Can resistant coral-*Symbiodinium* associations enable coral communities to survive climate change? A study of a site exposed to long-term hot water input

Climate change has led to a decline in the health of corals and coral reefs around the world. Studies have shown that, while some corals can cope with natural and anthropogenic stressors either through resistance mechanisms of coral hosts or through sustainable relationships with *Symbiodinium* clades or types, many coral species cannot. Here, we show that the corals present in a reef in southern Taiwan, and exposed to long-term elevated seawater temperatures due to the presence of a nuclear power plant outlet (NPP OL), are unique in terms of species and associated *Symbiodinium* types. At shallow depths (<3 m), eleven coral genera elsewhere in Kenting predominantly found with *Symbiodinium* types C1 and C3 (stress sensitive) were instead hosting *Symbiodinium* type D1a (stress tolerant) or a mixture of *Symbiodinium* type C1/C3/C21a/C15 and *Symbiodinium* type D1a. Of the 16 coral genera that dominate the local reefs, two that are apparently unable to associate with *Symbiodinium* type D1a are not present at NPP OL at depths of < 3 m. Two other genera present at NPP OL and other locations host a specific type of *Symbiodinium* type C15. These data imply that coral assemblages may have the capacity to maintain their presence at the generic level against long-term disturbances such as elevated seawater temperatures by acclimatization through successful association with a stress-tolerant *Symbiodinium* over time. However, at the community level it comes at the cost of some coral genera being lost, suggesting that species unable to associate with a stress-tolerant *Symbiodinium* are likely to become extinct locally and unfavorable shifts in coral communities are likely to occur under the impact of climate change.
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Introduction

As a result of global change, tropical ocean temperatures are predicted to rise between 1.0 to 3.0 °C by the end of this century (IPCC 2013) in addition to fluctuation resulting from an increase in minimum and maximum temperatures with daily minimum temperatures rising more rapidly than maximums (Traill et al. 2010; Vose et al. 2005). Evidence from research has shown a higher sensitivity to global warming in tropical species since they are exposed to narrower thermal niches (Kozak and Wiens 2007). Coral holobionts (in this paper refers to coral host + zooxanthellae, see Weber and Medina 2012) are known to be living at, or near, their tipping points (Donelson and Munday 2012; Monaco and Helmuth 2011) across a range of different thermal environments (Ladner et al. 2011). In the absence of significant mechanisms to resist stress, corals will likely face high levels of regional mortality through time (Ladner et al. 2011). For corals, it is now imperative to be able overcome the effects of climate change and continue to survive. The question remains as to whether every coral species can overcome the effects of climate change. It is necessary for the whole coral community in any given location to survive, as survival of only a few species cannot maintain the coral reef community. Marshall and Baird (2000) have suggested that the change in community structure and species diversity is a result of the differences among species in their susceptibilities to disturbance. The fact that relatively small excursions of seawater temperature can have large-scale impacts on coral survival indicates that reef-building corals are living close to their upper thermal limit (Fitt et al. 2001; Riegl et al. 2011). If environmental perturbations exceed the adaptive capacity of corals, it may result in a change in their communities over time such that species that are phenotypically plastic or can adapt genetically through time will become dominant. In a disturbed environment where organisms under constant stress or challenged by increasing stress over time (for example,
salinity or temperature increases), the number of individuals exposed to selection will be greater resulting in an overall shift in abundance and a change in composition (Bell 2012).

Over short periods of time (within a single generation), the potential for corals to acclimatize to climate change through phenotypic plasticity or by specific combinations with stress resistant *Symbiodinium* types thorough natural selection may be the over-riding determinant of survival (Marshall and Baird 2000). However, a beneficial association between a coral host and *Symbiodinium* is a rather complex and holistic process because the classification of *Symbiodinium* into functionally distinct evolutionary entities (using alpha-numeric designations equivalent to ‘species’) has shown them to belong to nine divergent phylogenetic ‘clades’ (A to I) (Pochon and Gates 2010). Corals associate with either single or multiple *Symbiodinium* clades, and occupy defined ecological niches and roles within and across coral hosts (LaJeunesse *et al.* 2010; Pochon and Gates 2010; Weber and Medina 2012) based on their physiological response, some of which includes photosynthetic efficiency (Iglesias-Peieto *et al.* 2004) and sensitivity or tolerance to heat stress (Baker 2003a,b; Jones *et al.* 2008; Little *et al.* 2004; Rown 2004; Sampayo *et al.* 2008; Warner *et al.* 2006), light adaptation. While spatial pattern in symbiont communities can be explained through identification at the clade level, the exclusion of intra-cladal differences more often obscures ecological patterns in *Symbiodinium* distribution (Tong *et al.* 2013). With increasing attention and advanced sequencing methods, diverse ‘sub species’ or ‘types’ have been discovered within the *Symbiodinium* clades, and various studies have discussed on length about the *Symbiodinium* type physiological differences and their contribution to coral host stress resistance. Similar to the case of *Symbiodinium* clade; geographic locations, local environmental conditions (differences in physical parameters such as temperature, light and turbidity) have been found to define the *Symbiodinium* type associated with coral hosts. For
example, corals present in shallow and turbid locations are found to be associated with
*Symbiodinium* type D1a (LaJeunesse et al. 2010) or the distribution of *Symbiodinium* types
according to different light environments (Farde et al. 2008). Thornhill et al. (2006) have shown
that *Orbicella annularis* from Florida Key were associated with *Symbiodinium* type D1a after the
1998 bleaching event and reverted back to being associated with *Symbiodinium* type B10 after
2002. Results from the laboratory experiments have shown difference in tolerance to temperature
stress (Brading et al. 2011; Fisher et al. 2012; Kramer et al. 2012; Wang et al. 2012). For
example, *Symbiodinium* type A1 was found to be more tolerant to temperature stress compared to
types C1 and B1 (Hawkins and Davy 2012) and *Symbiodinium* type C15 was more tolerant than
type C3 in terms of their photosynthetic efficiency. With respect to *Symbiodinium* type D1a;
studies have shown highest activation energy when subjected to temperature stress in freshly
isolated D1a when compared to B1, C1, C3 and C15 types (Wang et al. 2012). In a cold
temperature stress experiment, Thornhill et al. 2008 showed that *Symbiodinium* type B2
displayed rapid and full recovery at 10°C for 2-week period in their photochemical efficiency
compared to types A3, B1 and C2 (Fisher et al. 2012). These studies have revealed the
importance of *Symbiodinium* types in assisting the coral host resisting mechanisms to stress.

In addition to associating with a resistant *Symbiodinium* clade/type, corals can overcome
environmental perturbations, mainly seawater temperature fluctuations, by their ability to shuffle
between clades/types depending on the environmental and seasonal condition (LaJeunesse et al.
2004, Chen et al. 2005, Thornhill et al. 2006, Sampayo et al. 2008, LaJeunesse et al. 2010a,
Chen et al. 2005, Hsu et al. 2012, Keshavmurthy et al. 2012). It has often been found that corals
associating with stress resistant *Symbiodinium* clade D have experienced community changes that
resulted in some coral species being favored over others (Marshall and Baird 2000; Loya et al.
Studies have shown the possible ability of coral and various *Symbiodinium* combinations to respond to the effects of climate change as a result of a high degree of variation in coral and symbiont thermal tolerance and symbiont community shifts in response to thermal tolerance (Marshall and McCulloch, 2002; Hughes *et al.* 2003; Baker 2001; Baker *et al.* 2004; Berkelmans and Van Oppen 2006). Short-term field or laboratory studies that have been performed to understand the acclimatization and/or adaptation potential in corals to climate change (Meesters *et al.* 2002; Coles and Brown 2003; Ayre and Hughes 2004; Baker *et al.* 2004; Rowan 2004; Richier *et al.* 2005; Berkelmans and Van Oppen 2006; Gittenberger and Hoeksema 2006; Shaish *et al.* 2007; Strychar and Sammarco, 2009; Hennige *et al.* 2010; Barshis *et al.* 2010; Oliver and Palumbi 2011; Bellantuono *et al.* 2011; Keshavmurthy *et al.* 2012; Howells *et al.* 2013) have their own shortfalls. To understand more comprehensively the capacity of acclimatization and adaptation, a more effective way would be to conduct mesocosm experiments by mimicking different perturbations of climate change, but such experiments are difficult to conduct and suffer from logistical problems. Another way is to find a location that already is in a situation where seawater temperatures are similar to levels predicted for 2050 by IPCC and is also subjected to natural and anthropogenic disturbances over time.

In Kenting, southern Taiwan, there is an area that is influenced by constant hot-water effluent from a nuclear power plant located along the western side of Nanwan Bay that has been operating since 1984. At this nuclear power plant outlet (NPP OL), the average seawater temperature is 2.0°C higher than at other coral reef sites in Kenting (Fan *et al.* 1991; Pier 2011; also see Keshavmurthy *et al.* 2012). Hot water at the nuclear power plant (NPP) site is trapped and flows southwestward in Nanwan Bay because of a near-shore current and tides (Chiou *et al.* 1993). The hot water released in this area has had an impact on the marine ecology.
within the area of dispersal (Chiou et al. 1993; Hung and Huang 1998; Jan et al. 2001; Hwang et al. 2004). Recent studies on two coral species, *Isopora palifera* and *Platygyra verweyi* (sampling done on a local scale in Kenting, Taiwan), showed a differential trend in associating with *Symbiodinium* type D1a. For example, *P. verweyi*, distributed in shallow waters at depths of 20 meters, is associated with *Symbiodinium* type D1a, whereas it associates with *Symbiodinium* type C3 in cooler waters (Keshavmurthy et al. 2012). In the case of *Isopora palifera*, it overcomes the effect of hot water at NPP OL by shuffling its *Symbiodinium* clades seasonally (Hsu et al. 2012) and through the presence of exclusive stress-resistant haplotypes of the host (Hsu et al. 2012).

By utilizing Kenting and NPP OL as a natural mesocosm, we were able to investigate the present composition of the coral community and associated *Symbiodinium* types in sixteen genera of reef-building corals exposed to elevated seawater temperatures over the previous 26 years. For comparison, we sampled the same sixteen genera from seven adjacent sites that are not under the influence of the nuclear power plant’s hot water effluent to see how a long-term environmental perturbation (in this case seawater temperature) affected present day coral-*Symbiodinium* and coral host composition. Due to the lack of historical *Symbiodinium* composition data, and although it is not possible to show the process of acclimatization, we based our study on the hypothesis that the present composition of coral-*Symbiodinium* and coral hosts at the nuclear power plant location is the result of acclimative and/or adaptive phenotypic plasticity during 26 years of exposure to chronically elevated seawater temperatures. This could be due to the plasticity in the coral hosts or to associations with resistant *Symbiodinium* types. By conducting large-scale sampling from 16 coral genera, this study attempts to understand how
different coral species in a community might have adjusted or responded to long-term elevated seawater temperature stress. The possible outcomes are that either all corals exposed to chronic seawater temperature stress host only resistant types of *Symbiodinium*, or only those corals that are able to host resistant *Symbiodinium* types can survive. We discuss whether resistance at the genus or species level, if any, towards the long-term warming of seawater temperatures will enable corals at the community level to survive the effects of climate change.

**Material and Methods**

**Area description and Sampling**

Coral samples were obtained from eight sites present in Nanwan, Kenting located at the southern tip of Taiwan (Fig. 1). Of the eight sites, one was the nuclear power plant outlet (NPP OL), which was considered a natural mesocosm subject to long-term hot water perturbation. Seawater in the bay comes from the Kuroshio Current in winter and South China Sea current in summer. The average sea surface temperature in this bay is 29.0 °C in summer and 24.0 °C in winter. A total of 1913 coral colonies belonging to 60 species from 16 genera and representing 7 families were sampled in 2009 and 2010 from 3 m and 7 m depths from eight sites; Wanlitung (WLT), NPP OL, Houbihu (HBH), NPP Inlet (IL), Taioshih (TS), Tanziwan (TZW), Sanjiawan (SJW), and Longken (LK), including the reef near the NPP outlet (Fig. 1). Corals sampling was authorized by Kenting National Park project #488-100-01. For every coral genus sampled, a minimum of 15 colonies were collected from each location, if available, at each designated sampling depth. At some sampled sites, it was not possible to find even one colony of some genera, while at other sites the minimum sample was 1 and maximum was 35. Hence, it was not possible to maintain uniformity in sample number due to the uneven distribution of coral genera.
All samples were immediately fixed in 90% ethanol and stored until further analysis of *Symbiodinium* clades.

**DNA extraction**

DNA was extracted using the high-salt DNA extraction method that was modified according to Ferrara *et al.* (2006). Approximately 30 mg of coral were placed in 2 ml Eppendorf tubes into which 200 µl lysis buffer (1 M Tris-Boric, 0.5 M EDTA (pH 8), 20% sodium dodecylsulfate, and 5 M NaCl) and 10 µg of proteinase K (10 mg ml$^{-1}$) were added and incubated overnight in a 60 °C water bath. After incubation, an equal volume (210 µl) of NaCl (7 M) was added to the 2 ml tube and mixed gently, and the entire solution was transferred to extraction column tubes (GP™ Column, VIOGENE, USA) and subsequently centrifuged at 8000 g for 1 min. After discarding the flow-through, 500 µl of 70% EtOH was added and centrifuged at 8000 g for 5 min. This step was repeated twice. Finally, the extraction columns were transferred to new 1.5 ml Eppendorf tubes and incubated at 37 °C for 15 min. Preheated (60 °C) TE buffer (50 µl) was used for the final extraction step, and the column was centrifuged at 13,000 g for 3 min. All DNA samples were kept at -20 °C until further use.

**Restriction Fragment Length Polymorphism analysis of *Symbiodinium* clades**

Initially to assess the diversity of *Symbiodinium* at clade level, DNA from1913 coral samples were analyzed by Restriction Fragment Length Polymorphism (RFLP) technique. Both nuclear large-subunit ribosomal (nlSr) DNA and nuclear small-subunit ribosomal (nssr) DNA were used to investigate the clade diversity. The polymerase chain reaction (PCR) amplification of nlSrDNA and nssrDNA was modified from a previously published protocol (Chen *et al.* 2005).
The DNA concentration was adjusted to 30~50 ng µl⁻¹ for each PCR reaction, with a final concentration of 0.2 mM dNTP, 0.5 µM primer, 1x PCR buffer with 1.5 mM MgCl₂, and 2.5 units of Taq DNA polymerase (Invitrogen™, USA). PCR was performed by pre-denaturation at 95 ºC for 1 min followed by 30 cycles of denaturation at 94 ºC for 45 s, annealing at 50 ºC for 45 s, and extension at 72 ºC for 2 min. The final extension was performed at 72 ºC for 6 min. The D1 and D2 regions of Symbiodinium nlsrDNA were first amplified with the primer set (D1/D2 F: 5’-CCT CAG TAA TGG CGA ATG AAC A-3’ and D1/D2 R: 5’-CCT TGG TCC GTG TTT CAA GA-3’)(Loh et al. 2001), and the PCR products were then characterized using restriction enzyme Rsa I.

The nssrDNA of Symbiodinium was amplified using a host-excluding primer pair (ss5z: 5’-GCA GTT ATA RTT TAT TTG ATG GTY RCT GCT AC-3’ and ss3z: 5’-AGC ACT GCG TCA GTC CGA ATA ATT CAC CGG-3’), and the products were characterized using restriction enzymes Sau3A I and Taq I (Rowan & Powers 1991). All enzymes used for RFLP were purchased from MBI (Fermantas, Italy). Digested nlsrDNA PCR products were separated at 150 V by vertical gel electrophoresis for 3.5 h on a 5% acrylamide gel (30% acrylamide/bis-acrylamide (37.5:1), 10x TBE buffer, 25% ammonium persulfate, and TEMED). This was done to increase the clarity of the nlsrDNA band pattern, which sometimes was not clear on the agarose gels. The digestion products of nssrDNA PCR-RFLP were separated by gel electrophoresis on a 3% agarose gel for 3.5 h at 50 V.

DGGE analysis of Symbiodinium types

To assess the Symbiodinium diversity at type level, representative DNA samples from different band patterns from the RFLP analysis were picked and subjected to ribosomal internal transcribed spacer 2 region (ITS2) amplification using primers ITSintfor2: 5’-GAA TTG CAG
AAC TCC GTG-3′; ITS2clamp: 5′- CGC CCG CCG CGC CCC GCG CCC GTC CCG CGG
GAT CCA TAT GCT TAA GTT CAGC GGG T-3′ (Lajeunesse and Trench 2000). Each 50 µl
PCR reaction consisted of 50 ng genomic DNA, 1x PCR Buffer, 2.5 mM MgCl₂, 0.4 mM dNTPs,
0.4 µM of each primer, and 2 units of Taq polymerase (Invitrogen, USA). PCR was run on a Px2
thermal cycler (Thermo Scientific, USA) with touch-down PCR (LaJeunesse 2002) to ensure
specificity. The initial denaturing period was at 92 °C for 3 min, followed by 20 cycles of 30 s at
92 °C, and annealing conditions from 62 °C were decreased by 0.5 °C to the final annealing
temperature of 52 °C for 30 sec at 72 °C. Once the annealing temperature reached 52 °C, it was
maintained at that level for another 20 cycles, followed by a final extension period of 10 min at
72 °C. Each PCR product was loaded onto an acrylamide denaturing gradient gel (45-80%) and
then electrophoresed at 115V for 15 h using a CBS Scientific system (Del Mar, CA, USA). Gels
were stained with SYBR Green (Molecular Probes, Eugene, OR, USA) for 15 min and
photographed for further analysis. Band patterns were confirmed by sequencing the cut bands
from the DGGE gel.

Coral community data

Historical coral community data for NPP OL was obtained from various published
reports. Although it is difficult to compare historical data with the present data due to a change in
methodology over the years, it is still possible to get an idea of how the community readjusted or
redistributed over time. For 1986, we used community data previously published (Dai 1988, Dai
et al. 1998) that was obtained with quadrat sampling to estimate the percent cover of each species
at NPP OL. We compared this with the data from our own 2009-2010 survey using a 20 m
transect line at NPP OL. We also examined photographs taken of the coral community at NPP OL
in 1986 and 1995 and compared them with 2010 photographs obtained from the same locations. Due to problems in comparing the data, it was not possible to compare them quantitatively, so we only show the pictorial comparison of how some coral genera have been replaced over time.

**Historical and present day environmental data**

Seawater temperature data from 1982-1992 were obtained from the Taiwan Power Company’s eleven-year ecological survey of waters adjacent to the nuclear power plant. Data after 1995 was obtained from different sites recorded with temperature loggers (HOBO Pendant™, USA).

To analyze the relationship between *Symbiodinium* diversity trend and environmental parameters, data was also obtained as time series for sea surface temperature (SST which included; average temperature –Tavg, summer average temperature-Ts, winter average temperature-Tw), *chlorophyll a* (Cha-a), photosynthetically active radiation (PAR) and diffuse attenuation coefficient (Kd) over the time period 2002-2010 (at 4 km spatial resolution) from MODIS/aqua interface of the Giovanni online data system, developed and maintained by the NASA GES DISC.

For contour diagram of the seasonal seawater temperature across the Nanwan bay, seawater was collected using CTD at various sites between 2008-2010. The exact positions of sampling sites were located by global position system (GPS). The measurements of temperature were carried out with CTD instrument (Sea-Bird Model 19 plus) by the EPA/ROC (Taipei) on fishing boats. The precision for temperature was ±0.05°C. After the data output from CTD, contour maps were created using Surfer (Windows).
Statistical analysis

The nonparametric Mann–Whitney test from CTD, contour maps were created using Surfer (Windows). Sites were located by global position system (GPS). The mes in seawater temperatures between the two depths at NPP OL and between NPP OL and different sites (WLT, MBT, STZ, and IL). To compare *Symbiodinium* clade distributions, the Chi-Square test and single-factor between-subjects ANOVA (independent samples) were used. The ANOVA results were plotted as one-way diamond mean comparisons to see the differences in *Symbiodinium* composition at different sites between 3 m and 7 m and for total *Symbiodinium* C and *Symbiodinium* D composition at NPP OL (3 m and 7 m). The horizontal dashed line in the diamond plot is the overall mean (*i.e.*, grand mean). The line through the center of each diamond is the group mean. The top and bottom diamond vertices are the respective upper and lower 95% confidence limits (CI) about the group mean.

To understand the relationship between the environmental data, coral host and *Symbiodinium* at different sites and depths, similarity (Bray-Curtis) in the host and symbiont data (presence/absence) was analyzed using Principle Coordinate Analyses (PCO) and ANOSIM. The host and symbiont similarity matrices were analyzed for a significant relation (Spearman rho, rho \( \approx 0 \) indicates no relation is found, rho = 1 indicates a perfect relation) using RELATE. Distance-based analysis on a linear model (distLM) was used to model the relationship between the symbiont dissimilarity data and the environmental variables. To include the host effect on the symbiont matrix, PCO1 and PCO2 of the host presence/absence data (HPCO1 and HPCO2 for continued reference) were added as covariates to the environmental data matrix in subsequent linear regression data analyses (see Tonk et al. 2013b). In the distLM, marginal tests assessed the
importance of each variable separately and a forward search of the optimal fit based on an adjusted $R^2$ was used by sequentially adding environmental variables. The data was visualized with distance based redundancy analyses (dbRDA) ordination plots. Vector overlays using the environmental data and symbiont data separately as predictor variables (drawn as multiple partial correlations) were applied to visualize the effect, strength and direction of the different variables in the ordination plots. The symbiont distributions on a clade and type level were explained with environmental data (normalized), in which the collected host information included either on a species level or genus level. All symbiont and host data was transformed to relative abundance.

Results

Seawater temperatures in Kenting

Seawater temperature data from 1982-1992 (Fig. 2 A) shows that, prior to construction of the nuclear power plant, the average seawater temperature (26.0 °C) was the same at all locations where seawater temperature data could be obtained. However, after construction of the nuclear power plant in 1984, the average seawater temperature at NPP OL was consistently 2.0-3.0 °C higher (Fig. 2 A) than adjacent locations. Monthly and daily average seawater temperatures showed a similar trend (Fig. 2 B) (also see Keshavmurthy et al. 2012, Hsu et al. 2012). NPP OL
seawater temperatures at the 3 m depth were significantly higher than 7 m depths (p < 0.0001). The thermal effluent in NPP OL is restricted to the water column above 3m, and the temperature differential does not influence depths below 7m. Also, NPP OL experiences daily fluctuation of 8.0-10.0 ºC (for 6 hours) due to upwelling as a result of internal tides and waves generated at Luzon Strait, which directly affects the seawater present in Nanwan Bay in Kenting (Chen et al. 2004). Differences between daily average seawater temperatures at NPP OL and two relatively distant control sites (WLT and IL) were statistically significant (Mann-Whitney test, p < 0.0001), whereas those between NPP OL and two closer sites (MBT and STZ) were not statistically significant (Mann-Whitney test, p >0.001) (see also Keshavmurthy et al. 2012). Contour diagram of the seawater temperature in Nanwan showed consistent presence of hot water plume near the NPP OL irrespective of the seasons (Fig 3).

Coral - *Symbiodinium* associations

From the Restriction Fragment Length Polymorphism (RFLP) data, the dominant *Symbiodinium* clade associated with corals at eight sites in Kenting was *Symbiodinium* clades C (Fig. 4A). Our analysis of the *Symbiodinium* clade in coral genera at NPP OL showed that heat-tolerant *Symbiodinium* clade D was dominant in corals at the 3 m depth (Fig. 4A, B). Coral genera at the 7 m depth, however, were associated with *Symbiodinium* clade C at all study locations including NPP OL (Fig. 4A, B, Fig. 5). In contrast, *Symbiodinium* clade C was dominant at 7 m (199 out of 234 samples, 85%, Chi-square test: $X^2_{(0.01)}$: 0.0001) (Fig. 4A, B), with only five genera having a mixed composition of *Symbiodinium* clade C and *Symbiodinium* clade D (Fig. 4A, B). The difference in *Symbiodinium* clade D clade between 3 m and 7 m at NPP OL was significant (one way ANOVA; p = 0.001). A similar significant difference was also
observed in the presence of *Symbiodinium* clade C between 3 m and 7 m at NPP OL (**Fig. 5**).

Based on the results from ITS2 DGGE, the *Symbiodinium* types found in corals hosts belonged to type D1a (mainly found in coral hosts present in NPP OL 3m) and types C1, C3, C21a and C15. Since we have utilized both RFLP and DGGE for analysis, to avoid confusion, throughout the text we will follow the nomenclature used for DGGE and discuss the results using DGGE ITS2 *Symbiodinium* types.

Of sixteen genera (Fig. 4), twelve genera hosted significantly more *Symbiodinium*D type D1a at NPP OL 3m in response to thermal stress and mostly or only *Symbiodinium* types C1, C3, C21a and C15 at NPP OL 7m (*Acropora*, *Cyphastrea*, *Goniastrea*, *Isopora*, *Platygyra*, *Favites*, *Pocillopora*, *Acanthastrea*, *Leptoria*, *Montastraea*), except for *Pavona* and *Montipora* which had *Symbiodinium* types C1 and C15 respectively as the dominant clade at both NPP OL 3 m and 7 m. The genus *Porites* was specifically associated with *Symbiodinium* type C15 at all locations, and the weedy genus *Galaxea* was associated with *Symbiodinium* type D1a at all locations and rarely hosted *Symbiodinium* type C1 (Fig. 4B, also see Fig. S1). Finally, two genera, *Seriatopora* and *Stylophora*, were associated with *Symbiodinium* C type C1 and were entirely absent at the thermally stressed NPP OL site (Fig. 4C, see Fig. S1).

At sites other than NPP OL, the degree to which competent genera hosted *Symbiodinium* clade D or a combination of *Symbiodinium* clade D and clade C varied considerably. No *Acropora* samples were observed to do so, *Pocillopora* and *Platygyra* did so at only one other location, and other genera (*Cyphastrea*, *Goniastrea*, *Isopora*, *Pavona*, *Acanthastrea*, *Leptoria*, *Montastraea*) did so at multiple locations. However, genera *Cyphastrea*, *Isopora*, *Pavona*, and *Goniastrea* routinely hosted only *Symbiodinium* clade D at non-thermally stressed locations.
Species level comparisons among NPP OL (warm water influence) and WLT and SJW (sites without warm water influence) also showed that the 3 m site at NPP OL was dominated by *Symbiodinium* clade D, while at other sites and at NPP OL 7 m depths the dominant *Symbiodinium* belonged to clade C (Fig. 4C, Table S1, Fig. S1A, B).

**Coral Community**

A comparison of the 1986 coral community at 3 m depths at NPP OL to the 2010 community showed a difference in the dominant species (Table 1). While *Acropora* (24.20% of total coral cover) and *Montipora* (33.69% of total coral cover) dominated the shallow waters in 1986, *Montipora* (21.70% of total coral cover) and *Galaxea* (21.20% of total coral cover) dominated the 2010 coral community. Other genera (*Favites*, *Pavona*, and *Porites*) that were < 2% of the total abundance in 1986 had increased their relative abundance by 2010 (Table 1). By 2010, the presence of *Acropora* had been greatly reduced (0.70%) and *Seriatpora* and *Stylophora* were completely absent at 3 m depths at NPP OL (Fig. 6, Fig. 7, Table 1). Results from DistLM and Bray-Curtis similarity resemblance analysis on the coral community presence or absence at different locations also showed dominance of *Galaxea* at NPP OL 3m. (Fig, 8C)

**Relationship between sites, Symbiodinum distributions and environmental factors**

In terms of seawater temperature differences, there was a clear difference between NPP OL 3m and other sites with NPP OL 3m always appearing as an out group in the analysis (Fig. 8, Table 2). Due to its proximity with NPP OL, site TS also grouped with NPP OL 3m in DistLM analysis (Fig. 8A). Analysis also showed that several environmental parameters (Tavg - long term from 2002-2010) and Tsd), including the host, were responsible for driving the symbiont community. There was a clear pattern in terms of symbiont distribution (both on a cladal and type level) and
NPP OL 3m was significantly different in its symbiont composition (Fig. 8A, B, Table 2). The distribution of different general among the sites reflected the results of the present day coral community data. Genus Galaxea was mainly clustered with NPP OL 3m (Fig. 8C).

**Discussion**

This study observed the effect of chronically elevated seawater temperatures on the composition of coral hosts and coral-*Symbiodinium* in sixteen coral genera between NPP OL and adjacent control sites. Our results clearly showed the presence of more *Symbiodinium* clade D at NPP OL (3 m) compared with other sites, and *Symbiodinium* clade C was dominant at 7 m at all locations including NPP OL (Figs. 3, 4). Our results suggest that the present day coral host *Symbiodinium* combinations could be due to the long-term input of hot water. This can be seen by the dominance of *Symbiodinium* clade D in the coral hosts present near the NPP OL site. We posit that the observed composition of *Symbiodinium* associated with the corals at NPP OL (3 m) might have occurred over years of acclimatization of individual hosts and *Symbiodinium* clades exposed to higher and more variable temperatures and adaptation through selection and parallel evolution of resistant host-*Symbiodinium* combinations. A reef with a higher abundance of *Symbiodinium* clade D-dominated holobionts is assumed to have a higher tolerance to thermal stress (Ortiz et al. 2012). Alternatively, the present coral-*Symbiodinium* composition at NPP OL (3 m) might be a result of the adaptive plasticity of competent coral hosts and *Symbiodinium* either separately or in combination over time. We suggest that the only plasticity that predictably enhances fitness and is most likely to facilitate adaptive evolution on ecological timescales in new environments is that which places populations close enough to a new phenotypic optimum for directional selection to act (Ghalambor et al. 2007). Long-term environmental standing of
above average seawater temperatures at NPP OL 3m and other factors such as nutrient input into
the Nanwan might also be the reason for the present day structuring of the coral host and
Symbiodinium diversity. However, from our analysis, there was a clear relationship between the
environmental factors and distribution of Symbiodinium clades. The sampling sites at Nanwan,
although not very separated from each other, are different in the way they are affected mainly by
seawater temperature (Hsu et al. 2012). This is both due to the physical differences in
temperature fluctuations, internal waves, upwelling and the constant output of hot water into the
NPP OL site and surrounding areas (Hsu et al. 2012, Keshavmurthy et al. 2012). Such factors
have induced the present day Symbiodinium distribution in those coral hosts present at different
sites in Nanwan.

In studies conducted to date, the impact of thermally tolerant Symbiodinium has largely
been documented at the colony scale, and the consequences at the population and community
level within an ecosystem context are unknown (Ortiz et al. 2012). We show that at community
level, Symbiodinium type D1a was dominant in 12 of 16 genera living at the 3 m depth at NPP
OL (Fig. 4C, Fig. S1), whereas the same 12 genera at the 7 m depth at NPP OL were associated
only with Symbiodinium types C1, C3 and C21 (Fig. 4C, Fig. S1). The depth-related stratification
in the clade association at NPP OL might be due to decades of constant seawater temperature
elevation (2.0-3.0 °C). At generic level, most of the corals at NPP OL 3m hosted solely
Symbiodinium type D1a. Two genera Montipora and Porites associated mostly or solely with
Symbiodinium type C15. While the strategy of dominant 12 genera was their capacity to associate
with a stress-tolerant Symbiodinium clade, the strategy followed by Porites could be a result of a
stress-resistant mechanism. Genus Galaxea was abundant and associated with Symbiodinium type
D1a at almost every site where it was collected, indicating that Galaxea is able to tolerate
disturbances and thrive in perturbed environments. From the above observations we believe that such difference, despite constant warm water and a large daily fluctuation in the seawater temperature (Chen et al. 2004), is due to the ability of coral-Symbiodinium combinations to thrive or the host itself to be able to survive in such conditions. And because of inability of some genera to host Symbiodinium type D1a or any other resistant Symbiodinium combination, Stylophora and Seriatopora were absent from 3m at NPP OL.

Many previous studies have shown that the shuffling of Symbiodinium and/or host resistance mechanisms can confer resistance in corals to environmental stress. From the observations in this study and a recent study (Keshavmurthy et al. 2012), we suggest there is the possibility of a strategy shift (Done 1999) in corals present in NPP OL or other sites, although we could not demonstrate the shuffling of Symbiodinium in corals per se. NPP OL populations at 3 m would represent stressed populations that have evoked a dominance of stress tolerant Symbiodinium type D1a as one option during their adjustment to the upper limit of their thermal range. There are at least two other alternative explanations, however. First, Symbiodinium type D1a may be an opportunistic type (see Stat and Gates 2011) that occupies compromised coral hosts, resulting in a less than optimal symbiosis and reduced rates of host growth. In this case, NPP OL corals at 3 m are stressed and populated by less optimal varieties of Symbiodinium. Although corals associated with Symbiodinium type D1a may benefit in the short term by surviving bleaching, there are clearly trade-offs in terms of fitness that may have major implications for the long-term growth and survival of coral reefs (Stat and Gates 2011, also see Oritz et al. 2013), thereby negatively affecting the competitive ability of corals (Baker et al. 2013). For example, the association of A. tenuis juveniles with Symbiodinium D resulted in higher metabolic costs and lower physiological tolerances (Abrego et al. 2008). Second, new
host-*Symbiodinium* combinations could arise as a result of directional selection where all combinations of host-*Symbiodinium* arrive in the shallows at NPP OL but only those with significant associations with *Symbiodinium* D survive. The latter explanation would be a consequence of the fact that NPP OL is open to populations not experiencing the same levels of thermal stress, and hence the resulting composition of *Symbiodinium* is a consequence of active selection for the *Symbiodinium* that occupy these coral genera and not shuffling *per se*. However, Baker et al. (2013) have posited that acute disturbances or long-term environmental conditions are not sufficient to explain the composition of symbiont communities and their dynamics in any given location. The trend seen in the symbiont composition can be more explained by physical factors such as daily seawater temperature fluctuations as a result of tidal oscillations or internal waves induced upwelling, which act as acute and chronic stressors. This can be one explanation for the presence of dominant *Symbiodinium* type D1a in the coral hosts in NPP OL 3m. Constant hot water output and daily temperature fluctuations up to 10 °C (Chen et al. 2004) in the NPP OL site might have allowed *Symbiodinium* type D1a to be maintained at relatively high levels at NPP OL 3m. This also shows that *Symbiodinium* type D1a not necessarily be always opportunistic, thermally tolerant and transient during stress conditions, but also can be capable of long-term persistence on reefs with favorable conditions (Baker et al. 2013). All the hypotheses that we have put forth above should be considered as alternatives to explain the trend of existing coral-*Symbiodinium* associations at warm water-affected locations at NPP OL.

In the case of coral hosts, there were considerable changes in the types of dominant coral at 3 m while the composition at 7 m at NPP OL did not change over the years (Table 1, Fig. 6, Fig. 7). The present day framework of the NPP OL community could be due to a strategy shift between *Symbiodinium* and coral stages, where both transient and continual states exist in the
composition of associated *Symbiodinium* clades and coral genera. In the case of Kenting reefs, strategy shifting exists not only between the two water depths at NPP OL but also between the sites (see Kuo et al. 2012). Form the long term satellite seawater temperature data, the averages of temperature at NPP OL 3m is similar to the WLT site, however, the analysis results showed otherwise, with TS 7m as more similar to NPP OL 3m indicating some other factors in addition to the above mentioned environmental factors influencing the patterns seen in the host as well as *Symbiodinium* distributions. But the distLP analysis suggested that the environmental data more than sufficiently explains the variation seen in the symbiont community. The other explanation for the difference seen in the symbiont distribution might be the differences in the host distribution among the sites. But, our sampling design made sure to sample hosts as uniform as possible at all sites, except in such cases where a particular host was absent and could not be sampled. As mentioned above, apart from the environmental factors that were included in the analysis,

The differentiation between coral and *Symbiodinium* populations among the different sites at Kenting might have been a result of populations developing tolerance to along-shore gradients of environmental factors, including temperature and pollution. At scales of less than 1 km, differentiation can occur over short, vertical stress gradients along horizontal gradients of wave exposure covering a few hundred meters. Variations in seawater temperatures and anthropogenic changes in Kenting might have led to adaptive divergence in physiological traits among populations distributed across a variety of scales. Studies have shown that human and natural disturbances are similar at all the sites represented in this study (Meng et al. 2008; Kuo et al. 2012). Apart from long-term hot water perturbations at NPP OL, other areas are not much different in terms of exposure to stress at Kenting. While coral communities at other Kenting sites
show considerable changes over time (Kuo et al. 2012), the coral presence at NPP OL is still
diverse and there is no evidence of a shift in the community from corals to algae. Studies have
suggested that historical effects play an important role in determining the fate of individuals,
populations, and communities (from Marshall and Baird 2000). In case of corals, a historical
thermal exposure can influence their thermal tolerance (see Howells et al. 2013), and this could
be through acclimatization and selection for tolerant genotypes. Also, over longer periods of time
(between generations), selection may drive heritable changes in the mean phenotype, but in long-
lived individuals such as corals the genetic adaptation might be slow and occur over decadal time
scales (van Oppen et al. 2011; Császár et al. 2010; Hoegh-Guldberg et al. 2007; Aitken et al.
2008). However, genotypic variation among individuals allows the species to persist through the
expansion of genotypes better suited for a new climate (Richter et al. 2012; also see Kramer et al.
2010). van Oppen et al. (2011) suggested that somatic mutation during asexual reproduction
could aid corals in evolution and adaptation (see also Fautin 1997; Buddemeier et al. 1997).
Evolution within mitotic cell lineages, both in the coral host and Symbiodinium (Correa et al.
2010), might play a role in the adaptation of corals to climate change.

Conclusions

Our work and a previous study (Berkelmans and Van Oppen 2006) show that even if
several coral species could withstand temperature increases beyond 1.0-1.5 °C, the surviving
coral species may not be sufficient to maintain healthy reefs. Due to long-term acclimatization,
community level resistance to perturbations (in our case, seawater temperature) is possible
through association with stress resistant Symbiodinium clade D. Acclimatization is an important
strategy by which individuals can adjust phenotype to perform more optimally under changed
environmental conditions.

Studies have shown that in coral reefs the process of local adaptation and acclimatization to high average temperatures and recurrent thermal stress is possible (Rowan et al. 1997; Marshall and Baird 2000; Brown et al. 2002; Howells et al. 2011; 2013). There are limits to acclimatization, however, which are set by tradeoffs at various structural and functional levels that ultimately constrain the width of the thermal range of a given species (see Doney et al. 2012). These constraints highlight the limits of acclimatization, which ultimately results in shifting community composition as tolerance thresholds in the more vulnerable species of a given community are exceeded. Laboratory research has shown that genetic variation for plasticity exists (Barshis et al. 2010; Császár et al. 2010) and heritable plasticity can respond to artificial selection (Nussey et al. 2005). Given that corals are exposed to long-term anthropogenically driven environmental change (Hoegh-Guldberg 1999; Hoegh-Guldberg et al. 2007), it is imperative to obtain a better understanding of how natural selection acts on plasticity under altered levels of environmental variation in the wild (Nussey et al. 2005). Understanding the limits of these combinations will allow us to understand why some corals are 'winners' and others are 'losers' during the early stages of rapid anthropogenic climate change (see Loya et al. 2001).

To some extent, the development of an optimal combination of coral hosts (from around 800 species) and Symbiodinium (over 100 distinct genetic varieties from 4 major clades) is a complex (Goulet 2006) and stochastic process somewhat like a lottery (Jones 2008). Parmesan and Yohe (2003) provided evidence of ecosystems altered by climate change. Our results suggest that corals assemblages and their symbionts exposed to warmer waters are already undergoing alteration. Our data also suggest that not all coral species in a given community have the ability to acclimatize to survive in warmer water. Current evidence suggests that natural adaptation
within coral populations is unlikely to occur quickly enough to keep up with rapid changes in ocean temperatures, and although shuffling *Symbiodinium* clades could play an important role in extending the physiological performance of a coral species, this might not be the case at the community level, resulting in the loss of some species over time (Fig. 6). If the present trend of ocean warming and change continues, we might be looking at unsustainable restructuring of coral assemblages in a given coral community (Fig. 6), which in turn would cause irreversible damage to coral reef ecosystems.
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REFERENCES

Abrego D, Ulstrup K, Willis B, van Oppen M. 2008. Species endosymbionts and coral hosts define their bleaching response to heat the light stress. *Proceedings of the Royal Society B- Biological Sciences* 275:2273-2282.

Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications* 1:95-104.

Ayre D, Hughes T. 2004. Climate change, genotypic diversity and gene flow in reef-building corals. *Ecology Letters* 7:273-278.

Baird AH, Bhagooli R, Ralph PJ, Takahashi S. 2009. Coral bleaching: the role of the host. *Trends in Ecology and Evolution* 24: 16-20.

Baker A. 2001. Ecosystems: reef corals bleach to survive change. *Nature* 411:765 2001.

Baker A, Starger C, McClanahan T, Glynn P. 2004. Coral reefs: corals' adaptive response to climate change. *Nature* 430:741.

Baker AC, McClanahan TR, J SC, K BR. 2013. Long-term monitoring of algal symbiont communities in corals reveals stability is taxon dependent and driven by site-specific thermal regime. *Marine ecology progress series* 479:85-96.

Barshis DJ, Stillman JH, Gates RD, Toonen RJ, Smith LW, Birkeland C. 2010. Protein expression and genetic structure of the coral *Porites lobata* in an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity? *Molecular Ecology* 19:1705-1718.

Bellantunono AJ, Hoegh-Guldberg O, Rodriguez-Lanetty M. 2011. Resistance to thermal stress in corals without changes in symbiont composition. *Proceedings of the Royal Society - Biological Sciences* 279:1100-1107.

Bhagooli R, Yakovleva I. 2004. Differential bleaching susceptibility and mortality patterns among four corals in response to thermal stress. *Symbiosis* 37:121-133.

Bell G. 2012. Evolutionary rescue and the limits of adaptation. *Philosophical Transactions of the Royal Society B- Biological Sciences* 368:20120080.

Berkelmans R, Van Oppen MJH. 2006. The role of zooxanthellae in the thermal tolerance of corals: A “nugget of hope” for coral reefs in an era of climate change. *Proceedings of the Royal Society - Biological Sciences* 273:2305-2315.

Brown B. 1997. Adaptations of reef corals to physical environmental stress. *Advances in Marine Biology* 31:221-256.

Brown B, Downs C, Dunne R, Gibb S. 2002. Exploring the basis of thermotolerance in the reef coral *Goniastrea aspera*. *Marine Ecology Progress Series* 242:119-126.

Buddemeier RW, Fautin DG, Ware JR. 1997. Acclimation, adaptation and algal symbiosis in reef-building scleractinian corals. In *Coelenterate Biology: Proceedings of the Sixth International Congress of Coelenterate Biology*; Nationaal Natuurhistorisch Museum: Leiden, The Netherlands 385-401.

Chen C-T, Wang B-J, Hsing L-Y. 2004. Upwelling and degree of nutrient consumption in Nanwan Bay, Southern Taiwan. *Journal of Marine Science and Technology* 12:442-447.

Chen C, Wang J-T, Fang L-S, Yang Y-W. 2005. Fluctuating algal symbiont communities in *Acropora palifera* (Scleractinia: Acroporidae) from Taiwan. *Marine Ecology Progress Series* 263:261-269.
Chiou WD, Cheng LZ, Ou HC. 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. *Journal of Fisheries Society of Taiwan* 20:207-210.

Coles SL, Brown BE. 2003. Coral bleaching - capacity for acclimatization and adaptation. *Advances in Marine Biology* 46:183-214.

Correa AMS, Correa AMS, Baker AC, Baker AC. 2010. Disaster taxa in microbiologically mediated metazoans: how endosymbionts and environmental catastrophes influence the adaptive capacity of reef corals. *Global Change Biology* 17:68-77.

Császár NBM, Ralph PJ, Frankham R, Berkelmans R, van Oppen MJH. 2010. Estimating the potential for adaptation of corals to climate warming. *PLoS ONE* 5:e9751.

Dai C-F. 1988. Community ecology of corals on the fringing reefs of southern Taiwan. PhD thesis. Yale University.

Dai C-F, Chen Y-T, Kuo K-M, Chuang C-H. 1998. Changes of coral communities in Nanwan Bay, Kenting National Park: 1987-1996. *Journal of National Park Administration* 8:79-86.

Done T. 1999. Coral community adaptability to environmental change at the scales of regions, reefs and reef zones. *American Zoologist* 39:66-79.

Donelson JM, Munday PL. 2012. Thermal sensitivity does not determine acclimation capacity for a tropical reef fish. *Journal of Animal Ecology* doi: 10.1111/j.1365-2656.2012.01982.x.

Doney SC, Ruckelshaus M, Emmett Duffy J, et al. 2012. Climate Change Impacts on Marine Ecosystems. *Annual Review of Marine Science* 4:117-141.

Fan KL. 1991. The thermal effluent problems of three nuclear power plants in Taiwan. In: Oceanography of Asian Marginal Seas, (ed Takano K), Elsevier Oceanography Series, Amsterdam, The Netherlands 54:393-403.

Farde PR, Englebert N, Faria J, Visser PM, Bak RPM. 2008. Distribution and photobiology of *Symbiodinium* types in different light environments for three colour morphs of the coral *Madracis pharensis*: is there more to it than total irradiance? *Coral Reefs* 27: 913-925.

Fautin DG. 1997. Cnidarian reproduction: assumptions and their implications. In *Coelenterate Biology: Proceedings of the Sixth International Congress of Coelenterate Biology*; Naatboga Natuurhistorisch Museum: Leiden, The Netherlands, 151-162.

Ferrara GB, Murgia B, Parodi AM, et al. 2006. The assessment of DNA from marine organisms via a modified salting-out protocol. *Cellular & molecular biology letters* 11:155-161.

Fitt W, Brown B, Warner M, Dunne R. 2001. Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs* 20:51-65.

Ghalambor CK, McKay JK, Carroll SP, Reznick DN. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* 21:394-399.

Gittenberger A, Hoeksema BW. 2006. Phenotypic plasticity revealed by molecular studies on reef corals of *Fungia* (*Cycloseris*) spp. (*Scleractinia: Fungiidae*) near river outlets. *Contributions to Zoology* 75:195-201.

Goulet T. 2006. Most corals may not change their symbionts. *Marine Ecology Progress Series* 321:1-10.

Hennige S, Smith D, Walsh S. 2010. Acclimation and adaptation of scleractinian coral communities along environmental gradients within an Indonesian reef system. *Journal of Experimental Marine Biology and Ecology* 391:143-152.
Hoegh-Guldberg O. 1999. Climate Change, Coral Bleaching and the Future of the World's Coral Reefs. *Marine and Freshwater Research* 50:839-866.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, et al. 2007. Coral reefs under rapid climate change and ocean acidification. *Science* 318:1737fs und

Howells EJ, Beltrán VH, Larsen NW, Bay LK, Willis BL, Van Oppen MJH. 2011. Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change* DOI: 10.1038/NCLIMATE1330.

Howells EJ, Berkelmans R, van Oppen MJH, Willis BL, Bay LK. 2013. Historical thermal regimes define limits to coral acclimatization. *Ecology* 94:1078lls B

Howells EJ, Beltrán VH, Larsen NW, Bay LK, Willis BL, Van Oppen MJH. 2011. Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change* DOI: 10.1038/NCLIMATE1330.

Hwang R-L, Tsai C-C, Lee T-M. 2004. Assessment of temperature and nutrient limitation on seasonal dynamics among species of *Sargassum* from a coral reef in southern Taiwan. *Journal of Phycology* 40:463 of P

Keshavmurthy S, Hsu C-M, Kuo C-Y, Meng P-J, Chen CA. 2012. 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* 21:4393-4407.

Kozak KH, Wiens JJ. 2007. Climatic zonation drives latitudinal variation in speciation mechanisms. *Proceedings of the Royal Society - Biological Sciences* 274:2995gs of P

Kramer, Degen, Buschbom, Hickler, Thuiller, Sykes. 2010. Modelling exploration of the future of European beech (*Fagus sylvatica* L.) under climate change - Range, abundance, genetic diversity and adaptive response. *Forest Ecology and Management* 259:10Ecol

Kuo C-Y, Yuen YS, Meng P-J, et al. 2012. Recurrent disturbances and the degradation of hard coral communities in Taiwan. *PLoS ONE* 7:e44364.

Ladner JT, Barshis DJ, Palumbi SR. 2011. Protein evolution in two co-occurring types of *Symbiodinium*: an exploration into the genetic basis of thermal tolerance in *Symbiodinium*...
clade D. BMC Evolutionary Biology 12:217

LaJeunesse TC. 2001. Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus Symbiodinium using the ITS region: in search of a S region: in evel marker. Journal of Phycology 37: 866of Ph

LaJeunesse TC. 2002. Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Marine Biology 141:387iolog

LaJeunesse TC, Trench RK. 2000. Biogeography of two species of Symbiodinium (Freudenthal) inhabiting the intertidal sea anemone Anthopleura elegantissima (Brandt). Biological Bulletin 199:126−134

LaJeunesse TC, Bhagooli R, Hidaka M, DeVentier L, Done T, Schmidt GW, Fitt WK, Hoegh-Guldberg O. 2004. Closely related Symbiodinium spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients. Marine Ecology Progress Series 284: 147-161.

LaJeunesse TC, Pettay DT, Sampayo EM, Phongsuwan N, Brown B, et al. 2010a. 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. Journal of Biogeography 37: 785–800.

Little AF, MJH van Oppen, BL Willis. 2004. Flexibility in algal endosymbioses shapes growth in reef corals. Science 304:1492-1494.

Loya Y, Sakai K, Yamazato K, Nakano Y, Sambali H, Van Woesik R. 2001. Coral bleaching: the winners and the losers. Ecology Letters 4:122y Let

Marshall J, McCulloch M. 2002. An assessment of the Sr/Ca ratio in shallow water hermatypic corals as a proxy for sea surface temperature. Geochimica et Cosmochimica Acta 66:3263mica A

Marshall P, Baird A. 2000. Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs 19:155-163.

Meng P-J, Lee H-J, Wang J-T, Chen C-C, Lin H-J, Tew KS, Hsieh W-J. 2008. A long-term survey on anthropogenic impacts to the water quality of coral reefs, southern Taiwan. Environmental Pollution 156:67ment

Meesters EH, Nieuwland G, Duineveld G, Kok A, Bak R. 2002. RNA/DNA ratios of scleractinian corals suggest acclimatisation/adaptation in relation to light gradients and turbidity regimes. Marine Ecology Progress Series, 227 233ology Monaco CJ, Helmuth B. 2011. Tipping points, thresholds and the keystone role of physiology in marine climate change research. Advances in Marine Biology 60:123s in

Nussey DH, Postma E, Gienapp P, Visser ME. 2005. Selection on heritable phenotypic plasticity in a wild bird population. Science 310:304.

Oliver TA, Palumbi SR. 2011. Do fluctuating temperature environments elevate coral thermal tolerance? Coral Reefs 30:429eefsd

Ortiz JC, González-Rivero M, Mumby PJ. 2012. Can a thermally tolerant symbiont improve the future of Caribbean coral reefs? Global Change Biology 19:273Chang

Parmesan C, Yohe, G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37-42.

Pier J-J. 2011. Power uprate effect on thermal effluent of nuclear power plants in Taiwan. In: Nuclear Power—Operation, Safety and Environment (ed Tsvetkov P). InTech publication, Rijeka, Croatia. 287licatPochon X, Gates RD. 2010. A new Symbiodinium clade (Dinophyceae) from soritid foraminifera in Hawai‘i. Molecular Phylogenetics and
Evolution 56:492

Richter SS, Kipfer TT, Wohlgemuth TT, Guerrero CCC, Ghazoul JJ, Moser BB. 2012. Phenotypic plasticity facilitates resistance to climate change in a highly variable environment. Oecologia 169:269

Richier S, Furla P, Plantivaux A, Merle P-L, Allemand D. 2005. Symbiosis-induced adaptation to oxidative stress. The Journal of Experimental Biology 208:277

Riegl B, Purkis S, Al-Cibahy A, Abdel-Moati M. 2011. Present Limits to Heat-Adaptability in Corals and Population-Level Responses to Climate Extremes. PLoS ONE 6:e24802.

Rowan RR, Knowlton NN, Baker AA, Jara JJ. 1997. Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. Nature 388:265

Rowan R. 2004. Coral bleaching: thermal adaptation in reef coral symbionts. Nature 430:742

Rowan R, Powers DA. 1991. Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae). Marine Ecology Progress Series 71:65-73.

Shaish L, Abelson A, Rinkevich B. 2007. How plastic can phenotypic plasticity be? The branching coral Stylophora pistillata as a model system. PLoS ONE 2:e644.

Sampayo EM, Ridgeway T, Bongaerts P, Hoegh-Guldberg O. 2008. Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. Proceedings of the National Academy of Sciences 105: 10444 of the

Stat M, Gates RD. 2011. Clade D Symbiodinium in Scleractinian Corals: A e D emy of Sciencesy of corals are determined by fine-scale diJournal of Marine Biology 1urn

Strychar KB, Sammarco PW. 2009. Exaptation in corals to high seawater temperatures: Low concentrations of apoptotic and necrotic cells in host coral tissue under bleaching conditions. Journal of Experimental Marine Biology And Ecology 369:31 of

Thorntick DJ, LaJeunesse TC, Kemp DW, Fitt WK, Schmidt GW. 2006. Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. Marine Biology 148: 1887–1897

Tonk L, Sampayo EM, Weeks S, Magno-Canto M, Hoegh-Guldberg O. 2013. Host-Specific Interactions with Environmental Factors Shape the Distribution of Symbiodinium across the Great Barrier Reef. PLoS ONE 8(7): e68533.

Traill LW, Lim MLM, Sodhi NS, Bradshaw CJA. 2010. Mechanisms driving change: altered species interactions and ecosystem function through global warming. Journal of Animal Ecology 79:937

van Oppen MJH, Souter P, Howells EJ, Heyward A, Berkelmans R. 2011. Novel genetic diversity through somatic mutations: fuel for adaptation of reef corals? Diversity 3:405

Vose RS, Easterling DR, Gleason B. 2005. Maximum and minimum temperature trends for the globe: An update through 2004. Geophysical Research Letters 32:L23822.

Warner ME, LaJeunesse TC, Robison JD, Thur RM. 2006. The ecological distribution and comparative photobiology of symbiotic dinoflagellates from reef corals in Belize: Potential implications for coral bleaching. Limnology and Oceanography 51: 1887–1897

Weber M, Medina M. 2012. The role of microalgal symbionts (Symbiodinium) in holobiont physiology. Advances in Botanical Research 64:119
Table 1 (on next page)

Comparison of coral assemblages between 1986 and 2010 at NPP OL location.
Table 1.

| Year | Major assemblages at NPP OL (3m) | % of total cover | Minor assemblages at NPP OL (3m) | % of total cover | Reference |
|------|----------------------------------|------------------|----------------------------------|------------------|-----------|
| 1986 | **Acropora**                     | 24.20            | **Montastrea**                   | 3.35             | Dai, 1988 |
|      | **Montipora**                    | 33.69            | **Pocillopora**                  | 2.27             |           |
|      |                                  |                  | **Isopora**                      | 2.52             |           |
|      |                                  |                  | **Porites**                      | 1.84             |           |
|      |                                  |                  | **Seriatopora**                  | 1.58             |           |
|      |                                  |                  | **Stylophora**                   | 0.24             |           |
|      |                                  |                  | **Pavona**                       | 0.14             |           |
| 2010 | **Galaxea**                      | 21.20            | **Favites**                      | 5.40             | This study|
|      | **Montipora**                    | 21.70            | **Porites**                      | 3.00             |           |
|      |                                  |                  | **Pavona**                       | 3.30             |           |
|      |                                  |                  | **Acropora**                     | 0.70             |           |
|      |                                  |                  | **Seriatopora**                  | Not detected     |           |
|      |                                  |                  | **Stylophora**                   | Not detected     |           |
Table 2 (on next page)

Marginal tests with host genus HPCO1 and 2 and Symbiodinium
Table 2a. Marginal tests with host genus HPCO1 and 2 and *Symbiodinium* type

| Variable | Pseudo-F | P   | % variance explained |
|----------|----------|-----|----------------------|
| PAR(Kd)  | 0.83722  | 0.485 | 53.47                |
| Tw       | 0.62396  | 0.681 | 27.24                |
| Ts       | 1.5853   | 0.204 | 11.53                |
| Tavg     | 1.252    | 0.315 | 8.28                 |
| Tsd      | 1.3608   | 0.305 | 4.61                 |
| HPCO1ge  | 0.35728  | 0.801 | -1.13                |
| HPCO2ge  | 4.369    | 0.006 | -4.01                |

Table 2b. Marginal tests with host species HPCO1 and 2 *Symbiodinium* type

| Variable | Pseudo-F | P   | % variance explained |
|----------|----------|-----|----------------------|
| PAR(Kd)  | 0.83722  | 0.483 | 53.87                |
| Tw       | 0.62396  | 0.637 | 29.55                |
| Ts       | 1.5853   | 0.2   | 11.3                 |
| Tavg     | 1.252    | 0.314 | 5.26                 |
| Tsd      | 1.3608   | 0.27  | 3.72                 |
| HPCO1sp  | 1.6455   | 0.209 | -3.7                 |
| HPCO2sp  | 5.5322   | 0.001 |                     |

Table 2c. Marginal tests with host genus HPCO1 and 2 and *Symbiodinium* clade

| Variable | Pseudo-F | P   | % variance explained |
|----------|----------|-----|----------------------|
| PAR(Kd)  | 0.79276  | 0.482 | 96.98                |
| Tw       | 1.7847   | 0.182 | 3.82                 |
| Ts       | 3.766    | 0.071 | 0.58                 |
| Tavg     | 4.862    | 0.063 | 0.1                  |
| Tsd      | 11.06    | 0.029 | 0                    |
| HPCO1ge  | 1.4382   | 0.203 | -0.09                |
| HPCO2ge  | 1.8179   | 0.165 | -1.39                |

Table 2c. Marginal tests with host genus HPCO1 and 2 and *Symbiodinium* clade

| Variable | Pseudo-F | P   | % variance explained |
|----------|----------|-----|----------------------|
| PAR(Kd)  | 0.79276  | 0.473 | 96.86                |
| Tw       | 1.7847   | 0.197 | 3.88                 |
| Ts       | 3.766    | 0.055 | 0.59                 |
| Tavg     | 4.862    | 0.066 | 0.14                 |
| Tsd      | 11.06    | 0.031 | 0                    |
| HPCO1sp  | 0.3212   | 0.659 | -0.08                |
HPCO2sp  4.9074  0.057  -1.4
Figure 1

Map of the study area and sampling locations in Kenting, southern Taiwan.

Eight sampling sites including the nuclear power plant outlet (NPP OL) sites are shown by black stars.
Figure 2

Mean seawater temperatures

NPP OL (red line) and control (grey lines) locations measured at 3 m depth from 1986 to 1992 (A). Monthly average seawater temperatures at six locations including NPP OL (B).
Figure 3

Contour diagram for the seawater temperature at Nanwan.

The contour diagram of the sea surface temperature in Nanwan from May 2009 - May 2010. The red plume at the left is the constant hot water output from the nuclear power plant. The seawater near the nuclear power plant outlet are constantly hot irrespective of season.
Figure 4

*Symbiodinium* composition in 16 coral genera sampled at 3 m and 7 m seven locations and shown separately for NPP OL.

Distribution of *Symbiodinium* clades based on the restriction length fragment polymorphism (RFLP) at 3 m and 7 m in all coral hosts at 8 sites (A). Distribution of *Symbiodinium* clades in individual genera sampled from 8 sites (B) and distribution of *Symbiodinium* clades in 16 genera in NPP OL 3m and 7m (C). Brown bars = *Symbiodinium* clade C; light brown bars = *Symbiodinium* clades C+D, and yellow bars = *Symbiodinium* clade D.
Figure 5

One-way diamond mean plots of *Symbiodinium* clade C and *Symbiodinium* clade D composition at NPP OL (3 m and 7 m) (A). Comparison of total *Symbiodinium* clade C and *Symbiodinium* clade D composition at NPP OL (3 m and 7 m).
Figure 6

Coral communities at NPP OL over time showing the condition of reefs at 3 m and 7 m in 1986, 1995 and 2010
Figure 7

Coral host composition at 3 m and 7 m NPP OL site

Relative abundance of coral genera in 1986, 1995 and 2010 at NPP OL 3 m and 7 m. Black dotted line shows the change in *Acropora* abundance over time. Yellow dotted line shows the relative abundance of *Montipora* over time.
Figure 8

Distance based RDA plots

Environmental parameters and host genus information to *Symbiodinium* clades using the all the host genera (A). Environmental parameters and host genus information to *Symbiodinium* types using the all the host genera (B) and relation between genera and sampling sites (C). Biplot projections are shown for the effect of environmental factors including host data (HPCO1 and 2) and for the occurrence of a particular genera in relation to a sampling site. ‘the % of fitted’ explains the percentage of the variability in the original data explained by the axis, and ‘the % of total variation’ indicates the percentage of variation in the fitted matrix explained by the axis. Abbreviated site names are; Wanlitung (WLT), NPP OL, Houbihu (HBH), NPP Inlet (IL), Taioshih (TS), Tanziwan (TZW), Sanjiawan (SJW), and Longken (LK), and NPP Outlet (NPP OL)
