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Photosynthetic response of the Mediterranean zooxanthellate coral *Cladocora caespitosa* to the natural range of light and temperature

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SUMMARY

We investigated photoacclimation in the symbiotic Mediterranean coral *Cladocora caespitosa* by exposing it to three light levels (30, 80 and 250 μmol m^{-2} s^{-1}), which are in the range of those recorded for this species. The coral response to a change in both light and temperature was also assessed, by subjecting coral to two treatments corresponding to winter (14°C and 30 μmol m^{-2} s^{-1}) and summer (23°C and 250 μmol m^{-2} s^{-1}) conditions, as measured in the Ligurian Sea. Photosynthesis, measured using both respirometry and pulse amplitude modulated (PAM) fluorometry, revealed a linear relationship only at low light levels. At higher irradiance, relative electron transport rate (rETR) approached saturation more slowly than rates of oxygen production. At constant temperature, a change in light did not induce any change in zooxanthellae (zoox) and chlorophyll (Chl*a*+c_2) concentrations (mean 3.7×10^6 zoox cm^{-2} and 14.1 μg cm^{-2}, respectively); however, chlorophyll concentrations significantly increased under low light and temperature, probably in order to maintain a sufficient level of autotrophy. Maximal gross photosynthesis (Pg_{max}) as well as the saturation irradiance (E_k) and the respiration rate (R) were, however, significantly higher at 250 μmol m^{-2} s^{-1} compared to the lower light treatments, independently of temperature conditions. Acclimation to high light appeared to be partly driven by a change in the non-photochemical quenching (NPQ) capacity of the algal cells, and to a maximal rate of photon utilization. Conversely, under low light conditions, coral polyps presented a lower E_k, but also lower respiration rates, which correspond to a decrease in the energy expenditure. This ability to acclimate to different light conditions, might allow *C. caespitosa* to rapidly regulate its autotrophic rate in the different light conditions encountered in its natural habitats.

Key words: photoacclimation, photosynthesis, light, temperature, *Cladocora caespitosa*, temperate coral.

INTRODUCTION

Scleractinian corals, containing symbiotic dinoflagellates (commonly called zooxanthellae) and inhabiting tropical and temperate seas, experience a wide range of light habitats, from well lit to shaded environments (Iglesias-Prieto and Trench, 1997; Muller-Parker and Davy, 2001). In addition, temperate corals are exposed to marked seasonal variability in the main environmental parameters (Muller-Parker and Davy, 2001), including daily integrated irradiance fluxes that can be 4–7 times higher in summer than in winter. Coral photoacclimation has mainly been studied in tropical species, which adapt to the light level by changing zooxanthellae density and/or pigment concentration, in order to maintain optimal rates of photosynthesis (Falkowski and Dubinsky, 1981; Iglesias-Prieto and Trench, 1997). They also develop some protection against damages of excess light (Hoegh-Guldberg and Jones, 1999; Furla et al., 2005).

In contrast to tropical species, the response to a change in light intensity of temperate corals has received little attention. Such corals are different from their tropical counterparts, however, because they display greater plasticity in their association with symbionts (Szmant-Froelich and Pilson, 1984; Muller-Parker and Davy, 2001). Kevin and Hudson (Kevin and Hudson, 1979) were among the first to assess the effect of light (12 μmol m^{-2} s^{-1}) or dark conditions on the zooxanthellae density and photosynthesis of the temperate coral *Plesiastrea urvillei* (i.e. *P. versipora*). This coral retained zooxanthellae in the dark for more than 48 days and oxygen production was well adapted to the low light levels received by this species. The photosynthetic response of the ahermatypic temperate coral, *Astrangia danae*, to a change in temperature and light was then studied (Jacques and Pilson, 1980; Jacques et al., 1983). Corals were acclimated to 16 μmol m^{-2} s^{-1} and photosynthesis was measured at different light levels and seawater temperatures. It was shown that oxygen production increased with temperature, as well as with light up to 120 μmol m^{-2} s^{-1}. The dependency of photosynthesis on temperature was later confirmed in *P. versipora* (Howe and Marshall, 2001; Davy et al., 2006); however, in this species an impairment of photosynthesis could occur during heat-stress, temperatures of 28°C inducing a rapid decrease in the photosynthetic efficiency (Jones et al., 2000).

Among the few symbiotic corals inhabiting the Mediterranean, *Cladocora caespitosa* (Linnaeus 1767) is the most important constructional species, and can form structures comparable to tropical reefs (Schuhmacher and Zibrowius, 1985; Kružic and Požar-Domac, 2003). Large fossil formations dating from the late Pleistocene/early Holocene have been found all over the Mediterranean (Zibrowius, 1980; Aguirre and Jiménez, 1997; Peirano et al., 2004), and some large banks are still found in the Ligurian (Morri et al., 1994), Adriatic (Kružic and Požar-Domac, 2003) and Aegean seas (Kühlmann, 1996). This coral harbours symbiotic dinoflagellates in its tissue and lives at depths from 3 to

40 m, on hard or soft bottoms, and often in turbid environments (Peirano et al., 2005). Growth irradiance levels and temperatures measured in the Adriatic Sea ranged from ca. 20–250 μmol m^{-2} s^{-1} and from 12 to 24°C, respectively (Schiller, 1993). Despite its importance as one of the rare reef builders in the Mediterranean Sea, only two studies have measured the photosynthetic response of this coral to a change in light and/or temperature (Schiller, 1993; Rodolfo-Metalpa et al., 2008), or its response to abnormally high seawater temperatures (Rodolfo-Metalpa et al., 2006a; Rodolfo-Metalpa et al., 2006b). Schiller measured rates of photosynthesis at temperatures ranging from 9 to 21°C (Schiller, 1993), and at low temperatures high feeding levels increased zooxanthellae density and chlorophyll (chl) content (Rodolfo-Metalpa et al., 2008).

The first aim of this paper was to investigate the photoacclimation capacity of *C. caespitosa* to different light levels at the non-stressing temperature of 18°C. For this purpose, corals were cultured at three light levels (30, 80 and 250 μmol m^{-2} s^{-1}), corresponding to the range of irradiance previously described for this species (Schiller 1993; Peirano et al., 1999). We then examined the acclimation capacity of this Mediterranean symbiosis (both *in hospite* and on freshly isolated zooxanthellae) to an eightfold change in its growth irradiance. We also tested the photosynthetic response of *C. caespitosa* to two extreme situations of light and temperature, corresponding to 'winter' (14°C and 30 μmol m^{-2} s^{-1}) and 'summer' (23°C and 250 μmol m^{-2} s^{-1}) conditions (Schiller, 1993), to assess its acclimation to a changing environment. The second aim of this work was to evaluate the relationship between oxygen respirometry, which measures the net photosynthetic gas exchange over the whole coral, and fluorometry, which assesses gross photosynthetic electron transport from a small coral area. While respirometry has been extensively used and investigated, pulse amplitude modulated (PAM) fluorometry is a relatively new method of non-intrusive *in situ* measurement of photosynthetic parameters of the algal symbionts (Beer et al., 1998a; Beer et al., 1998b; Ralph et al., 1999). The comparison between the results of these two techniques on temperate corals is critical in order to validate the use of fluorescence as an accurate measurement of photosynthesis on symbiotic corals. However, to our knowledge, only one study has so far investigated this topic for a tropical species (Hoogenboom et al., 2006).

MATERIALS AND METHODS

Experimental design

Ten different colonies (ca. 20 cm in diameter) of *Cladocora caespitosa* L. were collected in the Gulf of La Spezia (Ligurian Sea; 44°03′N, 9°55′E), at 7–9 m depth in May 2006 (irradiance, 50 μmol m^{-2} s^{-1}; temperature, 18°C). They were brought back to the laboratory and divided into 50 nubbins (7–18 polyps) and 110 single polyps, which were carefully cleaned from epiphytes, associated fauna and sediment, and placed on PVC supports. Coral samples were then randomly divided into ten experimental tanks (15 l volume), continuously supplied with flowing seawater (2 l h^{-1}) and were maintained for 2 weeks at a similar irradiance (photoperiod: 12 h:12 h dark:light) and temperature to those measured at the collection site. HQI lamps were set up to emit the required right irradiance using plastic mesh and light was measured using a LI-COR underwater quantum sensor (Li-192; Lincoln, NE, USA). Temperature was kept constant in all tanks using heaters or a refrigerating system connected to electronic controllers.

Temperature and light conditions in the tanks were then gradually changed (by 1°C and 20–30 μmol m^{-2} s^{-1} per day, respectively) giving a total of five different treatments (two tanks per treatment). In the first three treatments (respectively called 18°C/30; 18°C/80; 18°C/250), samples were maintained at 18°C and at 30, 80 or 250 μmol m^{-2} s^{-1} (photoperiod: 12 h:12 h dark:light), in order to study the photoacclimation of this coral maintained at a constant temperature. Irradiance levels were chosen from the literature (Schiller 1993; Peirano et al., 1999) and our *in situ* observations (10–300 μmol m^{-2} s^{-1} throughout the year). In the other two treatments, samples were maintained either at 14°C and 30 μmol m^{-2} s^{-1} (called 14°C/30) or at 23°C and 250 μmol m^{-2} s^{-1} (called 23°C/250), in order to study the concurrent effect of light and temperature on the photosynthetic response of the corals, mimicking winter (14°C/30) and summer (23°C/250) conditions in the Ligurian Sea (Schiller, 1993; Peirano et al., 1999). In these treatments, photoperiods were 14 h:10 h dark:light and 8 h:14 h dark:light for winter and summer conditions, respectively. Corals were cultured under these five conditions for 4 weeks (the acclimation period) before measurements (described below) were performed. Preliminary experiments showed that this incubation length is sufficient for acclimation of photosynthesis and growth rates to new light and temperature conditions (Rodolfo-Metalpa et al., 2006b). During the whole incubation, corals were fed once a week with *Artemia salina* nauplii; feeding took place 3 days before recording any measurements of photosynthesis.

Chlorophyll and zooxanthellae measurements

Chlorophyll and zooxanthellae measurements were performed on five single polyps collected in each tank (N=10 for each treatment). Samples were frozen at –80°C and processed as described (Rodolfo-Metalpa et al., 2006a). Chl *a* and c_2 were determined according to the equations of Jeffrey and Humphrey (Jeffrey and Humphrey, 1975) using a spectrophotometer, and zooxanthellae were counted (number of fields=10, total counted >300 cells) using an inverse microscope (Leica, Wetzlar, Germany) and an improved version of the Histolab 5.2.3 image analysis software (Microvision, Every, France). Results were normalized to polyp surface area (Rodolfo-Metalpa et al., 2006a).

Fluorescence measurements and oxygen production in polyps

Pulse amplitude modulated (PAM) fluorometry assesses gross photosynthetic electron transport from a small area of the coral surface whereas oxygen respirometry measures gas exchange over the entire coral surface. With both techniques, the same light intensities were applied to the same three polyps randomly sampled in each tank (N=6 for each treatment) in order to establish the relationship between the gross photosynthesis (Pg) normalized to chl *a* and the relative electron transport rate (rETR) (Hoogenboom et al., 2006). This relationship was then fitted using a linear and a double exponential saturating function (Platt et al., 1980).

For measurements of rates of net photosynthesis (Pn) *versus* irradiance (E) and dark respiration (R), a random draw of sampling between tanks was used. Each polyp was placed in a closed thermostated Perspex chamber filled with 0.45 μm-filtered seawater continuously stirred with a stirring bar, and oxygen was measured using Clark-type electrodes connected to a Strathkelvin 928 oxygen meter and a computer. Electrodes were calibrated against O_2-free (using sodium dithionite) and air-saturated (100% O_2) seawater. The 100% O_2 concentration was calculated according to the experimental temperature and salinity values (table on: http://www.unisense.com/Default.aspx?ID=117). Polyps were allowed to acclimate for at least 15 min, and then subjected to the following light intensities: 0, 14, 44, 86, 158, 363, 493 and 739 μmol m^{-2} s^{-1}, as measured using a LiCor LI-192 underwater quantum sensor. Since light exposure

enhanced dark respiration in corals (Edmunds and Davies, 1988), this effect was measured in a preliminary experiment. Respiration rate increased when corals were exposed to increased light intensity from 14 to 170 μmol m^{-2} s^{-1} , and then remained stable at higher PAR (photosynthetically active radiation). Therefore, dark respiration in this experiment was measured at the end of the 158 μmol m^{-2} s^{-1} light intensity. Gross photosynthesis (Pg) was then calculated by summing the rates of *R* and Pn and plotted *versus* irradiance [P/E curves, formerly P/*I* (Falkowski and Raven, 1997)]. All measurements were normalized to chl *a* content [μg O_2 (μg chl *a*)$^{-1}$ h^{-1}] or to surface area (μg O_2 cm^{-2} h^{-1}). P/E curves were fitted with Pro Fit, Quantum Soft (Vetikon am See, Switzerland) software using the function of Harrison and Platt (Harrison and Platt, 1986) as described by Ralph and Gademann (Ralph and Gademann, 2005), for the determination of the maximum Pg rate (Pg_{max}), the saturation irradiance (E_k), and the initial slope of the curve (α).

The relative electron transport rate (rETR) was assessed using a Diving PAM (Walz GmbH, Effeltrich, Germany), on the same corals as those used for the photosynthetic rate measurements, by two different methods. The first method applied a saturation pulse to the corals incubated in the same chambers as those used for the oxygen photosynthetic measurements after 15 min of acclimation to each light level, by which time the photosystem was considered to be in steady state. The rETR was then calculated according to Ralph and Gademann (Ralph and Gademann, 2005) and the ETR/irradiance curves hereafter obtained will be called steady-state light curves (SLC). The second method assessed rETR using the rapid light curve (RLC) function of the PAM fluorometer, followed by 5 min relaxation in the dark (RLC+Rec), for corals sampled in the different conditions. 5–10 s after the coral was placed in the dark (Ralph and Gademann, 2005) the effective quantum yield ($\Delta F/F'_m$) and rETR were measured after exposure for 10 s to the same eight light intensities as those used for oxygen measurements (0, 14, 44, 86, 158, 363, 493 and 739 μmol m^{-2} s^{-1}). At the end of the last light level of the RLC, polyps remained in complete darkness and $\Delta F/F'_m$ was determined after 30 s, 1, 2 and 5 min. Non-photochemical quenching (NPQ), which is a measure of the thermal dissipation of the excess absorbed excitation energy, was also calculated during the RLC and relaxation periods. The kinetics of relaxation of NPQ allows the discrimination of the processes that led to the dissipation of excess absorbed light. Two main components of NPQ can be distinguished: (i) energy-dependent quenching (qE), which is a protective mechanism related to the build-up of the transthylakoid pH gradient, and which quickly relaxes after light exposure, and (ii) photoinhibitory quenching (qI), which results from damage to photosystems and relaxes much slowly [>10 min to several hours (Krause, 1988; Ralph and Gademann, 2005)] because it corresponds to a long-term photodamage. In non-stressful culture conditions, qE is expected to be the main source of NPQ and thus NPQ would dissipate quickly following exposure to a saturating light pulse, such as during the RLC. In contrast, if the coral experiences photodamage (e.g. excess light and temperature), NPQ relaxes much more slowly, even under dark conditions, indicating the temporary limited ability of the coral to recover.

Extraction of zooxanthellae and fluorescence measurements

rETR was also measured for the three light treatments (18°C/30; 18°C/80; 18°C/250) on freshly isolated zooxanthellae (FIZ) from three nubbins randomly sampled from each tank (*N*=6 for each treatment). This experiment was performed in order to compare the response of FIZ with *in hospite* zooxanthellae and assess if the animal pigments offer some protection to light. Zooxanthellae were detached from the skeleton by a gentle flow of air and re-suspended into 50 ml of 0.45 μm-filtered seawater. They were allowed to rest (under the treatment conditions) for 30 min before taking measurements. RLCs were performed by placing the 8 mm optic fibre in direct contact with a 10 ml glass chamber containing FIZ in suspension. 10 s later, FIZ were placed in the dark, $\Delta F/F'_m$ and rETR were measured by exposure for 10 s periods, to eight light intensities between 0 and 1064 μmol m^{-2} s^{-1}. RLCs were fitted as described for the P/E curves (see above). Such protocol gave very good initial $\Delta F/F'_m$ values (0.58±0.05, 0.57±0.009 and 0.65±0.09 for the three light levels), suggesting that the zooxanthellae had not suffered from the extraction procedure.

Statistical analysis

All data were tested for assumptions of normality and homoscedasticity by the Cochran test and were log-transformed when required. Data corresponding to each treatment were pooled since there was no significant difference between replicated tanks ($P>0.05$). One-way ANOVAs were used to test the effect of light (18°C/30; 18°C/80; 18°C/250) or to compare the two light and temperature treatments (14°C/30; 23°C/250). When ANOVAs showed significant differences, Tukey's honest significant difference test (HSD) attributed differences to specific factors and their interaction only. Statistical analyses were performed using STATISTICA® software (StatSoft, Tulsa, USA). Significant differences were assessed at $P<0.05$. All data were expressed as mean ± standard error (s.e.m.).

RESULTS

Zooxanthellae density and chlorophyll content

At the constant temperature of 18°C, zooxanthellae density and chl ($a+c_2$) concentrations per surface area (mean 3.7×10^6 zoox cm^{-2} and 14.1 μg cm^{-2}, respectively) remained unchanged between light levels (ANOVA, $P>0.05$, Fig. 1A,B). [Chl] per zooxanthellae was also comparable and equal to 4.48±0.40 pg chl *a* $zoox^{-1}$. However, when both temperature and light were changed, zooxanthellae density and chl concentration (Fig. 1A,B) significantly increased in the 14°C/30 treatment (ANOVA, $P<0.05$), but [chl] per zooxanthellae remained unchanged.

Photosynthesis measured using respirometry

Photosynthetic parameters, normalized to chl *a*, are presented in Table 1. At the constant temperature of 18°C, maximal rates of gross photosynthesis (Pg_{max}) and respiration (*R*), as well as the saturation irradiance (E_k), significantly increased with the light level. Similar results were obtained with data normalized to surface area (ANOVA, $P<0.05$; Fig. 2A). Indeed, corals maintained at 250 μmol m^{-2} s^{-1} showed the highest Pg_{max} (Tukey's HSD test: 30<80=250) and respiration rates (HSD test: 30<80<250, 9.7±1.2; 17.6±1.0 and 25.8±2.6 μg O_2 cm^{-2} h^{-1}, respectively). When both temperature and light were changed, corals maintained at 23°C/250 always showed significantly higher Pg_{max}, E_k and R, whether normalized to chl *a* (Table 1) or to surface area (ANOVA, $P<0.05$, Fig. 2A). Respiration rates measured at 14°C/30 and 23°C/250 were 8.2±0.9 and 28.02±3.02 μg O_2 cm^{-2} h^{-1}, respectively.

Photosynthesis measured using fluorometry

A plateau was not reached in any of the RLCs obtained with the whole polyp (Fig. 2B), therefore only values measured at the end of the RLC ($rETR_{max}$) were compared. At the constant temperature of 18°C, significant differences were obtained between light levels (ANOVA, $P<0.05$). $rETR_{max}$ was indeed significantly higher for

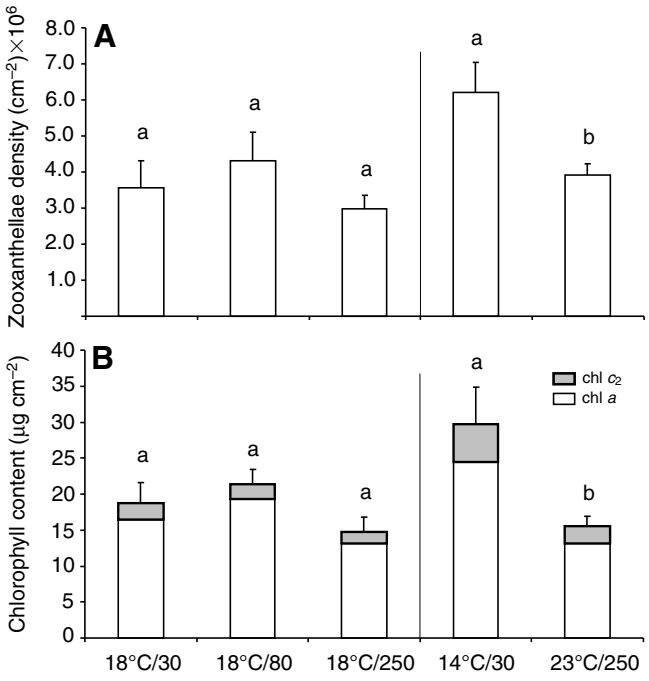

Fig. 1. (A) Zooxanthellae density and (B) chlorophyll content, measured for corals either maintained at 18°C under the three light intensities, 30, 80 and 250 µmol m^{-2} s^{-1} (18°C/30; 18°C/80; 18°C/250) or at 14°C and 30 µmol m^{-2} s^{-1} (14°C/30) and 23°C and 250 µmol m^{-2} s^{-1} (23°C/250). Data are presented as mean ± s.e.m. (N=10). Chlorophyll a and c_2 are shown separately but analysed as chl a+c_2. Means with different letters (a, b) are significantly different (P<0.05).

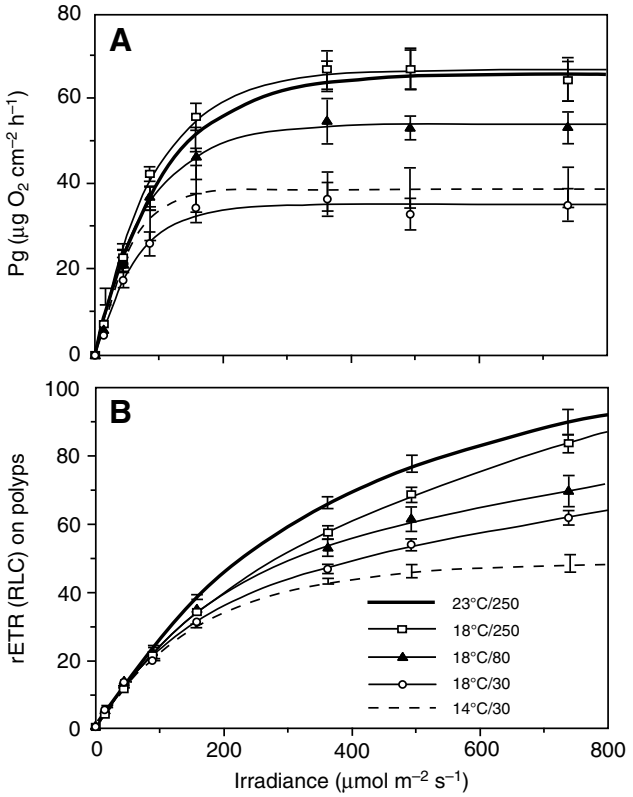

Fig. 2. Irradiance curves *vs* (A) Gross photosynthesis (Pg) and (B) relative electron transport rates (rETR; measured on the same polyps used in A). See Fig. 1 for explanation of treatments. Data are presented as mean ± s.e.m. (N=6). RLC, rapid light curve.

corals maintained at 250 µmol m^{-2} s^{-1} (Fig. 2B; HSD test: 30=80<250). These corals also showed the smallest increase in fluorescence (F) (Fig. 3B), corresponding to the largest decrease in maximum fluorescence yield (F'_m) and increase in NPQ (Fig. 3C,D). After relaxation, NPQ rapidly decreased by up to 65% of its maximal value, showing that most of quenching was energy-dependent (qE). Conversely, corals maintained at 30 µmol m^{-2} s^{-1} showed the highest increase in fluorescence yield (F), reflecting the closure of photosystems (photochemical quenching) but a reduced decline in F'_m, resulting in a limited development of NPQ (Fig. 3C,D). NPQ did not decrease during the whole relaxation period, suggesting that a significant fraction of the quenching was due to photoinhibitory quenching (qI) and not to energy dependent NPQ (qE). Results of RLCs measured at 14°C/30 and 23°C/250 are comparable to those obtained for the effect of light alone (30 and 250 µmol m^{-2} s^{-1}).

RLCs performed on zooxanthellae freshly isolated from corals and maintained at the three light levels (Fig. 4) showed that the plateau (ETR_{max}=95.9±4.3) was reached at an irradiance above 1000 µmol m^{-2} s^{-1}, with corresponding E_k values equal to 346±13 µmol m^{-2} s^{-1} for the three light treatments. Relaxation curves were in good agreement with those obtained for the RLCs performed on the entire association (results not shown).

Relationship between respirometry and fluorometry

The relationships between Pg and rETR are represented in Fig. 5A. For all treatments except 14°C/30, relations were linear for light intensities from 0 to 158 µmol m^{-2} s^{-1} (R^2=0.99), after which Pg began to saturate more rapidly than rETR. For the treatment

Table 1. Mean parameters of the P/E curves, normalised to chlorophyll *a* content, under various temperature and light intensity treatments

	Treatment						
	Light				Light and temperature		
	18°C/30	18°C/80	18°C/250	P value	14°C/30	23°C/250	P value
Pg_{max} (µg O_2 µg chl a^{-1} h^{-1})	2.18±0.47^a	2.31±0.48^a	5.09±0.61^b	**0.018**	1.55±0.19^a	4.54±0.56^b	**0.000**
E_k (µmol m^{-2} s^{-1})	76±2^a	78±1^a	105±7^b	**0.006**	50±4^a	115±4^b	**0.000**
α	0.06±0.005	0.06±0.003	0.05±0.008	0.46	0.03±0.015	0.05±0.005	0.27
R(–) (µg O_2 µg chl a^{-1} h^{-1})	1.25±0.14^a	1.36±0.13^{a,b}	2.18±0.27^b	**0.016**	0.34±0.05^a	2.38 ±0.28^b	**0.000**

Values are means ± s.e.m. (N=6). The effect of light at constant temperature (18°C) for three treatments (30, 80 and 250 µmol m^{-2} s^{-1}) and the concurrent effect of light and temperature (two treatments: 14°C/30 µmol m^{-2} s^{-1} and 23°C/250 µmol m^{-2} s^{-1}) were separately tested using one-way ANOVAs. When ANOVA showed significant differences (P<0.05, indicated in bold) a Tukey HSD test was used to attribute differences between specific factors. Means with different letters (a, b) are significantly different.

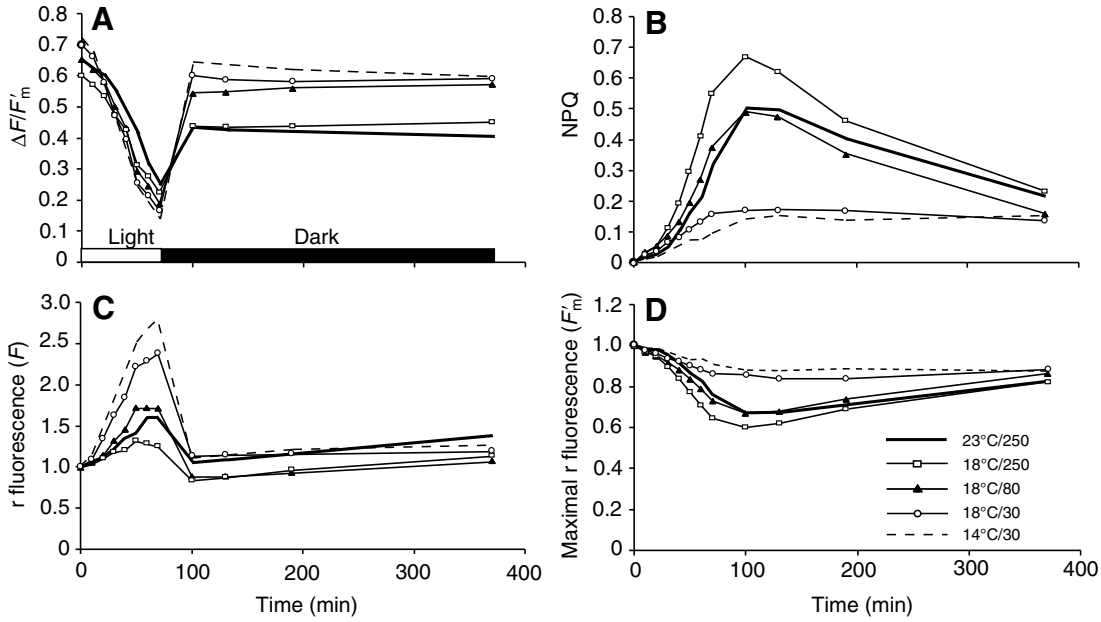

Fig. 3. Fluorescence parameters measured during rapid light curves (RLC) followed by 5 min relaxation in the dark (RLC+Rec). (A) Effective quantum yield (F_v/F'_m); (B) non-photochemical quenching (NPQ); (C) fluorescence (F); (D) maximum fluorescence (F'_m). F and F'_m are relative to the value at time 0.

14°C/30, the relationship was linear only between 0 and 86 μmol m^{-2} s^{-1}. Taking into account all light intensities (0–739 μmol m^{-2} s^{-1}) the relationship between Pg and rETR is well described by an exponentially saturating function (Fig. 5B) with R^2 ranging from 0.96 to 0.98.

DISCUSSION

Photoacclimation

The first aim of this study was to assess the acclimation of the Mediterranean coral *Cladocora caespitosa* to low and high photon flux. The symbiont response when light only was modified differed from that when light and temperature were both modified simultaneously. In corals maintained at a constant temperature (18°C), an eightfold increase in light intensity (30–250 μmol m^{-2} s^{-1}) did not induce any changes in the zooxanthellae density, or in the chl content per surface area or per zooxanthellae. Such stability in the symbiont and pigment contents was previously observed in the same coral species (Rodolfo-Metalpa et al., 2008), but the range of light investigated was smaller (50–120 μmol m^{-2} s^{-1}; 7-week acclimation). In the two experiments cited above zooxanthellae densities were similar to previous findings reported for the same species collected *in situ* [2.36±0.4×10^6 zoox cm^{-2} and 4.9 μg cm^{-2}, recalculated using a mean polyp surface of 0.7 cm^2 (Schiller, 1993)]. Other temperate species such as *Plesiastrea versipora* [0–10×10^6 zoox cm^{-2} (see Kevin and Hudson, 1979; Howe and

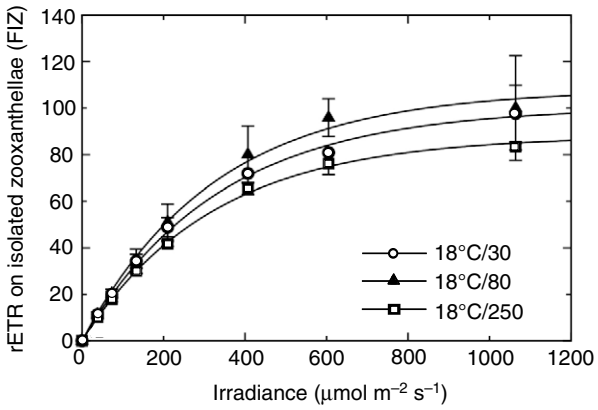

Fig. 4. Relative electron transport (rETR) rate measured on freshly isolated zooxanthellae from corals maintained at 18°C under the three light treatments (18°C/30; 18°C/80;18°C/250). Values are means ± s.e.m. (N=6).

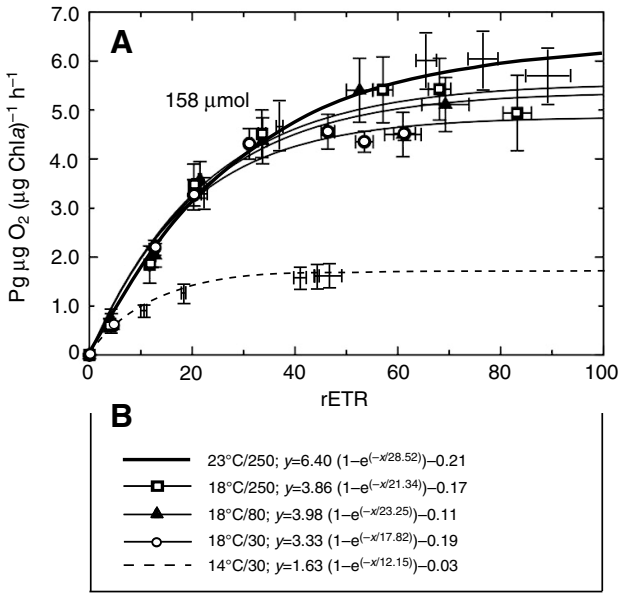

Fig. 5. (A) Relationship between the gross photosynthetic rates (Pg) and the rETR under the different temperature and light treatments (18°C/30; 18°C/80; 18°C/250; 23°C/250; 14°C/30). Data are mean ± s.e.m. (N=6). (B) The curvilinear equation for each treatment relationship.

Marshall, 2001)] and *Astrangia danae* [0–7×10^6 zoox cm^{-2} (see Szmant-Froelich and Pilson, 1980)] have a larger range of zooxanthellae density per surface area, perhaps due to the facultative symbiosis. Temperate sea anemones also have a stable symbiosis, since their zooxanthellae persist at high densities when exposed to a pronounced light gradient or to the dark for several weeks (Muller-Parker and Davy, 2001; Verde and McCloskey, 2002). The lack of change in symbiont density and chl content might have been for several reasons. (1) Lack of any photoacclimation with respect to pigment and cell densities when light is the only parameter changing. (2) A lower range of light intensities compared to that experienced by the corals *in situ*. In this experiment we used the upper light values reported for this coral (Schiller, 1993; Peirano et al., 1999), but since *C. caespitosa* can be distributed both in turbid as well as in sun washed habitats, it might experience higher light levels. (3) The acclimation period (4 weeks), which might be too short to allow a change in algal density since temperate symbioses seem to be particularly resilient to changes in their environment. This hypothesis seems unlikely, however, since a change in temperature induces rapid changes in zooxanthellae density and pigment concentration (Rodolfo-Metalpa et al., 2008) (and this study). In contrast to this temperate coral, most tropical corals quickly photoacclimate (within 1–2 weeks), presenting lower contents of chl and/or zooxanthellae in organisms adapted to high light (e.g. Chalker, 1981; Falkowski and Dubinsky, 1981; Iglesias-Prieto and Trench, 1994). Our results, however, confirm that symbiosis is affected by a change in temperature, since a significant increase in symbiont and total pigment concentration was observed in the low light/low temperature condition of this study, as well as in the low temperature treatments (both low and high light) in Rodolfo-Metalpa et al. (Rodolfo-Metalpa et al., 2008). In both studies, the corals were fed. This temperature-dependency of algal growth needs to be further investigated, since this trend was not observed in other tropical and temperate symbioses, in which algal concentrations either remained stable between 13 and 20°C [in *Anthopleura elegantissima* (Muller-Parker et al., 2007)] or increased with temperature increase [in *P. versipora* (Howe and Marshall, 2001)].

This study, however, demonstrates that the coral symbiosis of *C. caespitosa* photoacclimates rapidly to a wide range of irradiances by changing the rate of respiration and photosynthesis, and that the combination with both low and high temperatures did not change the pattern of photoacclimation (at least in the range of temperature investigated). Light did indeed affect the rates of photosynthesis, since Pg_{max} and E_k (normalized either to surface area or to mg chl *a*) were always higher in corals maintained at 250 than at 30 μmol m^{-2} s^{-1}. This is considered an 'optimization' process, since E_k (subsaturation irradiance) is one of the most reliable indicators of photoacclimation and reflects changes in the effective absorption cross section of the photosystems and in the minimal turnover time for carbon reduction (Kolber and Falkowski, 1993). Pg_{max} also reflects the number of PSUs, i.e. photosystem units, an increase in Pg_{max} corresponding to an increase in PSUs. *C. caespitosa* therefore rapidly acclimates to high photon flux, and this mechanism is further highlighted by the results obtained with RLCs of corals maintained at 250 μmol m^{-2} s^{-1}, which presented different evolutions of the F, F'_m and NPQ than the corals maintained at the lowest light levels. The relative constant fluorescence yield in the 250 μmol m^{-2} s^{-1} corals showed that there were almost no, or few, sink limitations to the photochemical pathway, even under very high irradiances, and that the sink could cope with the full range of light intensities (Ralph and Gademann, 2005). The decrease in F'_m (by 30–40%) was linked to the development of NPQ, which dissipates the incoming energy and prevents damage to the photochemical pathway. The light–dark relaxation kinetics showed that the photosystem recovered well from the light exposure since most of the NPQ (Fig. 3C), corresponding to the removal of the energy dependent NPQ (qE), relaxed in less than 5 min, leaving only ca. 20% or less associated to the inhibition quenching (qI). This ability of NPQ to return to low values is an additional indicator of the tolerance of the zooxanthellae of *C. caespitosa* to high light, and explains the increase in E_k observed in the photosynthetic curves. However, the quantum yield in these corals remained low relative to first values. Conversely, corals maintained at 30 μmol m^{-2} s^{-1} light intensity did not show a great ability to dissipate energy as heat, and most NPQ was due to photoinhibition (qI) and was not dissipated during the relaxation in the dark.

At high irradiance, acclimation processes in these temperate zooxanthellae therefore appear to be partly driven by a change in the NPQ capacity of the algal cells, and to a maximal rate of photon utilization, which results in a higher rate of light saturated photosynthesis. Following exposure to high light, these corals also largely increased their rates of respiration, suggesting a high capacity for metabolic activity once irradiance increases. Conversely, polyps maintained in the low light treatment had to maximize the capture of the low photon flux and minimize their energy costs (Anthony and Hoegh-Guldberg, 2003), and therefore presented a lower E_k but also lower respiration rates than corals maintained under high light. Respiration rates were also very low when both light and temperature were decreased. This is a common response of cnidarians maintained under low temperature (Jacques and Pilson, 1980; Verde and McCloskey, 2001) or low light (Falkowski and Dubinsky, 1981; Falkowski, 1990) conditions and corresponds to a decrease in the energy expenditure.

Whereas tropical symbioses often photoacclimate to different light levels by changing all autotrophic components, both increasing the algal density (Titlyanov et al., 2001), or the pigmentation per cell (Falkowski and Dubinsky, 1981) and the photosynthetic parameters of light saturation curves (Falkowski and Dubinsky, 1981; Mass et al., 2007), photoacclimation in *C. caespitosa* mostly involves a change in Pg_{max} and R, without any change in pigment concentration or zooxanthellae density. This acclimation can be achieved by changes in Rubisco levels, pigment ratios (diatoxanthin increasing from 26 to 34% of chlorophyll *a* for corals maintained at 30 and 250 μmol m^{-2} s^{-1} light intensity, respectively; P. J. Lopez, unpublished results), and also quantum yield of photosynthesis (i.e. the efficiency of the use of absorbed energy), as observed here and elsewhere (Stambler and Dubinsky, 2004). When both temperature and light are modified, however, photoacclimation goes through a change in all photosynthetic components.

Although photoacclimative responses observed in this study could be regarded as not being directly relevant to *in situ* conditions, they do provide information on the photo-physiological capacities of this Mediterranean coral. Rates of photosynthesis measured for *C. caespitosa*, both under low and high light conditions (36.7–78.4 μg O_2 cm^{-2} h^{-1} or 1.5–5.1 μg O_2 chl a^{-1} h^{-1}), are in the range of those measured for temperate (Kevin and Hudson, 1979; Schiller, 1993; Howe and Marshall, 2001) and also tropical corals (Lasker, 1981; Porter et al., 1984; Hoegh-Guldberg and Smith, 1989; Muscatine, 1990). Increased photosynthesis under high light/high temperature conditions might therefore allow *in situ* corals to increase their autotrophic acquisition of energy during the summer season, as already observed for other temperate corals (Howe and Marshall, 2001) and sea anemones (Verde and McCloskey, 2007). This season is commonly characterized by food shortage (Coma

and Ribes, 2003; Rossi et al., 2006) and increased autotrophy might help the corals in such conditions. Conversely, in winter, when PAR is low in the Mediterranean, the corals' capacity to acclimate to low light levels might allow them to maintain a non-negligible level of autotrophy.

Relationship between respirometry and fluorometry

The second aim of this study was to evaluate the functional relationship between measurements obtained with fluorometry and respirometry. Whereas P/E curves rapidly saturated (Fig. 2A) RLCs showed that saturation was not reached at ca. 800 μmol m^{-2} s^{-1} (Fig. 2B) as already observed in some high-light-adapted corals (Beer et al., 1998a; Ralph et al., 1999). This lack of saturation was not due to a limited exposure time to the actinic light during RLCs since steady-state light curves (SLC) did not saturate either (results not shown). Self-shading of the symbionts (Ralph et al., 1999), or shading by animal pigments (Klueter et al., 2006), also seem to be excluded, because RLCs performed on (optically thin) freshly isolated zooxanthellae (Fig. 4) showed that the plateau was reached at a high irradiance up to 1000 μmol m^{-2} s^{-1}. Additionally, RLCs on FIZ or on the entire symbiosis did not show photoinhibition, and did not present the typical pattern observed for temperate photosynthetic organisms such as macroalgae (Beer et al., 1998b; Figueroa et al., 2003). This could be due to the fact that *C. caespitosa* is in symbiosis with a *Symbiodinium* species belonging to the temperate clade A as almost all Mediterranean symbioses (Visram et al., 2006). This clade is widespread in the whole Mediterranean Sea, and it might be responsible for *C. caespitosa* high light resistance. Indeed, this species also shows exceptional plasticity in its habitat conditions, since it can also be found along the Lebanon and Israeli coasts where light levels can be much higher than those experienced in the Ligurian Sea.

The relationships between respirometry and fluorometry measurements were therefore non-linear, except at moderate light, from 0 to ca. 160 μmol m^{-2} s^{-1} (Fig. 5A). This result indicates that some oxygen evolved during the RLC was used before leaving the symbiosis or that less oxygen was produced per charge separation under high light. In a tightly coupled system, the photosynthesis products are expected to be rapidly respired by the host, and respiration rates are expected to increase with light intensity, leading to a more or less constant P/E curve. Therefore, the non-linearity could be due to an under-estimation of the light-enhanced respiration rates of the animal (Edmunds and Davies, 1988). If the saturation of Pg originates mainly from an increased utilisation of oxygen by the algae (and not the animal), two mechanisms leading to non-photosynthetic electron transport (Figueroa et al., 2003) are likely: the development of the Mehler cycle or photorespiration processes [due to an increased O_2 concentration in the cell (Kühl et al., 1995)]. Another explanation is cyclic flow of electrons around PSII (Falkowski et al., 1986; Prasil et al., 1996). This process does not require increase oxygen utilisation, but a reduced production of O_2 per charge separation at photosystem II (PSII). Such a mechanism has indeed been observed in diatoms at high light and proposed as a mechanism for photoprotection (Lavaud et al., 2002a; Lavaud et al., 2002b). We cannot, with the present data, evaluate which process(es) is/are responsible for this observation. Clearly, more focused studies on the photosynthetic and symbiosis physiology will be required to understand the differences between the fluorometric and the oxygen evolution measurements. Both measurements have their limitation and neither is infallible when it comes to estimating primary production. However, from our study it is evident that caution must be exercised when comparing ETR obtained by fluorometry to photosynthetic performances obtained by oxygen evolution of the temperate coral–algal complex as they clearly provide different information.

CONCLUSION

C. caespitosa has the capacity to rapidly acclimate to a wide range of light levels. The acclimation to high light is through the development of the non-photochemical quenching, which leads to higher Pg_{max}, E_k and R rates, and therefore an improved light harvesting and utilization. Acclimation to low light results in a reduction of all the photosynthetic parameters in order to maximize the capture of the low photon flux. In this study, however, *C. caespitosa* did not show photoacclimation in respect to pigments or cell densities, confirming previous findings on the peculiar stability of temperate symbioses over a wide range of light levels.

When both temperature and light conditions are modified, however, photoacclimation goes through a change in all photosynthetic components, including symbiont density and pigment concentration. Since rates of photosynthesis measured using respirometry saturated at lower light values compared to the electron transport rates, the relationship between respirometry and fluorometry was only linear at low light intensities; while different hypotheses were suggested to account for this difference, there is a clear need for further studies in this area. Until then the fluorometric tool remains an excellent probe of the physiology of the symbiosis and should be used to complement but not replace oxygen evolution measurements.

LIST OF ABBREVIATIONS

chl	chlorophyll
$\Delta F/F'_m$	effective quantum yield
E_k	saturation irradiance
F	fluorescence yield
FIZ	freshly isolated zooxanthellae
F'_m	maximum fluorescence yield
NPQ	non-photochemical quenching
PAM	pulse amplitude modulated
PAR	photosynthetically active radiation
Pg	gross photosynthesis
Pn	net photosynthesis
PSU	photosynthetic unit
qE	energy-dependent quenching
qI	photoinhibitory quenching
R	respiration rate
Rec	recovery
rETR	relative electron transport rate
RLC	rapid light curve function of the PAM fluorometer
SLC	steady-state light curve
α	initial slope of the curve
zoox	zooxanthellae

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