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Does thermal history influence the tolerance of temperate gorgonians to future warming?

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ABSTRACT

To date, several studies have provided evidence that thermal stress affects the growth, survival and physiology of tropical and temperate macroinvertebrate species. However, few studies have focused on subtidal temperate species and the potential differential thermal tolerances of populations dwelling under contrasting temperature conditions. To assess the role that environmental history has on the response of the temperate gorgonian *Eunicella singularis* to thermal stress, we compared populations dwelling in the coldest and warmest areas of the NW Mediterranean Sea. Our results show that *E. singularis* populations from both areas exhibited a high resistance to thermal stress; however, populations from warmer areas had an increased tolerance to thermal stress. Specifically, the upper thermal limits found for cold and warm populations were 28 and 29 °C, respectively. The higher resistance of *E. singularis* colonies to thermal stress found in this study compared to the field temperature conditions during recent mass mortality events highlights that performing further thermotolerance experiments under contrasting levels of feeding is necessary to fully assess the tolerance thresholds displayed by both study populations. To our knowledge, this study provides the first evidence for the role of thermal history in shaping the thermotolerance responses of Mediterranean marine invertebrates dwelling under contrasting temperature environments.

1. Introduction

Temperature is a key environmental factor that affects organisms at all organization levels by controlling their physiological and ecological processes (Calosi et al., 2008). As the mean and extreme temperatures increase in marine systems, the likelihood of the survival of a species or of a population is partially related to their physiological capacity to tolerate elevated temperatures (Hutchins, 1947). Differences in temperature tolerances between organisms can identify individual and ecological characteristics of "winners" and "losers" in a climate change context (Somero, 2010).

Marine benthic habitats, such as tropical and temperate reefs, are socially and economically important because they contribute crucial ecosystem services to the global economy (Costanza et al., 1997). However, these habitats suffer from dramatic increases in mass mortality events and diseases associated with ocean warming (Harvell et al., 1999, 2002; Doney et al., 2012). Among the most affected organisms are sessile invertebrate species, which play important roles in the structure and function of their habitats. Since organisms tend to have thermal tolerances that reflect the environment in which they are found, it is not surprising that recent research has focused on analyzing the thermal responses of marine invertebrates (species and/or populations) to different temperature regimes across varying temporal and spatial scales (Helmuth et al., 2002; Somero, 2005; Oliver and Palumbi, 2011). Several tropical invertebrate species and populations living in environments that have rapid thermal fluctuations and high maximum temperatures exhibit higher thermal tolerances than invertebrate species and populations living under more moderate temperature conditions (Warner et al., 1996; Castillo and Helmuth, 2005; Middlebrook et al., 2008; Oliver and Palumbi, 2011). However, the relationship between thermal history and thermal tolerance has rarely been examined in subtidal marine macroinvertebrates.

The Mediterranean Sea is one of the fastest warming regions affected by climate change (Bindoff et al., 2007; Burrows et al., 2011), and it has been greatly impacted by several human-mediated threats since ancient times (Coll et al., 2010). Moreover,

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the Mediterranean marine ecosystem, similar to other subtidal temperate ecosystems, experiences a narrower range of daily thermal fluctuations than intertidal reefs, where most of the thermal tolerance studies have been conducted. Given these facts, the Mediterranean Sea represents an excellent natural laboratory for exploring the responses of temperate marine biota impacted by climate change. The results of such explorations may provide new insights into the role of thermal histories on the resistance of marine invertebrates to thermal stress.

Several studies have provided evidence that thermal stress affects the growth, survival and physiology of zooxanthellate and azooxanthellate cnidarians inhabiting Mediterranean Sea subtidal reefs (Garrabou et al., 2009; Calvo et al., 2011). However, these studies have rarely examined the potential differential thermal tolerances at both inter- and intraspecific levels. The few studies that are available have considered the differences between shallow and deep populations from a single location and between populations dwelling in the coldest areas of the NW Mediterranean Sea (Rodolfo-Metalpa et al., 2006; Linares et al., 2008; Torrents et al., 2008; Coma et al., 2009; Ferrier-Pagès et al., 2009; Bensoussan et al., 2010; Pey et al., 2011).

This study focused on the temperate zooxanthellate gorgonian *E. singularis*, one of the species most affected by thermal anomalies in the NW Mediterranean Sea during the last few decades (Cerrano et al., 2000; Coma et al., 2006; Garrabou et al., 2009). In previous thermal tolerance studies, shallow *E. singularis* populations displayed a relatively high thermal tolerance. These populations only exhibited sublethal physiological effects after long-term exposure at 26 °C (Ferrier-Pagès et al., 2009; Previati et al., 2010), and they only suffered extensive damage when temperatures rose above 28 °C (Pey et al., 2011). As for other Mediterranean species, the *E. singularis* populations examined reside in the coldest areas of the NW Mediterranean Sea, such as the Gulf of Lions (Bensoussan et al., 2010; www.t-mednet.org). However, *E. singularis* populations are distributed throughout the entire Mediterranean Sea, and they experience contrasting thermal conditions. Here, we investigated the role of thermal history on the thermal stress responses of the temperate gorgonian *E. singularis*. In contrast to previous studies, we examined the populations dwelling in the warmest and the coldest areas of the NW Mediterranean Sea to experimentally test the hypothesis that they had differential thermal tolerance resistances.

2. Material and methods

2.1. Study locations

This study examined two populations of white gorgonian *E. singularis* that displayed differential thermal environments and were located in two areas of the NW Mediterranean Sea basin: the Medes Islands (L'Estartit, NE Spain) and Menorca Island (Balearic Islands, Spain) (Fig. 1a). Both populations, separated by approximately 230 km, exhibited different mortality responses during the two largest mass mortality events recorded in the Mediterranean Sea (recorded in 1999 and 2003), which were caused by positive thermal anomalies (Perez et al., 2000; Cerrano et al., 2000; Coma et al., 2006; Garrabou et al., 2009). While the Medes Islands population was not affected by the 1999 event (Linares et al., 2008, Fig. 1b), the Menorca Island population was significantly impacted by this event, losing approximately 50% of its population (Coma et al., 2006, Fig. 1c and d). The 2003 mass mortality event faintly impacted the Menorca Island population (affecting less than 10% of the population), and only slightly damaged the population inhabiting the Medes Islands (less than 5% of the population) (Garrabou et al., 2009, Fig. 1d). Data on temperature conditions concomitant with these two mortality events were not complete for both study areas (only weekly temperature records for 1999 and hourly records from 2002 were available for the Medes Islands, and no data were available for either year for Menorca Island), and thus, hinder precise comparative reports. However, data available for the Medes Islands indicate that temperatures rarely reached more than 24 °C at the depths where *E. singularis* dwelt in the summers of 1999 and 2003 (Fig. 2). This temperature is commonly recorded at Menorca Island (Fig. 2), and it is likely that during the temperature anomalies associated with mortality outbreaks, the temperatures reached higher maxima and/or warmer temperatures for longer periods of time. These data indicate that temperature conditions could explain differential mortality impacts that occurred during the 1999 and 2003 mass mortality events.

2.2. Temperature data

High-resolution hourly temperature recordings since 2002 and 2004 were obtained from the Medes Islands and Menorca Island, using *in situ* Stowaway Tidbits autonomous sensors installed at 20 and 25 m depth, respectively, where the sampled *E. singularis* populations dwelt. From these recordings, several temperature statistics were obtained to compare the temperature conditions recorded over the annual cycle (2005 and 2006) and during the summer periods for all available years (arbitrarily considered from July 1st to September 30th). Specifically, daily temperature means were calculated for the annual cycles, and the mean and maximum temperatures and mean percentage of summer periods with temperatures $\geq$23°, 24°, 25° and 26 °C recorded during the summer periods were calculated.

2.3. Experimental design

Experiments were carried out using apical tips (between 5 and 10 cm in length) sampled from each of 60 healthy white gorgonian colonies from each study location. Samples were randomly collected during SCUBA dives from populations at depths between 20 and 25 m (120 apical tips were collected). Apical tips from each colony were transported in portable coolers from the collection sites to the Experimental Aquarium Zone (ZAE) of the Institute of Marine Sciences in Barcelona in less than 24 h.

Each colony's apical tip (hereafter referred to as 'colony') was assembled on experimental plates. All experiments used the same two aquarium settings: control and treatment, into which 30 of 60 colonies from each population were placed. Ten colonies from each treatment were placed into control and treatment sets that were composed of three tanks (approximately 48 l). The tanks were supplied with Mediterranean seawater (salinity 38‰) that was continuously pumped from 20 m into the experimental aquarium facilities of the Institute of Marine Sciences. Upon arrival to the facilities, the seawater was filtered (up to 50 μm before reaching the experimental tanks). Each tank had an inlet pipe for the supply of fresh seawater and an outlet pipe. Both pipes remained open so that the tanks functioned as an open system. In the treatment setting, seawater was heated in a buffer tank equipped with submersible resistance heaters and was regulated by temperature controllers (Aqua Medic T controller). Irradiance was obtained from 30 W cool white fluorescent bulbs, which used an on/off regime of a 12 h light–dark cycle and was calibrated to match the light intensity that both populations received in their natural environment approximately 50 and 150 μmol photons m^{-2} s^{-1} (authors' unpublished data). Temperature and irradiance were measured every half hour in the control and treatment aquariums using HOBO Onset Pendant autonomous temperature and irradiance sensors. Experimental tanks

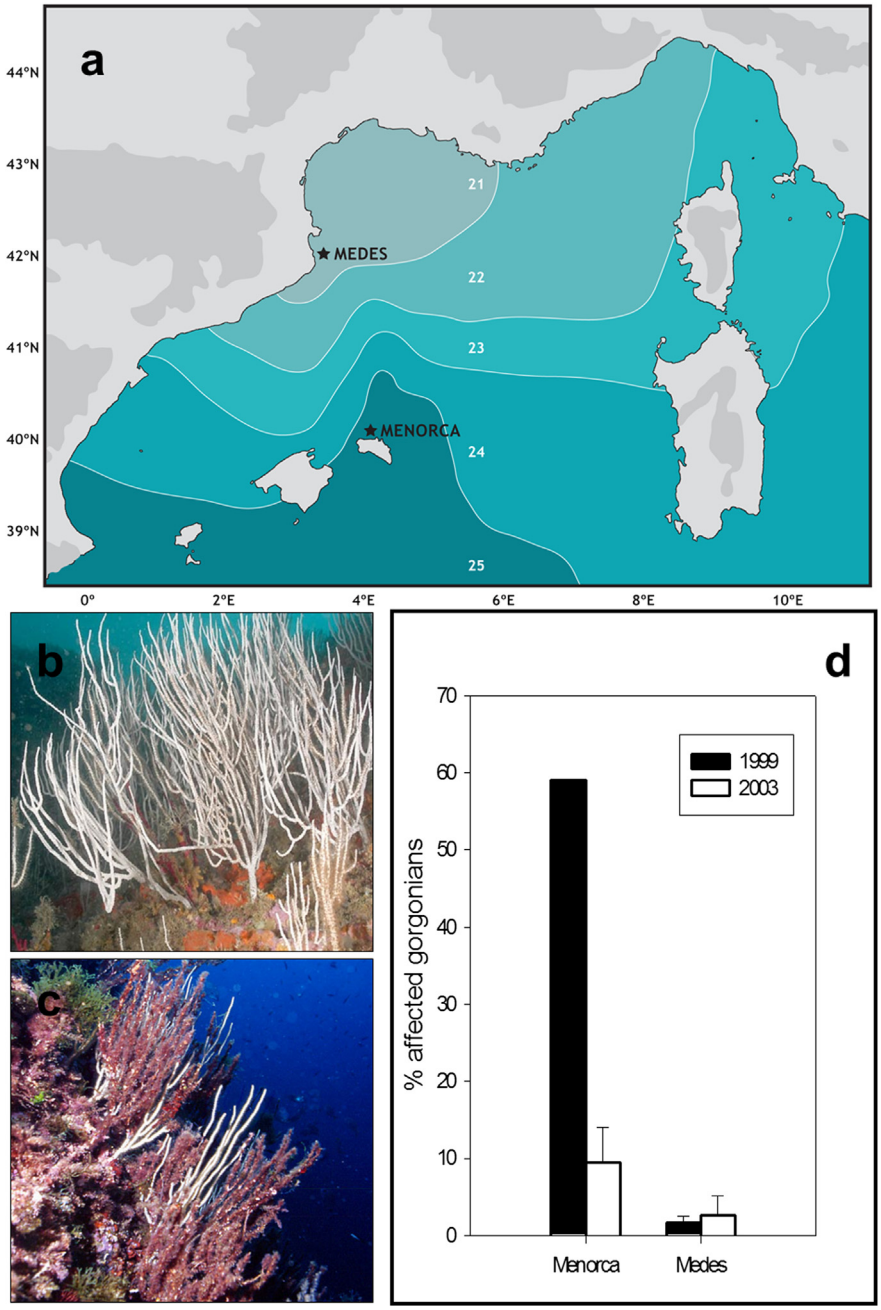

Fig. 1. a) Locations of the two study populations inhabiting the contrasting thermal environments. b) A healthy *Eunicella singularis* population on the Medes Islands. c) An *E. singularis* population affected by the 1999 mass mortality event on Menorca Island. d) The percentage of *E. singularis* colonies displaying >10% of injured tissue on Menorca Island and the Medes Islands MPA during the 1999 and 2003 mass mortality events (data recompiled from Coma et al. (2006), Linares et al. (2008), Garrabou et al. (2009).

were equipped with submersible pumps to facilitate water circulation. The submersible pumps provided a continuous circular current with a flow rate of approximately 60 l h^{-1}. Finally, 4 days per week colonies from both treatments were fed a supply of Ocean nutrition™ frozen Cyclops alternated with a supply of "Benthic nutrition" prepared aquarium food mixture. The supply of food pulses instead of constant food supplies was based on previous *in situ* and experimental studies (Rossi, 2002; Coma et al., 2009; Crisci, 2011). In our study, the frequency and amount of food (quantities were suggested by the manufacturer relative to volume of the tanks) together with the maintenance of the system in a closed mode for 4 h during feeding (the water circulation was provided solely by air pumps and food was retained in tanks), ensured that colonies in the feeding treatment were supplied with a surplus of food.

We ran two complementary thermotolerance experiments between July and October of 2010. Control tanks were always maintained between 17 and 18 °C. The treatment tanks' temperature ranged from 24 °C to 29 °C. During the first week, the temperature gradually increased from 17 °C to 24 °C. In the first experiment, the temperature was maintained at 24 °C for three days, then increased to 25 °C for one month and finally rose to 26 °C for an additional month. In the second experiment, the temperature increased from 17 °C to 24 °C in one day and then was maintained at 24 °C for three days. After that, the temperature increased by one degree every week, until a final temperature of 29 °C was reached.

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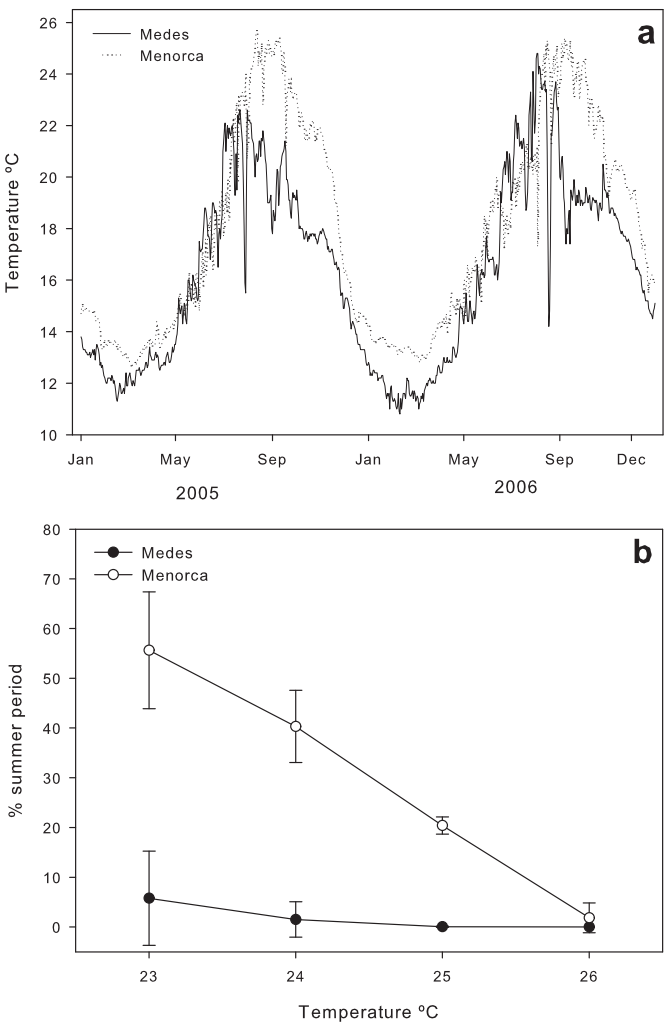

Fig. 2. a) Daily mean temperatures recorded during 2005 and 2006; obtained from hourly temperatures recorded at 20 m and 25 m depth at the Medes Islands and Menorca Island, respectively. b) Mean percentages of the summer period (from July to September) with temperatures above 23, 24, 25 and 26 °C during the last five years in the two study locations.

2.4. Response variables

Two response variables were used to assess potential differences in thermotolerance features between the study populations: 1) the percentage of necrosis of each colony (lethal effects), and 2) the mean biomass per colony (g DW/cm^2) for both experimental treatments (sublethal effects). Biomass is considered to be a good indicator of gorgonian fitness because it indicates the beginning of partial mortality when it falls below a critical threshold (Coma et al., 2009). Therefore, differences between colony weights were used to explore sublethal effects of prolonged thermal stress. At the end of the first experiment, surviving colonies were frozen and photographed. Images were processed using Seascape software, and the surface area of each colony was determined (Teixidó et al., 2011). The colonies were dried for 24 h at 90 °C and were weighed. Dry tissues of each colony were scraped off, and the denuded skeletal axis was weighed again. The tissue dry weight of each colony was obtained by subtracting the weight of the skeletal axis from the initial dry weight. Resulting measurements were used to establish the dry weight per unit area (g DW/cm^2), which was used in the analysis. The percentage of necrosis was estimated every 2–3 days during the first experiment and during the second experiment for temperatures up to 26 °C. After this point, necrosis rates were assessed daily until the experiment ended. Control experiments are not included in the figures, because all control colonies remained healthy throughout the experimental period (i.e., no tissue necrosis occurred).

2.5. Statistical analysis

We pooled data from the three replicate tanks together because previous analyses demonstrated that there were not significant differences in tank effects. Control experiments were not considered in the statistical analyses because all control colonies remained healthy throughout the experimental period (i.e., no tissue necrosis occurred).

For the first experiment, a one-way factor PERMANOVA was performed, treating population (Po) as a fixed factor and each time point as a separate variable to compare the *E. singularis* necrosis percentages of both populations at 25–26 °C. This multivariate approach was used instead of a repeated measures analysis of a single response variable using the Euclidean distance because it completely avoided having to consider any notions of sphericity and interdependence of replicates (Anderson, 2001).

In addition, the analysis of the differences in the biomass of the final colonies was carried out using only data from the first experiment, because the second experiment resulted in severe necrosis in most of the colonies. A two-way fixed ANOVA with population and treatment as fixed factors was used to examine the data. ANOVA analyses were performed using the STATISTICA (version 8.0, StatSoft).

For the second experiment, where three successive temperatures were tested, a two-factor PERMANOVA was performed, with population (Po) and temperature (Te) treated as fixed factors and each time point treated as a separate variable. Tissue necrosis rates were analyzed every three days during each experiment's threshold (day 1, 4 and 7). To simplify the analysis, pair-wise comparisons for the factor of interest (here, population) were performed separately for each temperature level. Differences that proved to be significant in the pair-wise comparison PERMANOVA test are represented with an asterisk in Fig. 2. Although our variables were univariate, a PERMANOVA analysis was used because the null distribution of the test statistic in a PERMANOVA analysis was produced by permutation. In this way, the usual normality and sphericity assumptions of ANOVA were avoided, and the interpretation of interaction terms within random factors was permitted (Anderson, 2001). Moreover, to avoid the problem of dependence associated with repeated measures, a multivariate approach to a repeated measures analysis of a single response variable was performed through the PERMANOVA, treating time points as variables and hence, not considering the time factor in the analysis. This approach completely avoided having to consider any notions of sphericity (Anderson et al., 2008). All analyses on necrosis rates (experiment 1 and 2) were carried out using the Primer v6 statistical package (Clarke and Gorley, 2006) in conjunction with the Windows PERMANOVA + module (Anderson et al., 2008).

3. Results

Daily mean temperature cycles showed contrasting conditions in the two study locations at 20–25 m, the intermediate depth that *E. singularis* inhabited (Fig. 2a). Although maximum temperatures recorded varied per year (Fig. 2a), Menorca Island had much warmer temperatures (maximum temperatures of 27 °C) than the North Catalan Coast (Medes Islands, maximum temperatures of 25.1 °C). In the waters around the Medes Islands, summertime

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temperatures were generally below 23 °C and rarely reached maximum values of 24.8 °C in warm years, such as in 2006 (Fig. 2a). On Menorca Island, *Eunicella* populations were subjected to temperatures higher than or equal to 23 °C during most of the summer (Fig. 2b). Moreover, on Menorca Island, almost 20% of the summertime temperatures were higher than or equal to 25 °C (Fig. 2b), while temperatures were never this high on the Medes Islands, even during warm summers, such as the one that occurred in 2006 (Fig. 2a).

In the first experiment, the two *E. singularis* populations did not exhibit any signs of necrosis (<5%) after two months of being exposed to 25 and 26 °C, and the main effect of location was not significant for necrosis (one-way PERMANOVA, $p = 1$; Table 1, Fig. 3a). In contrast, comparisons of biomass values (DW/cm^2) showed that the Menorca Island population had significantly lower values than the Medes Islands population (two-way ANOVA, $p < 0.05$; Fig. 3b). However, the main effect of treatment and the interaction between treatment and location were not significantly different with respect to biomass values (two-way ANOVA, $p = 0.107$ and $p = 0.507$, respectively; Fig. 3b).

In the second experiment, *Eunicella* populations displayed differences in both the magnitude and timing of necrosis, indicating differential critical temperature thresholds between the two populations (Fig. 3c). Initial observations of necrotic damage in the Medes Islands population appeared on the first days that temperatures reached 28 °C. This population also displayed a rapid increase in necrosis during exposure to this temperature. In contrast, necrosis started in the Menorca Island population when the population was subjected to 29 °C. Total tissue mortality or substantial injury (>80% of tissue death) occurred in most colonies of both populations only after being exposed to 29 °C for three days (Fig. 3c). The PERMANOVA statistical analysis of necrosis rates showed no significant differences between populations either at 27 °C, when the colonies were not affected ($p = 0.512$; Fig. 3c), or at 29 °C, when the colonies from both populations were largely affected ($p = 0.228$; Fig. 3c). In contrast, at 28 °C, the necrosis rate of *E. singularis* on the Medes Islands was significantly higher ($p = 0.042$) than the necrosis rate observed on Menorca Island (Fig. 3c, Table 1).

In both experimental sets, *E. singularis* populations submitted to control conditions (between 17 and 18 °C) remained healthy (no tissue necrosis observed) until the end of the experiments. Hence, as mentioned above, these data are not shown in the Figures and tests were not performed to examine the control experiments.

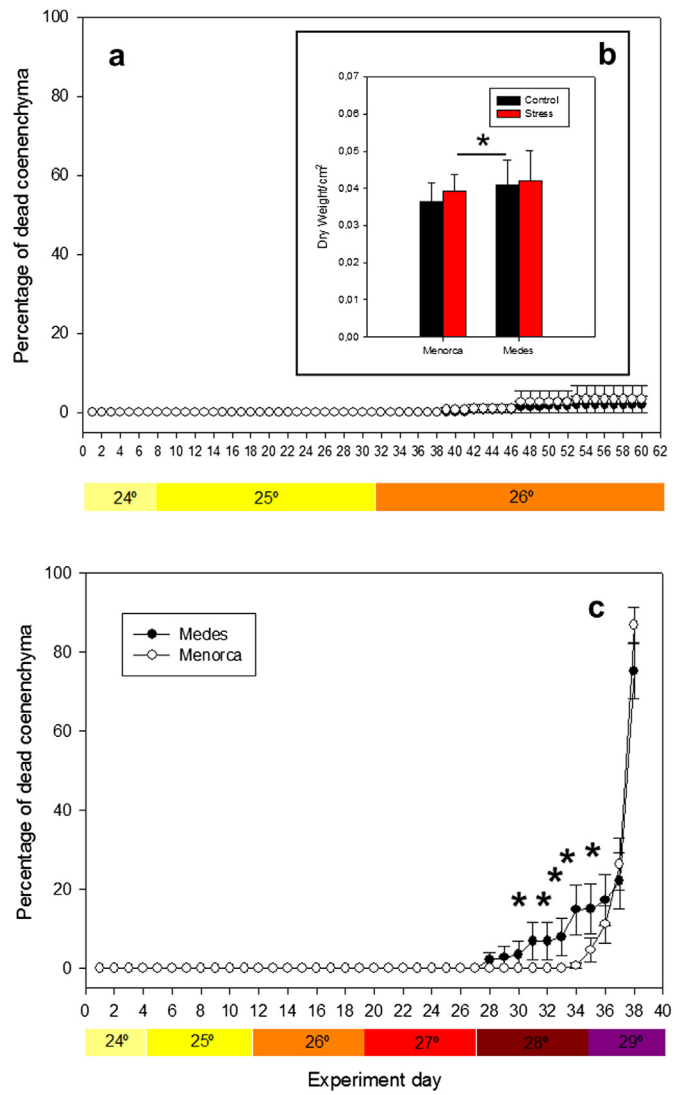

Fig. 3. a) The percentage of dead coenenchyma (mean ± SE) of *E. singularis* colonies from two populations submitted to different thermal stress conditions. b) The mean biomass (±SE) of *E. singularis* colonies from both study populations in the control and stress treatment groups following the first experiment. c) Percentage of dead coenenchyma (mean ± SE) of *E. singularis* colonies from two study populations submitted to different environmental stressors after the second experiment. Asterisks indicate significant differences obtained from a PERMANOVA statistical test.

4. Discussion

The results obtained from our experiments demonstrated differential thermotolerances of *Eunicella* populations subjected to hyperthermal conditions (upper thermal limits of 28 and 29 °C for the Medes Islands and Menorca Island populations, respectively). Although the lethal temperatures reported in this study have not been recorded at the depths where *E. singularis* dwell in both areas (Bensoussan et al., 2010; www.t-mednet.org), the differential response to the same hyperthermal conditions support the hypothesis that populations dwelling in warmer waters (such as on Menorca Island) have an increased tolerance to thermal stress. More precisely, a 1 °C temperature differential was detected between the first appearances of tissue necrosis in the two populations. The results found in this study agree with results for other Mediterranean anthozoans (Rodolfo-Metalpa et al., 2006; Torrents et al., 2008) and tropical corals (Middlebrook et al., 2008), demonstrating that shallow populations (acclimated to warm temperature conditions) have a higher tolerance to thermal stress than deep populations. However, these results contrast with previous observations for *E. singularis* populations, which noted that populations dwelling in deep habitats (with colder conditions)

Table 1
Permanova results obtained from the first and second experiment performed in this study. Po (population), Ti (time) and Te (treatment).

	Df	SS	MS	Pseudo-*F*	*P* (perm)
1st Experiment					
Po	1	47.083	47.083	0.11662	1
Res	58	23,417	403.75		
Total	259	23,465			
2nd Experiment					
Po	1	1141.5	1141.5	0.9663	0.356
Te	2	2.5543E5	1.2772e5	108.11	0.001
PoxTe	2	5841.4	2929.7	2.4724	0.026
Res	174	2.0555E5	1181.3		
Total	179	4.6796E5			

were less sensitive to thermal stress than populations inhabiting shallow habitats, which are acclimated to warmer conditions (Ferrier-Pagès et al., 2009; Pey et al., 2011).

In contrast, no significant effects or injuries were detected when *Eunicella* populations were submitted to long-term exposure in warm temperatures (25 and 26 °C). The absence of lethal or sublethal effects (in either necrosis rates or biomass), even after two consecutive months of exposure to warm conditions, is noteworthy because on the Medes Islands, temperatures above 25 °C have never been reported at 20 m depth, while the mean number of days with temperatures equal to or higher than 25 °C at 25 m depth are approximately 3-fold lower on Menorca Island than the temperature conditions used in our experiment. Our results on the absence of lethal and sublethal (biomass) effects at 25 and 26 °C are in partial agreement with previous studies on *E. singularis*, which maintained health for more than two weeks at 26 °C. These studies reported no sign of necrosis, but in contrast showed a decrease in calcification and photosynthesis rates, as well as an increase in levels of protein damage (Ferrier-Pagès et al., 2009; Pey, 2012). Regarding the lethal temperatures, our results are in agreement with those found in Pey (2012), which were between 27 and 28 °C; Pey (2012) studied populations from the coldest part of the NW Mediterranean Sea (Medes, NE Spain and Marseilles, SE France).

Anomalous warm temperatures triggered widespread mortality events in the NW Mediterranean Sea in 1999 and 2003 (Cerrano et al., 2000; Perez et al., 2000; Garrabou et al., 2009). Data reported from different affected areas of the NW Mediterranean Sea indicated that in 1999, the water column was largely stable, having temperature values of approximately 24 °C for several weeks (Cerrano et al., 2000; Romano et al., 2000; Perez et al. 2000). However, in the summer of 2003, temperature conditions reached extreme values of approximately 26–28 °C (Garrabou et al., 2009). These thermal conditions were concomitant with moderate to severe mass mortality impacts on *E. singularis* populations. However, as mentioned for the Medes Islands, the temperature records that were available suggest that the thermal stress never reached the abovementioned values in either 1999 or 2003, which corresponded with null or low incidences of widespread mass mortality events. Temperature records to further explore the relationship of the observed impacts on Menorca Island are lacking.

Temperature conditions recorded during the mass mortality events contrast with the lethal thermal thresholds observed in our model gorgonian species, as well as with similar studies with other species that examined the upper thermal limits in laboratory settings (Ferrier-Pagès et al., 2009; Previati et al., 2010; Pey, 2012). The difference in temperature conditions required for the initiation of necrosis in the natural environment versus in *ex situ* experiments suggests the importance of other factors. These factors have been highlighted in previous studies on mass mortality and bleaching events in temperate and tropical species and may include food availability (Coma et al., 2009; Connolly et al., 2012), pathogens (Bally and Garrabou, 2007) and/or genetic factors (Barshis et al., 2010).

A recent experimental study revealed that energetic constraints due to the combined effects of high metabolic activity and prolonged low food might be a primary trigger of mass mortality events (Coma et al., 2009). In fact, tropical coral species that can significantly increase their heterotrophic input of fixed carbon have an ecological advantage during thermal stress events for long-term survival (Grottoli et al., 2006). Likewise, food availability has the potential to increase corals' capacity to survive thermally induced bleaching and to ameliorate its sublethal effects (Connolly et al., 2012). These findings highlight the need to perform further thermotolerance experiments under different levels of feeding thresholds, including a starved control, to fully assess the tolerance thresholds displayed by both study populations.

Conversely, in our experimental study, *Eunicella* populations were provided with sufficient nutrition. This was because we were interested in exploring thermal tolerance features of both populations, and thus we needed to avoid any energetic constraints. Comparisons of biomass measurements between the control and thermal stress conditions were not statistically significant, which seems to corroborate that our feeding strategy efficiently avoided energy shortages. The only difference in biomass displayed at both locations was in agreement with the more oligotrophic conditions found on Menorca Island compared to the Medes Islands, which were influenced by seasonal inputs of organic matter from the nearby Ter River (Ribes et al., 1999). However, this higher physiological status did not result in a higher resistance to thermal stress.

Given the results obtained in this study, and the fact that only the host in these symbiotic gorgonians was considered, further research should investigate the role of symbionts enhancing local thermal resistance. To date, previous work showed that symbiotic diversity and the chlorophyll content of each zooxanthellae cell did not vary significantly between depths at different localities than our study sites (Forcioli et al., 2011). This absence of photoacclimation has led to the presumption that temperate symbiotic species may depend less on the photosynthetic activity of their zooxanthellae than their tropical counterparts (Muller-Parker and Davy, 2001; Forcioli et al., 2011). However, several questions about the role of symbionts on temperate species remain. Although a unique clade of *Symbiodinium* ("temperate A") has been identified for Mediterranean species (Visram et al., 2006), a recent study has shown divergent thermal tolerances in a generalist *Symbiodinium* type for a tropical coral from two different thermal environments (Howells et al., 2011). This finding demonstrates that *Symbiodinium* can adapt to local differences in thermal climates and that this adaptation shapes the fitness of coral hosts.

5. Conclusions

Our study is one of the first to provide evidence on the role of thermal histories in shaping thermotolerance responses of Mediterranean marine invertebrate species dwelling under contrasting temperature regimes. The differential response of populations to the same hyperthermal conditions (a 1 °C temperature differential between the first appearances of tissue necrosis in the two populations examined) support the hypothesis that populations dwelling in warmer waters, such as on Menorca Island, have an increased tolerance to thermal stress. However, the higher resistance of *E. singularis* colonies to thermal stress in comparison to the field observations highlights the need to perform further thermotolerance experiments under different levels of feeding rates to fully assess the tolerance thresholds displayed by both study populations. Comparative thermotolerance experimental studies represent a promising avenue of research to better understand the adaptation capability of marine populations to the expected ocean warming, and help managers to adopt measures to mitigate the effects of climate change. In short, we urgently need to better understand the responses (from the simplest, such as the one examined in this study, to the more complex), and the processes involved in temperate species responses to thermal stress.

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