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Growth and photosynthesis of two Mediterranean corals, *Cladocora caespitosa* and *Oculina patagonica*, under normal and elevated temperatures

Riccardo Rodolfo-Metalpa[1,*], Cécile Richard[1], Denis Allemand[1,2] and Christine Ferrier-Pagès[1]

[1]*Centre Scientifique de Monaco, Av. Saint Martin, MC-98000, Principality of Monaco and* [2]*UMR 1112 INRA-UNSA, Faculté des Sciences, Université de Nice Sophia-Antipolis, bp 71, F-06108, Nice Cedex 02, France*

*Author for correspondence (e-mail: rrmetalpa@centrescientifique.mc)

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Summary

The Ligurian Sea (NW Mediterranean) experienced warm summers in 1998, 1999 and from 2003 to 2005. The temperature was 1–3°C higher than the mean summer value (24°C) and remained high over a long period. During these summers, mass-mortality events, affecting several sessile benthic species, were reported. In the present study, we tested the long-term (3–7 weeks) effect of different temperatures (20°C measured in spring and autumn, 24°C observed in summer, and 26°C and 28°C abnormal summer values) on two Mediterranean corals, *Cladocora caespitosa* and *Oculina patagonica*. Growth rate, photosynthetic efficiency (F_v/F_m), relative electron transport rate (ETR), zooxanthellae and chlorophyll (chl) contents were measured during 48 days incubation. At 20°C, all parameters remained constant during the whole experiment for both species. At higher temperatures, most physiological parameters were affected by only 2–5 weeks at 24°C, and were severely depressed at higher temperatures. Small replicate samples (nubbins) of *O. patagonica* significantly decreased their zooxanthellae and chl concentrations at all temperatures, after 2 weeks of incubation. Their F_v/F_m values, as well as their growth rates, were also gradually reduced during the incubation at all temperatures. However, only a few nubbins maintained at 28°C showed signs of tissue necrosis after 34 days, and these gradually recovered tissue when temperature was returned to normal. In nubbins of *C. caespitosa*, chl and zooxanthellae concentrations decreased only after 34 days of incubation at 26°C and 28°C. At the same time, tissue necrosis was observed, explaining the loss of the symbionts. F_v/F_m was reduced only after 34 days of incubation at the different temperatures, and growth rate was first enhanced, before collapsing by 30% at 24°C and by 90–100% at 26°C and 28°C. All samples maintained at 26°C and 28°C had died, due to tissue necrosis, by the end of the experiment. Results obtained suggest that *O. patagonica* is more able than *C. caespitosa* to resist high temperature conditions because of its rapid bleaching capacity. In contrast, it seems that *C. caespitosa* is living close to its thermal limit during the summer period; therefore, a long-term increase at 24°C or above could be lethal for this coral, just as was observed *in situ* during the recent warm summers.

Key words: corals, Mediterranean Sea, temperature, growth, photosynthesis, PAM, *Cladocora caespitosa*, *Oculina patagonica*.

Introduction

Over the past 100 years, the world's climate has dramatically changed due to anthropogenic activities, and has warmed by ca. 0.6°C (Walther et al., 2002). Recent evidence has also shown a large-scale warming of the Mediterranean Sea (Bethoux et al., 1990; Walther et al., 2002), corresponding to a 0.3–0.7°C increase in the mean water temperature from 1961 to 1990 (Walther et al., 2002). In the Ligurian Sea (NW Mediterranean), at 10–20 m depth, summer water temperatures recorded usually reach a maximum of 24°C during 2–3 weeks (from 1992 to 1997 and 2000; Table 1). In contrast, during the summers of 1998, 1999 and 2003–2005, positive thermal anomalies occurred (Table 1) (Romano et al., 2000; André et al., 2004; Harmelin, 2004). In the summer of 1999, for instance, the long-term increase was characterized by a lowering of the thermocline (down to 40 m depth) and by mean temperatures of up to 24°C over at least 4 weeks between August and September (Fig. 1). In the summer of 2003, large temperature shifts, up to 7.5°C, were recorded within a few hours, reaching 27.2°C at 12 m depth (Harmelin, 2004). Long periods of elevated temperatures have also been observed more recently, in the summer of 2005, with mean temperatures at 11 m depth exceeding 24°C for 6 weeks (Fig. 1).

During these warm summer periods, several mass-mortality events, on both large and small spatial scales, have been reported in the Ligurian Sea, affecting scleractinian corals such as *Cladocora caespitosa* and *Balanophyllia europaea* (Rodolfo-Metalpa et al., 2000; Rodolfo-Metalpa et al., 2005), sponges, gorgonians (Cerrano et al., 2000; Cerrano et al., 2001; Garrabou et al., 2001), and other sessile benthic species (Perez

Table 1. *Summary of the length of warm periods in the NW Mediterranean Sea when the water temperature was 24°C or above at 10 m depth*

Year	Temperature (°C)	Period (weeks)	Month	Mortality reference
1992–1997	⩽24	2–3	July–August	
1998	⩾24	3	July–August	1
1999	24–25	5	August–September	2,3
2000	24–25	1–2	August	
2001	<24			
2002	<24			
2003	24–28	6	July–August	4
2004	24–26	5	July–August	
2005	24–26	6	July–August	5

Temperatures were registered by SOMLIT (Service d'Observation en Milieu Littoral, INSU-CNRS, Villefranche-sur-mer, France) during the summers 1992–2005.

Mass-mortality events of benthic organisms observed in the NW Mediterranean Sea reported by [1](Rodolfo-Metalpa et al., 2000), [2](Rodolfo-Metalpa et al., 2005), [3](Cerrano et al., 2000), [4](Harmelin, 2004), [5](R. Rodolfo-Metalpa, unpublished data).

et al., 2000). Several hypotheses have been proposed to explain these mortality events, such as high temperatures, pathogen contaminations, and energetic constraints (Cerrano et al., 2000; Garrabou et al., 2001; Coma and Ribes, 2003). Necrosis was observed in gorgonians and sponges when temperatures remained above or equal to 24°C over several weeks and coincided with the occurrence of opportunistic organisms (Cerrano et al., 2000; Cerrano et al., 2001). Energy shortage was also observed in taxa exhibiting summer dormancy such as anthozoans and sponges (Coma et al., 2000). Therefore, the elevated temperature, together with the stability of the water column, were suggested to be the most likely cause of benthic mortality because they affected a wide variety of taxa down to a depth of 40 m (above the lowered thermocline), over a large geographical area (Coma et al., 2002; Coma and Ribes, 2003; Linares et al., 2005).

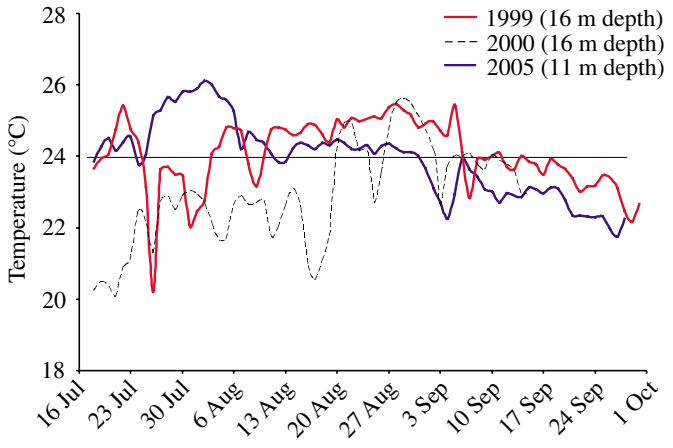

Fig. 1. Daily mean seawater temperature data for the summers 1999, 2000 and 2005 in the first 20 m in Monaco (Ligurian Sea). Hourly measurements were performed using Onset HOBO® water temperature pro data logger (unpublished data from the Oceanographic Museum of Monaco).

The response of temperate corals to temperature stress has been poorly studied (Jacques et al., 1983; Jones et al., 2000; Nakamura et al., 2003), particularly in the Mediterranean corals (Rodolfo-Metalpa et al., 2006). The only extended studies performed were to understand the bleaching of the coral *Oculina patagonica* along the Israeli coasts (e.g. Kushmaro et al., 1996). In this location, *O. patagonica* bleach during summer due to infection by *Vibrio shiloi*, which becomes virulent when the temperature rises above 26°C (Rosenberg and Falkovitz, 2004).

The aim of the present study was to monitor the response, in terms of photosynthesis and growth rate, of two symbiotic Mediterranean corals, *Cladocora caespitosa* (Linnaeus 1767) and *O. patagonica* (Angelis 1908), to different temperature conditions. *C. caespitosa* is a symbiotic scleractinian coral (Faviidae), native to the Mediterranean Sea (Zibrowius, 1980), which can form large banks of several m^2 (Peirano et al., 2001; Kružić and Pozar-Domac, 2003). In the Ligurian Sea, it is mainly distributed between 7 m and 15 m depth, and lives in turbid water at relatively low irradiance (Schiller, 1993; Peirano et al., 2005). *O. patagonica* is also a symbiotic scleractinian coral that is found, between 3 m and 10 m depth, in several Mediterranean Sea locations (Fine et al., 2001). The range of temperatures investigated was from 20°C to 26°C, 20°C being the temperature encountered in spring and autumn, and 24–26°C being recorded during recent warm summers (1999 and 2005). The length of exposure (3–7 weeks) was comparable to that experienced by the corals *in situ* (Fig. 1). Temperature was also increased up to 28°C, which is a normal summer temperature in the SE Mediterranean, where both corals are also found (Zibrowius, 1980).

Materials and methods

Collection and maintenance of corals

Several colonies of *Cladocora caespitosa* and *Oculina patagonica* were collected in the Ligurian Sea (NW

Mediterranean), respectively from the Bay of Fiascherino (Gulf of La Spezia, 44°03′N, 9°55′E) and Albissola (Gulf of Genoa, 44°17′N, 8°30′E), a distance of ca. 150 km from each other. They were then transported in aerated seawater, under reduced-light conditions, to the laboratory, where they were maintained in aquaria, continuously supplied with running Mediterranean seawater, pumped at 50 m depth. Water turnover rate in the aquaria was 20% h^{-1} and temperature was maintained constant at the temperature of collection (20°C). A submersible pump ensured seawater circulation in the tanks. Light intensity was provided by metal halide lamps (Philips, HPIT 400 W) during a 12 h:12 h dark:light photoperiod and measured using a Li-Cor underwater quantum sensor (LI-193SA; Li-Cor, Lincoln, NE, USA). Plastic mesh was used to adjust light intensity to 110 μmol photon m^{-2} s^{-1}, which is the mean irradiance calculated during the daylight period in the summer months (Peirano et al., 1999). Small replicate samples (nubbins) of *O. patagonica* were cut with pliers from mother colonies, attached to nylon threads and suspended in seawater. After ca. 3 weeks, tissue had re-covered the skeleton. Branches of *C. caespitosa* containing 10–15 polyps (for growth rates measurements) and single polyps (for the other measurements), referred to as nubbins, were detached from mother colonies, carefully cleaned of epiphytes, associated fauna and sediment using a brush, and placed on PVC supports. Twice a week, corals were abundantly fed with *Artemia salina* nauplii.

Experimental design

The experiment was designed to measure the growth and photosynthesis of corals maintained under different conditions: (i) at 20°C, which is the spring and autumn temperature; (ii) at 24°C, which is the mean summer temperature during a 2–3 week period in normal summers and more than 4 weeks in warm summers (Table 1; Fig. 1); (iii) at 26°C and 28°C, temperatures that might occur over a 2–3 week period in warm summers.

For this purpose, corals were randomly transferred into eight 15 l experimental tanks (2 tanks at each of 4 temperatures). Each tank contained 41 and 23 nubbins of *C. caespitosa* and *O. patagonica*, respectively. The experimental design was as described in Fig. 2. (a) Two tanks were maintained at 20°C throughout the whole experiment (48 days). (b) In two other tanks, temperature was slightly (by 1°C per day) increased from 20°C to 24°C and maintained during the 48 day period to mimic a long-term occurrence of high temperature as already observed *in situ* (Table 1, Fig. 1). (c) In the last four tanks, the temperature was first increased from 20°C to 24°C during a 14 day period (from T_0 to T_{14}), to mimic a normal summer temperature increase (Fig. 1); then, temperature was elevated at 26°C (in two tanks) and 28°C (in two other tanks) over an additional 3 week period, to mimic an abnormal warm summer (from T_{14} to T_{34}). The temperature was then returned to 24°C over two further weeks (T_{48}). Temperatures were kept constant using aquarium heaters connected to electronic controllers (±0.3°C accuracy). Small submersible pumps ensured seawater circulation in the tanks. Light intensity, seawater renewal and food level were maintained constant during the whole experiment, as described above.

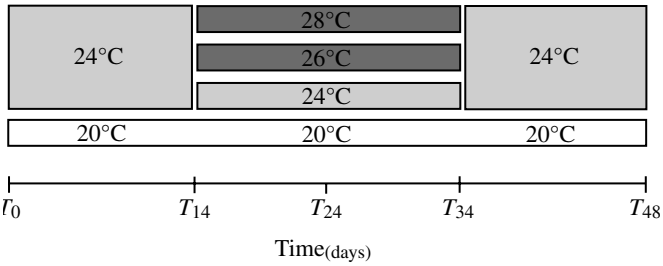

Fig. 2. Experimental design. See text for details.

Nubbins were sampled 5 times during the experiment, at the beginning (T_0), after 14 days (T_{14}), 24 days (T_{24}), 34 days (T_{34}) and 48 days (T_{48}), and several measurements (biomass parameters, rates of photosynthesis, respiration and growth, photosynthetic efficiency of the PSII and electron transport rate) were performed. During the entire experiment, corals were checked under a binocular for tissue degradation (necrosis or retraction into the polyps).

Biomass measurements

For each species, zooxanthellae density and chlorophyll contents (chl *a* and c_2) were measured for the 4 temperatures and the 5 sampling times. Three samples were randomly taken in each tank (N=6 samples per temperature). Samples were frozen at –80°C if not immediately processed. Coral tissue was detached using a Water Pick (Brown, Kronberg, Germany) in 0.45 μm filtered seawater. The slurry was homogenised using a Potter tissue grinder and a 2 ml sub-sample was taken for zooxanthellae density determination using an improved version of the Histolab® 5.2.3 image analysis software (Microvision, Every, France) (Rodolfo-Metalpa et al., 2006). For chl measurement, 10 ml sub-samples were centrifuged at 5000 ***g*** for 10 min at 4°C and the pellet containing the zooxanthellae was resuspended in 10 ml of pure acetone. Pigments were extracted at 4°C during 24 h. The extract was recentrifuged at 10 000 ***g*** for 15 min and chl *a* and c_2 were determined according to published methods (Jeffrey and Humphrey, 1975).

Data were normalized per skeletal surface area on both corals. For *O. patagonica* it was measured by the aluminum foil technique (Marsh, 1970), which has been found to be more accurate than the wax technique. For *C. caespitosa*, the mean polyp surface (*PS*) was calculated according to the following equation (Rodolfo-Metalpa et al., 2006): $PS=(2\pi R)H+\pi R^2$, where R is the polyp radius, and H the exosarc extension.

Growth rates

Six nubbins of *C. caespitosa* and five nubbins of *O. patagonica* were randomly chosen in each tank (N=12 and 10 for each temperature, respectively) and their growth rates were measured throughout the whole experiment on the same nubbins using the buoyant weight method (Davies, 1989). Daily growth rates (skeleton and tissue) of both corals were

calculated as the difference between two subsequent weights and normalized to the tissue surface area for both corals and by the initial weight for *O. patagonica*. Wet weight was converted into dry weight using an aragonite density of 2.93 g cm^{-3}.

Photosynthesis and respiration rates

These measurements were performed only for *C. caespitosa*, due to a lower number of samples of *O. patagonica*. Photosynthesis was assessed for the 4 temperatures and the 5 sampling times. Three nubbins were therefore taken in each tank (N=6) and were incubated in a glass thermostated chamber (from 20°C to 28°C, depending on the treatment) containing a Strathkelvin 928® oxygen electrode. The chamber was filled with 0.45 μm-filtered seawater continuously stirred with a stirring bar. The electrodes were calibrated before each experiment against air-saturated seawater and a saturated solution of sodium dithionite (zero oxygen). Samples were allowed to acclimate to chamber conditions and measurement started when polyps were expanded. Changes in dissolved oxygen concentrations were monitored on a computer during 15 min at the culture irradiance of 110 μmol photons m^{-2} s^{-1} (P_{110}) and in the dark (R). Light was provided by a metal halide lamp (Philips, HPIT 400 W, Guildford, Surrey, UK). Rates of P_{110} and R were estimated by regressing oxygen data against time, taking into account the seawater volume in the chamber. At the end of measurements, samples were frozen at –80°C for chl *a* measurements. Data were normalised by chl *a* content (μg O_2 μg chl a^{-1} h^{-1}) or by surface area (μg O_2 cm^{-2} h^{-1}).

Chlorophyll a fluorescence of PSII

Chl *a* fluorescence of PSII was always measured at the same time in the morning, during the whole experiment using a PAM fluorometer (DIVING-PAM, Walz, Germany). Five nubbins of *C. caespitosa* and three nubbins of *O. patagonica* were chosen in each tank (N=10 and 6 for each temperature, respectively) and followed during the whole experiment. The minimal (F_0 or F) and maximal (F_m or F_m') fluorescence yields were measured by applying a weak pulsed red light (max. intensity <1 μmol photon m^{-2} s^{-1}, width 3 μs, frequency 0.6 kHz), and a saturating pulse of actinic light (max. intensity >8000 μmol photon m^{-2} s^{-1}, width 800 ms), respectively. The maximum photosynthetic efficiency in dark-adapted corals ($F_v/F_m=(F_m–F_0)/F_m$, where F_v is the variable fluorescence) and the relative electron transport rate [ETR=$\Delta F/F_m' \times 0.5 \times$ PAR (photosynthetic active radiation), with $\Delta F=F_m'–F$] were used to assess the efficiency of the PSII between treatments. Rapid light curves (RLCs) were generated by illuminating corals for 10 s periods, eight times from 0 to 768 μmol photon m^{-2} s^{-1}. During measurements, the 8 mm optical fibre was maintained perpendicular to the coral's surface using a black-jacket at a fixed distance of 5 mm. Values of light intensities (PAR list) received by the corals during RLCs were obtained using the internal Light-Calibration program of the Diving-PAM. When F_v/F_m was <0.2 due to thermal stress, samples were considered dead and eliminated from the analysis.

Statistical analyses

Changes in growth rate, F_v/F_m and ETR_{max} were tested using repeated-measures ANOVAs since measurements were performed on the same corals. The following statistics were performed: (1) one-way ANOVAs testing the effect of time-exposure (T_0, T_{14}, T_{24}, T_{34}, T_{48}) on corals incubated at 20°C and at 24°C; (2) one-way ANOVAs testing the effect of time-exposure (T_{14}, T_{24} and T_{34}) on corals incubated at 26°C and at 28°C; (3) one-way ANOVAs testing the effect of decreased temperature from 26°C and 28°C to 24°C (from T_{34} to T_{48}). When ANOVA revealed significant differences (P<0.05), mean values were compared using Tukey HSD or Tukey HSD for unequal numbers (Spjotvoll/Stoline test).

Results

Measurements at 20°C and 24°C

At 20°C, all parameters tested remained constant for both corals, for the duration of the entire experiment (Table 2). The long-term exposure of *C. caespitosa* to 24°C had a gradual,

Table 2. *Summary of the results of the one-way ANOVAs testing the effect of exposure time to the different treatments on the physiological parameters of C. caespitosa*

	Temperature (°C)					
Treatment	20	24	26	28	26	28
Exposure time $T_{(days)}$	(T_0–T_{48})	(T_0–T_{48})	(T_{14}–T_{34})	(T_{14}–T_{34})	(T_{34}–T_{48})	(T_{34}–T_{48})
Growth rates (mg cm^{-2} day^{-1})	NS	<0.001	<0.001	<0.001	<0.01	<0.001
Photosynthesis (P_{110}) (μg O_2 μg chl a^{-1} h^{-1})	NS	<0.01	<0.001	<0.01	NS	NS
Respiration (R) (μg O_2 μg chl a^{-1} h^{-1})	NS	<0.01	<0.001	<0.05	NS	NS
F_v/F_m	NS	<0.05	<0.001	NS	NS	NS
ETR_{max}	NS	<0.05	<0.001	<0.001	NS	NS
Zooxanthellae (x cm^2)	NS	NS	<0.05	<0.001	<0.05	NS
Chl *a* (μg cm^{-2})	NS	NS	NS	NS	<0.05	NS
Chl c_2 (μg cm^{-2})	NS	NS	NS	NS	<0.01	NS

NS, non significant.

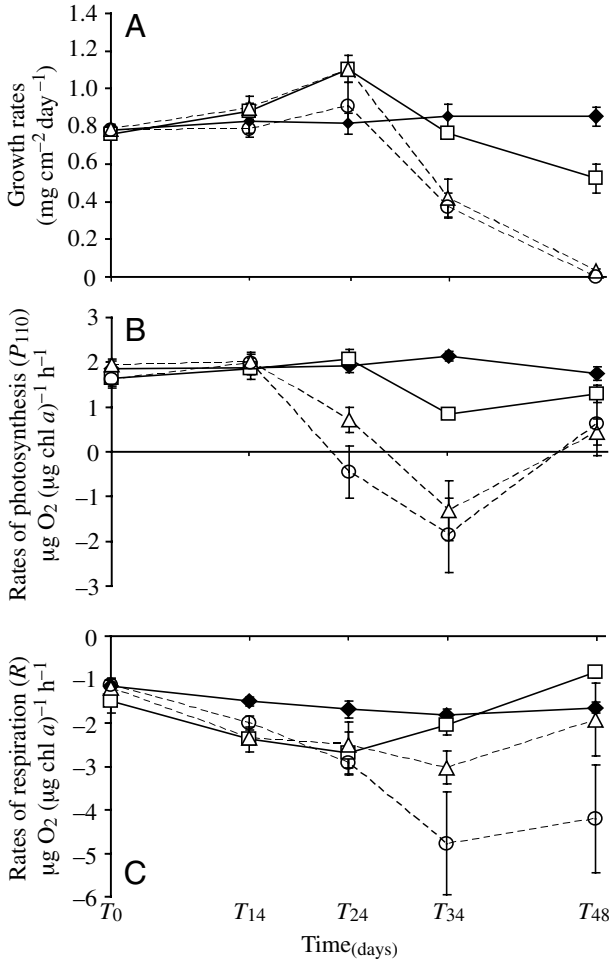

Fig. 3. *Cladocora caespitosa*. (A) Growth rates; (B) rates of photosynthesis (P_{110}) measured at the culture irradiance of 110 µmol photon m^{-2} s^{-1} and (C) rates of respiration in the dark. Temperatures were 20°C (filled diamonds), 24°C (open squares), 26°C (open triangles) and 28°C (open circles). Values are means ± s.e.m., N=12 (A), N=6 (B,C).

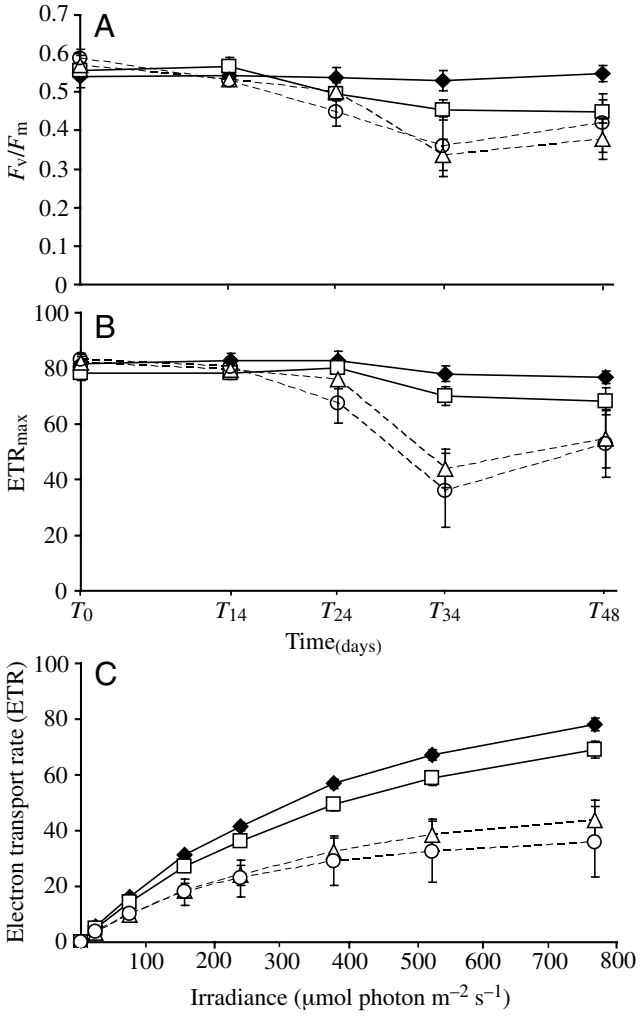

Fig. 4. *Cladocora caespitosa*. (A) Photosynthetic efficiency (F_v/F_m) measured on dark-adapted samples; (B) maximum electron transport rate (ETR$_{max}$); (C) Rapid light curves measured at T_{34}. Temperatures were 20°C (filled diamonds), 24°C (open squares), 26°C (open triangles) and 28°C (open circles). Values are means ± s.e.m., N=10.

negative impact on most parameters (Figs 3–5; Table 2). Rates of photosynthesis (P_{110}), F_v/F_m and ETR$_{max}$ (Fig. 3B, Fig. 4A,B), remained constant during the first 24 days, but significantly decreased between T_{24} and T_{48} by 19, 19 and 14%, respectively (Tukey test, P<0.05: T_{34}<T_{14}=T_{24} for P_{110}; T_{34}=T_{48}<T_0=T_{14} for F_v/F_m; T_{48}<T_{24} for ETR$_{max}$). Zooxanthellae density as well as chl a and c_2 content did not significantly change (Fig. 5A–C; Table 2). Growth and respiration rates (R) significantly increased from T_0 to T_{24} by ca. 45 and 82%, respectively, but were then reduced at T_{48} by ca. 33% and 21% of their initial values (Tukey test, P<0.05) (Fig. 3A,C). At the end of the incubation ca. 10% of polyps showed signs of tissue necrosis.

In *O. patagonica*, 24°C had a rapid effect on most parameters, which had already changed during the first 14 days (Figs 6–8; Table 3). Therefore, F_v/F_m and ETR$_{max}$ decreased by 29% and 33%, respectively, between T_0 and T_{14} (Fig. 6A,B), and there was also a 59% and 75% reduction in zooxanthellae and chl c_2 contents, respectively, (Fig. 7A,C; Tukey test, P<0.05). These parameters, however, did not show any further decrease from T_{14} to T_{48}, in contrast to *C. caespitosa* (Tukey test, P<0.05). At T_{24} growth rate significantly decreased and was reduced by ca. 70% at the end of the incubation (Tukey test; P<0.05: T_{34}=T_{48}<T_0=T_{14}=T_{24}; Fig. 8). No sign of necrosis was observed.

Measurements at 26°C and 28°C

From T_{14} to T_{34} temperature was elevated to 26°C and 28°C and most parameters significantly decreased in both corals (Table 2). For *C. caespitosa*, P_{110} was negative or null (Fig. 3B) because rates of respiration increased, despite a large variability (Fig. 3C). F_v/F_m and ETR$_{max}$ decreased by ca. 40–50% at both temperatures (Fig. 4A,B,C) although a significant difference in F_v/F_m was only found at 26°C (Tukey

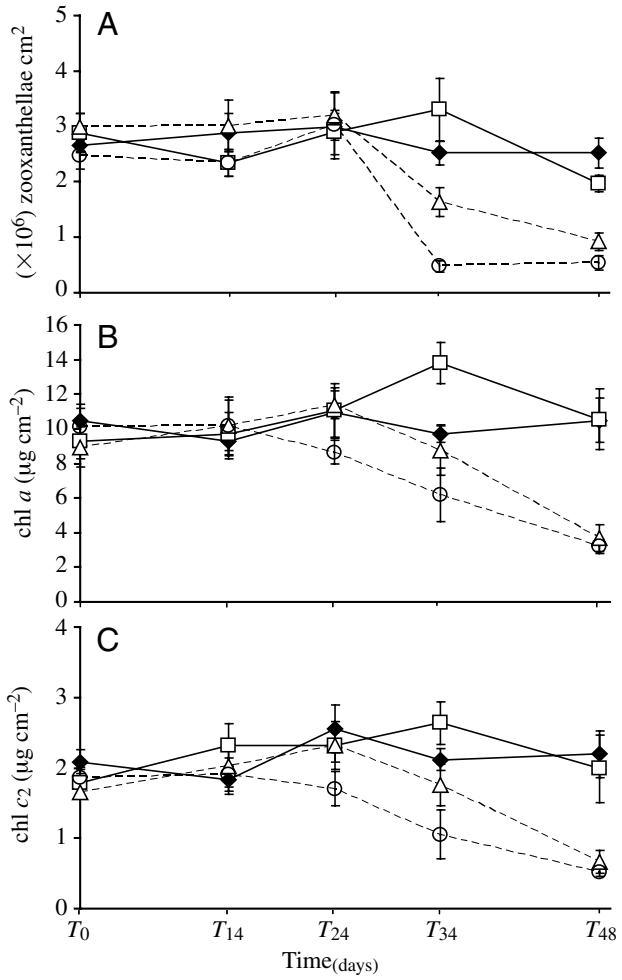

Fig. 5. *Cladocora caespitosa*. (A) Zooxanthellae density; (B) chlorophyll *a* and (C) chlorophyll c_2. Temperatures were 20°C (filled diamonds), 24°C (open squares), 26°C (open triangles) and 28°C (open circles). Values are means ± s.e.m., *N*=6.

test, $P<0.05$; Table 2). Zooxanthellae density was significantly reduced at T_{34} by 40% and 82%, for temperatures of 26°C and 28°C, respectively (Tukey test, $P<0.05$; Fig. 5A). No significant differences were found in chl *a* and c_2 content, however, due to the high variability (Fig. 5B,C; Table 2). Growth rates increased during the first 10 days of incubation (from T_{14} to T_{24}) by 45% and 18%, at 26°C and 28°C, respectively (Fig. 3A), but then drastically decreased at T_{34} by ca. 50% of their initial values (T_0) (Tukey test, $P<0.05$). Almost all samples presented signs of necrosis at T_{34} in parallel with zooxanthellae density decrease (Fig. 5A). Two and five nubbins out of ten maintained at 26°C and 28°C, respectively, died at T_{34}. Fewer than 5% of the polyps did not show necrosis but were pale (= bleached) at 28°C.

When the temperature was returned to 24°C, P_{110}, R, F_v/F_m and ETR_{max} did not show any further decrease (Fig. 3B,C and Fig. 4A,B; Table 2). Growth rates kept on decreasing, to become null at the end of the experiment (Table 2; Fig. 3A). 100% nubbins maintained at 26°C and 28°C underwent necrosis. As a consequence, zooxanthellae and chl content were 65–70% lower than the values measured at the beginning of the experiment (Fig. 5A–C).

In *O. patagonica*, no significant further change in F_v/F_m and ETR_{max} values was induced by an increase in temperature from 24°C to 26°C or 28°C (Fig. 6A,B; Table 3). A significant decrease was found in zooxanthellae and chl *a* and c_2 content from T_{14} to T_{24}, however (Tukey test, $P<0.05$: $T_{14}>T_{24}=T_{34}$; Fig. 7A–C). Growth rate was also significantly reduced at these temperatures (Tukey test, $P<0.05$; Fig. 8). Only two nubbins maintained at 28°C showed signs of tissue retraction. When temperature was returned to 24°C, there was a continuous decrease in growth rate, which was reduced by 80% at the end of the experiment for both 26°C and 28°C (Fig. 8; Table 3). Nubbins that showed signs of tissue retraction recovered from the stress by producing new tissue.

Discussion

The aim of the study was to test, for the first time, the response of two symbiotic Mediterranean corals to: (i) a 7 week exposure to the mean spring and autumn temperature of 20°C; (ii) a 7 week exposure to the mean summer temperature of 24°C; and (iii) a 3 week exposure to a 2–4°C increase in the mean summer temperature. These conditions are similar to situations occurring in the first 20 m depth of the Ligurian Sea. The coral response was assessed on both the rates of growth and photosynthesis. Results obtained have shown that, for the two corals studied, most of their physiological parameters were affected by as little as 2–5 weeks at 24°C, and were severely depressed at higher temperatures. The two corals, however, presented different responses to the stress. This study has also shown that *C. caespitosa* is living close to its thermal limit during the summer period and a long-term increase at 24°C or above could be lethal for it.

Growth and photosynthesis at 20°C

At 20°C, all the parameters measured remained constant during the incubation, suggesting that both corals were fully adapted to such conditions. Rates of photosynthesis and growth of Mediterranean corals under normal temperature and light conditions have been poorly investigated (Peirano et al., 1999; Peirano et al., 2005; Schiller, 1993). Comparison of the growth rate measurements with other studies performed on temperate corals is difficult, because they all used various growth indices, including linear extension rate (Schiller, 1993; Goffredo et al., 2004; Peirano et al., 2005), skeletal density pattern (Peirano et al., 1999), internal growth lines (Nagelkerken et al., 1997), incorporation of ^{45}Ca (Howe and Marshall, 2002; Marshall and Clode, 2004), or % wet mass (Miller, 1995).

Growth rates at 20°C of *O. patagonica* and *C. caespitosa* were equal to 0.2 and 0.8 mg cm^{-2} day^{-1}, respectively. If we assume that growth is sustained continuously throughout the year, we get a maximal calcium deposition of 0.73 and 2.92 kg $CaCO_3$ m^{-2} $year^{-1}$, respectively. An annual maximal production of 1.7 kg $CaCO_3$ m^{-2} $year^{-1}$ was measured in *C.*

Table 3. *Summary of the results of the one-way ANOVAs testing the effect of time-exposure at the different treatments, on the physiological parameters of O. patagonica*

	Temperature (°C)					
Treatment	20	24	26	28	26	28
Exposure time $T_{(days)}$	(T_0–T_{48})	(T_0–T_{48})	(T_{14}–T_{34})	(T_{14}–T_{34})	(T_{34}–T_{48})	(T_{34}–T_{48})
Growth rates (mg cm^{-2} day^{-1})	NS	<0.001	<0.001	<0.001	<0.001	<0.05
F_v/F_m	NS	<0.001	NS	NS	NS	NS
ETR_{max}	NS	<0.001	NS	NS	NS	NS
Zooxanthellae (x cm^2)	NS	<0.001	<0.001	NS	NS	NS
Chl *a* (μg cm^{-2})	NS	NS	<0.05	<0.05	NS	<0.05
Chl c_2 (μg cm^{-2})	NS	<0.001	<0.001	<0.05	NS	<0.05

NS, non significant.

caespitosa using sclerochronology (Peirano et al., 2001), a value slightly lower than our estimation. When compared to other temperate corals, similar growth rates were obtained for *Plesiastrea versipora* [0.15–0.18 mg cm^{-2} day^{-1} (Kevin and Hudson, 1979; Howe and Marshall, 2002)] and *Astrangia danae* [0.6 mg cm^{-2} day^{-1} (Jacques et al., 1983)]. Growth rates of the Mediterranean corals are therefore much lower (5 times) than those experienced by tropical scleractinian species [ca. 4 mg cm^{-2} day^{-1} (Lough and Barnes, 2000; Carricart-Garnivet, 2004)], despite the fact that they are symbiotic. Low growth rates seem to be a feature of temperate corals, and could be explained by several parameters such as temperature (Lough and Barnes, 2000), light (Bak, 1974) and carbonate saturation state (Kleypas et al., 1999), all of which are decreased in temperate compared to tropical waters.

In terms of photosynthesis/photosynthetic efficiency, to our knowledge there are few data available, either for Mediterranean (Schiller, 1993; Fine et al., 2004) or for other temperate corals (Jones et al., 2000; Howe and Marshall, 2001; Nakamura et al., 2003). At 20°C, the dark-adapted F_v/F_m of the symbiotic zooxanthellae of both corals (0.6–0.65) were in the range of values observed in marine algae (Büchel and Wilhelm, 1993) and in zooxanthellae symbiotic with tropical corals (e.g. Jones et al., 1998). The rate of photosynthesis measured for *C. caespitosa* at the culture irradiance of 110 μmol photons m^{-2} s^{-1} [2 μg O_2 μg chl a^{-1} h^{-1} or ca. 20 μg O_2 cm^{-2} h^{-1}] is also in the range of those previously measured for other temperate corals under conditions of similar irradiance (Schiller, 1993; Howe and Marshall, 2001).

Finally, *C. caespitosa* and *O. patagonica* contained high densities of zooxanthellae (3×10^6 and 16×10^6 zooxanthellae cm^{-2}, respectively) compared to tropical reef corals [usual range: 0.3–3.5$\times10^6$ zooxanthellae cm^{-2} (e.g. Hoegh-Guldberg and Smith, 1989; Muller-Parker et al., 1994; Stimson et al., 2002)]. This seems a typical feature of temperate corals (Kevin and Hudson, 1979; Jacques et al., 1983; Schiller, 1993; Howe and Marshall, 2001), adapted to a shade environment during most of the year (Muller-Parker and Davy, 2001). This symbiosis seems also to be stable, since the zooxanthellae density does not change with the season or the depth (Schiller, 1993). Despite the fact that Mediterranean corals contain a high density of zooxanthellae with a maximal photosynthetic efficiency, their growth rates are much lower than tropical corals, suggesting that fewer photosynthates are allocated for calcification in Mediterranean corals and may be used as energy to accommodate lower temperature conditions, or released as mucus. Indeed, at least for *C. caespitosa*, high rates of mucus release [44% of its respiration (Herndl and Velimirov, 1986)] seems to be an adaptation to the life in turbid waters (Schiller, 1993).

Growth and photosynthesis at high temperatures

The growth of tropical (Lough and Barnes, 2000; Carricart-Garnivet, 2004; Marshall and Clode, 2004; Edmunds, 2005) or temperate scleractinian corals (Jacques et al., 1983; Miller, 1995; Howe and Marshall, 2002; Peirano et al., 2005) has often been investigated under the normal range of temperatures experienced by corals in their natural environments. Few studies, however, have assessed the effect of a small but prolonged 1–2°C temperature increase above the optimum, and these studies have been performed only with tropical corals (Jokiel and Coles, 1977; Abramovitch-Gottlib et al., 2003; Reynaud et al., 2004). They demonstrated an impairment of skeletal growth at high temperatures. In the same way, the effect of elevated temperatures on photosynthesis of temperate scleractinian species has been poorly studied (Jones et al., 2000; Nakamura et al., 2003), in contrast to tropical corals (e.g. Coles and Jokiel, 1978; Baghooli and Hidaka, 2003; Hill et al., 2004).

In this study, *O. patagonica* showed a significant decrease in growth rate, when the temperature of 24°C was maintained for more than 3 weeks, suggesting that this maximum summer temperature is already a breaking point for the growth of this coral. A similar trend was observed in another temperate coral *Plesiastrea versipora* (Howe and Marshall, 2002), whose calcification was maximum at 3°C below the maximum summer temperature of 21°C. In parallel to the growth rate decline, there was also a significant decrease in the photosynthetic parameters (F_v/F_m, ETR) after 2 weeks at 24°C,

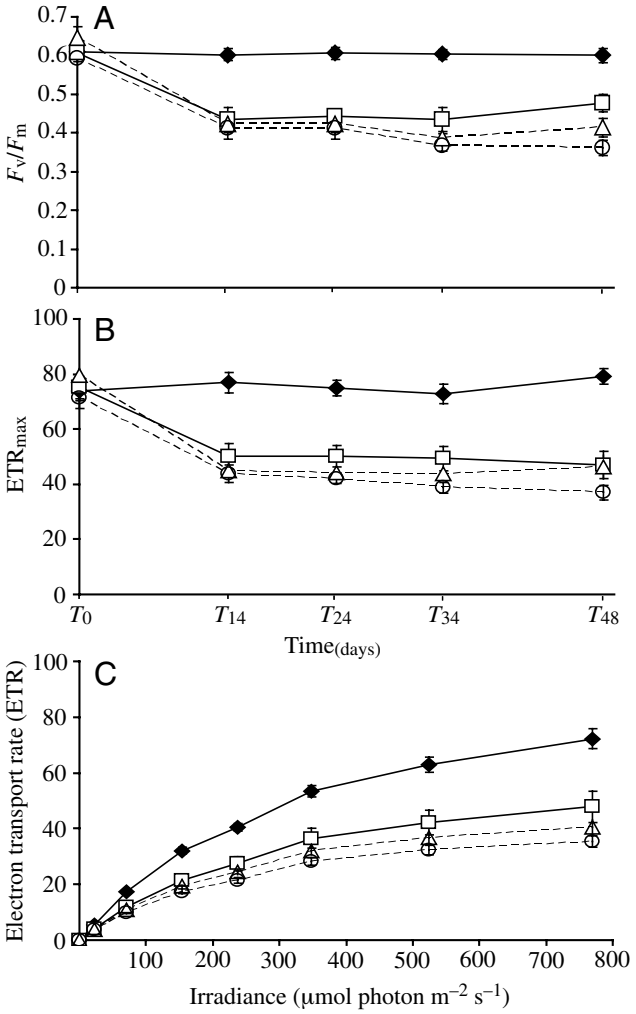

Fig. 6. *Oculina patagonica.* (A) Photosynthetic efficiency (F_v/F_m) measured on dark-adapted samples; (B) maximum electron transport rate (ETR_{max}); (C) Rapid light curves measured at T_{34}. Temperatures were 20°C (filled diamonds), 24°C (open squares), 26°C (open triangles) and 28°C (open circles). Values are means ± s.e.m., *N*=6.

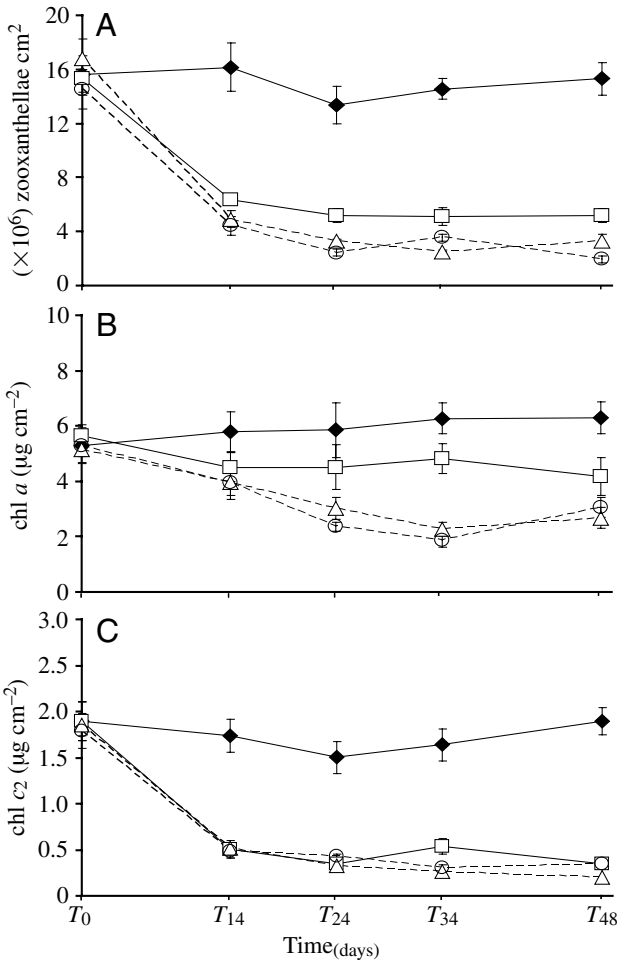

Fig. 7. *Oculina patagonica*. (A) Zooxanthellae density; (B) chlorophyll *a* and (C) chlorophyll c_2. Temperatures were 20°C (filled diamonds), 24°C (open squares), 26°C (open triangles) and 28°C (open circles). Values are means ± s.e.m., *N*=6.

suggesting a coupling between photosynthesis and calcification, as it has been already shown for other tropical (Gattuso et al., 1999) and temperate corals (Kevin and Hudson, 1979). The decrease in F/F_m (from ca. 0.6 to 0.4) coincided with the loss of zooxanthellae (from 16×10^6 to 4×10^6 cells cm^{-2}) and chl, suggesting that corals were stressed. This loss of symbionts occurred without any sign of necrosis and can be considered as a bleaching phenomenon. The decrease in growth and photosynthetic efficiency observed at 24°C (and above) is intriguing because this coral is also spread along the Israeli coasts, where it experiences, in the summer months, long-term exposure (4–6 months) to elevated temperatures (from 24°C to 30°C) (Shenkar et al., 2005). We may therefore be dealing with a different genetic population (either the algal or animal partner, or both), or there could have been some adaptive adjustments in the capability of the colonies to withstand high temperatures (Clausen and Roth, 1975). More comparative studies between the *Oculina* species originating from the Ligurian Sea and the coast of Israel are needed to understand their differences.

The results also show different responses of *C. caespitosa* compared to *O. patagonica* to the elevation in temperature. The first difference was in the pattern of growth response. In contrast to *O. patagonica*, the growth rate of *C. caespitosa* significantly increased during the first 3 weeks of temperature increase, especially at 24°C and 26°C, suggesting a temperature enhancement of growth in this coral. In general the temperature–growth response of corals is characterized by a minimum growth rate at the lowest temperature, enhancement up to a threshold temperature, and a decline thereafter (Edmunds, 2005). Accordingly, the positive effect of elevated temperature on growth rate of *C. caespitosa* was limited for the first 3 weeks, after which a decrease was observed. This may be due to a lack of energy to sustain these higher growth rates,

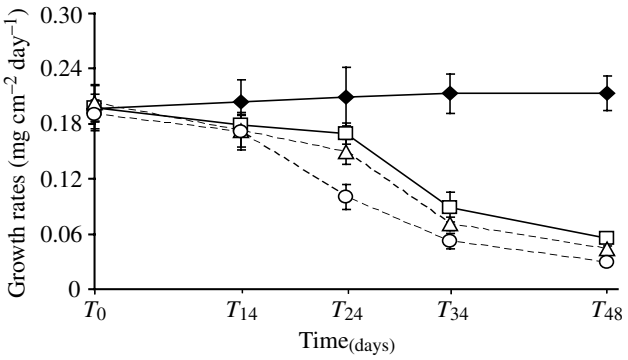

Fig. 8. Growth rates of *Oculina patagonica*. Temperatures were 20°C (filled diamonds), 24°C (open squares), 26°C (open triangles) and 28°C (open circles). Values are means ± s.e.m., *N*=10.

since the rates of photosynthesis, as well as the photosynthetic efficiency significantly decreased during, respectively, the third (for 26°C and 28°C) and the fourth (for 24°C) week of incubation.

The second difference was in the mortality response. In contrast to *O. patagonica*, which rapidly (after 2 weeks at 24°C) bleached but showed no sign of necrosis, *C. caespitosa* underwent necrosis after 5 weeks at elevated temperatures, leading to the loss of the coral tissue and its associated zooxanthellae. The process started with a retraction of the tissue inside the calyx of the polyp, leaving large zones of skeleton without tissue (Fig. 9A); this has been interpreted, when observed *in situ* and in summer, as a way of protecting the photosynthetic apparatus during short periods of high irradiance and temperature (Brown et al., 1994; Brown et al., 2002; Peirano et al., 2005). However, in the present study, the retraction was followed by tissue necrosis (Fig. 9B,C) and 100% mortality of the nubbins of *C. caespitosa* at the end of the incubation at 26°C and 28°C. During this experiment, *O. patagonica*, by remaining alive and without necrosis, was more able than *C. caespitosa* to resist to high temperature conditions. Its rapid capacity to bleaching was maybe one of the keys to its success.

During the last few years, studies on the effect of environmental changes on corals have mainly concerned tropical reef corals, because of the massive bleaching events occurring everywhere (Wilkinson et al., 2004). Less attention has been given to temperate marine organisms, and especially to the Mediterranean benthic fauna (Cerrano et al., 2000). However, mass mortality events in the Mediterranean Sea have increased during the last 10 years, presumably due to global warming. Average temperatures have already increased by 0.3–0.7°C (Walther et al., 2002) and might be higher in the next few years. The results obtained show that the two symbiotic scleractinian corals, especially *C. caespitosa*, which is endemic to the Mediterranean, may already suffer during warm summers. Further studies are required to provide a better knowledge of the ability of Mediterranean species to recover from such stress and perhaps adapt to it.

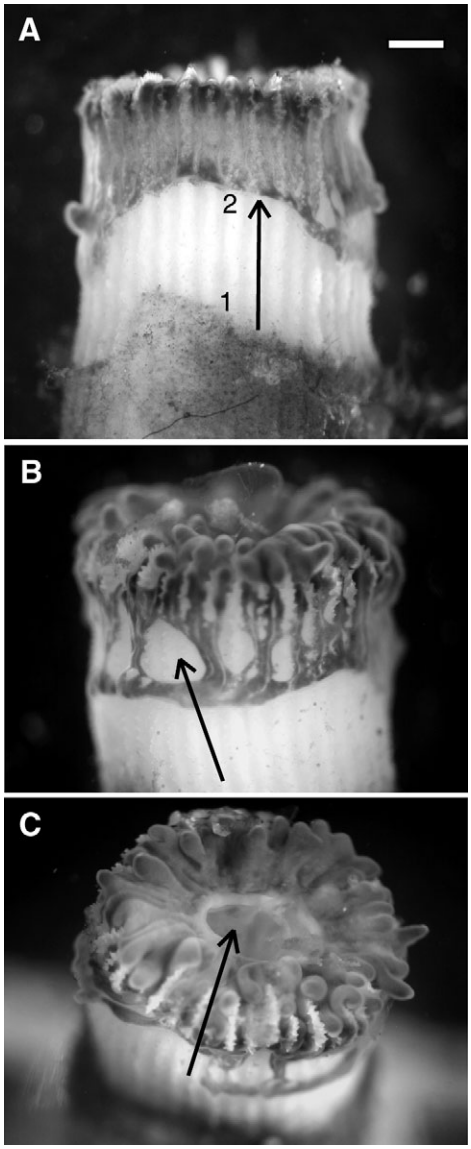

Fig. 9. Polyp of *Cladocora caespitosa*. Photographs taken at T_{34}, showing signs of tissue necrosis. (A) Tissue retraction started from the distal portion of the calyx; the arrow shows the direction (from 1 to 2); (B) tissue then underwent necrosis around the calyx edge (arrow); (C) necrosis finally reached the inside of the polyp; the arrow shows the lack of polyp tissue inside the corallite. Scale bar, 1 mm.

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