

Temporal and Spatial Variations in Symbiont Communities of Catch Bowl Coral *Isopora palifera* (Scleractinia: Acroporidae) on Reefs in Kenting National Park, Taiwan

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Chia-Min Hsu, Shashank Keshavmurthy, Vianney Denis, Chao-Yang Kuo, Jih-Terng Wang, Pei-Jie Meng, and Chaolun Allen Chen (2012) Temporal and spatial variations in symbiont communities of the catch bowl coral *Isopora palifera* (Scleractinia: Acroporidae) on reefs in Kenting National Park, Taiwan. *Zoological Studies* 51(8): 1343-1353. Acclimatization through *Symbiodinium* shuffling is one of potential mechanisms in reef-building corals to survive environmental stress. In our previous study, the catch bowl coral *Isopora palifera* in Tantzei Bay (TZB), Nanwan, Kenting National Park (KNP), southern Taiwan was demonstrated to shuffle thermal-tolerant *Symbiodinium* D1a and thermal-sensitive *Symbiodinium* C3 in response to seasonal variations in sea surface temperatures (SSTs) in 2000 and 2001. In this study, we reexamined the temporal dynamics of the *Symbiodinium* community of *I. palifera* in TZB in 2006-2009. In addition, spatial variations in *Symbiodinium* communities in *I. palifera* were also examined at 6 other sites of Nanwan, KNP in 2009, including a site located at a nuclear power plant outlet (NPP-OL) in southern Taiwan with a yearly mean SST 0.6-1.5°C higher compared to the other sites. Phylotyping and DNA sequence analyses of *Symbiodinium* ribosomal 28S and ITS2 markers showed that *I. palifera* colonies at TZB continued to show seasonal shuffling, but shifted to thermal-sensitive type C3 dominant in 2006-2009. This differed from the symbiont community originally dominated by the thermal-tolerant *Symbiodinium* D1a in 2000 and 2001 after the 1998 mass-bleaching event. Significant differences in spatial variations of the symbiont community in Nanwan were detected with *I. palifera* colonies at the NPP-OL dominated by *Symbiodinium* D1a. Our study results suggest that *I. palifera* can acclimatize to SST anomalies by shuffling to thermal-tolerant *Symbiodinium* D1a and can revert to thermal-sensitive C3 when the stress disappears, but will maintain the thermally tolerant *Symbiodinium* D1a as the dominant symbiont if the heat stress continues. <http://zoolstud.sinica.edu.tw/Journals/51.8/1343.pdf>

Key words: *Isopora palifera*, Symbiont shuffling, Temporal and spatial variations, Thermal tolerance, Acclimatization.

Reef-building corals and their endosymbiotic algae of the genus *Symbiodinium* suffer from a variety of stressors responsible for the worldwide decline in coral reefs (reviewed in Hughes et al.

2003, Bellwood et al. 2004, Hoegh-Guldberg et al. 2007). Elevated sea surface temperatures (SSTs) are one of the major stressors causing the breakdown of symbiosis between coral hosts

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and their symbionts resulting in coral bleaching (Wilkinson 1998, Hoegh-Guldberg 1999). Projections of rising SSTs for the 21st century according the different scenarios as a result of environmental change (IPCC 2007), that may result in increased frequency and severity of coral bleaching in the coming decades, may lead to the worldwide collapse of coral reef ecosystems (Hoegh-Guldberg et al. 2007).

There are at least 4 potential mechanisms for corals to respond to rising SSTs due to climate change: acclimatization, adaptation, migration, and extinction (reviewed in Buddemeir and Fautin 1993, Hoegh-Guldberg 1999, Hoegh-Guldberg et al. 2007 2008, Edmunds and Gates 2008, also see Kesahvmurthy et al. 2012). Among them, acclimatization through hosting or shuffling heat-tolerant *Symbiodinium* types has been documented in reef-building corals (Table 1) (Baker 2001, Baker et al. 2004, Berkelmans and van Oppen 2006, Thornhill et al. 2006a, Jones et al. 2008). With the aid of molecular genetic markers, 9 *Symbiodinium* “clades” (A-I) and numerous “types” have been confirmed in the last 2 decades (Rowan and Powers 1991, Rowan and Knowlton 1995, Pochon et al. 2004, Sampayo et al. 2008, Pochon and Gates 2010, LaJeunesse et al. 2010). Among diverse *Symbiodinium* types, clade D was proven to be heat-tolerant, whereas clade C is mostly heat-sensitive (Baker 2003a b, Baker et al. 2004, Ulstrup and van Oppen 2003, Fabricius et al. 2004, Little et al. 2004, Rowan 2004, Jones et

al. 2008, but see Tchernov et al. 2004, Sampayo et al. 2008). Corals hosting *Symbiodinium* clade D are usually found in reefs that experience higher SST anomalies compared to normal sites or other marginal reef habitats (Lien et al. 2007, Mostafavi et al. 2007, LaJeunesse et al. 2010, Oliver and Palumbi 2011a). Clade D-associated corals also showed higher survival rates under heat-treatment experiments (Edmunds 2005, Fitt et al. 2009, Oliver and Palumbi 2011b, Putnam and Edmunds 2011, Bellantuono et al. 2012). Nevertheless, multiple-year monitoring of *Symbiodinium* community dynamics shows that coral hosts tend to revert to clade C as the dominant symbiont by shuffling after the heat stress is removed (Thornhill et al. 2006a), suggesting that there is a negative effect on corals hosting only *Symbiodinium* clade D as the dominant symbiont. This scenario is supported by the monitoring of growth rates in coral juveniles infected with *Symbiodinium* clades C and D, in which corals infected with clade D showed significantly lower growth rates and lower photosynthetic product transportation compared to those infected with *Symbiodinium* clade C (Little et al. 2004, Abrego et al. 2008, Cantin et al. 2009, Jones et al. 2010).

Surveys of *Symbiodinium* diversity in Taiwan have shown that *Symbiodinium* clade C is the dominant symbiont associated with reef-building corals, while *Symbiodinium* clade D is associated with corals that inhabit shallow waters and reef edges in deep water in Kenting National Park

Table 1. Review and definitions of potential responses of corals to climate change

Response	Definition and mechanism	References
Acclimatization	Reversible phenotypic increase of stress tolerance in a natural environment without genetic changes, e.g., <i>Symbiodinium</i> shuffling to thermal-tolerant communities when facing a warmer environment	Coles and Brown 2003, Edmund and Gates 2008, Jones et al. 2008
Adaptation	Genotypic response under natural selection acting on biological units, e.g., genetic change of a coral host or its symbiotic partner when facing thermal stress	Coles and Brown 2003
Migration	Pole-ward distribution expansion beyond original historical range of species, e.g., dispersion of warm-adapted <i>Acropora</i> species from low latitudes to historically colder high-latitude areas	Hoegh-Guldberg et al. 2008, Baird et al. 2012
Extinction	Inability to disperse or adapt to the pace of climate change and facing a high extinction risk, e.g., endangered <i>Acropora</i> species in the Caribbean	Precht et al. 2002, Hoegh-Guldberg et al. 2008

(KNP) and the Penghu I. (Chen et al. 2005b). *Isopora palifera* is one of the scleractinian corals distributed in shallow water (< 3 m) of fringing reefs in Nanwan, KNP (Fig. 1). A population at Tantzei Bay (TZB) hosted both *Symbiodinium* C and D, either solely or simultaneously (Chen et al. 2005a b). Bimonthly sampling from Jan. 2000 to July 2001, 1.5 yr after the 1998 mass bleaching in Nanwan (Fujiwara et al. 2000), demonstrated that the symbiont community within *I. palifera* at TZB was highly dynamic with significant fluctuations; a drop in *Symbiodinium* clade D percentage occurred in May, which was correlated with a rise in SSTs in the late spring/early summer at the sampled reef (Chen et al. 2005a), suggesting that *I. palifera* has the potential to acclimatize by shuffling its symbiont community in response to thermal stress.

In this study, we reexamined the temporal dynamics of the *Symbiodinium* community of *I. palifera* in TZB in 2006-2009. In addition, spatial variations in *Symbiodinium* communities were also examined in *I. palifera* at 6 other sites in Nanwan in 2009, including a site located at the Third Nuclear Power Plant outlet (NPP-OL) in southern

Taiwan. The 3rd NPP was constructed in the late 1970s and began to operate in 1984. Historical data show that the NPP-OL had an average SST during summer 2.0-3.0°C higher than that at other coral reef sites in KNP (Fan 1991, Peir 2011, Keshavmurthy et al. 2012). The hot water at the NPP-OL is trapped and flows southwestward in Nanwan because of the near-shore current and tides (Chiou et al. 1993). The hot water released in this region has impacted the marine ecology within the area of dispersal (Chiou et al. 1993, Huang et al. 1998, Jan et al. 2001, Hwang et al. 2004). Bleaching of corals located at the NPP-OL caused by the outflow of thermally polluted water has been reported in the last 2 decades, including the most severe episode in 1998 (Wilkinson 1998). By combining temporal and spatial examinations of symbiont communities, results suggested that *I. palifera* can acclimatize to SST anomalies by shuffling to thermal-tolerant *Symbiodinium* types, and then revert to thermal-sensitive types when the stress disappears, but maintains thermal-tolerant *Symbiodinium* as the dominant symbiont in areas with a continuing presence of heat stress.

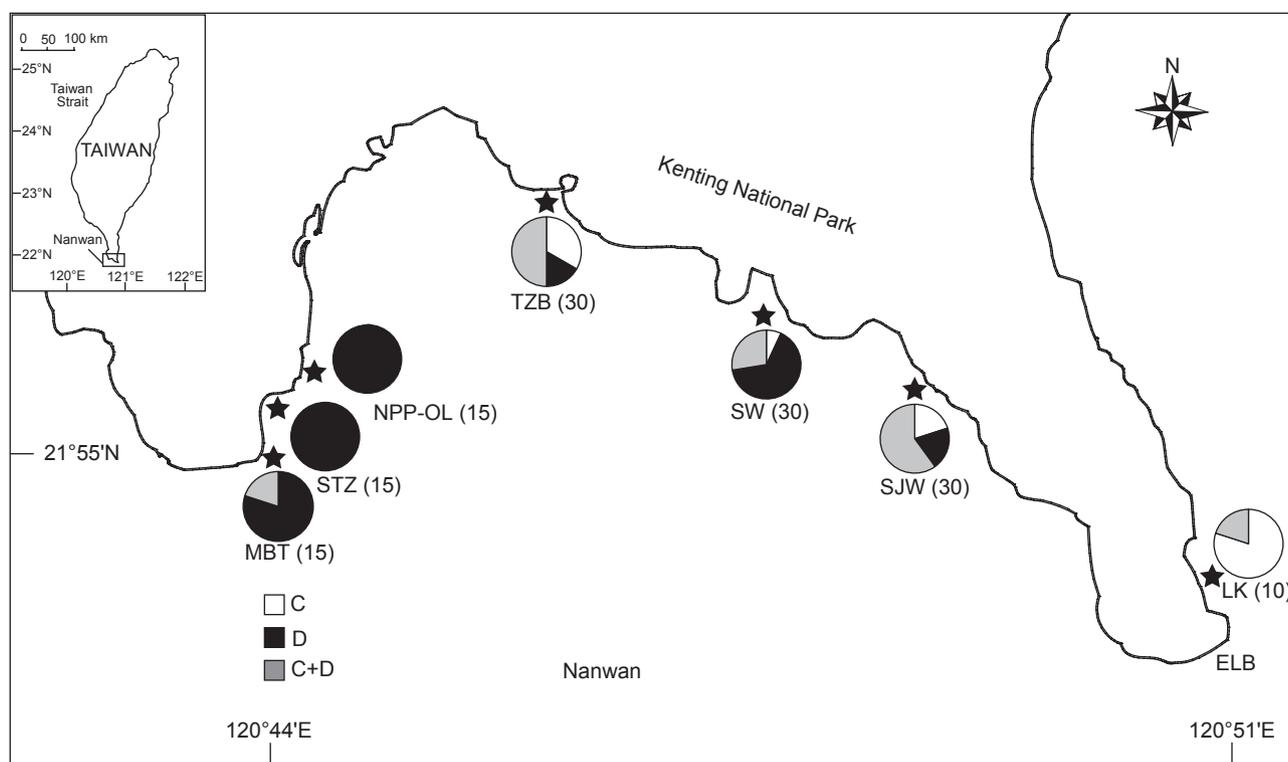


Fig. 1. *Symbiodinium* clades in *Isopora palifera* colonies at the study sites. Numbers of colonies sampled at each site are indicated in brackets. MBT, Maobitou; STZ, Siatanzai; NPP-OL, outlet of the nuclear power plant; TZB, Tantzei Bay; SW, Shiaowan; SJW, Shinjaowan; LK, Longken.

MATERIALS AND METHODS

Study sites, seawater surface temperatures, and sampling

Nanwan is a bay located in the central coast of KNP at the southern tip of Taiwan (Fig. 1). Two capes, Maobitou (MBT) and Eluanbi (ELB), form the borders of this bay, while the coast is lined by well-developed fringing reefs. In the sheltered area of this bay, a nuclear power plant was built in 1979 and began operation in 1984. The warm water generated by its cooling system is released into a small bay, the NPP-OL, and chronically affects the adjacent marine communities (Chiou et al. 1993, Huang et al. 1998, Jan et al. 2001, Hwang et al. 2004, Meng et al. 2008, Keshavmurthy et al. 2012). From 2006 to 2009, seawater temperatures at the NPP-OL and 4 other sites around this bay (Fig. 1), Tantzei Bay (TZB), Shiaowan (SW), Shinjaowan (SJW), and Longken (LK), were monitored at 60-min intervals using *in situ* temperature loggers (Hobo Pendant, Onset Corp., Cape Cod, Massachusetts, USA, with a precision of $\pm 0.5^\circ\text{C}$). Data from Chen et al. (2005a) were used to characterize temporal SSTs in 2000–2001. Due to exposure of shallow reefs to strong waves caused by the seasonal monsoons at some sites, it was not possible to set temperature loggers at the same depth at all sites. All samples were collected from *I. palifera* colonies living in shallow waters (< 3 m). For temporal variations in the *Symbiodinium* community, 32 colonies were tagged and sampled using 2-cm-diameter cores with a pneumatic drill connected to a regulator on a scuba diving tank bimonthly from Mar. 2006 to Nov. 2009 at TZB as described in Chen et al. (2005a). To investigate spatial variations in symbiont communities, 145 colonies were randomly sampled at 6 sites: MBT, Siatanzai (STZ), NPP-OL, SW, SJW, and LK in 2009 (Fig. 1). All samples were kept in 80% ethanol (EtOH) before the molecular genetic analyses.

Molecular phylotyping of *Symbiodinium* spp.

Symbiodinium was identified by analyzing the large subunit of ribosomal (18S) DNA modified from methods described by Chen et al. (2005a). Briefly, *Symbiodinium* DNA was extracted using a Viogene® Plant DNA extraction kit (Taipei, Taiwan). *Symbiodinium* clades were identified by a restriction fragment length polymorphism (RFLP) analysis by amplifying

28S ribosomal DNA with primers 28S zoox-D1/D2F (5'-CCTCAGTAATGGCGAATGAACA-3') and 28S zoox-D1/D2R (5'-CCTTGGTCCGTGTTTCAAGA-3') (Loi 1998) and using *Rsa* I as the restriction enzyme. Subsamples of known clades were then selected and checked for *Symbiodinium* type identification using denaturing gradient gel electrophoresis (DGGE) of the internal transcribed spacer 2 (ITS2) region (LaJeunesse 2002). Predominant bands were excised, dissolved in distilled water, sequenced after DNA amplification. Then *Symbiodinium* spp. was identified by BLASTn with sequences from the National Center for Biotechnology Information (NCBI) database. Data were compared with results in Chen et al. (2005a) in 2000–2001 to analyze temporal variations in the *Symbiodinium* community.

Statistical analysis

SSTs at the different sites were characterized by daily averages, daily fluctuations, and daily maximum/minimum values. Daily averages and fluctuations were calculated from Jan. 2007 to Jan. 2008 at the different monitoring sites and compared using a one-way analysis of variance (ANOVA) followed by Tukey post-hoc test. Temporal and spatial differences in the symbiont community were examined using Pearson Chi-squared test. Except when precise, data are presented as the mean \pm standard deviation (S.D.). All statistical analyses related to temperature and the symbiont community were performed using R (version 2.14.2) statistical software.

RESULTS

Seawater temperatures

Data loggers deposited at different sites in Nanwan of KNP recorded the maximum daily average SST of $32.2 \pm 1.1^\circ\text{C}$ at the NPP-OL on 26 July 2007 (Fig. 2). The daily average temperature at the NPP-OL was $> 30^\circ\text{C}$ from late June to early Aug. except for temperature drops due to disturbance by typhoons. Monthly means of daily averages ranged 25.5 ± 0.9 – $30.6 \pm 1.5^\circ\text{C}$ at the NPP-OL, 23.3 ± 0.6 – $30.0 \pm 1.2^\circ\text{C}$ at TZB, 24.4 ± 0.8 – $29.4 \pm 1.0^\circ\text{C}$ at SJW, and 24.5 ± 0.6 – $27.8 \pm 1.0^\circ\text{C}$ at LK (Fig. 2A–F). Differences between the warmest and coolest months in 2007 reached 6.7°C at TZB, 5.1°C at the NPP-OL, 5.0°C at SJW, and 3.3°C at LK (Fig. 2G–L). Mean daily temperature

fluctuations significantly differed among sites (one-way ANOVA, $F = 118.03$, $p < 0.05$), ranging between $2.73 \pm 1.23^\circ\text{C}$ at the NPP-OL and $0.92 \pm 0.43^\circ\text{C}$ at LK. Overall, the yearly mean SST at the NPP-OL was $0.6\text{-}1.5^\circ\text{C}$ higher compared to other sites in Nanwan during our study period (Fig. 2).

Temporal variations in the symbiont community in *Isopora palifera* at TZB

Isopora palifera collected in 2006-2009 at TZB was consistently associated with *Symbiodinium* clades C and D (Figs. 3, 4) compared to the same

population studied in 2000-2001 (6-9 yr ago) (Chen et al. 2005a). The ITS2-DGGE analysis further respectively identified the *Symbiodinium* clades to C3 and D1a type levels (*Symbiodinium trenchi*) (Fig. 3B, D). Due to the high resolution of ITS2-DGGE when compared to 28s rDNA-RFLP, it was possible to detect the presence of type C3 in coral samples hosting the dominant *Symbiodinium* clade D (Fig. 3), and the presence of heteroduplexes (Het in Fig. 3B, D) also confirmed the evidence of background type C3 in the ITS2-DGGEs fingerprint. No other *Symbiodinium* type was detected using ITS2-DGGE in any of the temporal

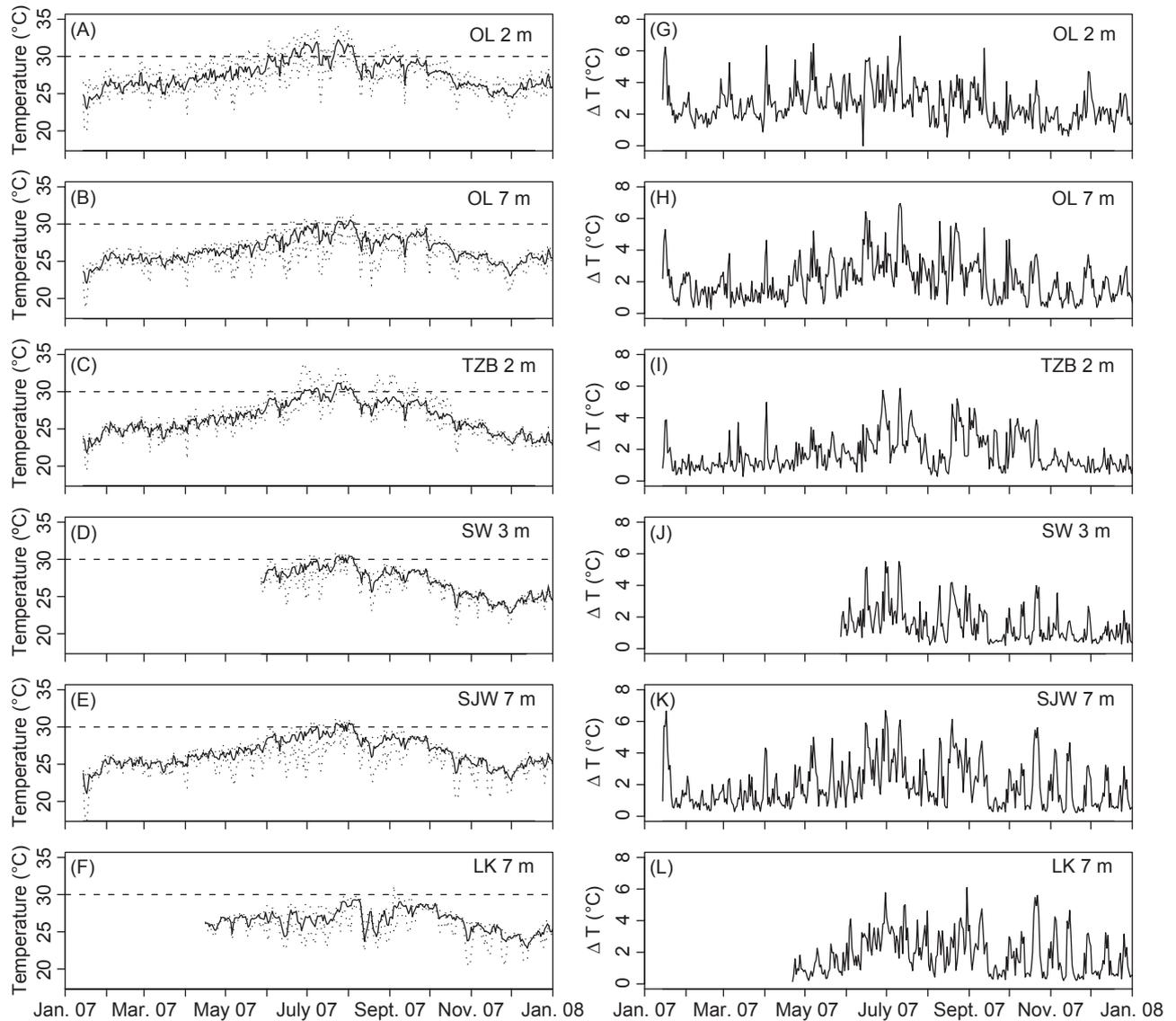


Fig. 2. Sea surface temperatures at the study sites from Jan. 2007 to Jan. 2008. (A-F) Daily mean seawater temperatures (continuous line) with maximum and minimum values (dotted lines); (G-L) daily temperature fluctuations (ΔT , daily maximum - daily minimum). For abbreviations see figure 1.

and spatial samples used in this study; thus, 28s rDNA-RFLP was applied to all samples to estimate symbiont compositions.

Bimonthly sampling showed that 33%-80% of *Symbiodinium* D1a dominated *I. palifera* colonies in 2000-2001 (Fig. 4A). In contrast, there were only 0%-12% of D1a-dominated colonies in 2006-2007 (Fig. 4B), and 0%-14% of D1a-dominated colonies in 2008-2009 (Fig. 4C).

The symbiont community of *I. palifera* significantly differed among bimonthly samples in 2006-2007 and 2008-2009 (Fig. 4B, C). The symbiont community was dominated by type C3, ranging from 32% in Mar. 2006 to 70% in

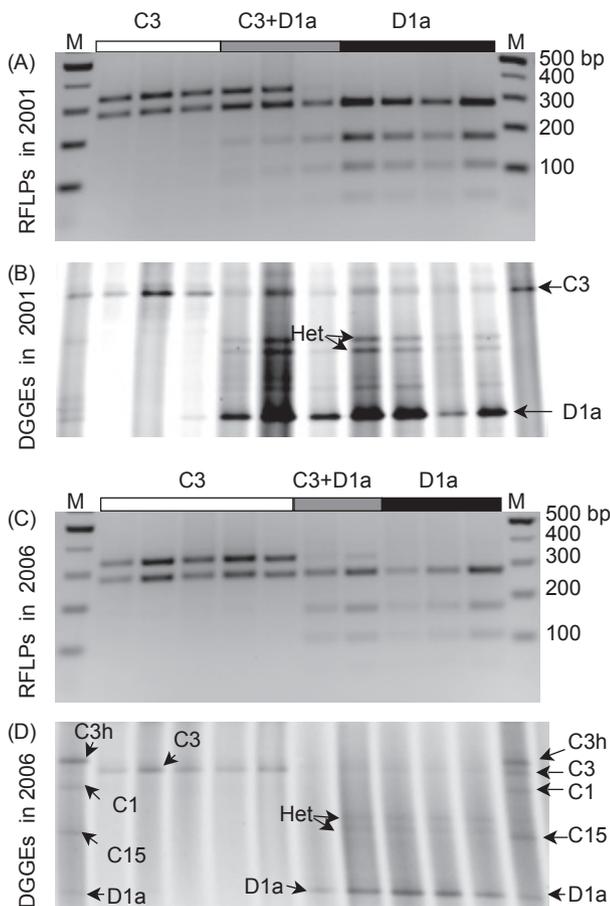


Fig. 3. *Symbiodinium* phylotyping of *Isopora palifera* using 28s rDNA-RFLP and ITS2-DGGE fingerprint analysis during 2 sampling periods. Samples used were the same for both techniques. (A) RFLP in 2001; (B) DGGE in 2001; (C) RFLP in 2006; (D) DGGE in 2006. Letters indicate different clades and types of *Symbiodinium*. A 100-bp ladder marker was used for the RFLP analysis, and PCR products of ITS2 from *Symbiodinium* DNA of C3h, C3, C1, C15, and D1a were pooled and run as a reference marker (M) for DGGE. Heteroduplexes in the DGGE gel are indicated by "Het".

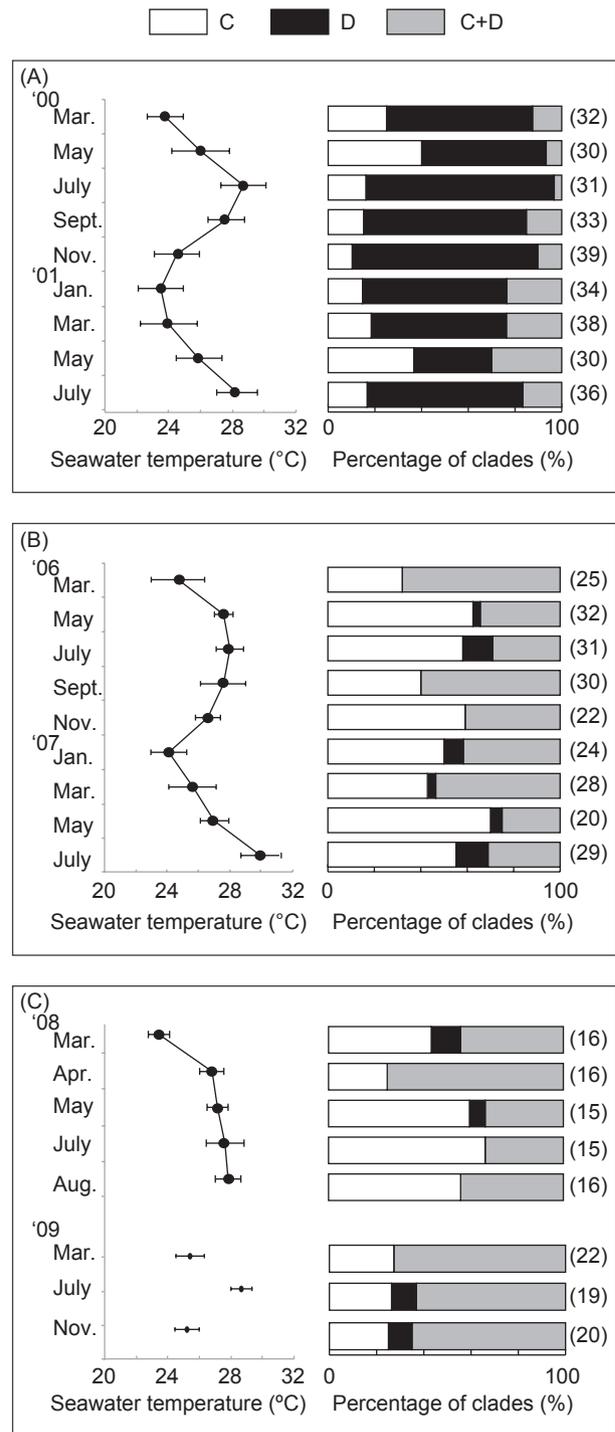


Fig. 4. Sea surface temperatures (mean \pm S.D., left) at Tantzei Bay and compositions of *Symbiodinium* clades (right) in *Isopora palifera* for the periods (A) 2000-2001 (data derived and modified from Chen et al. 2005a); (B) 2006-2007, and (C) 2008-2009. Numbers in brackets indicate the number of colonies sampled each month.

May 2007, and a mixture of types C3 and D1a, ranging from 68% in Mar. 2006 to 25% in May 2007 (Pearson Chi-square test, $\chi^2 = 8.73$, $d.f. = 2$, $p < 0.05$). Colonies with type D1a occurred in relatively low percentages, ranging from 0% in Mar. 2006 to 12% in July 2007 (Pearson Chi-square test, $\chi^2 = 8.88$, $d.f. = 2$, $p < 0.05$) (Fig. 4B). In 2008-2009, type C3 ranged 25% in Apr. 2008 to 66% in July 2008, and the mixture of types C3 and D1a ranged from 34% in July 2008 to 75% in Apr. 2008 (Pearson Chi-square test, $\chi^2 = 5.43$, $d.f. = 1$, $p < 0.05$). In 2009, type C3 was 27% and the mixture of types C3 and D1a was 73% in Mar. Type D1a in both 2008 and 2009 ranged 0%-14% (Fig. 4C).

Spatial variations of *Symbiodinium* clades associated with *Isopora palifera* at Nanwan

The *Symbiodinium* community in *I. palifera* showed significant differences among sites in reefs of KNP (Pearson Chi-square test, $\chi^2 = 93.46$, $d.f. = 12$, $p < 0.001$, Fig. 1). At LK, 80% of *I. palifera* colonies surveyed were dominantly associated with type C3 *Symbiodinium*, and 20% were associated with a mixture of C3 and D1a. At SJW, SW, and TZB, 60%, 26.7%, and 51.7% of sampled *I. palifera* colonies respectively hosted a mixture of types C3 and D1a. Colonies hosting the dominant D1a represented 16.6% of *I. palifera* colonies sampled at TZB, 20% at SJW, and 63.3% at SW. D1a was the dominant type identified in all colonies collected from the NPP-OL and STZ. The percentage of *I. palifera* associated with the mixture of C3 and D1a increased to 20% at the MBT, at the southwestern end of Nanwan (Fig. 1).

DISCUSSION

The data collected in this study clearly showed that (i) reef-building corals, such as *Isopora palifera* that harbors 2 *Symbiodinium* clades or types, can continually shuffle their symbiont communities through time regardless of the thermal tolerability of the *Symbiodinium* types; (ii) there were significant temporal variations in the association of thermal-sensitive and -tolerant *Symbiodinium* between years right after the 1998 mass bleaching and years without severe coral bleaching; and (iii) significant spatial variations in *I. palifera* symbiont communities may be related to different thermal histories at different sites of Nanwan, KNP.

Long-term stabilization and shuffling of the symbiont community in *I. palifera*

Our long-term monitoring data indicate stable associations of *Symbiodinium* C3 and D1a types in *I. palifera*. With application of advanced molecular techniques to detect background symbionts (Mieog et al. 2007, Silverstein et al. 2012), there is a consensus that corals can host multiple *Symbiodinium* clades or types; therefore, the potential for reef corals to adapt or acclimatize to environmental changes via symbiont community shifts may be more phylogenetically widespread. However, only a handful species of reef corals examined for their long-term response to stresses have shown distinct shuffling (Table 2) (Toller et al. 2001, Baker et al. 2004, Chen et al. 2005a, Thornhill et al. 2006a, Jones et al. 2008). For example, *Acropora millepora* in the Keppel Is., Australia shuffled its symbiont community from thermal-sensitive ITS1 type C2 (same as ITS2 C3, sensu LaJeunesse et al. (2003) and van Oppen et al. (2001)) to D or C1 after a bleaching event in 2006, suggesting that a change in the symbiont community structure, while it persisted, was likely to have substantially increased the thermal tolerance of this coral population (Jones et al. 2008). In the Caribbean, *Montastrea annularis* and *M. franksi* from the Florida Keys exhibited shifts in their associations which were attributed to recovery from the stress of a 1998 bleaching event (Thornhill et al. 2006a). In contrast, *A. cervicornis*, *A. palmata*, and *Siderastrea* spp. examined using repetitive sampling of labeled colonies from the Bahamas and Florida Keys showed little to no changes in their dominant symbionts even under the thermal effect of the 1998 mass bleaching. In *I. palifera* at the TZB, symbiont shuffling is a dynamic response to seasonal temperature fluctuations without the effect of natural coral bleaching events (Chen et al. 2005a). Thus, even if background symbionts might be detected by high-resolution molecular techniques, corals do not always need to shuffle their background symbionts in response to stresses of environmental change.

Shifts of dominant *Symbiodinium* and post-bleaching reversion in *I. palifera*

The dramatic shift of dominant symbionts in *I. palifera* from *Symbiodinium* D1a in 2000-2001 to C3 in 2006-2009 supports a scenario of post-bleaching reversion (Thornhill et al. 2006a). A 5-year monitoring of 3 *Montastrea* species, *M. annularis*,

M. franksi, and *M. faveolata*, in Florida showed changes in their symbiont compositions, and most notable was the disappearance of *Symbiodinium* D1a. This disappearance took place over a number of years after the 1998 mass bleaching event before stabilizing to the most common symbionts in the area; whereas another study on the same *Montastrea* spp. using experimentally induced bleaching showed no significant return to the original symbiont community after 9 mo (Toller et al. 2001). Although pre-1998 bleaching samples of *I. palifera* were not available in this study, our monitoring data in 2006–2009 suggested the similar disappearance of D1a as seen in *Montastrea* species. The post-bleaching reversion from *Symbiodinium* D1a to C3 also supported the consensus (reviewed in Stat and Gate 2011) that in the short term, corals associated with clade D benefit from higher thermal tolerance and

resistance to bleaching, but in the long term, they are negatively affected with respect to growth rates (Little et al. 2004, Jones et al. 2010) and transportation of photosynthetic products (Cantin et al. 2009) compared to conspecifics harboring clade C. Further investigations are underway to examine the life histories and physiological performances of *I. palifera* hosting different symbiont communities.

Spatial variations in the symbiont diversity of *I. palifera* and local thermal histories

Contrasting spatial variations in symbiont communities were probably observed in *I. palifera* due to exposure of coral holobionts to different thermal histories at different sites of Nanwan. *Isopora palifera* associated with the dominant *Symbiodinium* D1a occurred at sites with higher SST fluctuations (such as the NPP-OL and STZ),

Table 2. Review of cnidarian species that can and cannot “shuffle” temporally

Host species	<i>Symbiodinium</i> type	Study site	Shuffle	Reference
<i>Stephanocoenia intersepta</i> , <i>Porites astreoides</i> , <i>Diploria labyrinthiformis</i> , <i>Siderastrea siderea</i>	Clades A, B, C, D	San Blas Archipelago, Panama	Yes	Baker 2001
<i>Colpophyllia natans</i> , <i>Montastraea cavernosa</i>	Clades B, C	San Blas Archipelago, Panama	No	Baker 2001
<i>Montastraea annularis</i>	Clades A, B, C, D	San Blas Archipelago, Panama	Yes	Toller et al. 2001
<i>Oulastrea crispata</i>	Clade D	Penghu, Taiwan	No	Chen et al. 2003
<i>Plexaura kuna</i>	Clade B	San Blas Is., Panama	No	Goulet and Coffroth 2003
<i>Alveopora japonica</i> , <i>Heteractis</i> sp.	Clades C, F	Jeju I., Korea	No	Rodriguez-Lanetty et al. 2003
<i>Pocillopora verrucosa</i> , <i>Pavona gigantea</i>	C1c, D1 (ITS2)	La Gaviota I., Mexico	No	Iglesias-Prieto et al. 2004
<i>Isopora palifera</i>	Clades C, D	Kenting, Taiwan	Yes	Chen et al. 2005a
<i>Gorgonia ventalina</i>	Clade B	Florida Keys, USA	No	Kirk et al. 2005
<i>Acropora millepora</i>	C2, C2*, D (ITS1)	Great Barrier Reef, Australia	Yes	Berkelmans and van Oppen 2006
<i>Acropora palmata</i> , <i>Siderastrea siderea</i>	A3, C3 (ITS2)	Bahamas, Florida Keys, USA	No	Thornhill et al. 2006a
<i>Montastrea faveolata</i> , <i>M. annularis</i> , <i>M. franksi</i>	B1, B10, C3, C12, D1a (ITS2)	Florida Keys, USA	Yes	Thornhill et al. 2006a
<i>Agaricia agaricites</i> , <i>Porites astreoides</i> , <i>Siderastrea radicans</i>	A4a, B5, C3a (ITS2)	Bahamas, Florida Keys, USA	No	Thornhill et al. 2006b
<i>Zoanthus sansibaricus</i>	C1/C3-related (full-ITS)	Kagoshima Bay, Japan	No	Reimer et al. 2007
<i>Pavona decussata</i>	Clade C	Okinawa, Japan	No	Suwa et al. 2008
<i>Pavona divaricata</i>	Clades C, D	Okinawa, Japan	Yes	Suwa et al. 2008
<i>Condylactis gigantea</i>	Clades A, B	Bermuda	Yes	Venn et al. 2008
<i>Acropora millepora</i>	C1, C2, D, D* (ITS1)	Keppel Is., Australia	Yes	Jones et al. 2008
<i>Stylophora pistillata</i>	C8/a, C35/a, C78, C79 (ITS2)	Heron Is., Australia	Yes	Sampayo et al. 2008
<i>Acropora millepora</i> , <i>A. palifera</i> , <i>Favites abdita</i> , <i>Goniastrea favulus</i> , <i>Lobophyllia corymbosa</i> , <i>Montipora digitata</i> , <i>Pocillopora damicornis</i> , <i>Porites cylindrical</i> , <i>Seriatopora hystrix</i> , <i>Stylophora pistillata</i>	C3, C3n-t, C8/C8a/C79, C15, C21, C33, C42a-b, C78, D1, D1a-c (ITS2)	One Tree and Heron I., Australia	No	Stat et al. 2009
<i>Montastrea annularis</i>	A13, B1j, C3, C7-a, D1a (ITS2)	Barbados (Caribbean)	Yes	LaJeunesse et al. 2009
<i>Acropora aspera</i> , <i>A. formosa</i> , <i>A. millepora</i> , <i>Platygyra daedalea</i> , <i>Porites lutea</i> , <i>Porites</i> <i>cylindrica</i> , <i>Montipora digitata</i>	C3, C15 (ITS2)	Heron I., Australia	No	Fisher et al. 2012
<i>Acropora millepora</i>	C3 (ITS2)	Heron I., Australia	No	Bellantuono et al. 2012

and *Symbiodinium* C3 was dominant at sites with lower SST fluctuations (such as LK). *Isopora palifera* colonies associated with the mixture of C3 and D1a were found at sites with moderate fluctuations in SSTs. *Platygyra verweyi*, the other shallow-water coral species monitored at different sites of Nanwan, also showed an exclusively high incidence of *Symbiodinium* D1a at the NPP-OL due to high seawater temperatures and increased C3 dominance at other sites where seawater temperatures were lower (Keshavmurthy et al. 2012). Similar patterns of spatial variations in the symbiont community within a small geographic scale in response to different SST fluctuations were also documented in Pacific reefs (Fabricius et al. 2004, Oliver and Palumbi 2009 2011a). Thirteen non-poritid and 2 *Porites* species in a 0.5°C warmer lake in Palau associated with only *Symbiodinium* D showed no bleaching mortality during 1998, compared to their counterparts outside the lake (Fabricius et al. 2004). Eight of 9 coral species in a 1.5°C hotter lagoon hosted thermal-tolerant *Symbiodinium* D on the back reef of Ofu I., American Samoa (Oliver and Palumbi 2009 2011a). All of those studies suggested that environmentally variable microhabitats with distinct local histories of thermal variability might play a significant role in determining dominance of heat-tolerant types in the symbiont community, consequently increasing the coral-algal thermal tolerability.

The NPP-OL as a candidate site to study acclimatization and adaptation of reef corals under impacts of environmental change

Both this study on *I. palifera* and a previous study on *P. verweyi* (Keshavmurthy et al. 2012) in reefs of Nanwan showed that these 2 main reef-building corals responded to high SST stress at the NPP-OL by nearly exclusively hosting the heat-tolerant *Symbiodinium* D1a. This suggests that long-term (> 26 yr) thermally polluted water from the NPP-OL might have been the driving force for coral holobionts and even the coral community in this small area acclimatizing and even adapting to impacts of environmental change. Genetic analysis of the *P. verweyi* host showed no genetic differentiation between the NPP-OL and other sites. Thus, changes in the composition of *Symbiodinium* types among closely located sites in KNP suggest that *P. verweyi* might have acclimatized to the constant thermal stress by selective association with heat-tolerant

Symbiodinium types. However, the role of the host in adaptation was inconclusive (Keshavmurthy et al. 2012). Further studies examining host genetics of *I. palifera* and expanding to more coral taxa that occur at the NPP-OL and reference sites in Nanwan are underway to elucidate the phylogenetically widespread mechanisms in response to climate change.

In summary, temporal and spatial variations in symbiont communities were observed in *I. palifera* colonies by long-term monitoring (2000-2009) of the symbiont phylotype diversity on reefs in Nanwan, Kenting National Park, Taiwan. Our study results suggest that *I. palifera* acclimatizes to SST anomalies by shuffling to thermal-tolerant *Symbiodinium* and reverting to thermal-sensitive types over a period of several years as the stress disappears, but maintains thermal-tolerant *Symbiodinium* as the dominant symbiont if the heat stress continues.

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REFERENCES

- Abrego D, KE Ulstrup, BL Willis, MJH van Oppen. 2008. Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proc. R. Soc. Lond. B. Biol. Sci.* **275**: 2273-2282.
- Baird AH, B Sommer, JS Madin. 2012. Pole-ward range expansion of *Acropora* spp. along the east coast of Australia. *Coral Reefs* doi:10.1007/s00338-00012-00928-00336.
- Baker AC. 2001. Reef corals bleach to survive change. *Nature* **411**: 765-766.
- Baker AC. 2003a. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. *Annu. Rev. Ecol. Syst.* **34**: 661-689.
- Baker AC. 2003b. Symbiont diversity on coral reefs and its relationship to bleaching resistance and resilience. *In*

- E Rosenberg, Y Loya, eds. Coral health and disease. Berlin: Springer, pp. 177-191.
- Baker AC, CJ Starger, TR McClanahan, PW Glynn. 2004. Corals' adaptive response to climate change. *Nature* **430**: 741.
- Bellantuono AJ, O Hoegh-Guldberg, M Rodriguez-Lanetty. 2012. Resistance to thermal stress in corals without changes in symbiont composition. *Proc. R. Soc. Lond. B. Biol. Sci.* **279**: 1100-1107.
- Bellwood DR, TP Hughes, C Folke, M Nyström. 2004. Confronting the coral reef crisis. *Nature* **429**: 827-833.
- Berkelmans R, MJH van Oppen. 2006. 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. Lond. B. Biol. Sci.* **273**: 2305-2312.
- Buddemeier RW, DG Fautin. 1993. Coral bleaching as an adaptive mechanism: a testable hypothesis. *Bioscience* **43**: 320-326.
- Cantin NE, MJH van Oppen, BL Willis, JC Mieog, AP Negri. 2009. Juvenile corals can acquire more carbon from high-performance algal symbionts. *Coral Reefs* **28**: 405-414.
- Chen CA, KK Lam, Y Nakano, WS Tsai. 2003. 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.* **42**: 540-550.
- Chen CA, JT Wang, LS Fang, YW Yang. 2005a. Fluctuating algal symbiont communities in *Acropora palifera* (Scleractinia: Acroporidae) in Taiwan. *Mar. Ecol. Progr. Ser.* **295**: 113-121.
- Chen CA, YW Yang, NV Wei, WS Tsai, LS Fang. 2005b. Symbiont diversity in scleractinian corals from tropical reefs and subtropical non-reef communities in Taiwan. *Coral Reefs* **24**: 11-22.
- Chiou WD, LZ Cheng, HC Ou. 1993. 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. *J. Fish. Soc. Taiwan* **20**: 207-220.
- Coles SL, BE Brown. 2003. Coral bleaching - capacity for acclimatization and adaptation. *Adv. Mar. Biol.* **46**: 183-223.
- Edmunds PJ. 2005. The effect of sub-lethal increases in temperature on the growth and population trajectories of three scleractinian corals on the southern Great Barrier Reef. *Oecologia* **146**: 350-364.
- Edmunds PJ, RD Gates. 2008. Acclimatization in tropical reef corals. *Mar. Ecol. Progr. Ser.* **361**: 307-310.
- Fabricius KE, JC Mieog, PL Colin, D Idip, MJH van Oppen. 2004. Identity and diversity of coral endosymbionts (Zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. *Mol. Ecol.* **13**: 2445-2458.
- Fan KL. 1991. The thermal effluent problems of three nuclear power plants in Taiwan. *Elsevier Oceanogr. Ser.* **54**: 309-403.
- Fisher PL, MK Malme, S Dove. 2012. The effect of temperature stress on coral-*Symbiodinium* associations containing distinct symbiont types. *Coral Reefs* **31**: 473-485.
- Fitt KW, RD Gates, O Hoegh-Guldberg, JC Bythell, A Jatkar, AG Grottoli et al. 2009. Response of two species of Indo-Pacific corals, *Porites cylindrica* and *Stylophora pistillata*, to short-term thermal stress: The host does matter in determining the tolerance of corals to bleaching. *J. Exp. Mar. Biol. Ecol.* **373**: 102-110.
- Fujiwara S, ST Shibuno, K Mito, T Nakai, Y Sasaki, CF Dai, G Chen. 2000. Status of coral reefs of East and North Asia: China, Japan and Taiwan. In C Wilkinson, ed. Status of coral reefs of the world. Townsville, Australia: Australian Institute for Marine Science, pp. 131-140.
- Goulet TL, MA Coffroth. 2003. Stability of an octocoral-algal symbiosis over time and space. *Mar. Ecol. Progr. Ser.* **250**: 117-124.
- Hoegh-Guldberg O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshw. Res.* **50**: 839-866.
- Hoegh-Guldberg O, L Hughes, S McIntyre, DB Lindenmayer, C Parmesan, HP Possingham, CD Thomas. 2008. Assisted colonization and rapid climate change. *Science* **321**: 345-346.
- Hoegh-Guldberg O, PJ Mumby, AJ Hooten, RS Steneck, P Greenfield, E Gomez et al. 2007. Coral reefs under rapid climate change and ocean acidification. *Science* **318**: 1737-1742.
- Huang TC, CC Huang, KT Shao. 1998. Ecological survey of coastal water adjacent to nuclear power plants in Taiwan. *Chem. Ecol.* **15**: 129-142.
- Hughes TP, AH Baird, DR Bellwood, M Card, SR Connolly, C Folke et al. 2003. Climate change, human impacts, and the resilience of coral reefs. *Science* **301**: 929-933.
- Hwang RL, CC Tsai, TM Lee. 2004. Assessment of temperature and nutrient limitation on seasonal dynamics among species of *Sargassum* from a coral reef in southern Taiwan. *J. Phycol.* **40**: 463-473.
- Iglesias-Prieto R, VH Beltrán, TC LaJeunesse, H Reyes-Bonilla, PE Thomé. 2004. Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. *Proc. R. Soc. Lond. B. Biol. Sci.* **271**: 1757-1763.
- Jan RQ, JP Chen, CY Lin, KT Shao. 2001. Long-term monitoring of the coral reef fish communities around a nuclear power plant. *Aquat. Ecol.* **35**: 233-243.
- Jones A, R Berkelmans. 2010. Potential costs of acclimatization to warmer climate: growth of a reef corals with heat tolerant vs. sensitive symbiont types. *PLoS One* **5**: e10437.
- Jones AM, R Berkelmans, MJH van Oppen, JC Mieog, W Sinclair. 2008. A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proc. R. Soc. Lond. B. Biol. Sci.* **275**: 1359-1365.
- Keshavmurthy S, CM Hsu, CY Kuo, PJ Meng, JT Wang, CA Chen. 2012. Symbiont communities and host genetic structure of the brain coral *Platygyra verweyi*, at the outlet of a nuclear power plant and adjacent areas. *Mol. Ecol.* **21**: 4393-4407.
- Kirk NL, JR Ward, MA Coffroth. 2005. Stable *Symbiodinium* composition in the sea fan *Gorgonia ventalina* during temperature and disease stress. *Biol. Bull.* **209**: 227-234.
- LaJeunesse TC. 2002. Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Mar. Biol.* **141**: 387-400.
- LaJeunesse TC, WKW Loh, R van Woesik, O Hoegh-Guldberg, GW Schmidt, WK Fitt. 2003. Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. *Limnol. Oceanogr.* **48**: 2046-2054.
- LaJeunesse TC, RT Smith, J Finney, H Oxenford. 2009. Outbreak and persistence of opportunistic symbiotic dinoflagellates during the 2005 Caribbean mass coral 'bleaching' event. *Proc. R. Soc. Lond. B. Biol. Sci.* **276**:

- 4139-4148.
- LaJeunesse TC, DT Pettay, EM Sampayo, N Phongsuwan, B Brown, DO Obura et al. 2010. 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.* **37**: 785-800.
- Lien YT, Y Nakano, S Plathong, H Fukami, JT Wang, CA Chen. 2007. Occurrence of the putatively heat-tolerant *Symbiodinium* phylotype D in high-latitude outlying coral communities. *Coral Reefs* **26**: 35-44.
- Little AF, MJH van Oppen, BL Willis. 2004. Flexibility in algal endosymbioses shapes growth in reef corals. *Science* **304**: 1492-1494.
- Loi T. 1998. Molecular diversity of the predominant clade C zooxanthellae from scleractinian corals of the Great Barrier Reef. Honour's thesis, Department of Microbiology, Univ. of Sydney, Sydney, Australia.
- Meng PJ, HJ Lee, JT Wang, CC Chen, HJ Lin, KS Tew, WJ Hsieh. 2008. A long-term survey on anthropogenic impacts to the water quality of coral reefs, southern Taiwan. *Environ. Pollut.* **156**: 76-75.
- Mieog JC, van Oppen, NE Cantin, WT Stam, JL Olsen. 2007. 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* **26**: 449-457.
- Mostafavi PG, SMR Fatemi, MH Shahhosseiny, O Hoegh-Guldberg, WKW Loh. 2007. Predominance of clade D *Symbiodinium* in shallow-water reef-building corals off Kish and Larak Islands (Persian Gulf, Iran). *Mar. Biol.* **153**: 25-34.
- Oliver TA, SR Palumbi. 2009. Distributions of stress-resistant coral symbionts match environmental patterns at local but not regional scales. *Mar. Ecol. Progr. Ser.* **378**: 93-103.
- Oliver TA, SR Palumbi. 2011a. Many corals host thermally resistant symbionts in high-temperature habitat. *Coral Reefs* **30**: 241-250.
- Oliver TA, SR Palumbi. 2011b. Do fluctuating temperature environments elevate coral thermal tolerance? *Coral Reefs* **30**: 429-440.
- Peir JJ. 2011. Power uprate effect on thermal effluent of nuclear power plants in Taiwan. In P Tsvetkov, ed. Nuclear power - operation, safety and environment. Rijeka, Croatia: Tech Publication, pp. 287-302.
- Pochon X, RD Gates. 2010. A new *Symbiodinium* clade (Dinophyceae) from soritid foraminifera in Hawai'i. *Mol. Phylogenet. Evol.* **56**: 492-497.
- Pochon X, TC LaJeunesse, J Pawlowski. 2004. Biogeographic partitioning and host specialization among foraminiferan dinoflagellate symbionts (*Symbiodinium*; Dinophyta). *Mar. Biol.* **146**: 17-27.
- Precht WF, AW Bruckner, RB Aronson, RJ Bruckner. 2002. Endangered acroporid corals of the Caribbean. *Coral Reefs* **21**: 41-42.
- Putnam HM, PJ Edmunds. 2011. The physiological response of reef corals to diel fluctuations in seawater temperature. *J. Exp. Mar. Biol. Ecol.* **396**: 216-223.
- Reimer JD, S Ono, J Tsukahara, K Takishita, T Maruyama. 2007. Non-seasonal clade-specificity and subclade microvariation in symbiotic dinoflagellates (*Symbiodinium* spp.) in *Zoanthus sansibaricus* (Anthozoa: Hexacorallia) at Kagoshima Bay, Japan. *Phycol. Res.* **55**: 58-65.
- Rodrigues-Lanetty M, SJ Chang, JI Song. 2003. Specificity of two temperate dinoflagellate-anthozoan associations from the north-western Pacific Ocean. *Mar. Biol.* **143**: 1193-1199.
- Rowan R. 2004. Thermal adaptation in reef coral symbionts. *Nature* **430**: 742.
- Rowan R, N Knowlton. 1995. Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proc. Natl. Acad. Sci. USA* **92**: 2850-2853.
- Rowan R, DA Powers. 1991. Molecular genetic identification of symbiotic dinoflagellates (Zooxanthellae). *Mar. Ecol. Progr. Ser.* **71**: 65-73.
- Sampayo EM, T Ridgway, P Bongaerts, O Hoegh-Guldberg. 2008. Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. *Proc. Natl. Acad. Sci. USA* **105**: 10444-10449.
- Silverstein RN, AMS Correa, AC Baker. 2012. Specificity is rarely absolute in coral-algal symbiosis: implications for coral response to climate change. *Proc. R. Soc. Lond. B. Biol. Sci.* **279**: 2609-2618.
- Stat M, RD Gates. 2011. Clade D *Symbiodinium* in scleractinian corals: a "nugget" of hope, a selfish opportunist, an ominous sign, or all of the above? *J. Mar. Biol.* 2011 Article ID 730715. doi:10.1155/2011/730715.
- Stat M, WKW Loh, TC LaJeunesse, O Hoegh-Guldberg, DA Carter. 2009. Stability of coral-endosymbiont associations during and after a thermal stress event in the southern Great Barrier Reef. *Coral Reefs* **28**: 709-713.
- Suwa R, M Hirose, M Hidaka. 2008. Seasonal fluctuation in zooxanthellar genotype composition and photophysiology in the corals *Pavona divaricata* and *P. decussata*. *Mar. Ecol. Progr. Ser.* **361**: 129-137.
- Tchernov D, MY Gorbunov, C de Vargas, SN Yadav, AJ Milligan, M Häggblom, PG Falkowski. 2004. Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proc. Natl. Acad. Sci. USA* **101**: 13521-13535.
- Thornhill DJ, TC LaJeunesse, DW Kemp, WK Fitt, GW Schmidt. 2006a. Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. *Mar. Biol.* **148**: 711-722.
- Thornhill DJ, WK Fitt, GW Schmidt. 2006b. Highly stable symbioses among western Atlantic brooding corals. *Coral Reefs* **25**: 515-519.
- Toller WW, R Rowan, N Knowlton. 2001. Repopulation of zooxanthellae in the Caribbean coral *Montastraea annularis* and *M. faveolata* following experimental and disease-associated bleaching. *Biol. Bull.* **201**: 360-373.
- Ulstrup KE, MJH van Oppen. 2003. Geographic and habitat partitioning of genetically distinct zooxanthellae (*Symbiodinium*) in *Acropora* corals on the Great Barrier Reef. *Mol. Ecol.* **12**: 3477-3484.
- van Oppen MJH, FP Palstra, AM-T Piquet, DJ Miller. 2001. Patterns of coral-dinoflagellate associations in *Acropora*: significance of local availability and physiology of *Symbiodinium* strains and host-symbiont selectivity. *Proc. R. Soc. Lond. B. Biol. Sci.* **268**: 1759-1767.
- Venn AA, JE Loram, HG Trapido-Rosenthal, DA Joyce, AE Douglas. 2008. Importance of time and place: patterns in abundance of *Symbiodinium* clade A and B in the tropical sea anemone *Condylactis gigantea*. *Biol. Bull.* **215**: 243-252.
- Wilkinson C. 1998. The 1997-1998 mass bleaching event around the world. In CR Wilkinson, ed. Status of coral reefs of the world. Townsville, Australia: Australian Institute of Marine Science, pp. 15-38.