; Figure 4) with a higher host mortality after shuffling (2014RTE: 75% vs. 2015RTE: 0%; Figure 4), indicating that the prolonged thermal stress (MDH) can affect the success of shuffling of *P. verweyi* toward stress-tolerant symbiont type D1/D1a and the subsequent host mortality. Alternatively, symbiont shuffling could aid *P. verweyi* survival under moderate MDH stress (Figure 6), however, shuffling cannot guarantee the survival of *P. verweyi* since the survival rate in our study dramatically decreased under high MDH even though shuffling had occurred after bleaching (Figure 6). Association with D1/D1a symbiont appeared to be important for *P. verweyi* to survive thermal stress, but the benefit from D1/D1a may disappear if the level of thermal stress goes beyond a potential MDH threshold more than 5.68 (Figure 6).

The bleaching event of TWO and TIO accompanying by the decrease of almost all physiological parameters indicate that both groups indeed suffered physical stress during summer bleaching events (Figure 5a-e). The high mortality in those shuffled *P. verweyi* nubbins (Figure 4a) may be due to the insufficient time for *P. verweyi* host to establish a sufficient trophic interaction with the low-density symbiont type D1/D1a (7.4×10^4 to 1.8×10^5 cell cm^{-2}, Appendix A7). The trend of recovery in photochemical efficiency, and the comparable values of chlorophyll a concentrations per symbiont cell for TWO and TIO to NOO and each of their native group in September (Figure 5a, c; Appendix A7), when the relative abundances of stress-tolerant symbiont type D1/D1a

significantly increased, suggested that those symbiont type D1/D1a presented in TWO and TIO colonies may still remain functional under the prolonged thermal stress so that the mortality might be caused by the stress responses of *P. verweyi* host which may still need further investigation in future studies. Contrast to the stress responses on TWO and TIO, no sign of bleaching occurred to NOO in 2014RTE. Most of the parameters were stable throughout 9-month experiment period (Figure 5a-e). The different response of NOO clearly indicates the better thermal tolerance of *P. verweyi* population in NPP-OL, which may be due to the better thermal tolerance of D1/D1a symbiont[78] and/or the acclimatization/adaptation of *P. verweyi* host living in NPP-OL. With the limiting genetic markers, the population of *P. verweyi* in NPP-OL was suggested to have no genetic differences to WLT and NPP-IL[47, 92]. The pattern of NOO thus may likely to be due to the thermal tolerance of D1/D1a symbiont and the acclimatization of *P. verweyi* host while the adaptation was still inconclusive.

Our study design cannot, in an absolute term, to determine whether the symbiont shifting pattern occurring in TWO and TIO after bleaching were due to symbiont switching or shuffling. If the case in our study was due to switching, TWO and TIO should recognize and phagocytose the type D1/D1a symbiont from NPP-OL during summer bleaching. However, it may less likely be the case of our study due to the type D1/D1a symbiont density for most of the nubbins were already reaching the orders of magnitude of 10^4 to 10^5 cell/cm^2 (Appendix A4) within two month, assuming the symbiont uptake started from July when the signs of bleaching were first observed. Given the long doubling time (10 to 70 days) commonly required for *in hospite Symbiodinium*[62, 105, 106, 107], the density of the type D1/D1a symbiont may need at least the orders of magnitude of 100 to 1000 cell/cm^2 at the beginning of July. If TWO and TIO had gone through the mass expulsion of symbiont during the observed bleaching in

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July, the majority of the coral host gastrodermal cells that were containing type C3/C3cc symbiont may either detach from coral host[23] or degrade[108] resulting in only few host cells could acquire heterologous *Symbiodinium*. In addition, phagocytosis may have certain cost on host energy (lipid, protein, etc.) to increase the membrane surface area of gastrodermal cells. Whether TWO and TIO could retain enough energy for acquiring symbiont were in doubt. In contrast, shuffling could have higher chances to occur in our study since type D1/D1a symbiont could grow *in hospite* without extra energy to acquire from the environment. In this study, some of the TWO and TIO nubbins had harbored type D1/D1a symbiont in background level at the time of transplantation (Appendix A2, A4) as the source of shuffling. For the other nubbins of TWO and TIO that did not show background symbiont type at the beginning, we cannot rule out completely for those potentially present in extremely low abundance and were undetected by the qPCR assay. So here we use shuffling to explain our results instead of switching.

Results of this study suggest that a potential threshold of prolonged thermal stress may exist to determine the survival of coral host during the bleaching recovery phase. Few studies reported the host mortality after shuffling to a stress-tolerant symbiont type. Berkelmans and Van Oppen 2006 had conducted a heat stress tank experiment on shuffled (from type C2 dominant to clade D dominant) *Acropora millepora* colonies retrieving from a 9-month field transplantation and showed a drastic decline in physiological conditions of the symbiont clade D with minor mortality under 32°C for 15 days although it was suggested that all the colonies were not likely to survive if the temperature further increased[37]. Another study, Silverstein et al 2015, found no mortality among shuffled (from C3/C3cc to D1/D1a) *Montastraea cavernosa* after repeated heat stress tank experiment under 32°C for 10 days. However, none of them
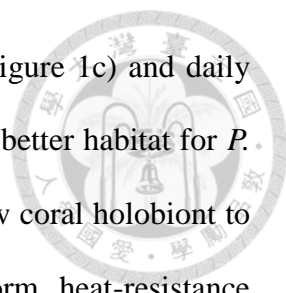
explore the limits of shuffling under long-term field conditions. In this study, we advance the current understanding of shuffling by applying MDH to assess the role of prolonged thermal stress that affects the process of coral shuffling and subsequent survival under field condition.
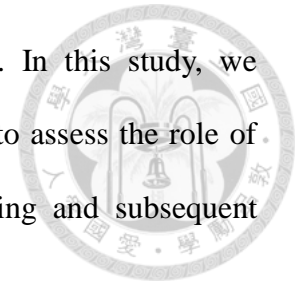
Corals living in thermally fluctuate habitat may show better tolerance to heat stress[94, 109, 110]. However, *P. verweyi* from NPP-IL, where the daily seawater temperature fluctuation can also be as high as NPP-OL in summer (Figure 1b), were all bleached and died which levels were similar to TWO in 2014RTE. Our results indicate that the previous exposure to high thermal fluctuation may not help *P. verweyi* to survive such high MDH occurred in 2014RTE (Figure 3a; Figure 4a; Figure 6)[94].
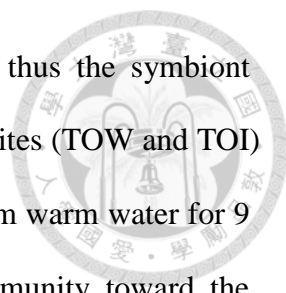
The prolonged thermal stress patterns demonstrated in this study thus may allow us to link *P. verweyi* survival in future warming scenarios. From the DHW projection of simulation model, the $1° \times 1°$ grid reef cell containing Nanwan is predicted to exceed 6°C-week at least twice during 2020 to 2030 in RCP8.5 scenario[2], and to exceed 8°C-week at least twice in the same time interval (2020 to 2030; Table 1). In 2015RTE, a case of maximum DHW=5.67, which represents a "shuffle and survive" scenario might happen at Nanwan before 2020 (Table 1), while another case of maximum DHW=10.43 representing a "shuffle but likely not survive" scenario, might occur at same site after but not far from 2030 (Table 1). However, a finer resolution may be needed in the future simulation model to have a better representation for Nanwan.

From the growth results of RTE between NPP-OL and WLT, *P. verweyi* nubbins associating with symbiont type D1/D1a (NOO, TOW) can have an equal or even higher tissue expansion rate (for about 2 times higher) than nubbins associating with symbiont type C3/C3cc (NWW). Furthermore, the tissue coverage growth of TOW was even higher (also about 2 times higher) than the native group of NPP-OL (Figure 7a)

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suggesting that WLT, where less seawater temperature extremes (Figure 1c) and daily seawater temperature fluctuations (Figure 1b) are present, may be a better habitat for *P. verweyi* compared NPP-OL in terms of tissue expansion which allow coral holobiont to allocate more energy to tissue expansion rather than to perform heat-resistance mechanisms (e.g. synthesize heat shock proteins[111]), or other factors such as nutrient[112, 113], heterotrophy[113, 114], and water velocity[115]. The higher tissue coverage growth rate for *P. verweyi* nubbins originated from the NPP-OL population may be due to a difference in the host performance itself and/or a difference in energy contribution of associating symbiont. However, given the evidence of higher photosynthates translocation of type D1/D1a symbiont than type C3/C3cc symbiont[73], the higher tissue coverage growth on the symbiont type D1/D1a dominant *P. verweyi* nubbins sourcing from the NPP-OL population in our study suggests a potential acclimatization and/or adaptation of *P. verweyi* host could exist.

Most of the previous studies have shown a negative trend in terms of growth in corals associating with symbiont clade D, which was lower than clade C dominant corals[10, 74, 75, 76]. Although Yuyama and Higuchi 2014[77] also illustrated a higher coral growth rate in *Acropora millepora* juveniles inoculated with symbiont clade D than with type C1 under laboratory condition for 2 months, such pattern with adult coral under field condition has not been documented. The comparable tissue coverage growth of NPP-OL native *P. verweyi* (NOO) with the WLT native ones (NWW) might be a result of physiological compensation of *P. verweyi* host which respond to the lower energy translocated from symbiont type D1/D1a. The *P. verweyi* nubbins transplanted from NPP-OL to WLT (TWO) then showed a boosted in tissue coverage growth rate that was two times higher than NOO when the thermal stress was removed. In this case, *P. verweyi* from NPP-OL might have already formed a sustainable symbiosis with

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symbiont type D1/D1a through acclimatization and/or adaptation thus the symbiont communities of *P. verweyi* transplanted from NPP-OL to other two sites (TOW and TOI) remained stable with symbiont type D1/D1a even after relieving from warm water for 9 and 7 month in our study as corals tend to shift symbiont community toward the thermal-susceptible type after thermal stress disappear[46, 79], although this could also be due to not enough time. Although there is no evidence to show the exact years for *P. verweyi* population of NPP-OL association with symbiont type D1/D1a, the symbiont community of *P. verweyi* at NPP-OL surveyed in 2009 and in our study (2014 and 2015; Figure 4a, b) showed an stable and almost complete dominance of symbiont type D1/D1a in shallow water (<3m) in recent years. However, given the fact that the shallow water (<3m) of NPP-OL has been affected by the warm water effluent from operation in 30 years ago, the time period for *P. verweyi* associating with symbiont type D1/D1a could be expected to be even longer than 6 years. The long-term thermal stress at NPP-OL could prevent the symbiont shifting toward more productive but thermal-susceptible symbiont types thus aid the development of the potential compensation mechanisms. Despite we observed a unique pattern in the case of transplantation between NPP-OL and WLT compare to other relating studies, however, this pattern did not apply to the RTE between NPP-OL and NPP-IL suggest that it may be influenced by potential environmental differences existing between WLT and NPP-IL. Since both sites show few incidences of extremely high temperatures (Figure 1c), the differences may lie in the complicated interactions of other factors (water velocity[115], nutrient[116, 117], upwelling, internal wave, bacterial community[69]) that need to be further investigated in the future.

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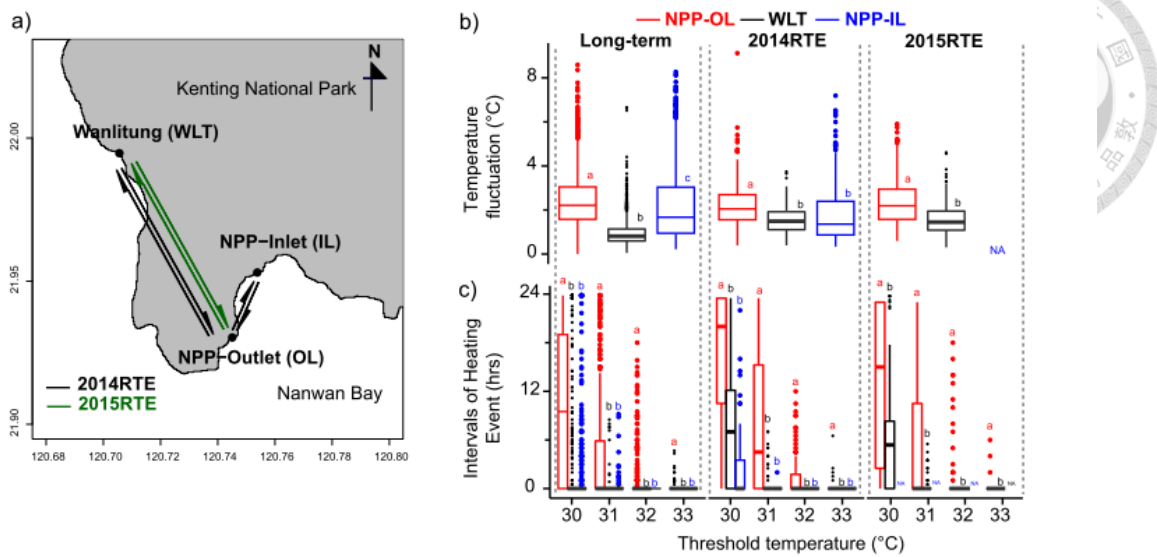
Figure 1 Diagram of Reciprocal Transplant Experiment (RTE) design and temperature

regimes of each study sites.

a) Study sites and RTE directions. Arrows in black indicate the directions of RTE for 2014 (5 colonies were transplanted for each site). Arrows in green indicate the directions of RTE for 2015 (30 colonies were transplanted for each site). b) Daily seawater temperature fluctuations by sites. c) Intervals of summer daily heating event that exceeds a given temperature threshold by sites. The intervals represent hours between the first and last record of temperatures that exceed a given threshold in each day. Data were subsetted with long-term temperature records (2007-2010 and 2013), 2014RTE (Febuary 2014 to January 2015), and 2015RTE (Febuary 2015 to November 2015) and plotted in each big column. NA represent no data were collected from NPP-IL in 2015RTE. Different lowercase letters indicate a significant difference in daily temperature ranges between sites (Kruskal-Wallis test, Dunn's post hoc test, Bonferroni adjusted p-values at α = 0.05). Bar within each box represents the median. Box boundaries represent the 75th (Q3) and 25th (Q1) percentiles. Upper whisker boundary represents the smaller value of the maximum or Q3+1.5*IQR, whereas lower whisker boundary represents the larger value of the minimum or Q1-1.5*IQR. Black dots represent the outliers that values exceed the range [Q1-1.5*IQR, Q3+1.5*IQR]. The interquartile range (IQR) = Q3-Q1.

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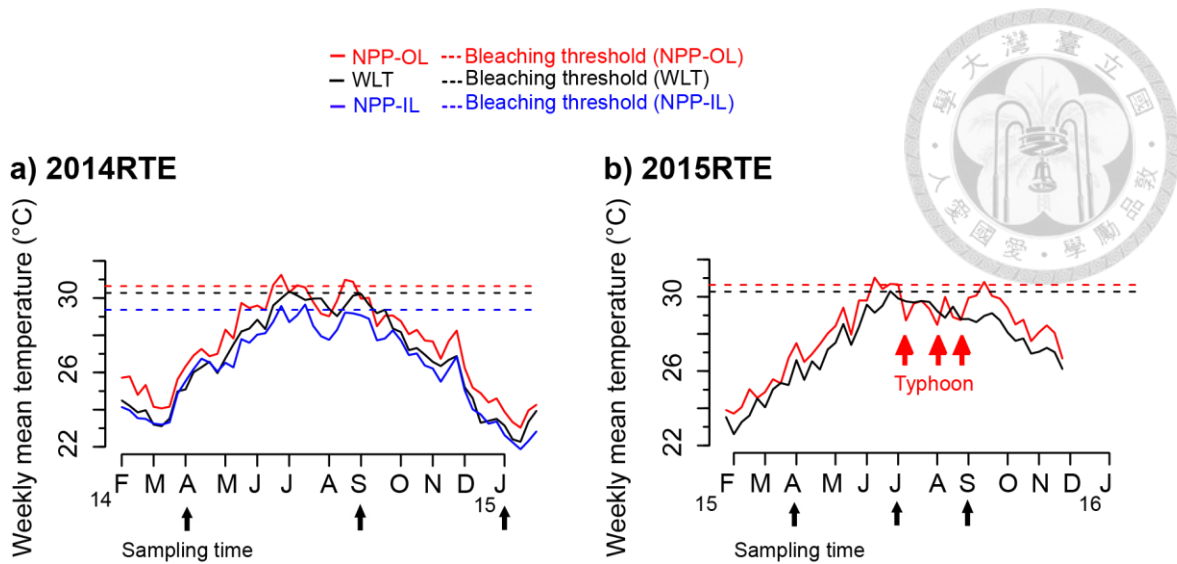
Figure 2   Time series records of *in situ* weekly mean temperature by sites.

a) Temperature recorded in 2014RTE. b) Temperature recorded in 2015RTE. The bleaching threshold (MMM+1) was determined from long-term data sets (2007-2010 and 2013) of each site.
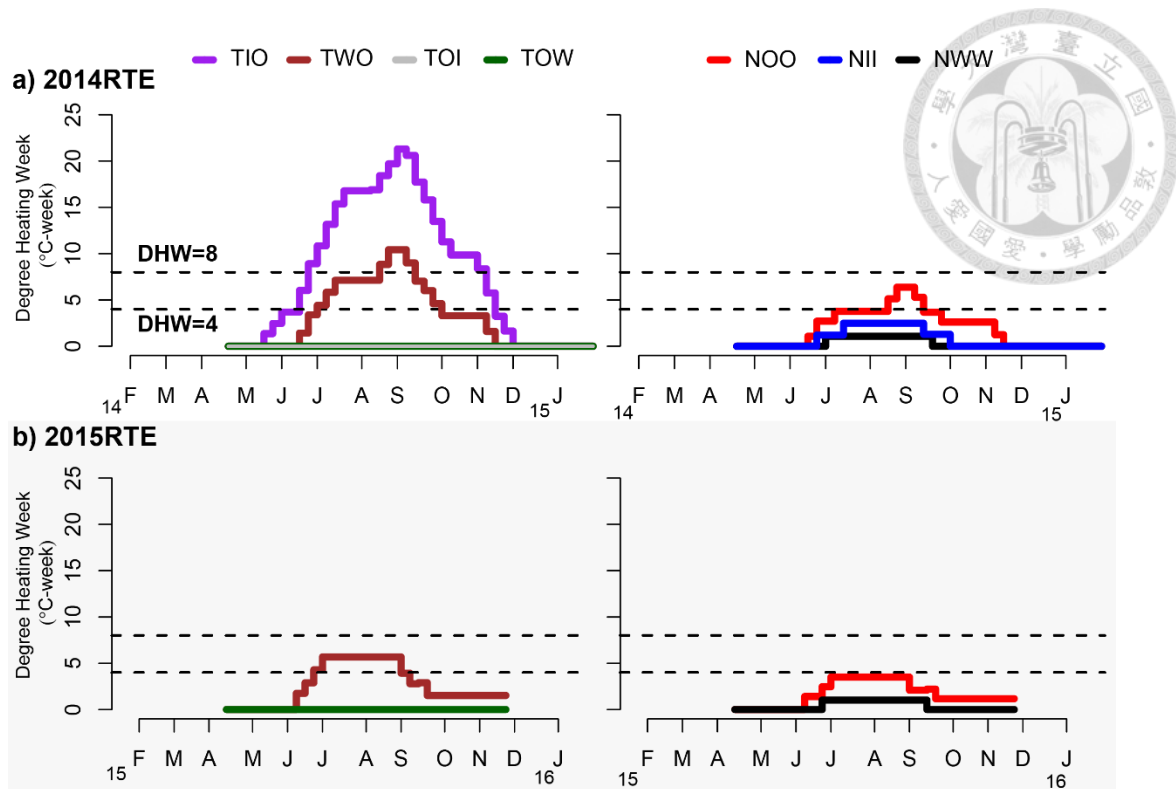
Figure 3 Time series of Degree Heating Week (DHW) for each experiment group.

a) Data calculated for 2014RTE. b) Data calculated for 2015RTE. Dotted lines indicate the alerting levels (DHW=4: Significant bleaching, DHW=8: Widespread bleaching and mortality can be expected). All transplant groups are shown in left part while all native groups are shown in right part. Abbreviations were described in abbreviation page.
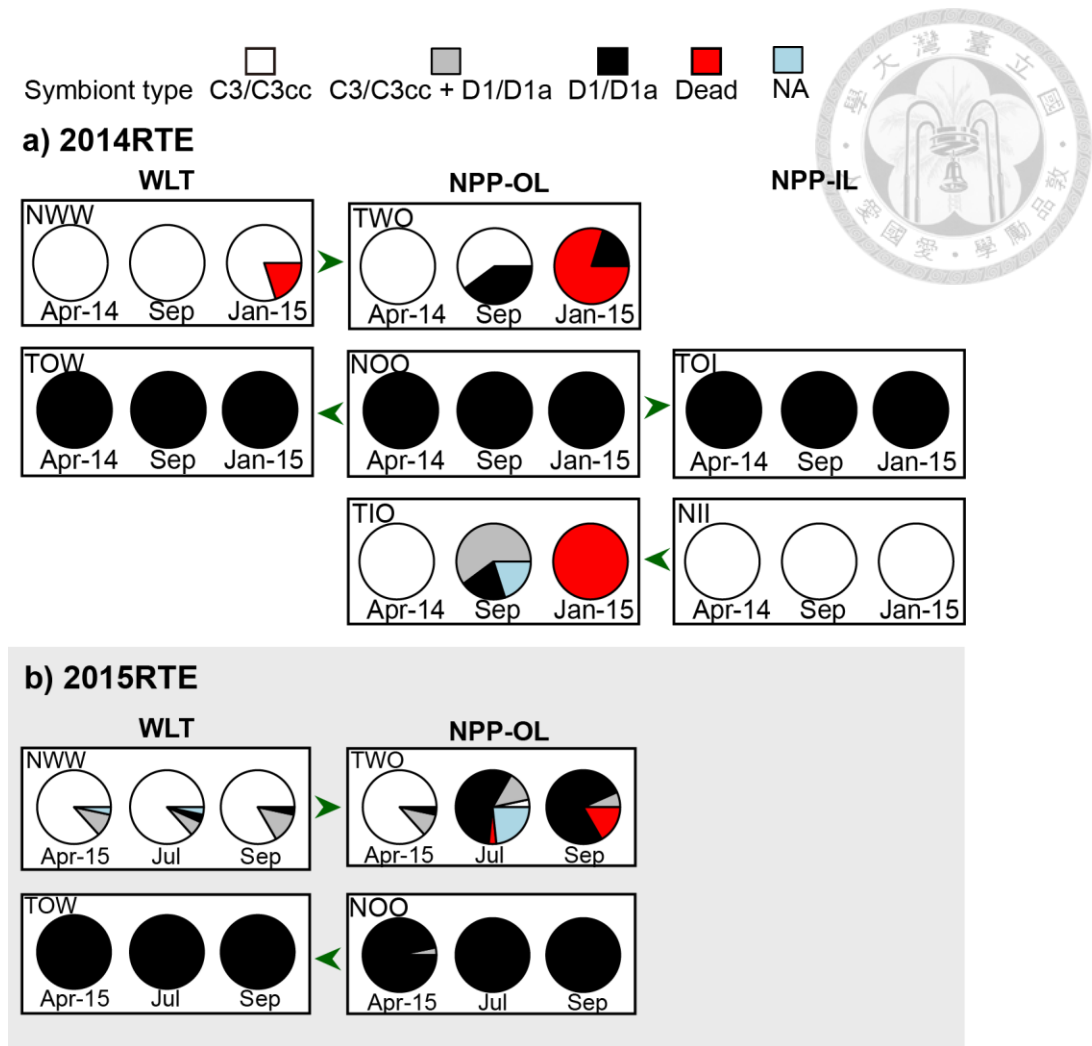
Figure 4 Dynamic of symbiont community for each experiment group through time.

a) 2014RTE; n=5 colonies for each group per sampling time; Appendix A2. b) 2015RTE; n=30 colonies for each group per sampling time; Appendix A3. NA indicates the colony that was still alive but both symbiont type cannot be detected. Arrows indicate the transplant directions. Abbreviations were described in abbreviation page.
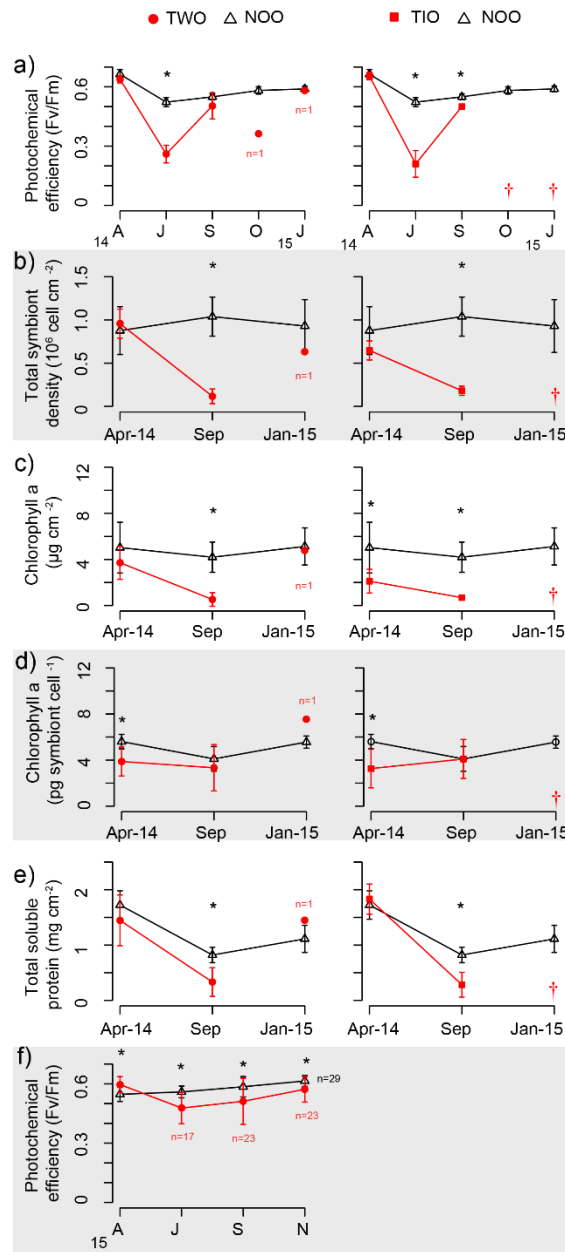
Figure 5 Physiological parameters of experiment groups located at NPP-OL at each

sampling time.

a) Total symbiont cell densities b) Chlorophyll a concentrations per cm^2 c) Chlorophyll a concentrations per symbiont cell d) Total soluble protein concentrations e) Dark-adapted photochemical efficiency measured in 2014RTE. (n=5 colonies for each group per sampling time unless stated otherwise). f) Photochemical efficiency measured in 2015RTE. (n=30 colonies for each group per sampling time unless stated otherwise). All data are presented in Mean ± SD. Asterisks represent a significant difference of total symbiont density between each transplant group and the native group on each month. The detailed statistics were described in Appendix A6, A8, and A10. †: All samples were dead. Abbreviations were described in abbreviation page.
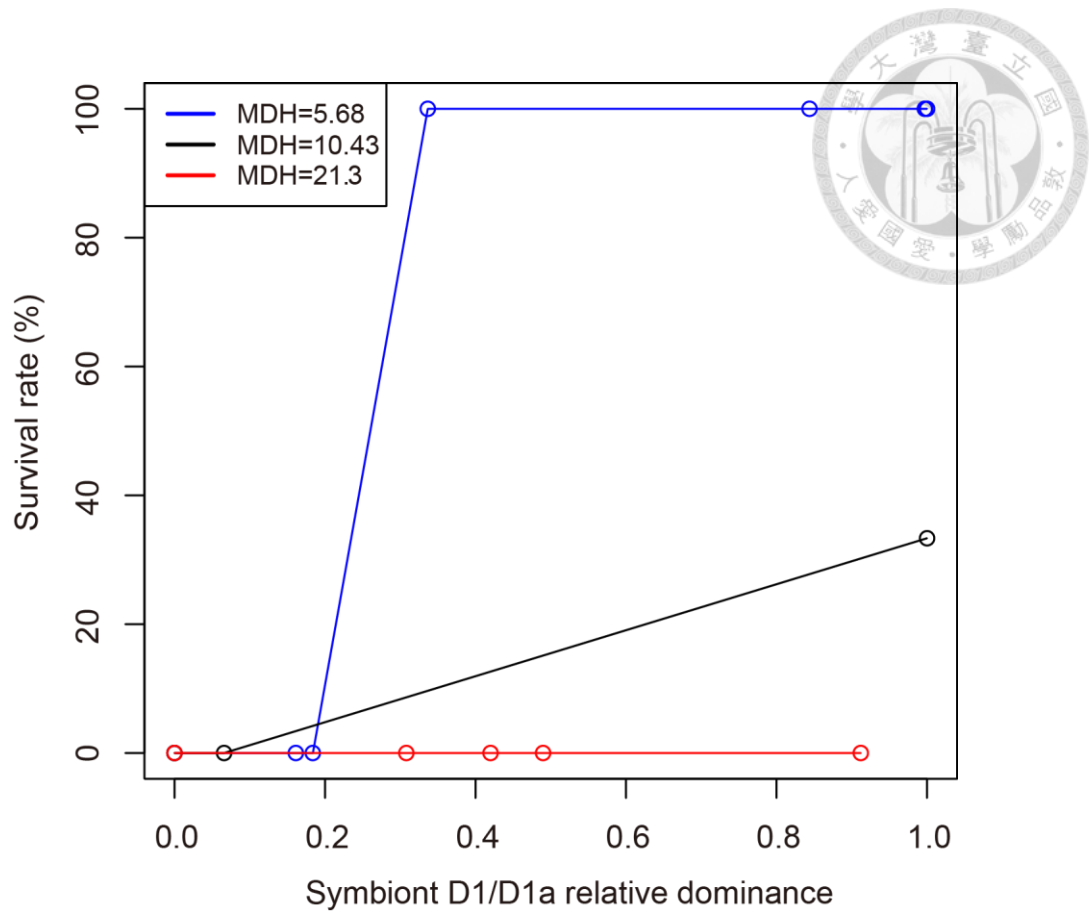
Figure 6 Survival rate of TWO and TIO with the different relative dominance of type

D1/D1a symbiont under different maximum Degree Heating Week (MDH).

Circles represent the symbiont type D1/D1a relative dominance of each colony at its last sampling time.

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Table 1   Future predictions of prolonged thermal stress events in Nanwan

RCP	DHW>6	DHW>8
2.6 2x	2010-20	2020-30
4.5 2x	2020-30	2030-40
6 2x	2020-30	2040-50
8.5 2x	**2020-30**	**2020-30**
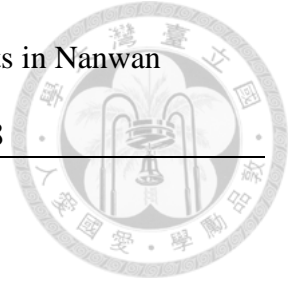
Future predictions for years that Degree Heating Week (DHW) may exceed certain levels at Nanwan bay, Kenting National Park under different emission scenarios based on IPCC AR5. 2x: The DHW conditions that may occur at least twice within given time interval. b) The maximum DHW occurred in each reciprocal transplant experiment (RTE), the rate of successful shuffling for *P. verweyi* transplanted into NPP-OL, and the subsequent host mortality rate after shuffling. Note that for the maximum DHW in 2014RTE, we only report the DHW for TWO (transplant group from WLT to NPP-OL) since a very high mortality already occurred under this situation.
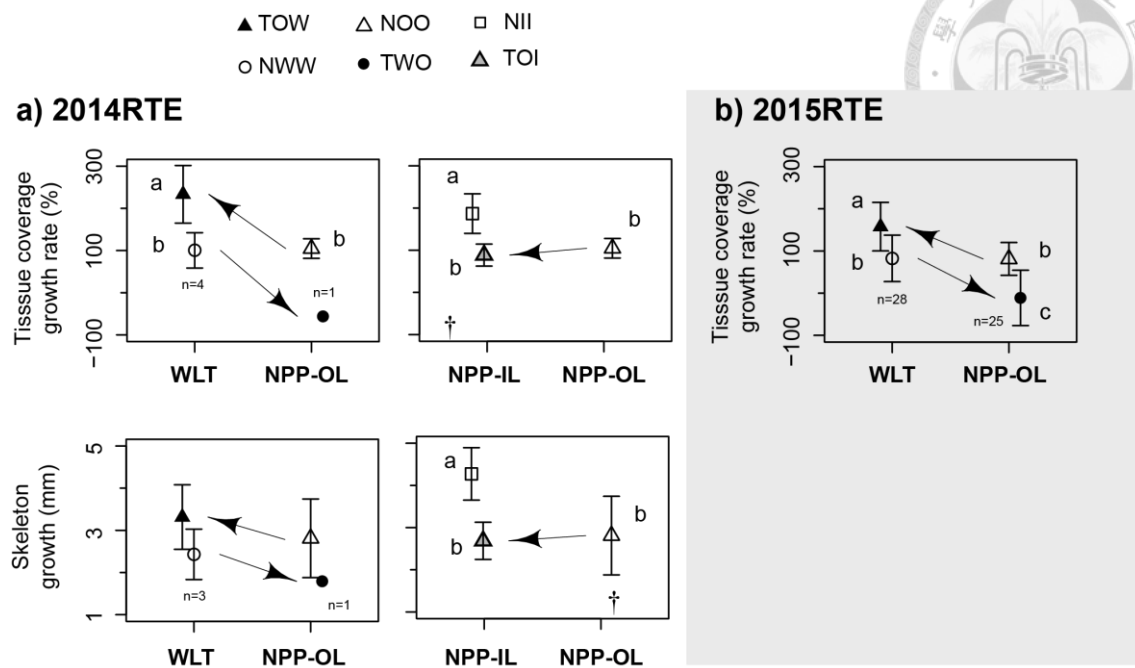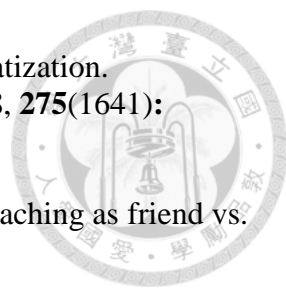
Figure 7 Tissue coverage growth and skeleton growth of each experiment group in

2014RTE and 2015RTE.
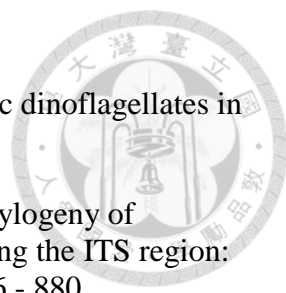
a) (Top) Tissue coverage growth and (Bottom) skeleton growth in 2014RTE (n=5 colonies for each group per sampling time unless stated otherwise). b) Tissue coverage growth in 2015RTE. Arrows represent the directions of transplantation. Data were measured at the end of each RTE relative to initial (n=30 colonies for each group per sampling time unless stated otherwise). Different lowercase letters indicate a significant difference between groups (Two-way ANOVA, Tukey's post hoc test, Bonferroni adjusted p-values at α=0.05). For tissue coverage growth in 2015RTE, there is an origin effect (F=62.163, p<0.001) and location effect (F=67.68, p<0.001) while no origin × location interaction (F=0.695, p=0.406). No statistics for origin versus location effect were conducted in 2014RTE because of lack of sample number caused by mortality. The detailed statistics were described in Appendix A12 to A14. †: All samples were dead. Abbreviations were described in abbreviation page.

References

1.   IPCC. *Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge University Press: Cambridge, United Kingdom and New York, NY, USA, 2013.

2.   van Hooidonk, R., Maynard, J. A., Manzello, D. & Planes, S. Opposite latitudinal gradients in projected ocean acidification and bleaching impacts on coral reefs. *Global Change Biol.* 2014, **20**(1)**:** 103-112.

3.   Hoegh-Guldberg, O. Coral reef sustainability through adaptation: glimmer of hope or persistent mirage? *Current Opinion in Environmental Sustainability* 2014, **7:** 127-133.

4.   Hoegh-Guldberg, O.*, et al.* Coral reefs under rapid climate change and ocean acidification. *Science* 2007, **318**(5857)**:** 1737-1742.

5.   Margulis, L. & Fester, R. *Symbiosis as a source of evolutionary innovation: speciation and morphogenesis*. MIT Press, 1991.

6.   Rowan, R. Review—diversity and eology of zooxanthellae on coral reefs. *J. Phycol.* 1998, **34**(3)**:** 407-417.

7.   Rohwer, F., Seguritan, V., Azam, F. & Knowlton, N. Diversity and distribution of coral-associated bacteria. *Mar. Ecol. Prog. Ser.* 2002, **243:** 1 - 10.

8.   Weber, M. X. & Medina, M. The role of microalgal symbionts (*Symbiodinium*) in holobiont physiology. In: Piganeau G (ed). *Advances in Botanical Research. Genomic insights into the biology of algae*. Academic Press, 2012.

9.   Jones, A. M. & Berkelmans, R. Tradeoffs to thermal acclimation: energetics and reproduction of a reef coral with heat tolerant *Symbiodinium* type-D. *Journal of Marine Biology* 2011, **2011:** 1-12.

10. Jones, A. & Berkelmans, R. Potential costs of acclimatization to a warmer climate: growth of a reef coral with heat tolerant vs. sensitive symbiont types. *PLoS ONE* 2010, **5**(5)**:** e10437.

11. Fitt, W. K., Spero, H. J., Halas, J., White, M. W. & Porter, J. W. Recovery of the coral *Montastrea annularis* in the Florida Keys after the 1987 Caribbean "bleaching event". *Coral Reefs* 1993, **12:** 57-64.

12. Thornhill, D., Fitt, W. & Schmidt, G. Highly stable symbioses among western Atlantic brooding corals. *Coral Reefs* 2006, **25**(4)**:** 515-519.

13. Jones, A. M., Berkelmans, R., van Oppen, M. J. H., Mieog, J. C. & Sinclair, W. A community change in the algal endosymbionts of a scleractinian coral

following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society B: Biological Sciences* 2008, **275**(1641)**:** 1359-1365.

14.   Suggett, D. J. & Smith, D. J. Interpreting the sign of coral bleaching as friend vs. foe. *Global Change Biol.* 2011, **17**(1)**:** 45-55.

15.   Jokiel, P. L. & Coles, S. L. Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. *Coral Reefs* 1990, **8:** 155-162.

16.   Yonge, C. & Nicholls, A. Studies on the physiology of corals. V. The effect of starvation in light and in darkness on the relationship between corals and zooxanthellae. *Gt. Barrier Reef Exped. Sci. Rep* 1931, **1:** 177-211.

17.   Jones, R. J. Coral bleaching, bleaching-induced mortality, and the adaptive significance of the bleaching response. *Mar Biol* 2008, **154**(1)**:** 65-80.

18.   Jones, R. J., Hoegh-Guldberg, O., Larkum, A. W. D. & Schreiber, U. Temperature-induced bleaching of corals begins with impairment of the CO2 fixation mechanism in zooxanthellae. *Plant, Cell Environ.* 1998, **21**(12)**:** 1219-1230.

19.   Warner, M. E., Fitt, W. K. & Schmidt, G. W. Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *Proceedings of the National Academy of Sciences* 1999, **96**(14)**:** 8007-8012.

20.   Jones, R. J. & Hoegh-Guldberg, O. Diurnal changes in the photochemical efficiency of the symbiotic dinoflagellates (*Dinophyceae*) of corals: photoprotection, photoinactivation and the relationship to coral bleaching. *Plant, Cell Environ.* 2001, **24**(1)**:** 89-99.

21.   Lesser, M. P. Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. *Limnol. Oceanogr.* 1996, **41**(2)**:** 271-283.

22.   Wooldridge, S. A. A new conceptual model for the warm-water breakdown of the coral–algae endosymbiosis. *Marine and Freshwater Research* 2009, **60**(6)**:** 483-496.

23.   Gates, R. D., Baghdasarian, G. & Muscatine, L. Temperature stress causes host cell detachment in symbiotic Cnidarians: implications for coral bleaching. *The Biological Bulletin* 1992, **182**(3)**:** 324-332.

24.   Cunning, R. & Baker, A. C. Excess algal symbionts increase the susceptibility of reef corals to bleaching. *Nature Climate Change* 2013, **3**(3)**:** 259-262.

25.   Pochon, X. & Gates, R. D. A new *Symbiodinium* clade (*Dinophyceae*) from soritid foraminifera in Hawai'i. *Molecular phylogenetics and evolution* 2010, **56**(1)**:** 492-497.

26.   Coffroth, M. A. & Santos, S. R. Genetic diversity of symbiotic dinoflagellates in the genus *Symbiodinium*. *Protist* 2005, **156**(1)**:** 19-34.

27.   LaJeunesse, T. Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: in search of a" species" level marker. *J. Phycol.* 2001, **37:** 866 - 880.

28.   Baker, A. C. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. *Annual Review of Ecology, Evolution, and Systematics* 2003, **34**(1)**:** 661-689.

29.   Correa, A. M. S. & Baker, A. C. Understanding diversity in coral-algal symbiosis: a cluster-based approach to interpreting fine-scale genetic variation in the genus *Symbiodinium*. *Coral Reefs* 2009, **28:** 81-93.

30.   Blackall, L. L., Wilson, B. & van Oppen, M. J. H. Coral—the world's most diverse symbiotic ecosystem. *Mol. Ecol.* 2015, **24**(21)**:** 5330-5347.

31.   LaJeunesse, T. C.*, et al.* Ecologically differentiated stress-tolerant endosymbionts in the dinoflagellate genus *Symbiodinium* (*Dinophyceae*) Clade D are different species. *Phycologia* 2014, **53**(4)**:** 305-319.

32.   Tonk, L., Bongaerts, P., Sampayo, E. M. & Hoegh-Guldberg, O. SymbioGBR: a web-based database of *Symbiodinium* associated with cnidarian hosts on the Great Barrier Reef. *BMC Ecol.* 2013, **13**(1)**:** 1-9.

33.   Lien, Y. T.*, et al.* Occurrence of the putatively heat-tolerant *Symbiodinium* phylotype D in high-latitudinal outlying coral communities. *Coral Reefs* 2007, **26**(1)**:** 35-44.

34.   Chen, C. A., Lam, K. K., Nakano, Y. & Tsai, W. S. A stable association of the stress-tolerant zooxanthellae, *Symbiodinium* clade D, with the low-temperature-tolerant coral, *Oulastrea crispata* (Scleractinia: Faviidae) in subtropical non-reefal coral communities. *Zool. Stud.* 2003, **42**(4)**:** 540-550.

35.   Toller, W. W., Rowan, R. & Knowlton, N. Repopulation of zooxanthellae in the Caribbean corals *Montastraea annularis* and *M. faveolata* following experimental and disease-associated bleaching. *The Biological Bulletin* 2001, **201**(3)**:** 360-373.

36.   Rowan, R. Thermal adaptation in reef coral symbionts. *Nature* 2004, **430:** 742.

37.   Berkelmans, R. & Van Oppen, M. The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope'for coral reefs in an era of climate change. *Proc R Soc B* 2006, **273:** 2305 - 2312.

38.   McGinty, E., Pieczonka, J. & Mydlarz, L. Variations in reactive oxygen release and antioxidant activity in multiple *Symbiodinium* types in response to elevated

temperature. *Microb Ecol* 2012, **64**(4)**:** 1000-1007.

39.   Wang, J. T., Meng, P. J., Chen, Y. Y. & Chen, A. C. Determination of the thermal tolerance of *Symbiodinium* using the activation energy for inhibiting photosystem II acitivity. *Zool. Stud.* 2012, **51**(2)**:** 137-142.

40.   Deschaseaux, E. S. M.*, et al.* Comparative response of DMS and DMSP concentrations in *Symbiodinium* clades C1 and D1 under thermal stress. *J. Exp. Mar. Biol. Ecol.* 2014, **459:** 181-189.

41.   Tchernov, D.*, et al.* Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings of the National Academy of Sciences of the United States of America* 2004, **101**(37)**:** 13531-13535.

42.   Iglesias-Prieto, R. & Trench, R. K. Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. *Marine Ecology Progress Series* 1994, **113**(1)**:** 163.

43.   Iglesias-Prieto, R. & Trench, R. K. Acclimation and adaptation to irradiance in symbiotic dinoflagellates. II. Response of chlorophyll-protein complexes to different photon-flux densities. *Mar Biol* 1997, **130**(1)**:** 23-33.

44.   Howells, E. J.*, et al.* Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change* 2012, **2:** 116-120.

45.   Silverstein, R. N., Cunning, R. & Baker, A. C. Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Global Change Biol.* 2015, **21**(1)**:** 236-249.

46.   Hsu, C. M.*, et al.* Temporal and spatial variations of symbiont communities in catch bowl coral, *Isopora palifera* (Scleractinia; Acroporidae), at reefs in Kenting National Park, Taiwan. *Zool. Stud.* 2012, **51**(8)**:** 1343-1353

47.   Keshavmurthy, S.*, et al.* Symbiont communities and host genetic structure of the brain coral *Platygyra verweyi*, at the outlet of a nuclear power plant and adjacent areas. *Molecular ecology* 2012, **21**(17)**:** 4393-4407.

48.   Keshavmurthy, S.*, et al.* Can resistant coral-*Symbiodinium* associations enable coral communities to survive climate change? A study of a site exposed to long-term hot water input. *Peer J* 2014, **2:** e327.

49.   Hume, B. C. C.*, et al. Symbiodinium thermophilum* sp. nov., a thermotolerant symbiotic alga prevalent in corals of the world's hottest sea, the Persian/Arabian Gulf. *Scientific Reports* 2015, **5:** 8562.

50.   Goulet, T. L. Most corals may not change their symbionts. *Mar. Ecol. Prog. Ser.* 2006, **321:** 1-7.

51.   Silverstein, R., Correa, A. & Baker, A. Specificity is rarely absolute in
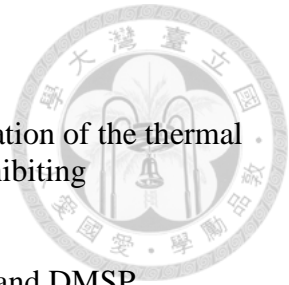
coral-algal symbiosis: implications for coral response to climate change. *Proc R Soc B Biol Sci* 2012, **279**(1738)**:** 2609 - 2618.
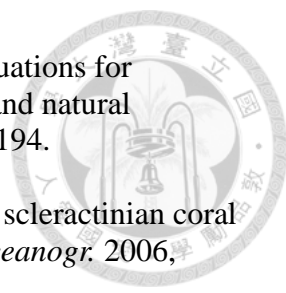
52. Mieog, J. C., Oppen, M. J. H., Cantin, N. E., Stam, W. T. & Olsen, J. L. Real-time PCR reveals a high incidence of *Symbiodinium* clade D at low levels in four scleractinian corals across the Great Barrier Reef: implications for symbiont shuffling. *Coral Reefs* 2007, **26**(3)**:** 449-457.

53. Yachi, S. & Loreau, M. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proceedings of the National Academy of Sciences of the United States of America* 1999, **96**(4)**:** 1463-1468.

54. Lee, M. J.*, et al.* Most low-abundance "background" *Symbiodinium* spp. are transitory and have minimal functional significance for symbiotic corals. *Microb Ecol* 2016**:** 1-13.

55. Salih, A., Larkum, A., Cox, G., Kuhl, M. & Hoegh-Guldberg, O. Fluorescent pigments in corals are photoprotective. *Nature* 2000, **408**(6814)**:** 850-853.

56. Mason, D. S., Schafer, F., Shick, J. M. & Dunlap, W. C. Ultraviolet radiation-absorbing mycosporine-like amino acids (MAAs) are acquired from their diet by medaka fish (*Oryzias latipes*) but not by SKH-1 hairless mice. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 1998, **120**(4)**:** 587-598.

57. Baird, A. H., Bhagooli, R., Ralph, P. J. & Takahashi, S. Coral bleaching: the role of the host. *Trends in ecology & evolution* 2009, **24**(1)**:** 16-20.

58. Lesser, M. P., Stochaj, W. R., Tapley, D. W. & Shick, J. M. Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. *Coral Reefs* 1990, **8**(4)**:** 225-232.

59. Buddemeier, R. & Fautin, D. Coral bleaching as an adaptive mechanism - a testable hypothesis. *Bioscience* 1993, **43**(5)**:** 320 - 326.

60. Buddemeier, R. W., Baker, A. C., Fautin, D. G. & Jacobs, R. The adaptive hypothesis of bleaching. In: Rosenberg E, Loya Y (eds). *Coral Health and Disease*. Springer Science & Business Media, 2004.

61. Lin, K. L., Wang, J. T. & Fang, L. S. Participation of glycoproteins on zooxanthellal cell walls in the establishment of a symbiotic relationship with the sea anemone, *Aiptasia pulchella*. *Zool. Stud.* 2000, **39**(3)**:** 172-178.

62. Davy, S. K., Allemand, D. & Weis, V. M. Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiology and molecular biology reviews : MMBR* 2012, **76**(2)**:** 229-261.

63. Fransolet, D., Roberty, S. & Plumier, J.-C. Establishment of endosymbiosis: the

case of cnidarians and *Symbiodinium*. *J. Exp. Mar. Biol. Ecol.* 2012, **420–421**(0)**:** 1-7.

64. Fitt, W. K. & Trench, R. K. Endocytosis of the symbiotic dinoflagellate *Symbiodinium microadriaticum* Freudenthal by endodermal cells of the scyphistomae of *Cassiopeia xamachana* and resistance of the algae to host digestion. *Journal of cell science* 1983, **64:** 195-212.

65. Hohman, T. C., McNeil, P. L. & Muscatine, L. Phagosome-lysosome fusion inhibited by algal symbionts of *Hydra viridis*. *The Journal of Cell Biology* 1982, **94**(1)**:** 56-63.

66. Muscatine, L. & Pool, R. R. Regulation of numbers of intracellular algae. *Proc R Soc Lond B Biol Sci* 1979, **204**(1155)**:** 131-139.

67. Coffroth, M. A., Poland, D. M., Petrou, E. L., Brazeau, D. A. & Holmberg, J. C. Environmental symbiont acquisition may not be the solution to warming seas for reef-building corals. *PLoS ONE* 2010, **5**(10)**:** e13258.

68. Cunning, R., Silverstein, R. N. & Baker, A. C. Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. *Proceedings of the Royal Society of London B: Biological Sciences* 2015, **282**(1809).

69. Rouzé, H., Lecellier, G., Saulnier, D. & Berteaux-Lecellier, V. *Symbiodinium* clades A and D differentially predispose *Acropora cytherea* to disease and *Vibrio* spp. colonization. *Ecology and Evolution* 2016, **6**(2)**:** 560-572.

70. LaJeunesse, T. C., Smith, R. T., Finney, J. & Oxenford, H. Outbreak and persistence of opportunistic symbiotic dinoflagellates during the 2005 Caribbean mass coral 'bleaching' event. *Proceedings of the Royal Society of London B: Biological Sciences* 2009, **276**(1676)**:** 4139-4148.

71. Howells, E. J., Berkelmans, R., van Oppen, M. J., Willis, B. L. & Bay, L. K. Historical thermal regimes define limits to coral acclimatization. *Ecology* 2013, **94**(5)**:** 1078-1088.

72. Baker, A., McClanahan, T., Starger, C. & Boonstra, R. Long-term monitoring of algal symbiont communities in corals reveals stability is taxon dependent and driven by site-specific thermal regime. *Mar. Ecol. Prog. Ser.* 2013, **479:** 85-97.

73. Cantin, N. E., van Oppen, M. J. H., Willis, B. L., Mieog, J. C. & Negri, A. P. Juvenile corals can acquire more carbon from high-performance algal symbionts. *Coral Reefs* 2009, **28**(2)**:** 405-414.

74. Little, A. F., van Oppen, M. J. H. & Willis, B. L. Flexibility in algal endosymbioses shapes growth in reef corals. *Science* 2004, **304**(5676)**:** 1492-1494.

75. Mieog, J. C., *et al.* The roles and interactions of symbiont, host and environment in defining coral fitness. *PLos ONE* 2009, **4**(7)**:** e6364.

76. Cunning, R., Gillette, P., Capo, T., Galvez, K. & Baker, A. C. Growth tradeoffs associated with thermotolerant symbionts in the coral *Pocillopora damicornis* are lost in warmer oceans. *Coral Reefs* 2015, **34**(1)**:** 155-160.

77. Yuyama, I. & Higuchi, T. Comparing the Effects of Symbiotic Algae *Symbiodinium* Clades C1 and D on Early Growth Stages of *Acropora tenuis*. *PLoS ONE* 2014, **9**(6)**:** e98999.

78. Stat, M. & Gates, R. D. Clade D *Symbiodinium* in scleractinian corals: a "nugget" of hope, a selfish opportunist, an ominous sign, or all of the above? *Journal of Marine Biology* 2011, **2011:** 1-9.

79. Thornhill, D., LaJeunesse, T., Kemp, D., Fitt, W. & Schmidt, G. Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. *Mar Biol* 2006, **148**(4)**:** 711-722.

80. Hughes, T. P*., et al.* Climate change, human impacts, and the resilience of coral reefs. *Science* 2003, **301**(5635)**:** 929-933.

81. Chiou, W. D., Cheng, L. Z. & Ou, H. C. Relationship between the dispersion of thermal effluent and the tidal current in the waters near the outlet of the third nuclear power plant in southern Taiwan. *Journal of Fisheries Society of Taiwan* 1993, **20:** 207-220.

82. Chou, Y., Lin, T. Y., Chen, C. T. A. & Liu, L. L. Effects of nuclear power plant thermal effluent on marine sessile invertebrate communities in southern Taiwan. *J. Mar. Sci. Technol.* 2004, **12**(5)**:** 448-452.

83. Chen, C. T. A., Wang, B. J., Wang, J. J., Tu, Y. Y. & Chang, W. C. Changes in seawater temperature over the past twenty-two years and the radioisotopes in a coral in Nanwan Bay near The Third Nuclear Power Plant. *Acta Oceanogr. Taiwanica* 2001, **39**(1)**:** 39-52.

84. Fan, K. L. The thermal effluent incident of the Third Nuclear Power Plant in southern Taiwan. *Acta Oceanogr. Taiwanica* 1988, **20:** 107-117.

85. Fan, K. L. The thermal effluent problems of three nuclear power plants in Taiwan. *Oceanogr. Ser.* 1991, **54:** 309-403.

86. Hung, T. C., Huang, C. C. & Shao, K. T. Ecological survey of coastal I water adjacent to Nuclear Power I Plants in Taiwan. *Chem. Ecol.* 1998, **15**(1-3)**:** 129-142.

87. Lee, H. J., Chao, S. Y., Fan, K. L., Wang, Y. H. & Liang, N. K. Tidally induced upwelling in a semi-enclosed basin: Nan Wan Bay. *J. Oceanogr.* 1997, **53:** 467-480.

88. Lee, H. J., Chao, S. Y. & Fan, K. L. Flood-ebb disparity of tidally induced recirculation eddies in a semi-enclosed basin: Nan Wan Bay. *Cont. Shelf Res.* 1999, **19:** 871-890.

89. IPCC. Summary for policymakers. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J*, et al.* (eds). *Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change.* Cambridge University Press: Cambridge, United Kingdom and New York, NY, USA, 2013, pp 1–30.

90. Wellington, G. M.*, et al.* Crisis on coral reefs linked to climate change. *Eos, Transactions American Geophysical Union* 2001, **82**(1)**:** 1-5.

91. Wijsman-Best, M. Biological results of the snellius expedition: XXVII. *Faviidae* collected by the snellius expedition. II. The genera *Favites*, *Goniastrea*, *Platygyra*, *Oulophyllia*, *Leptoria*, *Hydnophora* and *Caulastrea*. *Zoologische Mededelingen* 1976, **50**(4)**:** 45-63.

92. Lan, C. H. Host genetic and *Symbiodinium* composition in brain coral, *Platygyra verweyi* in Kenting, southern Taiwan. Master thesis, National Taiwan University, 2011.

93. Liu, G., Strong, A. E. & Skirving, W. Remote sensing of sea surface temperatures during 2002 Barrier Reef coral bleaching. *Eos, Transactions American Geophysical Union* 2003, **84**(15)**:** 137-141.

94. Schoepf, V., Stat, M., Falter, J. L. & McCulloch, M. T. Limits to the thermal tolerance of corals adapted to a highly fluctuating, naturally extreme temperature environment. *Scientific Reports* 2015, **5:** 17639.

95. LeGore, S., Marszalek, D. S., Danek, L. J., Tomlinson, M. S. & Hofmann, J. E. Effect of chemically dispersed oil on Aranian Gulf corals: a field experiment. *International Oil Spill Conference Proceedings* 1989, **1989**(1)**:** 375-380.

96. Ferrara, G. B.*, et al.* The assessment of DNA from marine organisms via a modified salting-out protocol. *Cell. Mol. Biol. Lett.* 2006**:** 150-160.

97. LaJeunesse, T. C. Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Mar Biol* 2002, **141:** 387-400.

98. Ulstrup, K. E. & Van Oppen, M. J. Geographic and habitat partitioning of genetically distinct zooxanthellae (*Symbiodinium*) in *Acropora* corals on the Great Barrier Reef. *Molecular ecology* 2003, **12**(12)**:** 3477-3484.

99. Rasmussen, R. Quantification on the LightCycler. In: Meuer S, Wittwer C, Nakagawara K-I (eds). *Rapid cycle real-time PCR: methods and applications.* Springer Berlin Heidelberg: Berlin, Heidelberg, 2001, pp 21-34.

100. Jeffrey, S. W. & Humphrey, G. F. New spectrophotometric equations for determining chlorophylls a, b, c1, c2 in higher plants, algae, and natural phytoplankton. *Biochem. Physiolo. Pflanzen* 1975, **167:** 191-194.

101. Dove, S.*, et al.* Response of holosymbiont pigments from the scleractinian coral *Montipora monasteriata* to short-term heat stress. *Limnol. Oceanogr.* 2006, **51**(2)**:** 1149-1158.

102. R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2014.

103. Box, G. E. P. & Cox, D. R. An analysis of transformations. *Journal of the Royal Statistical Society. Series B (Methodological)* 1964, **26**(2)**:** 211-252.

104. LaJeunesse, T. C.*, et al.* Long-standing environmental conditions, geographic isolation and host-symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus *Symbiodinium. J. Biogeogr.* 2010, **37**(5)**:** 785-800.

105. Wilkerson, F. P., Kobayashi, D. & Muscatine, L. Mitotic index and size of symbiotic algae in Caribbean reef corals. *Coral Reefs* 1988, **7**(1)**:** 29-36.

106. Wilkerson, F. P., Muller, G. & Muscatine, P. L. Temporal patterns of cell division in natural populations of endosymbiotic algae. *Limnol. Oceanogr.* 1983, **28**(5)**:** 1009-1014.

107. Jones, R. J. & Yellowlees, D. Regulation and control of intracellular algae (= zooxanthellae) in hard corals. *Philosophical Transactions of the Royal Society B: Biological Sciences* 1997, **352**(1352)**:** 457-468.

108. Dunn, S. R., Bythell, J. C., Le Tissier, M. D. A., Burnett, W. J. & Thomason, J. C. Programmed cell death and cell necrosis activity during hyperthermic stress-induced bleaching of the symbiotic sea anemone *Aiptasia* sp. *J. Exp. Mar. Biol. Ecol.* 2002, **272**(1)**:** 29-53.

109. Oliver, T. A. & Palumbi, S. R. Do fluctuating temperature environments elevate coral thermal tolerance? *Coral Reefs* 2011, **30**(2)**:** 429-440.

110. Palumbi, S. R., Barshis, D. J., Traylor-Knowles, N. & Bay, R. A. Mechanisms of reef coral resistance to future climate change. *Science* 2014, **344**(6186)**:** 895-898.

111. Brown, B. E., Downs, C. A., Dunne, R. P. & Gibb, S. W. Exploring the basis of thermotolerance in the reef coral *Goniastrea aspera*. *Mar. Ecol. Prog. Ser.* 2002, **242:** 119-129.

112. Tanaka, Y., Miyajima, T., Koike, I., Hayashibara, T. & Ogawa, H. Imbalanced coral growth between organic tissue and carbonate skeleton caused by nutrient enrichment. *Limnol. Oceanogr.* 2007, **52**(3)**:** 1139-1146.
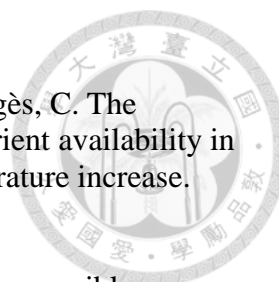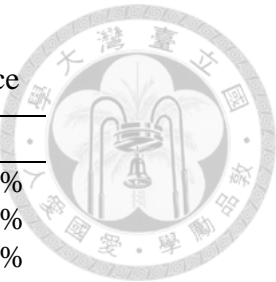
113. Ezzat, L., Towle, E., Irisson, J.-O., Langdon, C. & Ferrier-Pagès, C. The relationship between heterotrophic feeding and inorganic nutrient availability in the scleractinian coral *T. reniformis* under a short-term temperature increase. *Limnol. Oceanogr.* 2016, **61**(1)**:** 89-102.

114. Hughes, A. D. & Grottoli, A. G. Heterotrophic compensation: a possible mechanism for resilience of coral reefs to global warming or a sign of prolonged stress? *PLoS ONE* 2013, **8**(11)**:** e81172.

115. Smith, L. W., Wirshing, H. H., Baker, A. C. & Birkeland, C. Environmental versus genetic influences on growth rates of the corals *Pocillopora eydouxi* and *Porites lobata* (Anthozoa: Scleractinia). *Pac. Sci.* 2008, **62**(1)**:** 57-69.

116. Fabricius, K. E. Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Mar. Pollut. Bull.* 2005, **50**(2)**:** 125-146.

117. Meng, P. J.*, et al.* A long-term survey on anthropogenic impacts to the water quality of coral reefs, southern Taiwan. *Environ. Pollut.* 2008, **156**(1)**:** 67-75.

Appendix
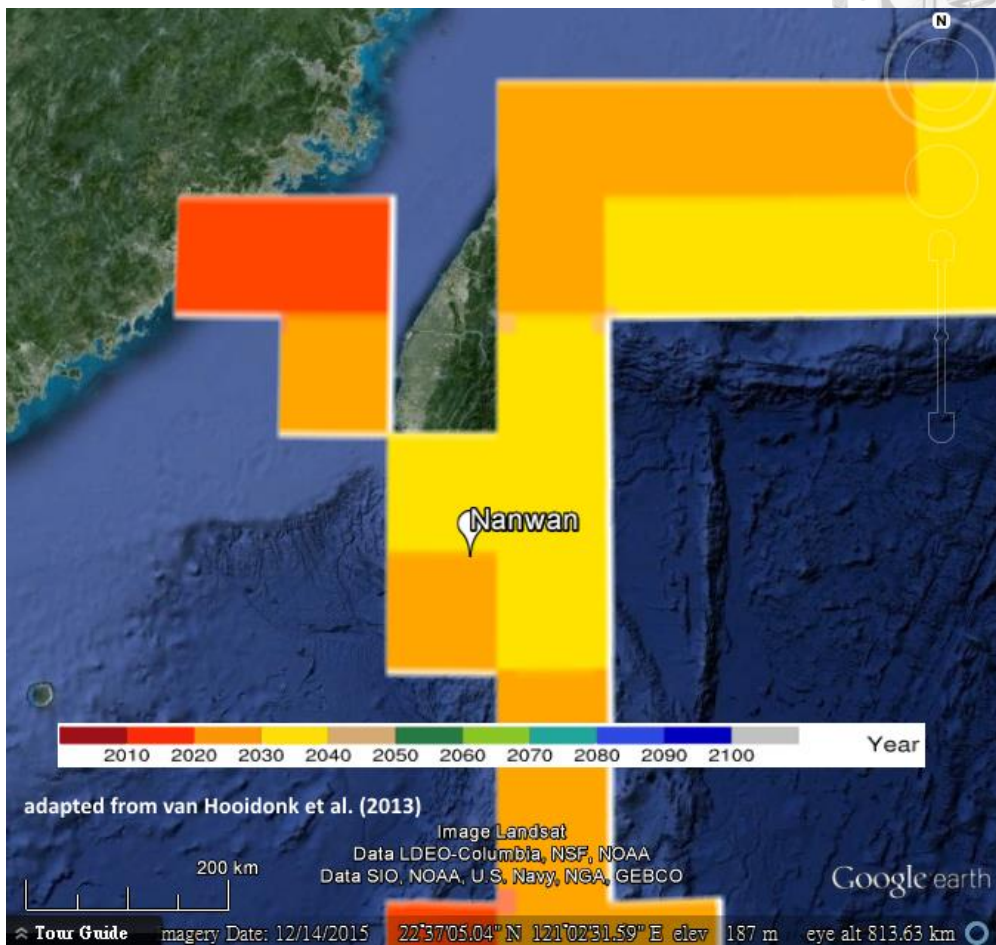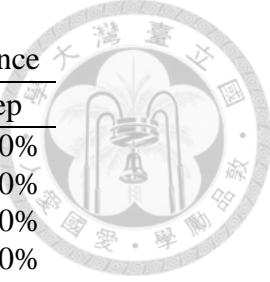
A1 Image of DHW projection (under RCP 6.0; DHW>6; might happen at least twice during projecting interval) on reef cell (each in 1° x 1° resolution grid) covering Nanwan.

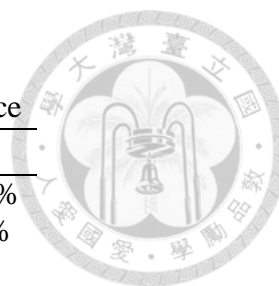| | | Symbiont types D1/D1a dominance | | |
group	colony	2014Apr	2014Sep	2015Jan
NOO	1	100.000%	100.000%	100.000%
NOO	2	100.000%	100.000%	100.000%
NOO	3	100.000%	100.000%	100.000%
NOO	4	100.000%	100.000%	100.000%
NOO	5	100.000%	100.000%	100.000%
TOW	1	100.000%	100.000%	98.061%
TOW	2	100.000%	100.000%	100.000%
TOW	3	100.000%	100.000%	100.000%
TOW	4	99.978%	100.000%	100.000%
TOW	5	100.000%	100.000%	100.000%
TOI	1	98.850%	100.000%	100.000%
TOI	2	100.000%	100.000%	100.000%
TOI	3	100.000%	100.000%	100.000%
TOI	4	100.000%	100.000%	100.000%
TOI	5	100.000%	99.556%	100.000%
NWW	1	0.000%	0.000%	0.000%
NWW	2	0.000%	0.000%	0.000%
NWW	3	0.000%	0.000%	0.000%
NWW	4	0.000%	3.799%	Dead
NWW	5	0.000%	0.000%	0.000%
TWO	1	0.017%	6.587%	Dead
TWO	2	0.000%	0.000%	Dead
TWO	3	0.000%	8.478%	100.000%
TWO	4	0.585%	100.000%	Dead
TWO	5	0.000%	100.000%	Dead
NII	1	0.000%	0.000%	0.023%
NII	2	0.000%	0.000%	0.000%
NII	3	0.000%	0.000%	0.000%
NII	4	0.000%	0.000%	0.000%
NII	5	0.001%	0.000%	0.000%
TIO	1	0.000%	49.022%	Dead
TIO	2	0.000%	30.783%	Dead
TIO	3	0.000%	41.984%	Dead
TIO	4	0.000%	91.156%	Dead
TIO	5	0.000%	NA	Dead

A2 Symbiont type D1/D1a dominance in total symbiont community in each group by time in 2014RTE. NA represents both symbiont type C3/C3cc and D1/D1a were undetected from a single colony. Abbreviations were described in abbreviation page.

(a)		Symbiont types D1/D1a dominance		
group	colony	2015Apr	2015Jul	2015Sep
NOO	1	100.000%	100.000%	100.000%
NOO	2	100.000%	100.000%	100.000%
NOO	3	100.000%	100.000%	100.000%
NOO	4	100.000%	100.000%	100.000%
NOO	5	100.000%	99.901%	100.000%
NOO	6	100.000%	100.000%	100.000%
NOO	7	100.000%	100.000%	100.000%
NOO	8	100.000%	100.000%	100.000%
NOO	9	100.000%	100.000%	100.000%
NOO	10	100.000%	100.000%	100.000%
NOO	11	100.000%	100.000%	100.000%
NOO	12	100.000%	100.000%	100.000%
NOO	13	100.000%	100.000%	100.000%
NOO	14	100.000%	100.000%	100.000%
NOO	15	100.000%	100.000%	100.000%
NOO	16	100.000%	100.000%	100.000%
NOO	17	80.645%	100.000%	100.000%
NOO	18	100.000%	100.000%	100.000%
NOO	19	100.000%	100.000%	100.000%
NOO	20	100.000%	100.000%	100.000%
NOO	21	100.000%	100.000%	100.000%
NOO	22	100.000%	100.000%	100.000%
NOO	23	100.000%	100.000%	100.000%
NOO	24	100.000%	100.000%	100.000%
NOO	25	100.000%	100.000%	100.000%
NOO	26	100.000%	100.000%	100.000%
NOO	27	100.000%	100.000%	100.000%
NOO	28	100.000%	100.000%	100.000%
NOO	29	100.000%	100.000%	100.000%
NOO	30	100.000%	100.000%	100.000%

A3　Symbiont type D1/D1a dominance in total symbiont community in each group by time in 2015RTE. (a) NOO (b) TOW (c) NWW (d) TWO. NA represents both symbiont type C3/C3cc and D1/D1a were undetected from a single colony. Abbreviations were described in abbreviation page.
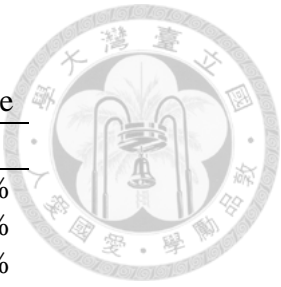
(b)		Symbiont types D1/D1a dominance		
group	colony	2015Apr	2015Jul	2015Sep
TOW	1	100.000%	100.000%	100.000%
TOW	2	100.000%	100.000%	100.000%
TOW	3	100.000%	100.000%	100.000%
TOW	4	100.000%	100.000%	100.000%
TOW	5	100.000%	100.000%	100.000%
TOW	6	100.000%	100.000%	100.000%
TOW	7	100.000%	100.000%	100.000%
TOW	8	100.000%	100.000%	100.000%
TOW	9	100.000%	100.000%	100.000%
TOW	10	100.000%	100.000%	100.000%
TOW	11	100.000%	100.000%	100.000%
TOW	12	100.000%	100.000%	100.000%
TOW	13	100.000%	100.000%	100.000%
TOW	14	100.000%	100.000%	100.000%
TOW	15	100.000%	100.000%	100.000%
TOW	16	100.000%	100.000%	100.000%
TOW	17	100.000%	100.000%	100.000%
TOW	18	100.000%	100.000%	100.000%
TOW	19	100.000%	100.000%	100.000%
TOW	20	100.000%	100.000%	100.000%
TOW	21	100.000%	100.000%	100.000%
TOW	22	99.828%	100.000%	100.000%
TOW	23	100.000%	100.000%	100.000%
TOW	24	100.000%	100.000%	100.000%
TOW	25	100.000%	100.000%	100.000%
TOW	26	100.000%	100.000%	100.000%
TOW	27	100.000%	100.000%	100.000%
TOW	28	100.000%	100.000%	99.966%
TOW	29	100.000%	100.000%	100.000%
TOW	30	100.000%	100.000%	100.000%

A3 (continued).
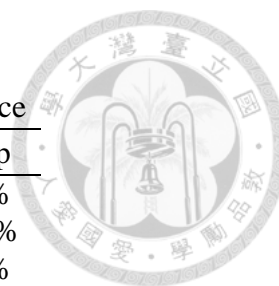
(c)		Symbiont types D1/D1a dominance		
group	colony	2015Apr	2015Jul	2015Sep
NWW	1	0.000%	0.000%	0.000%
NWW	2	12.711%	6.470%	28.460%
NWW	3	0.000%	0.000%	0.000%
NWW	4	0.000%	0.000%	0.020%
NWW	5	0.000%	0.000%	0.000%
NWW	6	0.000%	0.000%	0.000%
NWW	7	0.000%	4.963%	7.829%
NWW	8	NA	2.356%	22.971%
NWW	9	0.000%	NA	0.000%
NWW	10	0.000%	0.000%	0.000%
NWW	11	0.020%	0.000%	0.000%
NWW	12	0.022%	0.000%	0.000%
NWW	13	0.000%	0.000%	0.000%
NWW	14	0.000%	0.000%	0.000%
NWW	15	0.000%	0.000%	0.000%
NWW	16	0.000%	0.000%	0.000%
NWW	17	0.019%	0.000%	0.028%
NWW	18	0.000%	0.000%	0.000%
NWW	19	0.000%	0.000%	0.000%
NWW	20	0.000%	0.000%	0.000%
NWW	21	0.000%	0.000%	0.000%
NWW	22	0.198%	0.000%	0.000%
NWW	23	79.841%	88.243%	77.205%
NWW	24	8.851%	27.263%	44.608%
NWW	25	85.576%	100.000%	95.126%
NWW	26	0.040%	0.000%	0.466%
NWW	27	0.000%	0.000%	0.000%
NWW	28	0.000%	0.000%	0.000%
NWW	29	0.000%	0.000%	0.000%
NWW	30	0.000%	0.000%	0.000%

A3 (continued).

(d)		Symbiont types D1/D1a dominance		
group	colony	2015Apr	2015Jul	2015Sep
TWO	1	0.000%	100.000%	100.000%
TWO	2	39.585%	100.000%	99.712%
TWO	3	0.000%	18.386%	Dead
TWO	4	0.000%	0.000%	Dead
TWO	5	0.000%	NA	84.376%
TWO	6	0.000%	NA	33.657%
TWO	7	4.207%	100.000%	100.000%
TWO	8	0.558%	100.000%	100.000%
TWO	9	0.000%	NA	Dead
TWO	10	0.000%	NA	100.000%
TWO	11	0.000%	100.000%	100.000%
TWO	12	0.000%	NA	Dead
TWO	13	0.000%	100.000%	100.000%
TWO	14	0.000%	100.000%	100.000%
TWO	15	0.000%	44.115%	100.000%
TWO	16	0.000%	100.000%	100.000%
TWO	17	0.000%	86.786%	100.000%
TWO	18	0.000%	100.000%	100.000%
TWO	19	100.000%	100.000%	100.000%
TWO	20	0.054%	100.000%	100.000%
TWO	21	0.000%	NA	100.000%
TWO	22	0.038%	NA	100.000%
TWO	23	0.120%	100.000%	100.000%
TWO	24	0.150%	100.000%	100.000%
TWO	25	0.000%	100.000%	100.000%
TWO	26	0.000%	100.000%	100.000%
TWO	27	0.000%	100.000%	100.000%
TWO	28	0.000%	77.905%	100.000%
TWO	29	82.421%	100.000%	100.000%
TWO	30	16.122%	Dead	Dead

A3 (continued).
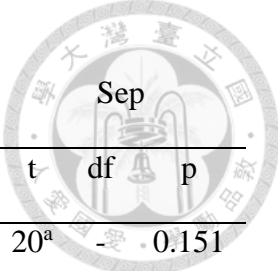
group	colony	symbiont types C3/C3cc	symbiont types D1/D1a	time
TWO	1	1024701	178	2014Apr
TWO	2	973367	0	2014Apr
TWO	3	1099996	0	2014Apr
TWO	4	662035	3896	2014Apr
TWO	5	1020263	0	2014Apr
TIO	1	549727	0	2014Apr
TIO	2	598196	0	2014Apr
TIO	3	722107	0	2014Apr
TIO	4	802871	0	2014Apr
TIO	5	564307	0	2014Apr
TWO	1	41962	2960	2014Sep
TWO	2	229146	0	2014Sep
TWO	3	43323	4014	2014Sep
TWO	4	0	74546	2014Sep
TWO	5	0	184983	2014Sep
TIO	1	113244	108803	2014Sep
TIO	2	78489	34935	2014Sep
TIO	3	141991	102821	2014Sep
TIO	4	13313	137975	2014Sep
TIO	5	0	0	2014Sep
TWO	3	0	631195	2015Jan

A4 Symbiont cell densities of symbiont types C3/C3cc and types D1/D1a in 2014RTE, values were estimated by multiplying total symbiont cell densities obtaining from cell counting to types C3/C3cc : D1/D1a ratio obtaining from qPCR. Abbreviations were described in abbreviation page.

parameters	TWO			TIO			NOO		
	t	df	**p**	t	df	**p**	t	df	**p**
Photochemical efficiency (Fv/Fm)									
April vs. July	16.01	4	**<0.001**	12	4	**<0.001**	25.63	4	**<0.001**
April vs. September	4.33	4	**0.012**	23.83	4	**<0.001**	17.64	4	**<0.001**
April vs. October	-	-	-	-	-	-	6.23	4	**0.003**
April vs. January	-	-	-	-	-	-	11.7	4	**<0.001**
July vs. September	-5.07	4	**0.007**	-8.43	4	**0.001**	-6.16	4	**0.004**
July vs. October	-	-	-	-	-	-	-5.91	4	**0.004**
July vs. January	-	-	-	-	-	-	-13.95	4	**<0.001**
September vs. October	-	-	-	-	-	-	3.97	4	**0.017**
September vs. January	-	-	-	-	-	-	-12.64	4	**<0.001**
October vs. January	-	-	-	-	-	-	-0.96	4	0.391
Total symbiont density (cell cm-2)									
April vs. September	10.20	4	**<0.001**	8.58	4	**0.001**	-1.33	4	0.255
April vs. January	-	-	-	-	-	-	-4.07	4	0.705
September vs. January	-	-	-	-	-	-	0.62	4	0.569
Chlorophyll a (μg cm -2)									
April vs. September	5.65	4	**0.005**	2.67	4	0.06	1.17	4	0.306
April vs. January	-	-	-	-	-	-	-0.12	4	0.911
September vs. January	-	-	-	-	-	-	-1.75	4	0.156
Chlorophyll a (pg symbiont cell -1)									
April vs. September	0.73	4	0.502	-0.732	4	0.505	2.92	4	**0.043**
April vs. January	-	-	-	-	-	-	0.11	4	0.914
September vs. January	-	-	-	-	-	-	-2.6	4	0.06
Total soluble protein (mg cm-2)									
April vs. September	7.10	4	**0.002**	10.42	4	**<0.001**	7.40	4	**0.002**
April vs. January	-	-	-	-	-	-	2.96	4	**0.042**
September vs. January	-	-	-	-	-	-	-2.53	4	0.065

A5 Paired t-test results of photochemical efficiency and other physiological parameters in 2014RTE. P-values in boldface highlighting the significant differences between time. Dash lines represent no statistics were performed due to high mortality.
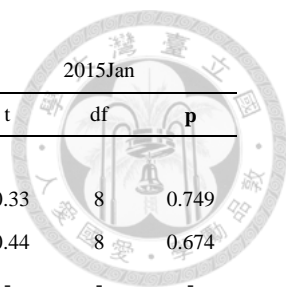
Photochemical efficiency	Apr-14			July			Sep		
	t	df	p	t	df	p	t	df	p
TWO vs NWW	9[a]	-	0.548	13.13	8	**<0.001**	20[a]	-	0.151
TIO vs NII	0.00	8	0.997	10.02	4.59	**<0.001**	6.33	8	**<0.001**
TWO vs NOO	1.86	8	0.100	11.68	8	**<0.001**	1.54	4.49	0.191
TIO vs NOO	0.70	8	0.501	9.81	8	**<0.001**	6.24	8	**<0.001**

A6 Student's t-test results of photochemical efficiency through time in 2014RTE. No statistics were performed for TWO and TIO after October due to insufficient sample number causing by mortality. [a]: Wilcoxon rank sum test was performed since data failed to meet homogeneity of variance even after transformation. †: All the samples were dead. Abbreviations were described in abbreviation page.
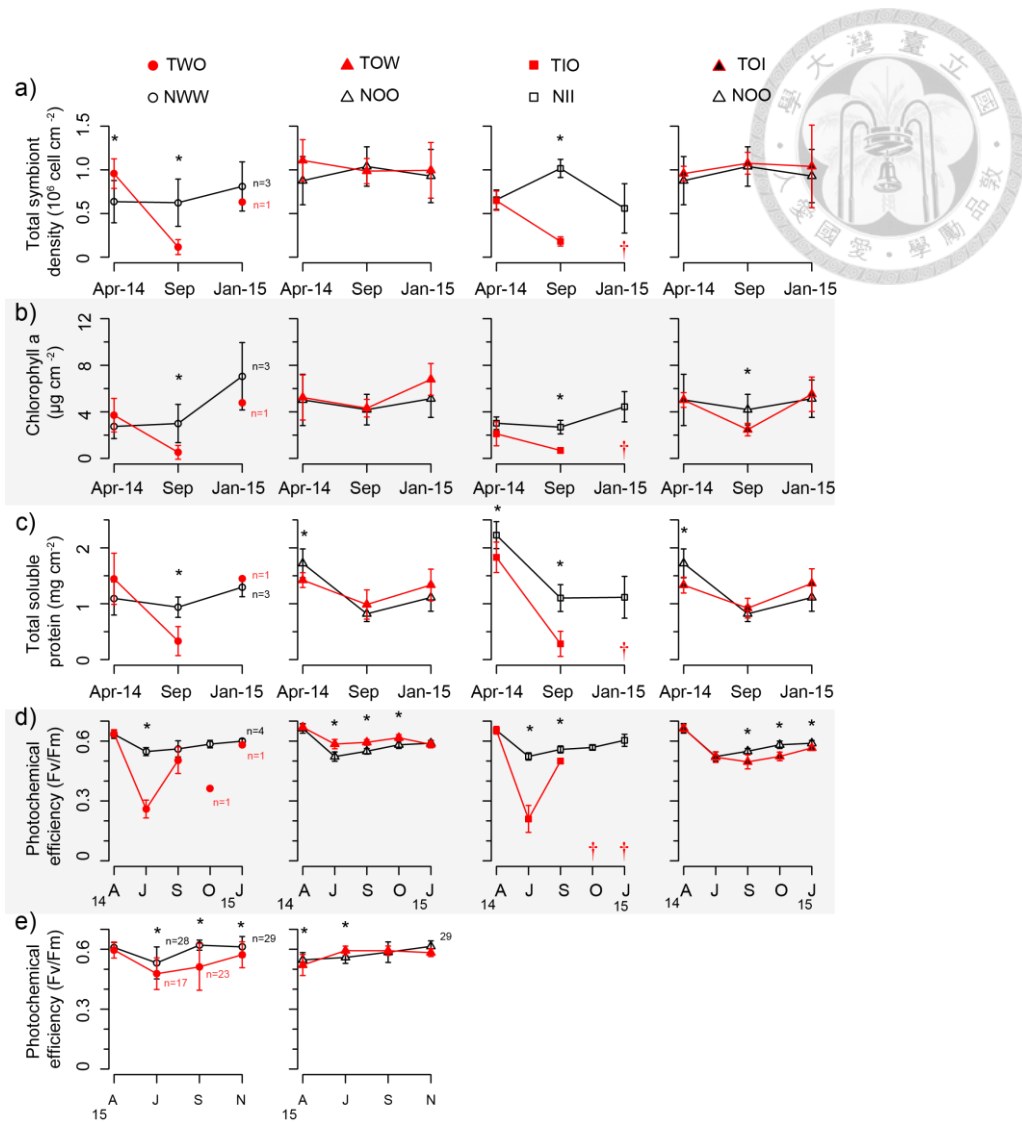
A7 Physiological parameters through sampling time. a) Total symbiont cell densities b) Chlorophyll a concentrations per cm^2 c) Total soluble protein concentrations d) Dark-adapted photochemical efficiency measured in 2014RTE. (n=5 colonies for each group per sampling time unless stated otherwise). e) Photochemical efficiency measured in 2015RTE. (n=30 colonies for each group per sampling time unless stated otherwise). All data are presented in Mean ± SD. Asterisks represent a significant difference of total symbiont density between each transplant group and the native group on each month. The detailed statistics were described in Appendix A6, A8, and A10. †: All the samples were dead. Abbreviations were described in abbreviation page.

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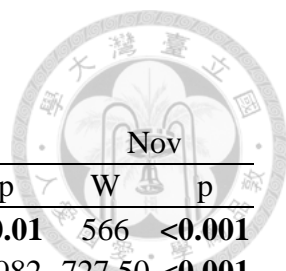| parameters	2014Apr			2014Sep			2015Jan		
	t	df	**p**	t	df	**p**	t	df	**p**
Total symbiont density									
TOW vs. NOO	-1.81[a]	8	0.108	0.44	8	0.671	-0.33	8	0.749
TOI vs. NOO	-1.30[a]	4.6	0.255	-0.32	8	0.757	-0.44	8	0.674
TWO vs. NWW	-2.43	8	**0.041**	4.00	4.79	**0.011**	-	-	-
TIO vs. NII	0.15	8	0.885	15.96	8	**<0.001**	†	†	†
TWO vs. NOO	-0.55	8	0.595	8.55	8	**<0.001**	-	-	-
TIO vs. NOO	2.18[a]	8	0.060	9.89[a]	8	**<0.001**	†	†	†
Chlorophyll a per cm²									
TOW vs. NOO	-0.16	8	0.879	-0.18	8	0.862	-1.76	8	0.117
TOI vs. NOO	W=7[b]	-	0.301	2.71	8	**0.028**	-0.39	8	0.706
TWO vs. NWW	-1.22	8	0.257	3.16	8	**0.013**	-	-	-
TIO vs. NII	1.74	8	0.119	7.18	8	**<0.001**	†	†	†
TWO vs. NOO	1.48[a]	8	0.177	5.70	8	**<0.001**	-	-	-
TIO vs. NOO	2.95[a]	8	0.019	5.89	4.23	**0.003**	†	†	†
Chlorophyll a per cell									
TOW vs. NOO	1.61	8	0.146	-0.52	8	0.620	-1.19 [a]	8	0.269
TOI vs. NOO	1.10	8	0.302	3.42	8	**0.009**	-0.26	8	0.804
TWO vs. NWW	0.63	8	0.547	0.85	8	0.418	-	-	-
TIO vs. NII	1.64	8	0.141	-1.90	4.38	0.124	†	†	†
TWO vs. NOO	2.75	8	0.025	0.76	8	0.470	-	-	-
TIO vs. NOO	2.93	8	0.019	0.01	8	0.992	†	†	†
Total soluble protein									
TOW vs. NOO	2.32	8	**0.049**	-1.25	8	0.246	-1.34	8	0.219
TOI vs. NOO	2.97	8	**0.018**	-1.00	8	0.346	-1.57	8	0.156
TWO vs. NWW	-1.44	8	0.188	4.29	8	**0.003**	-	-	-
TIO vs. NII	2.58	8	**0.032**	5.58	8	**<0.001**	†	†	†
TWO vs. NOO	1.18	8	**0.273**	4.55	8	**0.002**	-	-	-
TIO vs. NOO	-0.64	8	**0.540**	3.71	8	**0.006**	†	†	†

A8 Student's t-test results for physiology parameters for transplant groups compare to native groups through time in 2014RTE. No statistics were performed between TWO and NWW on January 2015 due to insufficient sample number (n=1) for TWO causing by mortality. [a]: Data were Box-cox transformed. [b]: Wilcoxon rank sum test was performed since data failed to meet homogeneity of variance even after transformation. †: All the samples were dead. Abbreviations were described in abbreviation page.
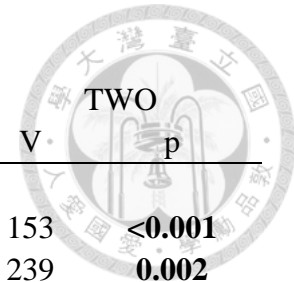
parameters	V	p
Photochemical efficiency (Fv/Fm)		
April vs July	153	**<0.001**
April vs September	239	**0.002**
April vs November	228	**0.027**
July vs September	22	0.365
July vs November	1	**<0.001**
September vs November	65	**0.025**

A9　Paired t-test results of photochemical efficiency in 2015RTE. P-values in boldface highlighting the significant differences between time. Dash lines represent no statistics were performed due to high mortality.

parameters	2015Apr		July		Sep		Nov	
Photochemical efficiency	W	p	W	p	W	p	W	p
TWO vs. NOO	105	**<0.001**	436	**<0.001**	505	**<0.01**	566	**<0.001**
TOW vs. NOO	633.00	**0.007**	154.5	**<0.001**	448.00	0.982	727.50	**<0.001**
TWO vs. NWW	523.50	0.280	344.5	**0.013**	622.00	**<0.001**	545.00	**<0.001**

A10 Wilcoxon rank sum test result of photochemical efficiency through time in 2015RTE. Abbreviations were described in abbreviation page.

(a)

	df	SS	MS	F	p
Group	4	8.05	2.0124	10.66	**0.00011**
Residuals	19	3.585	0.1887		

(b)

	NOO	TOW	TOI	NWW	TWO	NII	TIO
NOO		**0.001625**	1	1	NA	0.075209	NA
TOW	**0.001625**		**0.0004**	**0.00183**	NA	1	NA
TOI	1	**0.000436**		1	NA	**0.02001**	NA
NWW	1	**0.001829**	1		NA	0.06813	NA
TWO	NA	NA	NA	NA		NA	NA
NII	0.075209	1	**0.02**	0.06813	NA		NA
TIO	NA	NA	NA	NA	NA	NA	

A11 (a) One-way ANOVA table and (b) Tukey's post hoc test table for tissue coverage growth in 2014RTE and Bonferroni corrected p-values were reported. NA represent no statistic were performed due to lack of sample number causing by mortality. Abbreviations were described in abbreviation page.

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(a)

	df	SS	MS	F	p
Group	4	10.962	2.7405	5.742	**0.00333**
Residuals	19	9.068	0.4772		

(b)

	NOO	TOW	TOI	NWW	TWO	NII	TIO
NOO		1	1	1	NA	**0.03383**	NA
TOW	1		1	0.40678	NA	0.41072	NA
TOI	1	1		1	NA	**0.01772**	NA
NWW	1	0.40678	1		NA	**0.00421**	NA
TWO	NA	NA	NA	NA		NA	NA
NII	**0.03383**	0.41072	**0.01772**	**0.00421**	NA		NA
TIO	NA	NA	NA	NA	NA	NA	

A12 (a) One-way ANOVA and (b) Tukey's post hoc test results for skeleton growth in 2014RTE and Bonferroni corrected p-values were reported. NA represent no statistic were performed due to lack of sample number causing by mortality. Abbreviations were described in abbreviation page.

(a)

	df	SS	MS	F	p
Group	3	38.91	12.971	43.51	**<0.001**
Residuals	109	32.49	0.298		

(b)

	NOO	TOW	NWW	TWO
NOO		**<0.001**	1	**<0.001**
TOW	**<0.001**		**<0.001**	**<0.001**
NWW	1	**<0.001**		**<0.001**
TWO	**<0.001**	**<0.001**	**<0.001**	

A13 (a) One-way ANOVA and (b) Tukey's post hoc test results for tissue coverage growth in 2015RTE and Bonferroni corrected p-values were reported. Abbreviations were described in abbreviation page.

	df	SS	MS	F	p
origin	1	18.53	18.531	62.163	**<0.001**
location	1	20.18	20.176	67.681	**<0.001**
origin × location	1	0.21	0.207	0.695	0.406
Residuals	109	32.49	0.298		
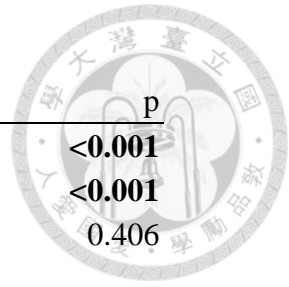
A14 Two-way ANOVA result of origin versus the location effect of tissue coverage growth of *P. verweyi* in 2015RTE between NPP-OL and WLT. The p-values in boldface represent a significant difference in the factor.