Thermally tolerant corals have limited capacity to acclimatize to future warming

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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 in the Mediterranean 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.*

121 *patagonica* is evidently able to acclimatize to a wide range of temperatures, studies
122 performed in different regions have reported vastly different physiological responses to
123 exposure to high temperatures. In Israel, laboratory and field studies of *O. patagonica* have
124 shown that it often bleaches during the summer (e.g. Fine & Loya, 1995; Kushamaro *et al*.,
125 1996) and recovers during the winter (Shenkar *et al*., 2005, 2006). In contrast, bleaching has
126 never been reported for colonies either in the northern region, where corals have instead
127 suffered mass mortality at the end of particularly warm summers (e.g. Cerrano *et al*., 2000;
128 Perez *et al*., 2000; Garrabou *et al*., 2009), or along the Spanish and Catalan coasts (Serrano *et*
129 *al*., 2013). Abnormal summer temperatures have also been demonstrated to be the causative
130 agent of the tissue breakdown and mortality (without bleaching) for this and other coral
131 species in the north Mediterranean (e.g., Cerrano *et al.*, 2000; Rodolfo-Metalpa *et al*., 2006a,
132 2008; Kersting *et al*., 2013).

133 The present distribution of *O. patagonica* encompasses regions with very different
134 thermal regimes, both in terms of the duration of the warm season and the temperature range.
135 Moreover, contrasting physiological responses to high temperature stress exhibited at
136 different locations throughout this coral species' geographic range suggest that there is strong
137 environmental control over thermal performance. Consequently we aimed to quantify how
138 thermal physiology varies among colonies of *O. patagonica* sampled from different locations
139 within the Mediterranean, and to investigate whether differences in thermal performance
140 (optimal temperature, temperature tolerance and temperature thresholds) were consistent with
141 regional differences in temperature regimes. To do so, we experimentally assessed plasticity
142 in symbiont identity, density, and photosynthetic properties, together with changes in host
143 tissue biomass in response to warming from 20 to 32°C, for colonies originating from four
144 geographically distinct locations (over a 12° latitudinal and a 35° longitudinal gradient),
145 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. 1): 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. For the northern location of 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). 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. 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:

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

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

293 (LRT) to determine whether including tank provided a significantly better fit to the data.
294 There was no evidence of tank effects for 5 of the 6 variables (LRT, $p > 0.42$ for each of
295 protein concentration, respiration rate, symbiont density, chl concentration and
296 photosynthetic yield but $p < 0.01$ for net photosynthesis rate) supporting our treatment of
297 coral fragments as independent replicates. Second, to determine whether one or more of the
298 fitted parameters (M_x, T_{opt} or T_{tol}) varied among the different coral populations we fitted Eq.
299 1 to all the data (i.e., pooled across locations, model 1 with 3 coefficients). Subsequently, we
300 fixed two of the fitted parameters as equal to the parameter estimated from the pooled data
301 and re-fit the model to estimate population specific parameters one at a time. For example,
302 we fixed T_{opt} and T_{tol} at the values estimated from the pooled data and re-fit M_x by population
303 (model 2 with 6 estimated parameters), and so on for T_{opt} (model 3, 6 parameters) and T_{tol}
304 (model 4, 6 parameters). Finally, we re-fit the model allowing all parameters to vary by
305 location (model 5, 12 parameters). Models were fit to data using least-squares non-linear
306 regression implemented in R version 3.0 (The R Foundation for Statistical Computing) using
307 'nls' (see Electronic Supplement A). The same parameter estimation procedure was
308 conducted for all 6 of the measured response variables (symbiont density, chl concentration,
309 maximum photochemical yield, holobiont photosynthesis, holobiont respiration and holobiont
310 protein content). An information theoretic model-selection approach was used to determine
311 which of models 1 – 5 was most likely given the data. To do this, we extracted the negative
312 log-likelihood for each model fit and calculated Akaike Information Criterion values (AIC)
313 for each model given the number of fitted parameters. AIC values were then converted to
314 Akaike weights (see Burnham & Anderson, 2002) to determine the relative support for each
315 model with respect to each physiological response variable. Consequently, we were able to
316 assess which, if any, aspects of thermal performance varied between local populations of *O.*
317 *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 chlorophyll 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.

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

343 Summer seawater temperatures differed substantially between locations, showing a
344 gradient of increasing temperature from Albissola to Haifa (Fig. 2). Mean (and maximum)
345 seawater temperatures from 1st June to 20th September 2009 were 24.4°C (28.08°C), 25.1°C
346 (28.06°C), 26.8°C (29.45°C) and 28.0°C (30.7°C) for Albissola, Portman, Alicante and
347 Haifa, respectively. The sampled locations also differed in their length of exposure to
348 temperatures. During the four months of measurement, 67%, 74%, 85% and 100% of the
349 temperature records were above 24°C at Albissola, Portman, Alicante and Haifa,
350 respectively. Moreover, temperatures higher than 27°C were more frequent in Alicante and
351 Haifa (64 and 81%, respectively) than in Albissola and Portman (2 and 26% of records,
352 respectively). Temperatures reaching 29°C were occasionally recorded in Alicante (2%) and
353 frequently in Haifa (49%) but never recorded at the other two locations.

354
355 *End-of-the-summer coral tissue appearance*

356 At the end of summer, coral tissue appearance differed among the four populations
357 (Fig. 3), and also among colonies within each population. Colonies from Alicante and Haifa
358 showed a variety of appearances including: white or slightly brown-colored with their
359 tentacles retracted (Fig. 3a); fully bleached with expanded transparent polyps (Fig. 3b); rarely
360 showing patchy areas of tissue loss (denuded skeleton; i.e., tissue breakdown) and areas
361 where polyps were normally pigmented and sometimes expanded (as in Fig. 3c); live brown
362 polyps but without any connecting tissue between polyps and with the whole skeleton
363 completely denuded; or healthy without any sign of bleaching or tissue loss. In contrast,
364 colonies from Albissola and Portman were never found to be bleached but they were found
365 with several visible patches of denuded skeleton (Fig. 3c). In Albissola, where colonies were
366 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* (3 from each of 4 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. 4). The magnitude of the decline in both symbiont density and chl concentration was highest for colonies from Albissola and Portman (Fig. 4a-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. 4k).

391 Although an approximately 4-fold decrease in the *Symbiodinium* and chl contents occurred in
392 nubbins from all locations no tissue necrosis was observed, even at the highest temperature.

393 We found very little support for geographic variation in thermal tolerance with respect
394 to the symbiont-related traits (i.e., symbiont density, chl and photochemical efficiency,
395 Electronic Supplement B). For symbiont density, the formal model selection procedure
396 showed negligible support for among-location variation in either the optimal temperature
397 (T_{opt}) or the thermal tolerance range (T_{tol}, Electronic Supplement B). Maximum symbiont
398 density, however, did vary among locations and declined in response to increasing local
399 average summer temperatures (Fig. 5a). Similar patterns of variation were observed for chl
400 concentration (Fig. 5b, e, h) and photochemical efficiency (Fig. 5c, f, i). For these two traits,
401 there was model support for among-location variation in the maximum value (M_x) of these
402 traits (wAIC = 0.14 for chl and 0.66 for photochemical efficiency), and for variation in each
403 of M_x, T_{tol} and T_{opt} (wAIC = 0.86 for chl and 0.34 for photochemical efficiency).
404 Nevertheless, for both chl concentration and photochemical efficiency, variance in the
405 location-specific estimates of T_{opt} and T_{tol} largely overlapped with the variance in the overall
406 fitted estimate (pooled over location, model 1) for these parameters (i.e., error bars on points
407 lie within shaded region, Fig. 5d-i). Moreover, there was relatively high variation in
408 parameter estimates for these traits (particularly for photochemical efficiency), both overall
409 and by-location. This variation reflects the fact that, for these traits, our data mostly lie above
410 the optimal temperature (Fig. 4e-l); the absence of data from below the optimal temperature
411 leads to high variance in the estimates of T_{opt} and $T_{tol,}$ and some ambiguity regarding whether
412 these parameters vary among locations. Finally, neither T_{opt} nor T_{tol} varied consistently in
413 response to mean summer temperatures experienced *in situ* (Fig. 5e-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. 6). Parameters describing these curves were estimated with relatively high precision due to exposure of corals to temperatures above and below the optimal temperature (Fig. 7, shaded regions and error bars are narrow compared with those in Fig. 5). Similar to our findings regarding model support for among-location variation in M_x for symbiont density, chl concentration and photochemical efficiency (Fig. 5 and 6), mean values for respiration, photosynthesis and protein content varied among the four sampling locations (M_x, Electronic Supplement B). Moreover, M_x for each of these physiological traits tended to decrease in response to increased mean summer temperatures *in situ* (Fig. 7a-c), although the high rate of photosynthesis at the optimal temperature for colonies collected from Haifa was contrary to this trend (Fig. 7a). 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, Electronic Supplement B), 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. 7g-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. 7e-f, error bars on points do not overlap shaded

439 regions). In addition, there was minimal (but non-negligible) model support for among-
440 location variation in T_{opt} only (Electronic Supplement B) indicating that the optimal
441 temperature for respiration, in particular, varies among populations. Finally, T_{opt} for
442 photosynthesis, respiration and protein did tend to increase with increasing mean summer
443 temperatures experienced by the source populations (Fig. 7d-f), although only up to a
444 threshold ambient temperature between 27 - 28°C.

445

446 ## Discussion

447 Understanding the ability of corals to resist bleaching due to thermal stress is critical
448 for predicting how reefs will change in response to rising global temperatures. By
449 investigating thermal performance in respect to multiple host- and symbiont-related
450 physiological processes we here show that, given that there are no changes in the type of
451 symbiont hosted by coral colonies, the stony coral *Oculina patagonica* has limited capacity to
452 adjust its physiology to match local temperature regimes. This interpretation is supported by a
453 formal model selection procedure that showed minimal support for variation in overall
454 thermal tolerance, or in thermal optima, among resident populations of corals that were
455 acclimatized/adapted to different environmental temperature regimes in the field. Symbiont
456 thermal physiology, in particular, was consistent among colonies sourced from different
457 regions of the Mediterranean. Importantly, we showed that populations living in nutrient-rich
458 and cold coastal areas, with very high symbiont densities (Albissola and Portman), undergo
459 dramatic tissue breakdown in response to increasing ocean temperatures during summer,
460 instead of the gradual loss of symbionts that is observed in colonies of populations in warmer
461 environments, with lower symbiont concentrations (Alicante and Haifa). Nevertheless, tissue
462 breakdown was not observed in response to warming in the laboratory, suggesting either that
463 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 genetic analysis found 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 skeletal surface area were clearly 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

488 we suggest that further research testing for interactions between temperature and other
489 environmental variables would be informative.

490 A first explanation of the differential responses to temperature stress (i.e., bleaching
491 versus tissue necrosis) that we observed at our study locations *in situ*, can be due to the
492 occurrence of a microbial agent (*Vibrio spp.)* in populations exposed to higher and persistent
493 temperatures (i.e., Haifa and Alicante). Indeed, both *in situ* and laboratory studies have
494 shown that the bleaching response in the eastern Mediterranean can be explained by the
495 interaction between high temperature and increased virulence of *Vibrio shiloi* (Kushamaro *et*
496 *al*., 1996), although other studies have shown seasonal bleaching without the presence of the
497 bacterium (Ainsworth & Hoegh-Guldberg, 2008; Ainsworth *et al*., 2008). Regardless of
498 whether bleaching of colonies is directly caused by the action of a bacterial disease, annual
499 bleaching along the Israeli coast is certainly temperature dependent, occurring when ambient
500 temperatures rise above ~26°C to reach 30-31°C (Shenkar *et al*., 2006). Although there is
501 ongoing debate regarding the role of coral disease as a driver of coral bleaching, if disease
502 virulence increases above a temperature threshold that is only reached in the southern part of
503 the Mediterranean (i.e. Israel), and where temperatures are locally higher than normal such as
504 in the Alicante harbour (Spain), this mechanism could explain the differential responses to
505 temperature stress that we observed at our study locations. Nonetheless, the repeated cycle of
506 annual bleaching and recovery during winter (Shenkar *et al*., 2006; Armoza-Zvuloni *et al*.
507 2011) supports our interpretation that *O. patagonica* has limited capacity to acclimatize or
508 adapt to thermal stress.

509 Alternatively, the differential responses to temperature stress between northern and
510 southern *O. patagonica* populations can be related to differences in the amplitude and
511 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. 2), and likely in parallel with the highest irradiance levels, the high metabolic activity of symbionts in combination with very high symbiont densities, potentially causes tissue breakdown due to accumulated oxidative-stress-associated tissue damage. Under such conditions, the coral host appears to be unable to expel symbionts rapidly enough to prevent severe oxidative damage, and tissue breakdown occurs before bleaching can be performed. 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 high metabolic activity, due to elevated temperature, combines with food/nutrient shortage to cause tissue breakdown (e.g. Coma *et al*., 2009, but see also Ezzat *et al*., 2013). Additional studies in the laboratory and the field are required to tease apart the direction and magnitude of these environmental interactions as determinants 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). In contrast, other research on tropical corals has shown that previous exposure to thermal stress can slightly enhance symbiont photosynthesis during subsequent exposure to high temperature, but that this does not mitigate loss of symbionts (Middlebrook *et al*., 2008). 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

562 host and symbiont-associated physiological traits, varies systematically with geographic
563 location in a scleractinian coral. We have uniquely shown that thermal tolerance breadth is
564 independent of local environmental conditions, and that *O. patagonica* has limited capacity,
565 overall, to adjust its thermal physiology to match the temperature within its local
566 environment.

567 Our study used a 'space for time' substitution to gain insight into the thermal
568 adaptation capacity of our study species. Despite among-location variation in mean summer
569 temperatures of approximately 3.5°C, a range comparable to the predicted average global
570 warming by the end of the 21st century range (Meehl *et al*., 2007), we found very little
571 support for substantial geographic variation in host and symbiont thermal physiology in
572 response to spatial variation in ocean temperature. Indeed, our study demonstrates that the
573 broad thermal tolerance of *O. patagonica* does not translate into a high capacity for thermal
574 acclimatization. Therefore, as *O. patagonica* is a relatively new member of the Mediterranean
575 coral fauna and local populations of this species have had limited time for genetic divergence,
576 our results also indicate that coral populations need to remain isolated for a long period of
577 time for thermal adaptation to take place. For *O. patagonica*, mortality associated with
578 thermal stress seems to manifest primarily as tissue breakdown (partial mortality) under
579 moderate but prolonged warming which does not impair symbiont photosynthesis and,
580 therefore, does not lead to bleaching. Nonetheless, the increased metabolic activity of the
581 high symbiont densities during warm summers causes oxidative damage to coral tissues
582 resulting in tissue breakdown. Clearly, global warming at the rate expected under most model
583 scenarios is likely to causes repeat incidents of local partial and whole colony mortality in the
584 Mediterranean (e.g. Garrabou *et al*., 2009) and drives a gradual range contraction of
585 Mediterranean coral populations.

586

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Figure Legends

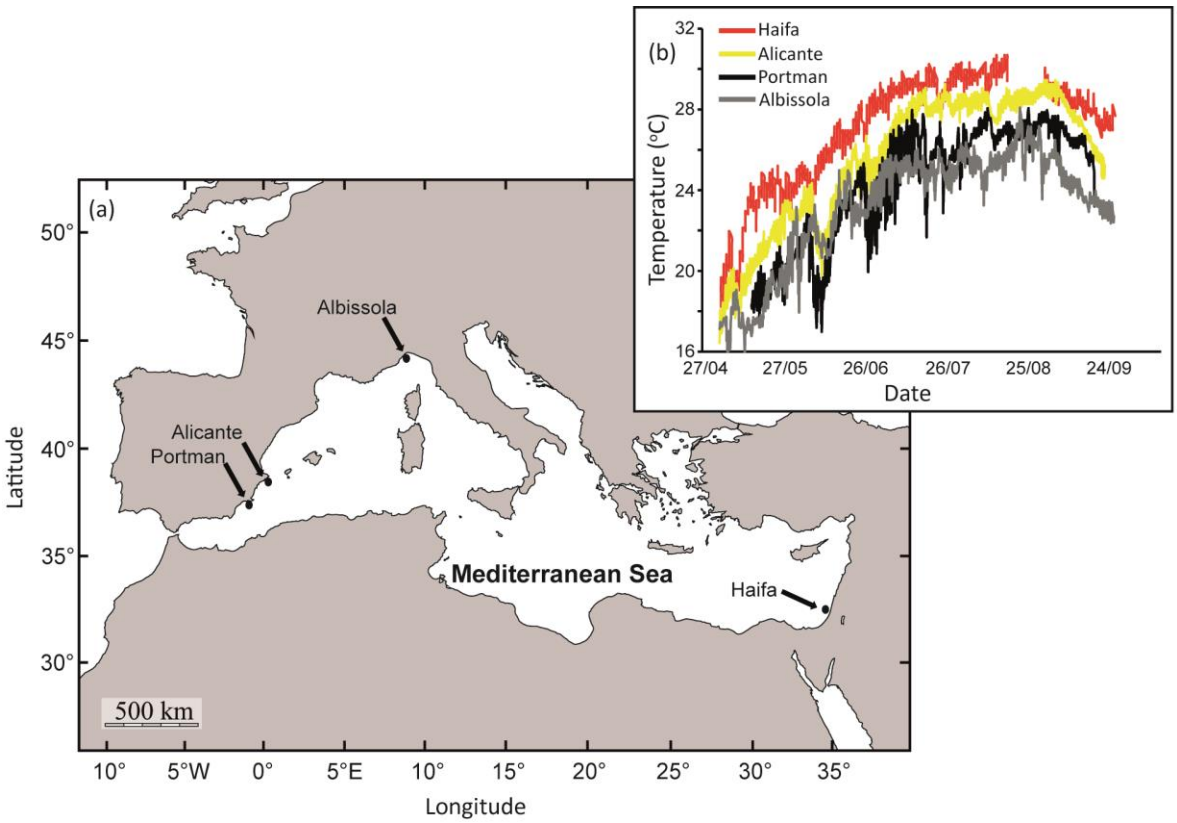
Figure 1: (a) Geographical locations of four populations of *Oculina patagonica* that were surveyed and sampled in the present study and (b) variation in spring and summer seawater temperatures within *Oculina patagonica* habitats at the sampling locations. Data are hourly measurements made during 2009.

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.

867 Figure 1



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871 Figure 2

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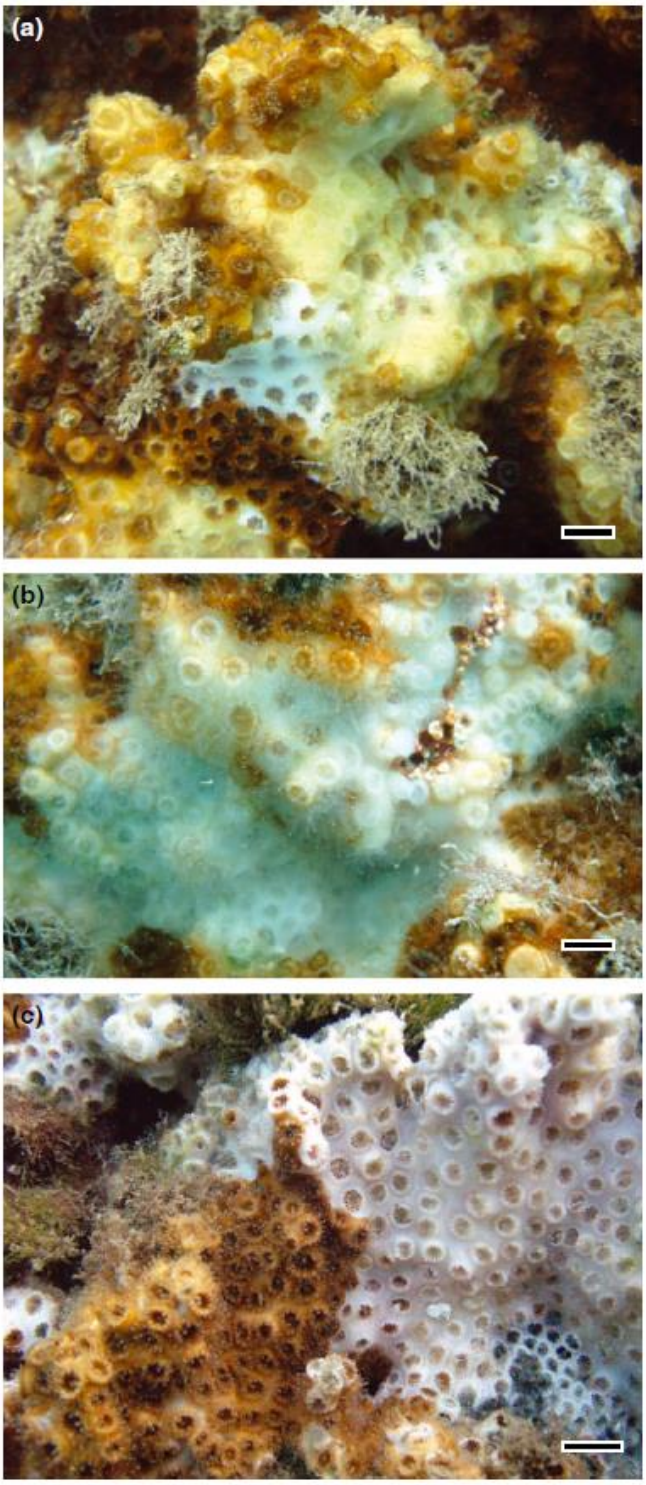

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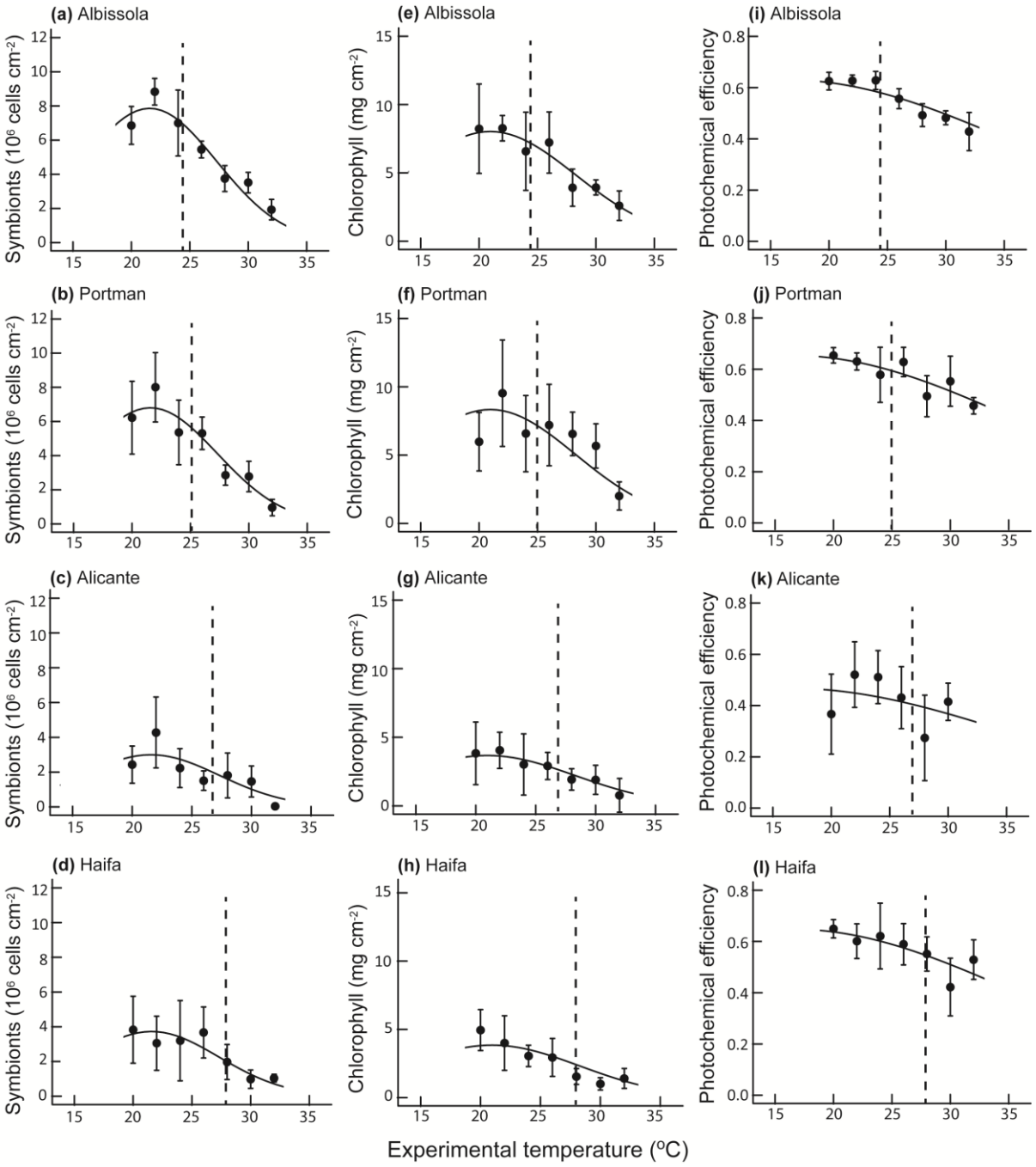

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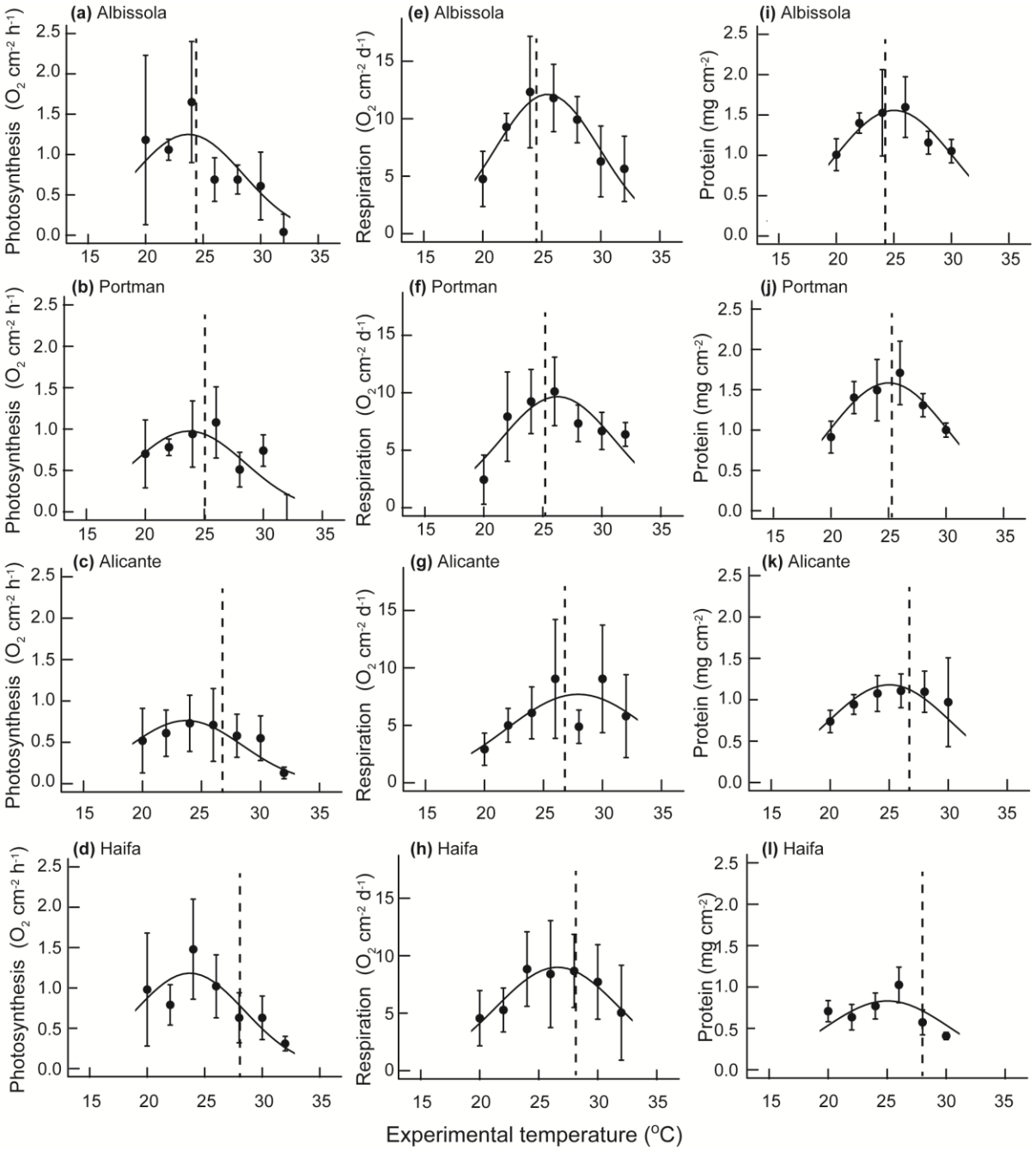
877 Figure 4



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