Received Date : 30-Jul-2013

Revised Date : 21-Feb-2014

Accepted Date : 25-Feb-2014

Article type : Primary Research Articles

Thermally tolerant corals have limited capacity to acclimatize to future warming

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/gcb.12571

Running head: Thermal acclimation by scleractinian corals

Keywords: thermal adaptation, physiological plasticity, climate change, ecological energetics, Mediterranean Sea, invasive species, coral bleaching

Type of paper: Primary Research Article

Abstract

Thermal stress affects organism performance differently depending on the ambient temperature to which they are acclimatized, which varies along latitudinal gradients. This study investigated whether differences in physiological responses to temperature are consistent with regional differences in temperature regimes for the stony coral *Oculina patagonica*. To resolve this question we experimentally assessed how colonies originating from four different locations characterized by >3°C variation in mean maximum annual temperature responded to warming from 20 to 32°C. We assessed plasticity in symbiont identity, density, and photosynthetic properties, together with changes in host tissue biomass. Results show that, without changes in the type of symbiont hosted by coral colonies, *O. patagonica* has limited capacity to acclimatize to future warming. We found little evidence of variation in overall thermal tolerance, or in thermal optima, in response to spatial variation in ambient temperature. Given that the invader *O. patagonica* is a relatively new member of the Mediterranean coral fauna our results also suggest that coral populations may need to remain isolated for a long period of time for thermal adaptation to potentially take place. Our study

indicates that for *O. patagonica*, mortality associated with thermal stress manifests primarily through tissue breakdown under moderate but prolonged warming (which does not impair symbiont photosynthesis and, therefore, does not lead to bleaching). Consequently, projected global warming is likely to causes repeat incidents of partial and whole colony mortality and might drive a gradual range contraction of Mediterranean corals.

Introduction

The capacity of species to persist throughout periods of global warming is influenced by their ability to adjust their physiology to cope with increased temperature (e.g., Stillman, 2003; Chown & Gaston, 2008). However, the process of physiological adjustment to temperature is species-specific, and is also governed by the particular environmental conditions to which local populations have become acclimatized and/or adapted (West & Salm, 2003; Angilletta, 2009). For instance, changes in metabolic physiology drive differences in the relationship between temperature and swimming speed in species of frogs that inhabit different locations along an elevation gradient (Navas, 1996). Similarly, the effect of temperature on various measures of organism performance (hereafter, 'thermal performance') differs according to incubation or culture temperature for Antarctic bivalves (Morley *et al*., 2012), soil microbes (Crowther & Bradford, 2013), and along a latitudinal gradient for damselflies (Dinh Van *et al*., 2013). Such occurrences of within-species variation in thermal performance, driven by differences in the temperature regime experienced by local populations, provide insight into how the thermal tolerance of species might evolve under global warming.

Although coral reefs have persisted throughout periods of rapid environmental change during their evolutionary history, their existence is currently threatened by ongoing increases in global temperature caused by the unprecedented rise in anthropogenic carbon dioxide

emissions (Salomon *et al*., 2009). From 1871-2007, average tropical (30ºN-30ºS) sea surface temperatures (SST) have increased by approximately 0.51ºC, at a rate of 0.04ºC/decade (Meehl *et al*., 2007). Current projections of the average global warming by the end of the 21st century range from 1.8ºC to 4.0ºC (Meehl *et al*., 2007), depending on the climate model used. Increased seawater temperature (alone or in combination with light intensity and/or nutrient imbalance, see Hoegh-Guldberg, 1999 and Wiedenmann *et al*., 2013 respectively) reduces the productivity of coral symbioses, and can cause mass coral 'bleaching' events. Such bleaching involves either the loss of coral photosymbionts ('zooxanthellae' in the dinoflagellate genus *Symbiodinium*) and/or degradation of their photosynthetic pigment complexes. Although a variety of interacting factors, including pollution, overfishing and ocean acidification, are recognised as threats to the persistence of coral reef ecosystems into the future (Bellwood *et al*., 2004), the rise in ocean temperature is generally considered to be the factor with the most immediate and catastrophic impact on coral populations (Great Barrier Reef Marine Park Authority, 2009). In this study, we aimed to determine whether thermal performance of the stony coral *Oculina patagonica* differed between populations located along a latitudinal temperature gradient in order to gain insight into the potential for corals to acclimatize or adapt to global warming.

Likely in response to the devastating effects of coral bleaching events on reef ecosystems, research into the thermal biology of corals has tended to focus on quantifying maximum temperature thresholds for coral bleaching and survival (Coles *et al*., 1976; Brown *et al*., 2000; Fitt *et al*., 2001; Maynard *et al*., 2008). Additionally, there has been a strong focus on investigating how maximum thermal thresholds depend upon the identity of the different *Symbiodinium* clades hosted by coral colonies and/or populations (e.g. Rowan *et al.,* 1997; Baker *et al*., 2004; Jones *et al*., 2008). However, there are numerous tradeoffs inherent in adjusting different aspects of thermal performance (e.g., increasing heat tolerance tends to

decrease cold tolerance, see Angilletta, 2009). Consequently, investigating just one aspect of thermal performance (e.g., the bleaching threshold) provides a limited understanding of how ambient temperatures affect coral populations. Resolving whether corals can acclimatize to global warming requires additional quantification of the optimal temperature for holobiont outcomes such as growth, and the temperature range over which these outcomes are positive. In the case of growth, and in contrast to extensive documentation of maximum thermal thresholds for corals, geographic variation in the optimal temperature for coral growth has been demonstrated for only one species (*Pocillopora damicornis*, Clausen & Roth, 1975). In contrast, a study of *Orbicella annularis* (formerly *Montastraea annularis,* Budd *et al.,* 2012) found no indication of differences in the optimal temperature for net productivity among sites (lagoon *versus* outer reef) despite differences in ambient temperature regimes (Castillo & Helmuth, 2005). Clearly, understanding the factors that control thermal performance of corals requires further research. Such studies are likely to be particularly informative in sub-tropical and temperate areas due to the large variation in ambient temperature experienced by resident species in those locations. Moreover, temperate locations, like the Mediterranean Sea, are likely to be among the regions most affected by climate change (IPCC, 2007; Coll *et al*., 2010).

Among the Mediterranean corals, *O. patagonica* is a recent immigrant from the cold/temperate south Atlantic (Zibrowius, 1974) and now inhabits both the northern cold regions (Ligurian Sea, SST 13-26°C) and the warm south-eastern regions (Levantine coast, SST: 16-32°C). Although originally discovered in the Ligurian Sea (NW Mediterranean) in 1966 (Zibrowius, 1974), it has mostly developed along the Spanish and Catalan coasts (Zibrowius & Ramos, 1983; Rubio Portillo *et al*., 2013; Serrano *et al*., 2013), suggesting that the species first settled in Spain before spreading to other regions of the Mediterranean, such as along the Israeli coasts (Fine & Loya, 1995; Fine *et al*., 2001), the Levantine (Bitar &

Zibrowius, 1997; Çinar *et al*., 2006), Aegean (Salomidi *et al.,* 2006; 2013), northern African coasts (Sartoretto *et al*., 2008), and the Ligurian Sea (see review by Fine *et al.,* 2001). It is also spreading geographically from the initial population established along the Spanish coast, with new colonies observed throughout the Mediterranean Sea, and with its presence at previously un-occupied sites all along the Spanish and Catalan coasts increasing during the last decade (Coma *et al*., 2011; Serrano *et al*., 2013, Rubio Portillo *et al*., 2013). Although *O. patagonica* is evidently able to acclimatize to a wide range of temperatures, studies performed in different regions have reported vastly different physiological responses to exposure to high temperatures. In Israel, laboratory and field studies of *O. patagonica* have shown that it often bleaches during the summer (e.g. Fine & Loya, 1995; Kushamaro *et al*., 1996) and recovers during the winter (Shenkar *et al*., 2005, 2006). In contrast, bleaching has never been reported for colonies either from cooler regions, where corals have instead suffered mass mortality at the end of particularly warm summers (e.g. Cerrano *et al*., 2000; Perez *et al*., 2000; Garrabou *et al*., 2009), or along the Spanish and Catalan coasts (Serrano *et al*., 2013). Abnormal summer temperatures have also been demonstrated to be the causative agent of the tissue breakdown and mortality (without bleaching) for this and other coral species in the north Mediterranean (e.g., Cerrano *et al.*, 2000; Rodolfo-Metalpa *et al*., 2006a, 2008; Kersting *et al*., 2013).

The present distribution of *O. patagonica* encompasses regions with very different thermal regimes, both in terms of the duration of the warm season and the temperature range. Moreover, contrasting physiological responses to high temperature stress exhibited at different locations throughout this coral species' geographic range suggest that there is strong environmental control over thermal performance. Consequently we aimed to quantify how thermal physiology varies among colonies of *O. patagonica* sampled from different locations within the Mediterranean, and to investigate whether differences in thermal performance

(optimal temperature, temperature tolerance and temperature thresholds) were consistent with regional differences in temperature regimes. To do so, we experimentally assessed plasticity in symbiont identity, density, and photosynthetic properties, together with changes in host tissue biomass in response to warming from 20 to 32°C, for colonies originating from four geographically distinct locations (over a 12° latitudinal and a 35° longitudinal gradient), using a common garden experimental approach. In addition, at the end of the summer, we recorded the occurrence of bleaching and/or tissue breakdown for coral colonies in the field at each of the four locations. Understanding the mechanisms that underlie geographic variation in the capacity for thermal tolerance can improve our ability to project the responses of coral populations to climate change.

Materials and Methods

Study locations, measurements and sampling

Field work for this study, including coral collection, was conducted at four locations (Fig. 1a): Albissola, Italy, in the Ligurian Sea (44°19'19" N, 8°29'55" E), Alicante and Portman, Spain, in the Balearic Sea (38°20'05"N, 00°29'23 W and 37°34'45"N, 00°50'39" W respectively) and Haifa, Israel (32°30'23" N, 34°53'30" E). During the end of summer 2009 (September-October) photographic surveys with an underwater Sony DSC-N2 digital camera were carried out to record the occurrence of bleaching and/or tissue breakdown for all colonies encountered at the four locations. In Albissola, the occurrence of bleaching and/or tissue breakdown on colonies was also monitored regularly during the summer seasons between 2003 and 2011. We note that we did not formally survey the density of colonies at each site because this has been documented in previous studies (e.g., Shenkar *et al.* 2005, 2006; Rubio Portillo *et al*., 2013; Serrano *et al*., 2013). In addition, we collected coral

samples for the thermal stress experiment and for the characterization of *Symbiodinium* communities in colonies at each location at 3 m depth. We also deployed Onset HOBO® Pro data loggers from June to September 2009 to record (hourly) seawater temperature at 3 m depth in the coral habitats at each location.

Response to thermal stress: experimental set-up

During October 2009, healthy samples (N = 336 nubbins, 2-5 cm^2 in size, 10-20 polyps $nubbin^{-1}$) were collected from multiple encrusting colonies of *O. patagonica* from each of the four study locations (30 from each location). Nubbins were transported back to the laboratory at the Centre Scientifique de Monaco (CSM), within one to three days of collection, where they were equally and randomly divided among eight 18 L flow-through aquaria (two replicates per sampling location, 42 nubbins per aquarium, Supplementary Information A, Fig 1A). Nubbins were widely spaced within aquaria so as not to shade or contact each other. Seawater flow within these aquaria came from a continuous supply of seawater into the CSM laboratories that is pumped from 50 m depth in the Mediterranean, and was supplied to aquaria with a turnover rate of 30% h^{-1}. Light-intensity was provided using metal halide lamps and neutral-density shade screens, and was carefully measured using a Li–Cor 4π spherical underwater quantum sensor (LI–193SA) to ensure a consistent level of 70 µmol photons m^{-2} s^{-1} for all tanks, with a 12h light: 12h dark photoperiod. Light levels in the Mediterranean Sea vary with season and water depth and the light intensity used in our experiment was selected to be non-stressful and ecologically relevant based on previous experiments on temperate corals (e.g.: Rodolfo-Metalpa *et al.*, 2008; Linares *et al.*, 2013; Ezzat *et al.*, 2013). Light was rigorously controlled to enable us to assess the effect of only one stressor (i.e., temperature) on coral performance. Also, since temperature was different between locations during collection (20-24°C), we gradually acclimated (1°C per

day for four days) all samples to an initial temperature of 20°C which represents the lowest temperature recorded in October in the northwest Mediterranean. This temperature was kept constant for ~three weeks before starting the first measurement, and served as a control temperature and to minimise any effects of prior colony health. Subsequently, temperature was gradually increased to a maximum of 32°C in steps of 2°C (7 levels) over a 14-week period (Supplementary Information A, Fig 1A). We included the temperature of 32ºC for consistency with the wider coral thermal tolerance literature. This temperature regime was designed to enable measurement of coral physiological response to a large range of temperatures, comparable to those in the Mediterranean during the spring and summer seasons. Each temperature step was maintained for two weeks to acclimate the corals to the temperature treatment, and the increase in temperature between steps was implemented over four days (a ramping rate of 0.5°C day^{-1}). This experimental design enabled us to monitor the cumulative effects of increasing ocean temperature on coral health, similar to the ocean warming observed during spring-summer in the Mediterranean Sea. We note that, during coral bleaching events, both the magnitude of the temperature increase and the duration of exposure to increased temperatures determine when corals bleach (e.g., Berkelmans, 2002). Temperature was controlled to within ± 0.1°C using temperature controllers (Corema) connected to 300 W submersible heaters. Submersible pumps (Micro-jet, Aquarium Systems) ensured water circulation inside the aquaria and corals were fed twice weekly with *Artemia salina* naupli.

Photosynthetic and respiration rates

At the end of every 2-week period of constant temperature exposure, photosynthesis and respiration rates were measured for 6 nubbins from each location (three nubbins from each of two tanks per location), and nubbins were then frozen at -20°C for subsequent

measurements of symbiont density and chlorophyll concentration (chl). Rates of respiration and photosynthesis were measured using a set of three closed thermostated Perspex chambers filled with ~50 mL of seawater coupled with a Strathkelvin oxygen electrode system (Strathkelvin 928 oxygen meter with computer interface). Chambers were maintained at the relevant treatment temperature using a minichiller. Oxygen electrodes were calibrated at the relevant treatment temperature using N_2- and air-bubbled enriched seawater as 0% and 100% oxygen saturation values respectively. The chambers were continuously stirred using magnetic stirrers, and light was provided by a HQI metal halide lamp with all measurements made at a light intensity of 70 µmol photons m^{-2} s^{-1}. During each 20-min incubation, corals were allowed to acclimate within the chambers for at least 10 min prior to measurement, and their net photosynthesis (P_n) was measured first, followed by the respiration (R) in the dark. We therefore calculated and compared between populations the gross photosynthesis as $P_g = P_n - R$. Photosynthetic rates were normalized to surface area measured using the aluminum foil method (Marsh, 1970). Measurements of colony photosynthesis using oxygen respirometry were supplemented with measurements of symbiont photochemical efficiency obtained using pulse amplitude modulated (PAM) fluorometry. To do this, we measured dark-adapted maximum photosynthetic yield (i.e., dark-adapted Fv/Fm, the maximum efficiency of light use for photosynthesis, see Maxwell & Johnson, 2000) of coral nubbins at the end of every 2-week period of temperature exposure using a Dual PAM fluorometer (Walz GmbH, Effeltrich, Germany). At each sampling time, Fv/Fm was measured after a 15-min dark acclimation period (after Hoegh-Guldberg & Jones, 1999) for six additional nubbins (three nubbins from each of two tanks) for each location. These nubbins were subsequently frozen at -80°C for protein assays.

Symbiodinium densities, chlorophyll, and protein content

Symbiodinium densities and chl $a + c_2$ concentrations were determined for all samples used for the photosynthesis measurements during the thermal stress experiment (N = 168, 6 replicate nubbins from 4 locations at 7 temperatures). Tissues were separated from the skeleton using an air-pick and homogenised in 7 mL of GF/C (Whatman) filtered seawater using a hand-held Potter tissue grinder. A sub-sample (1 mL) of this tissue slurry was used to measure the density of *Symbiodinium* while the remaining homogenate was used to measure chl $a + c_2$ concentration. At least 300 *Symbiodinium* cells were counted in 10 sedimentation chambers of known volume, using an inverse microscope (Leica, Wetzlar, Germany) and the Histolab 5.2.3 image analysis software (Microvision, Every, France). The remaining tissue slurry was centrifuged at 8,000 g for 10 min and the supernatant discarded. The *Symbiodinium* pellet was then re-suspended in 5 mL of acetone and kept in the dark for 24 h at 4°C. Samples were centrifuged for 15 min at 11,000 g and absorbance measured at three wavelengths (750, 663, 630 nm) on a spectrophotometer (SAFAS), and the equations of Jeffrey & Humphrey (1975) were used to calculate chl concentrations based on these absorbance readings. In addition, the samples used for the photochemistry measurements (N = 168) were assayed for total protein content using a bicinchoninic acid protein assay (Uptima, Interchim). For this purpose, each sample was treated with 1N sodium hydroxide for 30 minutes at 90°C. The slurry was then incubated in 96-well microplates with a dye reagent (Uptima Reagents, Interchim) for 30 min at 60°C. Protein standards across a range of concentrations from 0 to 2,000 µg ml^{-1} were also prepared using Bovine Serum Albumin (BSA, Interchim). Protein concentrations were finally determined by reading the absorbance at 560 nm relative to that of the protein standards using the GENESIS program (Kontron Instruments). All measurements were normalized to the nubbin surface area.

Genetic identification of Symbiodinium

Three nubbins from each of the four locations were collected during October 2009 and immediately frozen at -80°C. Frozen samples were processed by airpiking tissue from samples and extracting DNA from these blastates using a SOIL DNA Kit according to the manufacturer instructions. The ITS-2 region was amplified from each sample using the *Symbiodinium*-specific primers 'ITSintfor2' and 'ITS2clamp' (LaJeunesse & Trench, 2000) with the following profile: an initial denaturing step of 94°C for 3 min, followed by 35 cycles of 1 min at 94°C, 1 min at 58°C, and 1 min at 74°C, followed by a single cycle of 7 min at 74°C. Products were electrophoresed on 1.2% agarose gels to check for amplification success. *Symbiodinium* amplicons were then separated using denaturing gradient gel electrophoresis (DGGE, 35-75% gradient, Sunnucks, 2000) on a CBS Scientific system (Del Mar, CA, USA). Prominent bands characteristic of unique profiles (as described by LaJeunesse, 2002) were excised and re-amplified using the same primer set (without the GC clamp) under the conditions described above. Sequencing was performed using a Big Dye Terminator v. 3.1 cycle sequencing kit and an Applied Biosystems 3730xl DNA Analyzer (Foster City, CA, USA). Sequences were assembled and edited using the Vector NTI™ Advance 10 software (Invitrogen, Carlsbad, CA, USA) and then identified using BLAST searches against known sequences on GenBank.

Data analysis

To assess whether the temperature response of *O. patagonica* varied among the four study populations, we fitted thermal performance curves to the data for each physiological process, and then used a formal model selection procedure to determine whether the data supported geographic differences in the shape of the fitted curves. Previous studies indicate that a Gaussian curve is the most parsimonious function to describe the relationship between

temperature and physiological performance (Angilletta, 2006). Hence, we fitted the following equation to our data:

$$P_x = M_x \exp [-0.5(\text{abs } (T-T_{opt}))/T_{tol})^2] \qquad \text{Equation 1}$$

where P_x is the temperature (T) dependent physiological response, M_x is the maximum value of that response, T_{opt} is the optimal temperature (i.e., the mean of the function) and T_{tol} indicates the breadth of the thermal response (i.e., the standard deviation of the function). First, we tested for tank effects by fitting Eq 1 to the data for each response variable both separately for each tank and aggregated across tanks and then using a likelihood ratio test (LRT) to determine whether including tank provided a significantly better fit to the data. There was no evidence of tank effects for 5 of the 6 variables (LRT, $p > 0.42$ for each of protein concentration, respiration rate, symbiont density, chl concentration and photosynthetic yield but $p < 0.01$ for net photosynthesis rate) supporting our treatment of coral fragments as independent replicates.

To determine whether one or more of the fitted parameters (M_x, T_{opt} or T_{tol}) varied among the different coral populations we fitted Eq. 1 to all the data (i.e., pooled across locations, model 1 with 3 coefficients). Subsequently, we fixed two of the fitted parameters as equal to the parameter estimated from the pooled data and re-fit the model to estimate population specific parameters one at a time. For example, we fixed T_{opt} and T_{tol} at the values estimated from the pooled data and re-fit M_x by population (model 2 with 6 estimated parameters), and so on for T_{opt} (model 3, 6 parameters) and T_{tol} (model 4, 6 parameters). Finally, we re-fit the model allowing all parameters to vary by location (model 5, 12 parameters). Models were fit to data using least-squares non-linear regression implemented in R version 3.0 (The R Foundation for Statistical Computing) using 'nls' (see Supplementary

Information B). The same parameter estimation procedure was conducted for all 6 of the measured response variables (symbiont density, chl concentration, maximum photochemical yield, holobiont photosynthesis, holobiont respiration and holobiont protein content). An information theoretic model-selection approach was used to determine which of models 1 – 5 was most likely given the data. To do this, we extracted the negative log-likelihood for each model fit and calculated Akaike Information Criterion values (AIC) for each model given the number of fitted parameters. AIC values were then converted to Akaike weights (see Burnham & Anderson, 2002) to determine the relative support for each model with respect to each physiological response variable. Consequently, we were able to assess which, if any, aspects of thermal performance varied between local populations of *O. patagonica*. Based on these analyses we were able to detect: i) thermal adaptation as an increase in T_{opt} coincident with an increase in mean environmental temperature; ii) among-location differences in thermal tolerance through differences in T_{tol}; and iii) among-location differences in trait values when colonies were at their optimal temperature through differences in M_x. Finally, to gain additional insight into effects of sampling location on coral health, we used one-way ANOVA to test whether there were among-location differences in values of key physiological traits at the control temperature (20°C) and after 14 weeks of cumulative heat stress (at the end of the experiment). For these analyses, data were square root transformed (for the chl data) or log transformed (for the net photosynthesis data) to meet ANOVA assumptions of homogeneity of variance and normality of residuals, as visually assessed by inspection of normal QQ plots and residuals versus fitted values.

Results

Overall abundance of Oculina patagonica at the four study locations

Encrusting colonies of *O. patagonica* were found at all four study locations between depths of 0.5 to 6 m and tended to be more common on sub-vertical rocky areas of the substratum

compared with horizontal areas. Tens of isolated small colonies, approximately 10-30 cm in diameter, were found in Haifa and in Portman, while in Albissola only four colonies, three of ~50 cm in diameter and one very large colony covering around 5-6 m^2 (Zibrowius, 1974), were observed. In Alicante, colonies covered approximately 50-60% of an artificial wall inside a large harbour (~200 m long) at depths up to 6 m (see Fine & Loya, 1995; Izquierdo *et al*., 2007; Rubio Portillo, 2013). Sampling locations at Haifa, Albissola and Portman were well circulated, whereas the location at Alicante was more enclosed, likely with prolonged water retention.

Among-location variation in spring-summer temperature regimes in situ

Summer seawater temperatures differed substantially between locations, showing a gradient of increasing temperature from Albissola to Haifa (Fig. 1b). Mean (and maximum) seawater temperatures from 1st June to 20th September 2009 were 24.4°C (28.08°C), 25.1°C (28.06°C), 26.8°C (29.45°C) and 28.0°C (30.7°C) for Albissola, Portman, Alicante and Haifa, respectively. The sampled locations also differed in their length of exposure to temperatures. During the four months of measurement, 67%, 74%, 85% and 100% of the temperature records were above 24°C at Albissola, Portman, Alicante and Haifa, respectively. Moreover, temperatures higher than 27°C were more frequent in Alicante and Haifa (64 and 81%, respectively) than in Albissola and Portman (2 and 26% of records, respectively). Temperatures reaching 29°C were occasionally recorded in Alicante (2%) and frequently in Haifa (49%) but never recorded at the other two locations.

End-of-the-summer coral tissue appearance

At the end of summer, coral tissue appearance differed among the four populations (Fig. 2), and also among colonies within each population. Colonies from Alicante and Haifa showed a

variety of appearances including: white or slightly brown-colored with their tentacles retracted (Fig. 2a); fully bleached with expanded transparent polyps (Fig. 2b); rarely showing patchy areas of tissue loss (denuded skeleton; i.e., tissue breakdown) and areas where polyps were normally pigmented and sometimes expanded (as in Fig. 2c); live brown polyps but without any connecting tissue between polyps and with the whole skeleton completely denuded; or healthy without any sign of bleaching or tissue loss. In contrast, colonies from Albissola and Portman were never found to be bleached but they were found with several visible patches of denuded skeleton (Fig. 2c). In Albissola, where colonies were monitored regularly over an extended time period (2003-2011) tissue breakdown, involving surface areas of 9 to 300 cm^2 was observed at the end of the summers 2005, 2006, 2008, 2009, 2010 and 2011. At this location, areas of denuded skeleton were gradually covered by fouling organisms and were slowly partially recovered by new tissue (see also Rodolfo-Metalpa *et al*., 2008).

Genetic identification of Symbiodinium

All 12 colonies of *O. patagonica* (three from each of four locations) were characterized by a single dominant band on DGGE gels which, when excised and sequenced, was a 100% match to *Symbiodinium* B2.

Response of colonies to experimental thermal stress

Over the course of 14 weeks of gradually increasing experimental temperatures, we observed a general decline in symbiont density, chl concentration and photochemical efficiency for colonies of *O. patagonica* from each of the four locations (Fig. 3). The magnitude of the decline in both symbiont density and chl concentration was highest for colonies from Albissola and Portman (Fig. 3a-h), although analysis of variance indicated that the

cumulative effect of temperature stress on chl content was consistent among locations (two-way ANOVA, site x temperature interaction, $F_{(3,40)} = 1.1$, $p = 0.36$). Corals from Albissola and Portman also had the highest initial levels for these variables at 20-22°C (~8 x 10^6 cells cm^{-2} and 10 mg cm^{-2} at Albissola and Portman compared with ~3 x 10^6 cells cm^{-2} and 5 mg cm^{-2} at Alicante and Haifa for symbiont density and chl concentration respectively). In contrast, both the initial photochemical yield and the rate of decline in yield during experimental heating were approximately consistent among the populations, although there was high within-population variability for this variable for colonies from Alicante (Fig. 3k). Although an approximately 4-fold decrease in the *Symbiodinium* and chl contents occurred in nubbins from all locations no tissue necrosis was observed, even at the highest temperature.

We found very little support for geographic variation in thermal tolerance with respect to the symbiont-related traits (i.e., symbiont density, chl and photochemical efficiency, Supplementary Information C, Table C1). For symbiont density, the formal model selection procedure showed negligible support for among-location variation in either the optimal temperature (T_{opt}) or the thermal tolerance range (T_{tol}, Supplementary Information C, Table C1). Maximum symbiont density, however, did vary among locations and declined in response to increasing local average summer temperatures (Supplementary Information C, Fig C1a). Similar patterns of variation were observed for chl concentration (Fig C1b, e, h) and photochemical efficiency (Fig. C1c, f, i). For these two traits, there was model support for among-location variation in the maximum value (M_x) of these traits (wAIC = 0.14 for chl and 0.66 for photochemical efficiency), and for variation in each of M_x, T_{tol} and T_{opt} (wAIC = 0.86 for chl and 0.34 for photochemical efficiency). Nevertheless, for both chl concentration and photochemical efficiency, variance in the location-specific estimates of T_{opt} and T_{tol}

largely overlapped with the variance in the overall fitted estimate (pooled over location, model 1) for these parameters (i.e., error bars on points lie within shaded region, Fig. C1d-i). Moreover, there was relatively high variation in parameter estimates for these traits (particularly for photochemical efficiency), both overall and by-location. This variation reflects the fact that, for these traits, our data mostly lie above the optimal temperature (Fig. 4e-l); the absence of data from below the optimal temperature leads to high variance in the estimates of T_{opt} and $T_{tol,}$ and some ambiguity regarding whether these parameters vary among locations. Uncertainty in the shape of the thermal performance curve was particularly pronounced for the chl content of colonies collected from Haifa because the mean of the values at 20°C was higher than the mean at 22°C (and similarly, for the photochemical yield of samples from Haifa and Portman). We note, however, that when compared to the fit of a linear regression, Eq 1 provided an equally good fit to the photosynthetic yield data (F-test, $p > 0.17$), and a better fit (F-test, $p < 0.001$) to the chl data. Overall, neither the optimal temperature (T_{opt}) nor the overall thermal tolerance (T_{tol}) varied consistently in response to mean summer temperatures experienced *in situ* (Supplementary Information C, Fig. C1e-f and h-i).

Each of photosynthesis rate, respiration rate and tissue biomass (measured here as protein content) displayed a clear hump-shaped relationship with increasing temperature during the 14-week experimental heating period (Fig. 4). Parameters describing these curves were estimated with relatively high precision due to exposure of corals to temperatures above and below the optimal temperature (Supplementary Information C, Fig. C2, shaded regions and error bars are narrow compared with those in Fig. C1). Similar to our findings regarding model support for among-location variation in M_x for symbiont density, chl concentration and photochemical efficiency (Fig. 3 and C1), mean values for respiration, photosynthesis and protein content varied among the four sampling locations (M_x, Supplementary

Information C, Table C1 and Fig C2). Moreover, M_x for each of these physiological traits tended to decrease in response to increased mean summer temperatures *in situ* (Fig. C2a-c), although the high rate of photosynthesis at the optimal temperature for colonies collected from Haifa was contrary to this trend (Fig. C2a). In contrast, analysis of variance indicated that initial photosynthesis rate (at the control temperature) was higher than at the end of the experiment (two-way ANOVA, temperature effect $F_{(1,40)} = 49$, $p < 0.001$) but this effect was consistent among sites (two-way ANOVA, site x temperature effect $F_{(3,40)} = 1.3$, $p = 0.29$). Hence, exploring the full thermal performance curve revealed differences among populations that were not evident when physiology was compared between the initial (control) and final (heated) groups alone.

Although there was a degree of support for differences in each of M_x, T_{tol} and T_{opt} among locations (model 5, wAIC = 0.27 for respiration, 0.28 for photosynthesis and 0.38 for protein, Supplementary Information C, Table C1), variance in location-specific estimates for T_{tol} all overlapped with the variance in the overall fitted estimate (error bars on points overlap shaded regions in Fig. C2g-i). In contrast, our data indicate that the optimal temperature for respiration and protein, processes that are both dominated by host instead of symbiont physiology, does vary among locations (Fig. C2e-f, error bars on points do not overlap shaded regions). In addition, there was minimal (but non-negligible) model support for among-location variation in T_{opt} only (Supplementary Information C, Table C1) indicating that the optimal temperature for respiration, in particular, varies among populations. Finally, T_{opt} for photosynthesis, respiration and protein did tend to increase with increasing mean summer temperatures experienced by the source populations (Fig. C2d-f) although only up to a threshold ambient temperature between 27 – 28°C.

Discussion

Understanding the ability of corals to resist bleaching due to thermal stress is critical for predicting how reefs will change in response to rising global temperatures. By investigating thermal performance in respect to multiple host- and symbiont-related physiological processes we here show that, given that there are no changes in the type of symbiont hosted by coral colonies, the stony coral *Oculina patagonica* has limited capacity to adjust its physiology to match local temperature regimes. This interpretation is supported by a formal model selection procedure that showed minimal support for variation in overall thermal tolerance, or in thermal optima, among resident populations of corals that were acclimatized/adapted to different temperature regimes in the field. Symbiont thermal physiology, in particular, was consistent among colonies sourced from different regions of the Mediterranean. In contrast, we show that populations living in cold coastal areas have very high symbiont densities (Albissola and Portman) and undergo dramatic tissue breakdown in response to increasing ocean temperatures during summer, instead of the gradual loss of symbionts that is observed in colonies of populations in warmer environments, with lower symbiont concentrations (Alicante and Haifa). Nevertheless, tissue breakdown was not observed in response to warming in the laboratory, suggesting either that this phenomenon only occurs under specific warming regimes, or that temperature interacts with other environmental factors (such as light intensity, food shortage and ambient nutrient levels) in the field to cause tissue breakdown instead of symbiont loss. Overall, our results confirm that *O. patagonica* is able to tolerate a wide range of environmental temperatures and we show, for the first time, that the ability of this species to adjust its physiology according to local environmental temperature (i.e., acclimatize or adapt) is minimal.

Oculina patagonica is considered an immigrant species that has invaded the Mediterranean from the temperate SW Atlantic (Zibrowius, 1974). As a result of being recent

immigrants, it is likely that Mediterranean *O. patagonica* at these geographically distinct sites are closely related to one another. However, differences in the observed responses to temperature (bleaching in the south, *versus* tissue breakdown in the north) raise the question of how and why genetically similar corals respond differently to thermal stress. Because the symbiosis between *Oculina* and *Symbiodinium* is not considered obligate (*Oculina* can readily be found in an aposymbiotic state elsewhere in the Atlantic: Reed *et al*., 1981, and in Mediterranean caves: Koren & Rosenberg, 2008), expulsion of symbionts might be an expected general response when symbionts are no longer beneficial to the host. In other coral species increased thermal tolerance can be achieved by hosting different *Symbiodinium* that are more tolerant of high irradiance and temperature (e.g. Buddemeier & Fautin, 1993; Jones *et al*., 2008), or through local adaptation of symbionts (Howells *et al*., 2012). However, our analyses indicated that colonies of *O. patagonica* from the four Mediterranean populations all host the same *Symbiodinium* type (B2) so, clearly, differences in symbiont type cannot explain these patterns. However, symbiont densities per unit surface area were twice as high in colonies from the coolest sites (Rodolfo-Metalpa *et al*., 2008; Movilla *et al*., 2012) than in colonies from the warmest sites (Shenkar *et al*., 2005, 2006). Identifying the mechanisms underlying this difference is beyond the scope of our study and we suggest that further research testing for interactions between temperature and other environmental variables would be informative.

A first explanation of the differential responses to temperature stress (i.e., bleaching versus tissue necrosis) that we observed at our study locations *in situ*, can be due to the occurrence of a microbial agent (*Vibrio spp.)* in populations exposed to higher and persistent temperatures (i.e., Haifa and Alicante). Indeed, both *in situ* and laboratory studies have shown that the bleaching response in the eastern Mediterranean can be explained by the interaction between high temperature and increased virulence of *Vibrio shiloi* (Kushamaro *et*

al., 1996), although other studies have shown seasonal bleaching without the presence of the bacterium (Ainsworth & Hoegh-Guldberg, 2008; Ainsworth *et al*., 2008). Regardless of whether bleaching of colonies is directly caused by the action of a bacterial disease, annual bleaching along the Israeli coast is certainly temperature dependent, occurring when ambient temperatures rise above ~26°C to reach 30-31°C (Shenkar *et al*., 2006). Although there is ongoing debate regarding the role of coral disease as a driver of coral bleaching, if disease virulence increases above a temperature threshold that is only reached in the southern part of the Mediterranean (i.e. Israel), and where temperatures are locally higher than normal such as in the Alicante harbour (Spain), this mechanism could explain the differential responses to temperature stress that we observed at our study locations. Nonetheless, the repeated cycle of annual bleaching and recovery during winter (Shenkar *et al*., 2006; Armoza-Zvuloni *et al*. 2011) supports our interpretation that *O. patagonica* has limited capacity to acclimatize or adapt to thermal stress.

Alternatively, the differential responses to temperature stress between *O. patagonica* populations from locations with different temperature regimes can be related to differences in the amplitude and duration of warming between these regions. In corals from the Ligurian Sea, *Symbiodinium* density generally increases from January-February to June (20 – 21°C), in parallel to a gradual increase in temperature during spring as well as to the supply of nutrients from the winter mixing of deep waters. It then suddenly decreases in September concomitant with a rapid increase in temperature up to 25°C (Rodolfo-Metalpa *et al*., 2008). In contrast, *Symbiodinium* density in corals from Israel increases until March (20 – 21°C) and then gradually decreases reaching near zero concentrations in September concomitant with a gradual increase in temperature up to 30°C (Shenkar *et al*., 2006). Therefore, the decrease in

symbiont density in Israel begins 2 – 3 months sooner than in the Ligurian Sea, allowing gradual expulsion of symbionts to occur which would mitigate oxidative stress caused by high symbiont densities at high temperature and irradiance levels (e.g., Lesser, 1996; Cunning & Baker, 2013). Several studies, including this one, have established that *Symbiodinium* within *O. patagonica* maintain high rates of photosynthesis under experimental conditions of 24 – 26°C (Rodolfo-Metalpa *et al*., 2006b). Therefore, when temperatures >24°C persist in the field for >6 weeks (as in Albissola, see Fig. 1b) the high metabolic activity of symbionts combined with very high symbiont densities, potentially causes tissue breakdown due to accumulated oxidative-stress. Under such conditions, the coral host appears to be unable to expel symbionts rapidly enough to prevent severe oxidative damage, and tissue breakdown precedes bleaching. Clearly, prolonged exposure to high temperatures that remain below the threshold for bleaching may lead to cumulative thermal stress that is equally damaging to host physiology as the bleaching that occurs at higher temperatures.

Congruent with the second hypothesis (i.e., that duration and amplitude of the stress control whether temperature stress results in bleaching or tissue breakdown), we did not observe any signs of tissue breakdown in response to warming in the laboratory. Although we cannot definitively identify which environmental variable might interact with temperature to cause tissue breakdown in the field, we suggest that location specific light intensity is the most likely co-factor because, for corals, effects of thermal stress are generally more severe under high light intensities (e.g. Lesser, 1996). Our experimental irradiance was lower than the maximum observed in the field (Rodolfo-Metalpa *et al*., 2008), likely reducing the severity of thermal stress. Conversely, exacerbation of oxidative stress due to poor gas exchange

between coral tissue and seawater under low water flow conditions (e.g. Finelli *et al*., 2006) is not consistent with our results because tissue breakdown was not severe under the low flow conditions within Alicante harbour. Finally, it is possible that the corals sampled for our experiments (collected in autumn) were pre-conditioned to tolerate warming. For example, some tropical corals have slightly higher resistance to subsequent thermal stress when they have been pre-acclimated to higher temperature (Middlebrook *et al*., 2008). Additional studies in the laboratory and the field are required to tease apart the relative importance of the rate of warming, the prior occurrence of abnormally high temperatures, and/or other environmental interactions, as correlates of coral thermal stress responses.

There is increasing evidence of variation in the capacity for thermal acclimatization between populations (e.g. Seebacher *et al*., 2012), such that the response of individuals to temperature cannot be considered to be consistent throughout a species' geographic range. For instance, studies of within-species variation in thermal thresholds of corals have revealed that temperate gorgonians from populations in warmer regions of the Mediterranean have higher tolerance to thermal stress than those from cooler regions (Linares *et al*., 2013), and that bleaching susceptibility can vary in response to changes in either or both of symbiont type and environmental conditions along latitudinal gradients (Ulstrup *et al*., 2006).To our knowledge, the present study is the first to determine whether and how the optimal temperature and the breadth of the thermal 'window' (T_{tol}, *sensu* Pörtner, 2009), for various host and symbiont-associated physiological traits, varies systematically with geographic location in a scleractinian coral. Our results demonstrate that summer ocean temperatures in the Mediterranean are well above the temperature that maximises the chl content and photochemical efficiency of symbionts within *O. patagonica*. Although this meant that the

optimal temperature for these traits could not be quantified precisely at all of our sampling locations, this confirms that conditions in the Mediterranean are likely to become increasingly detrimental to the growth and survival of scleractinian corals. Overall, we have uniquely shown that thermal tolerance breadth is independent of local environmental conditions, and that *O. patagonica* has limited capacity, overall, to adjust its thermal physiology to match the temperature within its local environment.

Our study used a 'space for time' substitution to gain insight into the thermal adaptation capacity of our study species. Despite among-location variation in mean summer temperatures of approximately 3.5°C, a range comparable to the predicted average global warming by the end of the 21st century range (Meehl *et al*., 2007), we found very little support for substantial geographic variation in host and symbiont thermal physiology in response to spatial variation in ocean temperature. Indeed, our study demonstrates that the broad thermal tolerance of *O. patagonica* does not translate into a high capacity for thermal acclimatization. Therefore, as *O. patagonica* is a relatively new member of the Mediterranean coral fauna and local populations of this species have had limited time for genetic divergence, our results also indicate that coral populations need to remain isolated for a long period of time for thermal adaptation to take place. For *O. patagonica*, mortality associated with thermal stress seems to manifest primarily as tissue breakdown (partial mortality) under moderate but prolonged warming which does not impair symbiont photosynthesis and, therefore, does not lead to bleaching. Nonetheless, the increased metabolic activity of the high symbiont densities during warm summers causes oxidative damage to coral tissues resulting in tissue breakdown. Clearly, global warming at the rate expected under most model scenarios is likely to causes repeat incidents of local partial and whole colony mortality in the

Mediterranean (e.g. Garrabou *et al*., 2009) and drives a gradual range contraction of Mediterranean coral populations.

Acknowledgements

This study was partially funded by the Journal of Experimental Biology Travelling fellowship (RR-M), by the Centre Scientifique de Monaco, and James Cook University (MH). We thank Emily Tripp for assistance with DGGE gels, Andrés Izquierdo and Rami Tzadok for assistance during sample collection in Alicante and Israel, respectively. Two anonymous reviewers provided constructive comments that improved the manuscript. The work contributes to the EU project: `Mediterranean Sea Acidification under a changing climate' (MedSeA; grant agreement 265103).

References

Ainsworth TD, Hoegh-Guldberg H (2008) Cellular processes of bleaching in the Mediterranean coral *Oculina patagonica*. *Coral Reefs*, **27**, 593–597.

Ainsworth TD, Roff G, Fine M, Hoegh-Guldberg O (2008) Bacteria are not the primary cause of bleaching in the Mediterranean coral *Oculina patagonica*. *The ISME Journal*, **2**, 67–73.

Angilletta MJ (2006) Estimating and comparing thermal performance curves. *Journal of Thermal Biology*, **31**, 541–545.

Angilletta MJ (2009) Thermal adaptation a theoretical and empirical synthesis. OxfordUniversity Press New York, pp. 289.

Armoza-Zvuloni R, Segal R, Kramarsky-Winter E, Loya Y. (2011) Repeated bleaching events may result in high tolerance and notable gametogenesis in stony corals: *Oculina patagonica* as a model. *Marine Ecology Progress Series*, ***426***, 149-159.

Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Coral reefs: corals' adaptive response to climate change. *Nature* **430 (7001)**, 741–741

Bellwood DR, Hughes TP, Folke C, Nystrom M (2004) Confronting the coral reef crisis. *Nature*, **429**, 827–833.

Berkelmans R (2002) Time-integrated thermal bleaching thresholds of reefs and their variation on the Great Barrier Reef. *Marine Ecology Progress Series*, **229**, 73-82.

Bitar G, Zibrowius H (1997) Scleractinian corals from Lebanon, eastern Mediterranean, including a non-lessepsian invading species (Cnidaria: Scleractinia). *Scientia Marina*, **61**, 227–231.

Brown BE, Dunne RP, Goodson MS, Douglas AE (2000) Bleaching patterns in reef corals. *Nature*, **404**, 142–143.

Budd AF, Fukami H, Smith ND, Knowlton N (2012) Taxonomic classification of the reef coral family Mussidae (Cnidaria: Anthozoa: Scleractinia). *Zoological Journal of the Linnean Society* **166**, 465–529.

Buddemeier RW, Fautin DG (1993) Coral bleaching as an adaptive mechanism. *Bioscience*, **43**, 320–326.

Burnham KP, Anderson DR (2002) Model selection and inference: a practical information theoretic approach, 2nd edn. Springer-Verlag, New York, NY.

Castillo KD, Helmuth BST (2005) Influence of thermal history on the response of *Montastraea annularis* to short-term temperature exposure. *Marine Biology*, **148**, 261–270.

Cerrano C, Bavestrello G, Bianchi CN, Cattaneo-Vietti R, Bava S, Morganti C, Morri C, Picco P, Sara G, Schiaparelli S, Siccardi A, Sponga F (2000) A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (NW Mediterranean), summer 1999. *Ecology Letters*, **3**, 284–293.

Chown SL, Gaston KJ (2008) Macrophysiology for a changing world. *Proceedings of the Royal Society B – Biological Sciences*, **275**, 1469 – 1478.

Clausen CD, Roth AA (1975) Effect of temperature and temperature adaptation on calcification rate in the hermatypic coral *Pocillopora damicornis. Marine Biology*, **33**, 93–100.

Çinar ME, Bilecenoglu M, Ozturk B, Can A (2006) New records of alien species on the Levantine coast of Turkey. *Aquatic Invasions*, **1**, 84–90.

Coles S, Jokiel PL, Lewis CR (1976) Thermal tolerance in tropical versus subtropical Pacific reef corals. *Pacific Science*, **30**, 159–166.

Coll M, Piroddi C, Steenbeek J, et al. (2010) The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. PLoS ONE, **5**, e11842.

Coma R, Ribes M, Serrano E, Jiménez E, Salat J, Pascual J (2009) Global warming-enhanced stratification and mass mortality events in the Mediterranean. *Proceedings of the National Academy of Science*, **106**, 6176–6181.

Coma R, Serrano E, Linares C, Ribes M, Diaz D, Ballesteros E (2011) Sea urchins predation facilitates coral invasion in a marine reserve. PLoS ONE 6(7): e22017.

Cook CB, Muller-Parker G, Orlandini CD (1994). Ammonium enhancement of dark carbon fixation and nitrogen limitation in zooxanthellae symbiotic with the reef corals *Madracis mirabilis* and *Montastrea annularis*. *Marine Biology*, *118*(1), 157-165.

Cowther TW, Bradford MA (2013) Thermal acclimation in widespread heterotrophic soil microbes. *Ecology Letters*, **16**, 469–477.

Cunning R, Baker AC (2012) Excess algal symbionts increase the susceptibility of the reef corals to bleaching. *Nature Climate Change,* **3**, 259–262.

Dinh Van K, Janssens L, Debecker S, De Jong M, Lambret P, Nilsson-Ortman V, Bervoets L, Stoks R (2013) Susceptibility to a metal under global warming is shaped by thermal adaptation along a latitudinal gradient. *Global Change Biology*, doi:10.1111/gcb.12243.

Ezzat L, Merle P-L, Furla P, Buttler A, Ferrier-Pagés C (2013) The response of the Mediterranean gorgonian *Eunicella singularis* to thermal stress is independent of its nutritional regime. *PLoS ONE,* **8(5)**, e64370. doi:10.1371/journal.pone.0064370.

Fine M, Loya Y (1995) The coral *Oculina patagonica* a new immigrant to the Mediterranean coast of Israel. *Israel Journal of Zoology*, **41**, 84.

Fine M, Zibrowius H, Loya Y (2001) *Oculina patagonica*: a non lessepsian scleractinian coral invading the Mediterranean Sea. *Marine Biology,* **138**, 1195–1203.

Finelli CM, Helmuth BST, Pentcheff ND, Wethey DS (2006) Water flow influences oxygen transport and photosynthetic efficiency in corals. *Coral Reefs*, **25**, 47–57.

Fitt WK, Brown BE, Warner ME, Dunne RP (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs*,**20**, 51–65.

Fitt WK, McFarland FK, Warner ME, Chilcoat GC (2000) Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnology and oceanography*, *45*(3), 677-685.

Garrabou J, Coma R, Bensoussan N, Bally M, Chevaldonne P, Cigliano M, Diaz D, Harmelin JG, Gambi MC, Kersting DK, Ledoux JB, Lejeusne C, Linares C, Marschal C, Perez T, Ribes M, Romano JC, Serrano E, Teixido N, Torrents O, Zabala M, Zuberer F, Cerrano

C (2009) Mass mortality in north-western Mediterranean rocky benthic communities: effects of the 2003 heat wave. *Global Change Biology*, **15**, 1090–1103.

Godinot C, Ferrier-Pages C. Montagna P, Grover R (2011) Tissue and skeletal changes in the scleractinian coral *Stylophora pistillata* Esper 1797 under phosphate enrichment. *Journal of Experimental Marine Biology and Ecology*, **409(1)**, 200-207.

Great Barrier Reef Marine Park Authority (2009) Great Barrier Reef Outlook Report 2009. Great Barrier Reef Marine Park Authority, p 212 (www.gbrmpa.gov.au).

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, Jones RJ (1999) Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. *Marine Ecology Progress Series*, **183**, 73-86.

Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL (2012) Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change*, **2**, 116–120.

IPCC (2007) Climate change 2007: the physical sciences basis. Summary for policymakers. Contribution of working group I to the fourth assessment report. The Intergovernmental Panel on Climate Change, http://www.ipcc.ch/SPM2feb07.pdf.

Izquierdo A, Loya A, Díaz-Valdés M, Ramos-Esplá A (2007) Non indigenous species at the Alicante harbour (SE-Spain): *Oculina patagonica* de Angelis 1908, and *Botrycapulus aculeatus* (Gmelin, 1791). *Rapport de la Commission Internationale Exploration Scientifique de la Mer Mediterranée,* **38,** 506.

Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochemistry Physiology Pflanzen*, **167**, 191–194.

Jones AM, Berkelmans R, van Oppen MJH, Mieog JC, Sinclair W (2008) A community shift in the symbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society B – Biological Sciences*, **275**, 1359–1365.

Kersting DK, Bensoussan N, Linares C (2013) Long-Term Responses of the Endemic Reef-Builder *Cladocora caespitosa* to Mediterranean Warming. PLoS ONE 8(8): e70820. doi:10.1371/journal.pone.0070820.

Koren O *&* Rosenberg E (2008) Bacteria Associated with the Bleached and Cave Coral *Oculina patagonica Microbial Ecology,* **55**, 523–529.

Krom MD, Kress N, Brenner S, Gordon LI (1991) Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. *Limnology and Oceanography*, *36*(3), 424-432

Kushmaro A, Loya Y, Fine M, Rosenberg E (1996) Bacterial infection and coral bleaching. *Nature*, **380**, 96.

LaJeunesse TC (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine Biology* **141**, 387–400.

LaJeunesse TC & Trench RK (2000) Biogeography of two species of *Symbiodinium* (Freudenthal) inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt). *Biological Bulletin,* **199 (2)**, 126–134.

Lesser MP (1996) Elevated temperatures and ultraviolet radiation causes oxidativestress and inhibits photosynthesis in symbiotic dinoflagellates. *Limnology & Oceanography*, **41**, 271–283.

Linares C, Cebrian E, Kipson S, Garrabou J (2013) Does thermal history influence the tolerance to future warming events by temperate gorgonians? M*arine Environmental Research*, doi: 10.1016/j.marenvres.2013.01.009.

Marsh YA (1970) Primary productivity of reef-building calcareous and red algae. *Ecology*, **55**, 225–263

Maynard, JA, Turner, PJ, Anthony, KRN, et al. (2008). Reeftemp: An interactive monitoring system for coral bleaching using high-resolution sst and improved stress predictors. *Geophysical Research Letters*, **35**, L05603.

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany*, **51**, 659–668.

Meehl G, Arblaster JM, Tebaldi C (2007) Contributions of natural and anthropogenic forcing to changes in temperature extremes over the United States. *Geophysical Research Letters*, **34**, L19709.

Middlebrook R, Hoegh-Guldberg O, Leggat W (2008) The effect of thermal history on the susceptibility of reef-building corals to thermal stress. *The Journal of Experimental Biology* **211**, 1050–1056.

Morley SA, Hirse T, Thorne MAS, Portner HO, Peck LS (2012) Physiological plasticity, long term resistance or acclimation to temperature in the Antarctic bivalve, *Laternula elliptica*. *Comparative Biochemistry and Physiology A*, **162**, 16–21.

Movilla J, Calvo E, Pelejero C, Coma R, Serrano E, Fernández-Vallejo P, Ribes M (2012) Calcification reduction and recovery in native and non-native Mediterranean corals in

response to ocean acidification. *Journal of Experimental Marine Biology and Ecology* **438**, 144-158.

Navas CA (1996) Metabolic physiology, locomotor performance, and thermal niche breadth in neotropical anurans. *Physiological Zoology*, **69**, 1481–1501.

Perez T, Garrabou J, Sartoretto S, Harmelin JG, Francour P, Vacelet J (2000) Massive mortality of marine invertebrates: an unprecedented event in northwestern Mediterranean. *Compte Rendu Academie Science / Science de la Vie*, **323**, 853–865.

Pörtner H-O (2009) Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *The Journal of Experimental Biology,* **213**, 881–893.

Reed JK (1981) *In situ* growth rates of the scleractinian coral *Oculina varicosa* with zooxanthellae on 6-m reefs and without on 80-m banks. *Proceeding of the 4th International Coral Reef Symposium,* **2**, 201–206.

Rodolfo-Metalpa R, Reynaud S, Allemand D, Ferrier-Pagès C (2008) Temporal and depth response of two temperate corals, *Cladocora caespitosa* and *Oculina patagonica* from the North Mediterranean Sea. *Marine Ecology Progress Series*, **31**, 447–456.

Rodolfo-Metalpa R, Richard C, Allemand D, Ferrier-Pagès C (2006a) Growth and photosynthesis of two Mediterranean corals *Cladocora caespitosa* and *Oculina patagonica* under normal and elevated temperatures. *Journal of Experimental Biology*, **209**, 4546–4556.

Rodolfo-Metalpa R, Richard C, Allemand D, Bianchi CN, Morri C, Ferrier-Pagès C (2006b) Response of zooxanthellae in symbiosis with the Mediterranean corals *Cladocora caespitosa* and *Oculina patagonica* to elevated temperatures. *Marine Biology*, **150**, 45–55.

Rowan R, Knowlton N, Baker A, Jara J (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature*, **388**, 265–269.

Rubio Portillo E, Vázquez-Luis M, Izquierdo Muñoz A, Ramos Esplá A A (2013). Distribution patterns of alien coral *Oculina patagonica* De Angelis D´Ossat, 1908 in western Mediterranean Sea, *Journal of Sea Research,* doi:10.1016/j.seares.2013.07.007.

Salomidi M, Bellou N, Pancucci-Papadopoulou MA, Zibrowius H (2006) First observation of an invasive scleractinian coral in Greek waters. *41st European Marine Biology Symposium*, Cork, Ireland, 4-8 September 2006.

Salomidi M, Katsanevakis S, Issaris Y, Tsiamis K, Katsiaras N (2013). Anthropogenic disturbance of coastal habitats promotes the spread of the introduced scleractinian coral *Oculina patagonica* in the Mediterranean Sea. *Biological Invasions*, **15(9)**, 1961–1971.

Salomon S, Plattnerb G-K, Knuttic R, Friedlingstein P (2009). Irreversible climate change due to carbon dioxide emissions. *Proceedings of the National Academy of Science*, **106**, 1704–1709.

Sartoretto S, Harmelin JC, Bachet F, Bejaoui N, Lebrun O, Zibrowius H (2008) The alien coral *Oculina patagonica* De Angelis, 1908 (Cnidaria, Scleractinia) in Algeria and Tunisia. *Aquatic Invasions*, **3**, 173–180.

Seebacher F, Holmes S, Roosen NJ, Nouvian M, Wilson RS, Ward AJW (2012) Capacity for thermal acclimation differs between populations and phylogenetic lineages within a species. *Functional Ecology*, **26**, 1418–1428.

Serrano E, Coma R, Ribes M, Weitzmann B, García M, Ballesteros E (2013) Rapid northward spread of a zooxanthellate coral enhanced by artificial structures and sea warming in the western Mediterranean. PLoS ONE **8(1),** e52739.

Shenkar N, Fine M, Loya Y (2005) Size matters: bleaching dynamics of the coral *Oculina patagonica*. *Marine Ecology Progress Series*, **294**, 181–188.

Shenkar N, Fine M, Kramarsky-Winter E, Loya Y (2006) Population dynamics of zooxanthellae during a bacterial bleaching event. *Coral Reefs*,**25**, 223–227.

Stillman JH (2003) Acclimation capacity underlies susceptibility to climate change. *Science,* **301**, 65.

Sunnuck P (2000) Efficient genetic markers for population biology. *Trends in Ecology & Evolution*, **15(5)**, 199–203.

Ulstrup KE, Berkelmans R, Ralph PJ, van Oppen JH (2006) Variation in bleaching sensitivity of two coral species across a latitudinal gradient on the Great Barrier Reef: the role of zooxanthellae *Marine Ecology Progress Series* **314**, 135–148.

Wiedenmann J, D'Angelo C, Smith EG, Hunt AN, Legiret FE, Postle AD, Achterberg EP (2013) Nutrient enrichment can increase the susceptibility of reef corals to bleaching. *Nature Climate Change*, **3**, 160–164.

West JM, Salm RV (2003) Resistance and resilience to coral bleaching: Implications for coral reef conservation and management. *Conservation Biology*, **17**, 956–967.

Zibrowius H (1974) *Oculina patagonica*, scléractiniaire hermatypique introduit en Méditerranée. *Helgolander wiss Meeresunters*, **26**,153–173.

Zibrowius H, Ramos-Esplà AA (1983) *Oculina patagonica*, scléractiniaire exotique

en Méditerranée - nouvelles observations dans le Sud-Est de l'Espagne. *Rapport de la Commission Internationale Exploration Scientifique de la Mer Mediterranée* **28**, 297–301.

Figure Legends

Figure 1: (a) Geographical locations of four populations of *Oculina patagonica* that were surveyed and sampled in the present study and (b) variation in spring and summer seawater temperatures within *Oculina patagonica* habitats at the sampling locations. Data are hourly measurements made during 2009.

Figure 2: *In situ* images of *Oculina patagonica* at the end of summer (October 2009) at four study locations in the Mediterranean Sea. Colonies from Alicante (a) and Haifa (b) showed both patches of denuded skeleton and bleaching, while colonies from Albissola and Portman (c) showed only patches of denuded skeleton. Scale bars are 1 cm.

Figure 3: Thermal performance curves describing variation in symbiont density (a-d), chlorophyll concentration (e-h) and maximum photochemical efficiency (i-l) for four populations of *Oculina patagonica* under experimental warming. Data points are means (n = 6) and error bars show standard deviation. Fitted curves are non-linear regressions showing the best-supported model and dashed lines indicate the average summer temperature at each location.

Figure 4: Thermal performance curves describing variation in photosynthesis rate (a-d), dark respiration rate (e-h) and protein content (i-l) of coral host and symbionts combined, for four populations of *Oculina patagonica* under experimental warming. Data points are means (n = 6) and error bars show standard deviation. Fitted curves are non-linear regressions showing

the best-supported model and dashed lines indicate the average summer temperature at each location.