RESEARCH ARTICLE

Response of zooxanthellae in symbiosis with the Mediterranean corals *Cladocora caespitosa* and *Oculina patagonica* to elevated temperatures

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Abstract Scleractinian symbiotic corals living in the Ligurian Sea (NW Mediterranean Sea) have experienced warm summers during the last decade, with temperatures rapidly increasing, within a few days, to 3–4°C above the mean value of 24°C. The effect of elevated temperatures on the photosynthetic efficiency of zooxanthellae in symbiosis with temperate corals has not been well investigated. In this study, the corals, *Cladocora caespitosa* and *Oculina patagonica* were collected in the Ligurian Sea (44°N, 9°E), maintained during 2 weeks at the mean summer temperature of 24°C and then exposed during 48 h to temperatures of 24 (control), 27, 29 and 32°C. Chlorophyll (chl) fluorescence parameters [F_v/F_m, electron transport rate (ETR), non-photochemical quenching (NPQ)] were measured using pulse amplitude modulated (PAM) fluorimetry before, during the thermal increase, and after 1 and 7 days of recovery (corals maintained at 24°C). Zooxanthellae showed a broad tolerance to temperature increase, since their density remained unchanged and there was no significant reduction in their maximum quantum yield (F_v/F_m) or ETR up to 29°C. This temperature corresponded to a 5°C increase compared to the mean summer temperature (24°C) in the Ligurian Sea. At 32°C, there was a significant decrease in chl contents for both corals. This decrease was due to a reduction in the chl/zooxanthellae content. For *C. caespitosa*, there was also a decrease in ETR_{max}, not associated with a change in F_v/F_m or in the non-photochemical quenching (NPQ); for *O. patagonica*, both ETR_{max} and F_v/F_m significantly decreased, and NPQ_{max} showed a significant increase. Damages to the photosystem II appeared to be reversible in both corals, since F_v/F_m values returned to normal after 1 day at 24°C. Zooxanthellae in symbiosis with the Mediterranean corals investigated can therefore be considered as resistant to short-term increases in temperature, even well above the maximum temperatures experienced by these corals in summer.

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Introduction

The Ligurian Sea (NW Mediterranean Sea) hosts several symbiotic scleractinian coral species such as *Cladocora caespitosa* and *Oculina patagonica*. *C. caespitosa* (Faviidae), which is one of the main endemic Mediterranean coral species, is abundant both in past and recent times (Peirano et al. 1998) and can form extensive bioherms (Schumacher and Zibrowius 1985). It is commonly distributed from 5 to 20 m depth in turbid water, although it can sometimes reach depths of 40 m (Morri et al. 1994). The largest living banks, corresponding to reef-like structures, are found in the
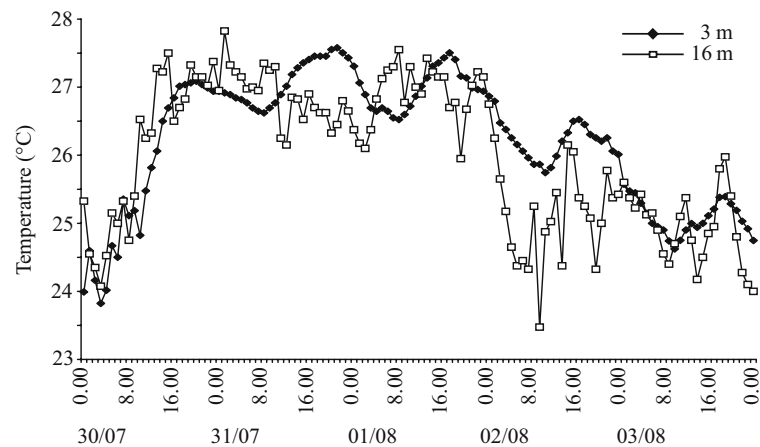
Adriatic Sea (Kružić and Požar-Domac 2003). *O. patagonica* (Oculinidae) is an immigrant scleractinian coral supposed to be originating from the Southwest Atlantic (Zibrowius 1974). Encrusting colonies cover hard substrates at a depth range of 0.5–10 m. While this species is very abundant along the Israeli and Spanish coasts, it is less expanded in the Ligurian Sea (see Zibrowius 1980; Fine et al. 2001 for a review).

Both species experience large changes in temperature throughout the year with mean values of 12 and 24°C during winter and summer months, respectively (Picco 1990; SOMLIT, Service d'Observation en Milieu Littoral, INSU-CNRS, Villefranche-sur-mer). During previous summer months (1997–2005), several mortality events occurred in the Ligurian Sea, which affected sponges, gorgonians (Cerrano et al. 2000; Coma et al. 2000; Garrabou et al. 2001; Linares et al. 2005), small sessile benthic organisms (Perez et al. 2000) and coral species (Rodolfo-Metalpa et al. 2000). These mortality events were coincident with registered positive thermal anomalies (Romano et al. 2000; Harmelin 2004) due to increases in Mediterranean sea surface temperatures (SST) (Bethoux et al. 1990; Walther et al. 2002). These SST anomalies were characterised either by long-term increases of 1–2°C above mean values (22–24°C in the upper 20 m) or short-term increases of 3–4°C. Long-term increases, such as those observed during the summer 1999, are characterised by the lowering of the thermocline and temperatures up to 24°C at 40 m depth for more than 1 month (Cerrano et al. 2000; Romano et al. 2000). Short-term temperature increases, which occurred during the summer 2003, were associated with large temperature shifts up to 7.5°C within a few hours (Harmelin 2004). Therefore, temperature values at 12 m reached 27.2°C at some moment. Temperatures at 3 and 16 m, where the corals are usually found, rapidly increased within 1 day from ca. 24°C, the usual summer temperature, to ca.

28°C and remained at this value for 2 days before decreasing again to 24°C (Fig. 1). A relationship between elevated temperatures and mortality of the benthic fauna was only suggested, but cannot be demonstrated, due to the poor knowledge of the effects of temperature and of the degree of its variation on physiological processes of the affected species (Coma et al. 2000).

In tropical scleractinian corals, numerous studies have shown that short-term increases in temperature, especially when combined with high-light intensity, decrease the photosynthesis/photosynthetic efficiency of the symbiotic zooxanthellae and most often lead to coral bleaching events (Jones et al. 1998; Fitt et al. 2001; Bhagooli and Hidaka 2004; Hill et al. 2004). In contrast, few studies have investigated the response of temperate coral symbionts to temperature increases above their normal range (Jacques et al. 1983; Ben-Haim et al. 1999; Jones et al. 2000; Nakamura et al. 2003). Conversely to Jacques et al. (1983), which showed an increase in photosynthesis up to 27°C in *Astrangia danae*, Jones et al. (2000) as well as Nakamura et al. (2003) showed that the photosynthesis/photosynthetic efficiency of *Plesiastrea versipora* and *Acropora pruinosa*, decreased after exposure to 28°C for 2–4 days. Ben-Haim et al. (1999) investigated the inhibition of photosynthesis of the coral *O. patagonica* after its infection by *Vibrio shiloi*. The vibrio infection induced bleaching of the coral (Kushmaro et al. 1996), which occurred during long exposures (>72 h) to high temperatures (Rosenberg and Falkovitz 2004).

The aim of this study was to investigate the effect of a short-term (from 5 to 48 h) increase in temperature on the photosynthetic efficiency of zooxanthellae in symbiosis with the two Mediterranean corals *C. caespitosa* and *O. patagonica*. The range of temperatures investigated (from 24 to 29°C) was comparable to those experienced by the corals in the Ligurian Sea

during warm summers. Temperature was also increased up to 32°C to assess the resistance of the corals to extreme value.

Materials and methods

Samples collection and acclimation

Seven colonies (ca. 20 cm in diameter) of *C. caespitosa* were randomly collected in the Bay of Fiascherino (Gulf of La Spezia, 44°03′N, 9°55′E) at 7 m depth. At this location, the species is very abundant and well distributed. It thrives in turbid waters and under low light conditions (from 50 to 110 μmol photons m^{-2} s^{-1}, Peirano et al. 1999, 2005). One hundred samples of *O. patagonica* were collected at Albissola (Gulf of Genoa, 44°17′N, 8°30′E) at 3 m depth. At this location, *O. patagonica* covers 5–6 m^2 of surface area and is growing on a vertical cliff facing north, which prevents exposure to direct sunlight. For both corals, seawater temperature was 20°C at the time of collection.

Samples were transported in aerated seawater under reduced-light conditions to the laboratory where the experiments were conducted. 252 nubbins (single polyp) of *C. caespitosa* were made from mother colonies, carefully cleaned of epiphytes and placed on PVC supports. 204 nubbins (8–23 cm^2) of *O. patagonica*, made from samples, were attached to a nylon wire and suspended in an aquarium (300 l) until tissue covered the denuded skeleton. The aquaria were continuously supplied with Mediterranean seawater (20°C) pumped from 50 m depth in front of the Oceanographic Museum. Renewal rate in the aquarium was 5 l h^{-1}. Light was provided by metal halide lamps (Philips, HPIT 400 W) on a 12:12 h (light:dark) photoperiod. Plastic mesh was used to set-up light intensity to the summer value of 110 μmol photon m^{-2} s^{-1}. Irradiance was measured using a Li-Cor underwater quantum sensor (LI-193SA). All corals were fed twice a week with *Artemia salina* nauplii.

Experimental set-up

The 252 nubbins of *C. caespitosa* and 204 of *O. patagonica* were randomly transferred to eight experimental tanks (15 l). Temperature in the tanks was slowly (1°C per day) increased from 20 to 24°C (mean summer temperature) and corals were maintained 2 weeks under these summer conditions (according to the *in situ* temperature sensors deployed by SOMLIT, corals are exposed to this temperature for ca. 3 weeks). In order to ensure that no change occurred in both corals, chlorophyll (chl) fluorescence parameters [F_v/F_m, electron transport rate (ETR), non-photochemical quenching (NPQ)] were measured on 50 nubbins both before and at the end of this control period. In addition, 20 nubbins were randomly collected and frozen at –80°C in order to determine protein and chl contents ($n = 10$), and zooxanthellae density.

Temperatures were then increased (1°C per hour) to 24 (control), 27, 29 and 32°C and maintained for 48 h. Two tanks for each treatment were used and temperature was maintained constant using aquarium heaters connected to electronic controllers (±0.2°C). At the end of the treatment, corals were slowly returned to the control temperature of 24°C for a recovery period. Chl fluorescence parameters (F_v/F_m, ETR, NPQ) were measured (as described below) on five nubbins of each species in each tank after 5, 24 and 48 h of treatment as well as during the recovery period (after 1 and 7 days). Three nubbins of each species were also collected in each tank after 5 and 48 h of treatment, and after 1 and 7 days of recovery for measurements of zooxanthellae density and chl content (see below). Similarly, three nubbins of *C. caespitosa* were frozen at –80°C for protein measurements. During the entire experiment, corals were also observed under a microscope for tissue degradation (necrosis or retraction into the polyps).

Chl fluorescence measurements

Photosynthetic efficiency of photosystem II (PSII) was measured on dark-adapted nubbins of *C. caespitosa* and *O. patagonica* (five replicates per tank and for each exposure time), using a PAM fluorometer (DIVING-PAM, Walz, Germany, Schreiber et al. 1986). During measurements, a black jacket of neoprene was mounted on the free-end of the 8 mm optical fibre in order to keep a fixed distance of 5 mm from the coral surface. The minimal (F_0) and maximal (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 ofactinic light (max intensity >8,000 μmol photon m^{-2} s^{-1}, width 800 ms), respectively. The maximum photosynthetic efficiency F_v/F_m = $(F_m–F_0)/F_m$ in dark-adapted corals and the relative ETR (ETR = F_v/F_m $0.5 \times$ PAR) were used to assess the efficiency of the PSII. The heat-dissipation process of excessive absorbed energy from PSII was reported as NPQ values (NPQ = $(F_m–F_m')/F_m'$, F_m' is the maximal fluorescence yield at a given light intensity). Rapid light curves (RLCs) were generated by illuminating dark-adapted corals from 0 to 835 μmol photon m^{-2} s^{-1} (PAR) in eight steps of 10 s. ETR_{max},

NPQ$_{max}$, obtained at the maximal light intensity of the RLC, were compared between treatments.

Data normalisation

In this study, results of *C. caespitosa* were normalised by surface area, in order to be compared with other corals. Individual polyps are present on the apical part of the tubular corallites, which have their own walls independent from the others (Zibrowius 1980). Polyp surface (PS) was therefore estimated, on all polyps, taking into account the mean diameter of the corallite (4–5 mm), as well as the exosarc extension around the corallite (3–5 mm), according to the following equation: $PS = (2\pi R)H + \pi R^2$, where R represent the polyp radius, and H is the exosarc extension, measured with a calliper. This method yielded results superior to the aluminium foil or the wax techniques, which are both difficult to apply to polyps that have a small exosarc extension. Results of *O. patagonica* are normalised per surface area, which was estimated using the aluminium foil method (Marsh 1970).

Laboratory analysis

In order to determine zooxanthellae density and chl *a*, c_2 concentrations, coral tissue was detached from the skeleton with a jet of re-circulated filtered seawater (0.45 μm pore size). The slurry that was produced from this procedure was homogenised using a potter tissue grinder and the volume of the homogenate recorded. A sub-sample of the homogenates was taken for zooxanthellae counting (ten separate chamber counts) using an improved version of the Histolab® 5.2.3 image analysis software (Microvision, Every, France). This method was compared to the haemocytometer method using six nubbins of *O. patagonica* with different zooxanthellae densities and for which five replicate counts were performed. No difference was found between the two techniques (one-way ANOVA, $P > 0.05$, $n = 6$). For chl measurements, 10 ml of the homogenate were centrifuged at 5,000 g for 10 min at 4°C and the supernatant was discarded leaving the algal pellet. This pellet was re-suspended in 10 ml of pure acetone, and pigments were extracted at 4°C during 24 h in the dark. The extract was re-centrifuged at 10,000 g for 10 min, the supernatant containing the pigments was collected, and absorbances were measured according to Jeffrey and Humphrey (1975).

Nubbins for protein estimation were treated in 1 N NaOH at 90°C for 30 min and the protein content was measured using the BCA assay Kit (Interchim, Smith et al. 1985). The standard curve was established with bovine serum albumin and the absorbance was measured with a multiscan bichromatic spectrophotometer (Labsystem®, Helsinki, Finland).

Statistical analysis

All data were tested for assumptions of normality and homoscedasticity by the Cochran' test and were transformed when required. One-way ANOVA showed no significant difference between replicated tanks, used for each temperature ($P > 0.05$). Therefore, data corresponding to each temperature were pooled for the analysis. One-way ANOVAs were used to test the effect of temperature change from 20 to 24°C during the control period. Two-way ANOVAs with fixed effect were performed on zooxanthellae densities, chl *a*, c_2 and protein contents, between temperatures (24, 27, 29 and 32°C) and exposure time (5 and 48 h) using STATISTICA® software (StatSoft, OK, USA). The same analysis was made on the chl fluorescence measurements (F_v/F_m, ETR$_{max}$ and NPQ$_{max}$), at each exposure time (5, 24 and 48 h). When ANOVAs showed significant differences, Tukey honest test (HSD) was used to attribute differences between specific factor, or their interaction only. Student's *t*-tests were used to compare data obtained during control and recovery periods. Statistical significance levels were defined as follow: n.s., not significant ($P > 0.05$); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. All data were expressed as mean ± standard deviation (SD).

Results

During the control period, no significant change occurred neither in the photosynthetic efficiency measured (F_v/F_m, ETR$_{max}$ and NPQ$_{max}$) nor in the zooxanthellae and chl contents (Table 1). Therefore, *C. caespitosa* contained $3.6 \pm 0.9 \times 10^6$ zooxanthellae cm^{-2}, 9.8 ± 1.9 and 1.8 ± 0.4 μg chl *a* and c_2 cm^{-2}, respectively. The mean surface area of a polyp was 0.78 ± 0.13 cm^{-2}. Protein content was equal to 2.3 ± 0.7 mg cm^{-2}. *O. patagonica* contained significantly more zooxanthellae ($10.1 \pm 2.9 \times 10^6$ zooxanthellae cm^{-2}), but a smaller amount of chls (5.7 ± 2.1 and 1.2 ± 0.2 μg chl *a* and c_2 cm^{-2}, respectively) than *C. caespitosa*. The protein content was equal to 3.4 ± 0.6 mg cm^{-2}. F_v/F_m of symbiotic dinoflagellates of both corals (0.598 ± 0.037 and 0.666 ± 0.018 for *O. patagonica* and *C. caespitosa*, respectively) were in the range of values already reported for tropical corals. The RLC curves showed ETR$_{max}$ values ranging

Table 1 Results of one-way ANOVA comparing zooxanthellae density (*zoox density*), chlorophylls (*chl*) contents and chl fluorescence variables (F_v/F_m, ETR_{max}, NPQ_{max}) measured at the beginning and at the end of the control period

Parameters	Cladocora caespitosa			Oculina patagonica		
	df	F-ratio	p-value	df	F-ratio	p-value
Zoox density ($\times 10^6$ cm^{-2})	(1,18)	0.069	0.79	(1,18)	0.029	0.86
Chl a (µg cm^{-2})	(1,18)	1.117	0.30	(1,18)	0.301	0.59
Chl c_2 (µg cm^{-2})	(1,18)	2.747	0.11	(1,18)	0.594	0.45
F_v/F_m	(1,48)	0.239	0.62	(1,48)	0.328	0.56
ETR_{max}	(1,48)	0.035	0.85	(1,48)	0.180	0.67
NPQ_{max}	(1,48)	0.072	0.79	(1,48)	0.081	0.81

between 57.8 ± 8.3 for *O. patagonica* and 78.6 ± 2.3 for *C. caespitosa*. The NPQ_{max} values at the beginning of the experiment were similar between the species (ca. 0.25).

During the experiment, no effect of temperature (24, 27, 28 and 32°C) and exposure time (5 and 48 h) was found on protein content of *C. caespitosa* nor on zooxanthellae density of both corals (Table 2; Fig. 2a, b). However, there was a significant effect of temperature, time or both on the chl concentration (cm^{-2}) of the corals (Table 2). Indeed, for *C. caespitosa*, chl a and c_2 cm^{-2} significantly decreased (Tukey test, $P < 0.05$) by 49 and 46%, respectively, after 48 h

Table 2 Summary of the results of the two-way ANOVAs testing the effect of temperature and time on the zooxanthellae density (*zoox density*), chlorophylls (*chl*), protein contents and chl a fluorescence parameters (F_v/F_m, ETR_{max}, NPQ_{max}) of in hospite zooxanthellae of *C. caespitosa* and *O. patagonica*

Parameters	Temperature	Time	Interaction
Cladocora caespitosa			
Zoox density ($\times 10^6$ cells cm^{-2})	NS	NS	NS
Chl a (µg cm^{-2})	*	***	**
Chl c_2 (µg cm^{-2})	**	*	*
Chl a (µg cell^{-1})	**	NS	NS
Chl c_2 (µg cell^{-1})	***	NS	NS
Protein (µg cm^{-2})	NS	NS	NS
F_v/F_m	NS	NS	NS
ETR_{max}	**	NS	NS
NPQ_{max}	NS	NS	NS
Oculina patagonica			
Zoox density ($\times 10^6$ cells cm^{-2})	NS	NS	NS
Chl a (µg cm^{-2})	NS	NS	NS
Chl c_2 (µg cm^{-2})	***	NS	NS
Chl a (µg cell^{-1})	NS	NS	NS
Chl c_2 (µg cell^{-1})	***	***	**
F_v/F_m	***	NS	NS
ETR_{max}	*	NS	NS
NPQ_{max}	***	NS	NS

NS no significant difference

*$P < 0.05$

**$P < 0.01$

***$P < 0.001$

at 32°C (Fig. 3a, b). As a consequence, there was a significant effect of temperature alone of the amount of chl a and c_2 per zooxanthellae (Table 2; Fig. 3c, d). For *O. patagonica* only temperature has a significant effect on chl c_2 cm^{-2} (Table 2), which drastically decreased (by 60%) at 29 and 32°C right after 5 h incubation (Tukey test, $P < 0.05$, Fig. 4a, b) and remained low during the whole experiment without further decrease. There was therefore a combined effect of temperature and time on the chl c_2 content per zooxanthellae (Table 2; Fig. 4d). Chl c_2 started to decrease at 29 and 32°C after 5 h incubation and also decreased at 27°C after 48 h incubation (Tukey test, $P < 0.05$).

F_v/F_m of *C. caespitosa* was not affected by temperature and exposure time (Fig. 5a; Table 2). Conversely temperature significantly reduced the F_v/F_m of *O. patagonica* (Table 2), which decreased at 32°C by 9% after 5 h incubation (Fig. 6a; Tukey test, $P < 0.05$).

Only temperature had a significant effect on the ETR_{max} values of both corals (Figs. 5b, 6b) exposed to 32°C (Table 2; Tukey test, $P < 0.05$). This effect was rapid, since a 19 and 27% decrease (for *C. caespitosa* and *O. patagonica*, respectively) occurred immediately after 5 h incubation and remained constant after 24 and 48 h incubation at 32°C (results not shown). The NPQ_{max} values of both corals rapidly increased to a constant value after 5 h at 32°C (Figs. 5c, 6c). However this increase was significant only for *O. patagonica* and was due to the temperature effect (Table 2; Tukey test, $P < 0.05$).

Throughout the entire experiment the animal part of the coral did not show any sign of stress, since there was no visible necrosis or tissue loss as observed under a binocular microscope. The absence of tissue loss was indicated by the stability in the protein content on *C. caespitosa*.

During the recovery period, parameters of corals that were not affected during the experiment (24, 27 and 29°C) remained constant and not significantly different from the control period (data not shown). For *C. caespitosa* having experienced 32°C, contents of chl
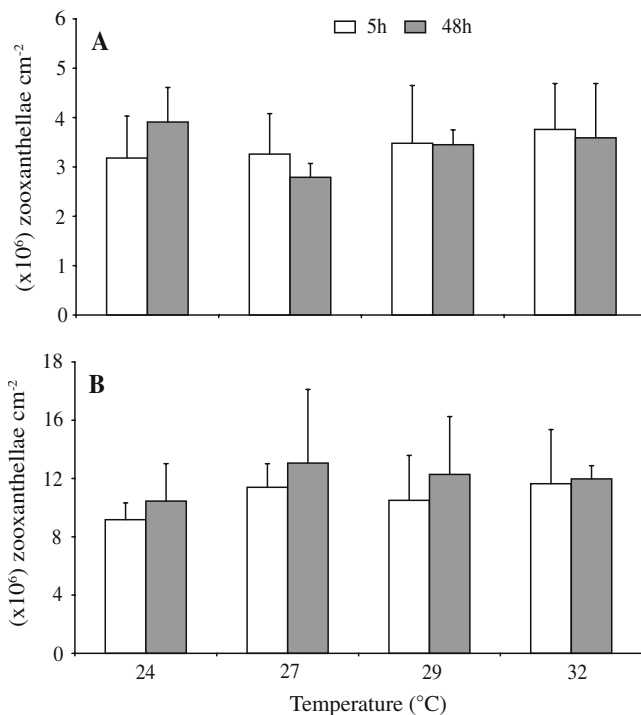
Fig. 2 Zooxanthellae density. **a** *Cladocora caespitosa*, **b** *Oculina patagonica*, after 5 and 48 h of incubation at 24°C (control), 27, 29 and 32°C and after 7 days of recovery at 24°C. Significant differences were assessed with a two-ways ANOVAs followed by a Tukey test (Table 2). Means values ± SD, $n = 6$

a and c_2 (expressed per cm^{-2} and zooxanthellae) (Fig. 3) recovered after 7 days (t-test, $n = 6$, $P > 0.05$). ETR_{max} and NPQ_{max} values (Fig. 5b, c) returned to their initial value after 1 day of recovery (t-test, $n = 10$, $P > 0.05$). For *O. patagonica* maintained at 29 and 32°C, chl c_2 content, expressed either per cm^{-2} or per

zooxanthellae (Fig. 4b, d) did not reach their initial value and remained significantly lower (t-test, $n = 6$, $P < 0.05$) even after 7 days of recovery. Conversely, ETR_{max} and NPQ_{max} values (Fig. 6b, c) returned to their initial value after 1 day of recovery (t-test, $n = 10$, $P > 0.05$).

Discussion

Few studies have investigated the effect of a short-term increase in temperature on the photosynthetic response of zooxanthellae in symbiosis with temperate corals (Jacques et al. 1983; Jones et al. 2000; Nakamura et al. 2003). The corals studied, *C. caespitosa* and *O. patagonica*, contained high zooxanthellae densities (3.6 and 10.1×10^6 cells cm^{-2}) as well as high chl a content (5.7 and 9.8 μg cm^{-2}) compared to tropical symbiotic corals (Stimson et al. 2002), but these values are in agreement with those already reported in other temperate corals (Jacques et al. 1983; Schiller 1993; Howe and Marshall 2001). This is a feature of shade-adapted anthozoans (Muller-Parker and Davy 2001). Indeed, these corals are particularly abundant in turbid shallow waters, living under light levels ranging from 50 to 110 μmol photons m^{-2} s^{-1} (Peirano et al. 1999, 2005). Both corals also presented relatively high protein contents compared to those measured in other scleractinian corals (Houlbrèque et al. 2003), maybe due to larger zooplankton ingestion. It has indeed been suggested that temperate suspension feeders, living in low-light environment, should rely more on the heterotrophic feeding than tropical ones (Piniak 2002). During the control period, the dark-adapted F_v/F_m of

Fig. 3 *Cladocora caespitosa*. **a** chl a cm^{-2}, **b** chl $c_2.cm^{-2}$, **c** chl a $cell^{-1}$, **d** chl c_2 $cell^{-1}$ after 5 and 48 h of incubation at 24°C (control), 27, 29 and 32°C and after 7 days of recovery at 24°C. Significant differences were assessed with a two-ways ANOVAs followed by a Tukey test (Table 2). Means values ± SD, $n = 6$. *Bars with different letters (a–d) are significantly different*
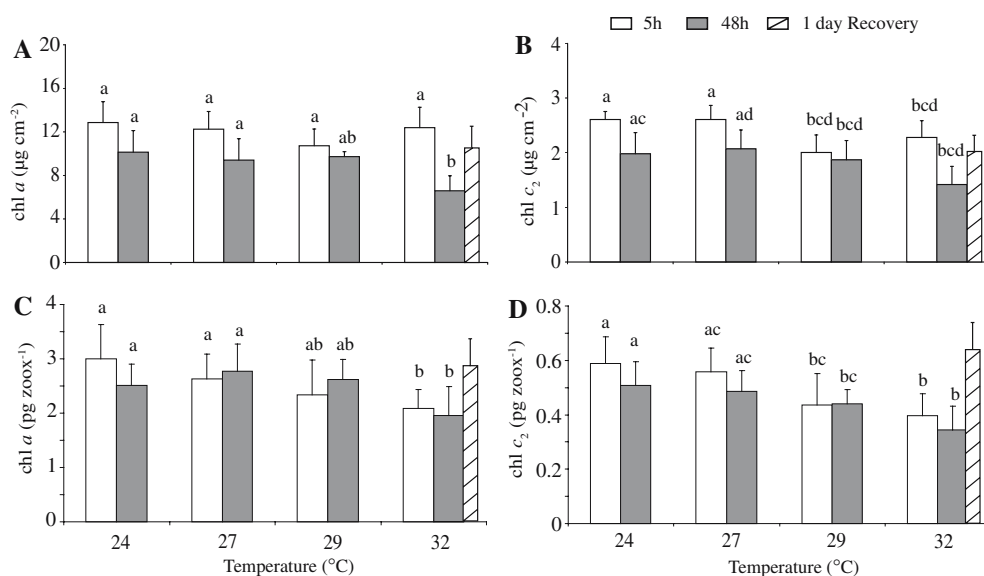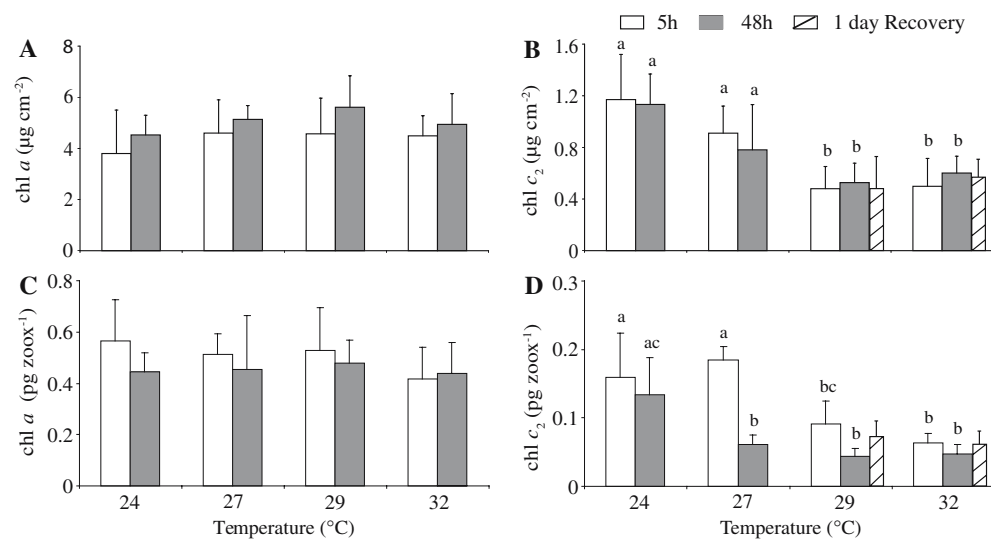
Fig. 4 *Oculina patagonica.*
Same legend as Fig. 3

the symbiotic zooxanthellae of both corals (0.6–0.65) was in the range of values normally observed in marine algae (Buchel and Wilhelm 1993). The NPQ values during this period were low, showing that the corals were not stressed.

Temperate zooxanthellae have been suggested to show a broad tolerance to temperature changes (Muller-Parker and Davy 2001). The effect of abnormal elevations in temperature on photosynthesis have however been poorly tested. In the temperate coral *P. versipora*, from South Australia, a significant long-term reduction in the photosynthetic efficiency (F_v/F_m) was observed when corals were maintained 48 h at 28°C (i.e. 4°C above ambient temperature; Jones et al. 2000). Such reduction was followed by the loss of algae (bleaching). The temperate zooxanthellae inhabiting Mediterranean corals therefore seem particularly resistant to elevation in temperature since there was no significant reduction in F_v/F_m or in the ETR_{max} (Figs. 5b, 6b) up to 29°C during 2 days. This temperature corresponds to a 5°C increase compared to the mean summer temperature (24°C). Elevation of the temperature up to 32°C did not induce algal loss nor mortality of the corals. This later temperature has never been registered in the Ligurian Sea. However, it might occur along the Israeli coasts, where both corals are also thriving (Zibrowius 1980; Fine et al. 2001).

The response of the corals to a temperature increase up to 32°C highlighted biological differences between them. For *C. caespitosa*, we found no significant reduction in the maximal photosynthetic efficiency (F_v/F_m) associated with a decrease of the ETR and an increase in the NPQ, although not significant (Figs. 5a–c). These observations indicate that the PSII of zooxanthellae in symbiosis with *C. caespitosa*, exposed 48 h to 32°C, was

not damaged but became unable to tolerate the high level of incoming energy and therefore had to resort to excess energy dissipation pathways such as NPQ for photoprotection (Warner et al. 1996). These results are in agreement with the conclusions of Jones et al. (1998), which suggest that damage to PSII is a secondary effect of temperature stress. Indeed, in this study, Jones et al. (1998) proposed that NPQ increase is induced by a limitation in assimilatory electron flow and is the first step of reactions at the PSII level following heat stress beyond which the electron transport chain becomes over-reduced and significant damage to PSII occurs. The latter phenomenon seems to happen for *O. patagonica* in our study because we found a significant decrease in F_v/F_m and ETR_{max} together with an important elevation of the NPQ values (Fig. 6a–c). This is the first major difference between the two corals, even if the temperature-induced changes appeared to be reversible in both corals: photosynthetic efficiency values returned to normal after a recovery period of 1 day.

In terms of "symbiosis", no loss in zooxanthellae density was observed, but both corals experienced a small bleaching since the level of chl per zooxanthellae (Figs. 3, 4) significantly decreased at high temperatures (Hoegh-Guldberg and Smith 1989). Indeed, *C. caespitosa* lose both chl a and c_2 (per cm^{-2} or per algal cell) when maintained during 2 days at 32°C, but recovered its initial levels when conditions returned to normal. Conversely, *O. patagonica* only lose chl c_2 (per cm^{-2} or per algal), but both at 29 and 32°C (Fig. 4b, d) and did not recover its initial level after 7 days under normal conditions. *O. patagonica*, is known to frequently bleach along the Israeli coasts (Fine et al. 2001) due to the infection by *V. shiloi* at high temperatures. In the

present study, bleaching in *O. patagonica* was not severe maybe due to: (a) the short-term increase in temperature, too short to activate *V. shiloi*, the causative agent of bleaching (Kushmaro et al. 1996); (b) the small size of the colonies, as it has recently been demonstrated that bleaching mostly affects larger colonies (Shenkar et al. 2005); (c) a different symbiont genotype contained in the two population of north and south Mediterranean *O. patagonica*.
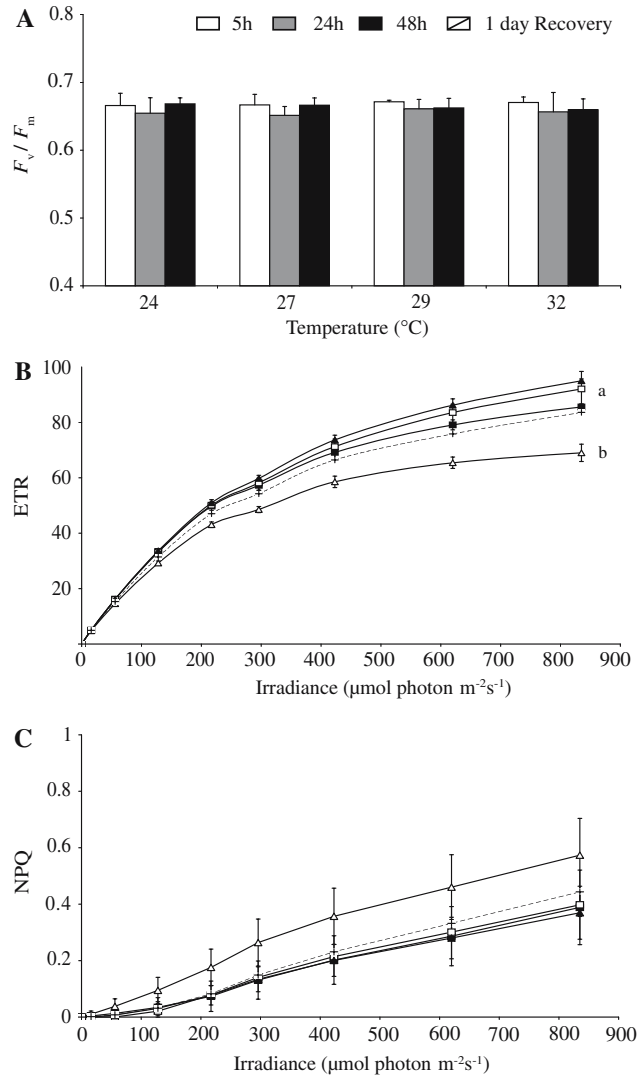
In tropical corals, the response of zooxanthellae to temperature increase is different depending on whether they are exposed to low or high levels of irradiance (Bhagooli and Hidaka 2004). A decrease in the photosynthetic efficiency usually occurs under conditions of elevated temperature and light. In this experiment, we used the highest irradiance known to be experienced by *C. caespitosa* in the Ligurian Sea during the summer season (110 µmol photons m^{-2} s^{-1}, Peirano et al. 1999). This coral is adapted to these low light levels since an I_k (saturation threshold irradiance) of 31–98 µmol photons m^{-2} s^{-1} has been calculated from its photosynthetic curves (Schiller 1993). Our light level is also similar to the one used by Jones et al. (2000) for testing the effect of temperature on the temperate coral *P. versipora* (150–175 µmol photon m^{-2} s^{-1}).

Fig. 5 *Cladocora caespitosa*. Chl fluorescence parameters (F_v/F_m, ETR, NPQ) on dark-adapted nubbins. **a** F_v/F_m as a function of temperature (24, 27, 29 and 32°C) at three incubation time (5, 24 and 48 h) and after 1 day of recovery at 24°C, **b** ETR and **c** NPQ as a function of irradiance, following 5 h incubation at 24°C (*filled square*), 27°C (*open square*), 29°C (*filled triangle*) and 32°C (*open triangle*) and after 1 day of recovery at 24°C (*plus*). Significant differences were assessed with a two-way ANOVAs followed by a Tukey test (Table 2). Means values ± SD, *n* = 10. *Bars* with different letters (a–d) are significantly different
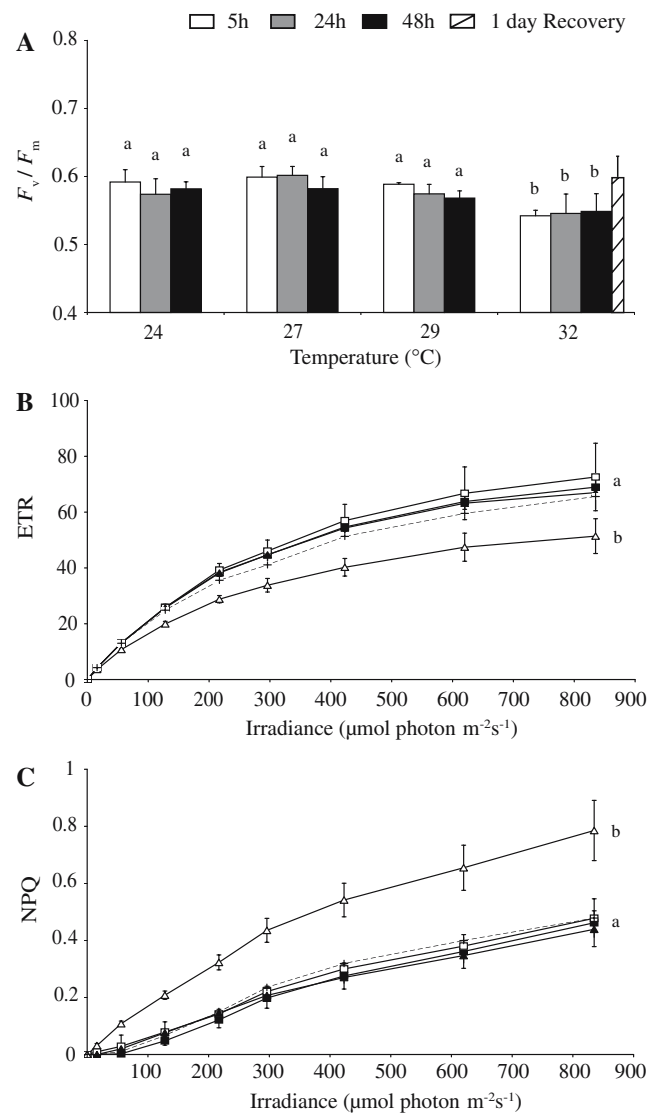

Fig. 6 *Oculina patagonica*. Same legend as Fig. 5

P. versipora significantly reduced its photosynthetic efficiency and bleached, suggesting that this light level was already stressful for this coral.

Zooxanthellae in symbiosis with the two Mediterranean corals investigated here can be considered resistant to short-term increases in temperature, even well above the maximum temperatures ever experienced by the corals in their natural habitat. In tropical symbiotic associations, the host is often selective in its choice of algal partner, containing different algal genotypes (or clades) depending on the environmental conditions (Rowan et al. 1997). It has been observed, for example, in *Montastrea*, that zooxanthellae of ribotype C have a lower tolerance than ribotypes A and B to elevated temperature/irradiance (Rowan et al. 1997). Zooxanthellae in the Mediterranean corals *C. caespitosa* and *O. patagonica* may have gained some genetic adaptations to large and transient changes in temperature, even above the typical maximum. Those in symbiosis with *C. caespitosa* belong to the clade A Temperate (D. Forcioli, personal communication), as most of the other zooxanthellae in symbiosis with Mediterranean sea anemones (Savage et al. 2002). There is therefore a high level of specificity in this temperate symbiosis, as has been observed in other temperate associations (Savage et al. 2002; Rodriguez-Lanetty et al. 2003). However, little knowledge exists on this type of symbiosis and especially on the physiology of the clade A Temperate, which might explain their particular resistance to large temperature fluctuations. More generally, the biochemical basis of the variation among genetically distinct zooxanthellae in susceptibility to bleaching is unknown. It has been suggested, however, that selective pressures in temperate environments have granted symbiosis specificity an adaptive value (Rodriguez-Lanetty et al. 2003). In the tropics, it has been observed that corals having high zooxanthellae densities and protein contents were more resistant to bleaching and mortality than corals with low levels in both parameters (Stimson et al. 2002). This has been explained by an enhanced biochemical protection against damage by radiant energy and the ability of coral tissue to produce antioxydant enzymes and heat shock proteins, which protect both partners of the symbiosis from temperature stress (Brown et al. 2002). Therefore, specific adaptations of Mediterranean clades to temperature, as well as high zooxanthellae densities and protein contents, might make Mediterranean zooxanthellae more resistant to short-term temperature increases. This observation is consistent with the fact that *C. caespitosa* died by losing tissue but not bleached during the mortality events of these recent summers (Rodolfo-Metalpa et al. 2000,

2005); this trend is therefore different from the temperature-induced bleaching in tropical corals (Hoegh-Gulberg 1999). This difference, however, needs more investigation to be fully understood in both its physiological mechanisms and ecological implications.

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