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REPORT

Relationship between symbiont density and photosynthetic carbon acquisition in the temperate coral *Cladocora caespitosa*

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Abstract This study quantified variation in net photosynthetic carbon gain in response to natural fluctuations in symbiont density for the Mediterranean coral *Cladocora caespitosa*, and evaluated which density maximized photosynthetic carbon acquisition. To do this, carbon acquisition was modeled as an explicit function of symbiont density. The model was parameterized using measurements of rates of photosynthesis and respiration for small colonies with a broad range of zooxanthella concentrations. Results demonstrate that rates of net photosynthesis increase asymptotically with symbiont density, whereas rates of respiration increase linearly. In combination, these functional responses meant that colony energy acquisition decreased at both low and at very high zooxanthella densities. However, there was a wide range of symbiont densities for which net daily photosynthesis was approximately equivalent. Therefore, significant changes in symbiont density do not necessarily cause a change in autotrophic energy acquisition by the colony. Model estimates of the optimal range of cell densities corresponded well with independent observations of symbiont concentrations obtained from field and laboratory studies of healthy colonies. Overall, this study demonstrates that the seasonal fluctuations, in symbiont numbers observed in healthy colonies of the Mediterranean coral investigated, do not have a strong effect on photosynthetic energy acquisition.

Keywords Scleractinian coral · Photosynthesis · Respiration · Energy balance · Symbiosis · Optimality model

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Introduction

The phenomenon of coral bleaching is recognized as one of the key threats to the persistence of coral reefs (e.g., Bellwood et al. 2006). Bleaching is a loss of symbiotic dinoflagellates, commonly known as zooxanthellae, from coral tissue and/or a reduction in symbiont pigmentation (e.g., Hoegh-Guldberg 1999). For coral colonies, varying the symbiont population influences the amount of carbon (energy) that is photosynthetically fixed by the symbionts and translocated to the coral host (e.g., Hoegh-Guldberg and Smith 1989). Of primary concern is the potential for the breakdown of the coral-zooxanthellae symbiosis to cause colony mortality (Marshall and Baird 2000; Loya et al. 2001), heighten the susceptibility of corals to disease (e.g., Muller et al. 2008) and reduce the future reproductive output of colonies (Baird and Marshall 2002).

Despite the observed negative effects of coral bleaching, such events occur against a background of natural fluctuations in symbiont numbers. Field studies demonstrate annual variation in zooxanthella density of between two and 10-fold, both in tropical (Brown et al. 1999; Fagoonee et al. 1999; Fitt et al. 2000) and temperate species (Rodolfo-Metalpa et al. 2008a). Moreover, symbiont concentrations vary two to fivefold between colonies sampled at a given time point (Stimson 1997; Fagoonee et al. 1999).

Habitat-related variation in zooxanthella density is also apparent for tropical (Fitt et al. 2000) and Mediterranean (Shenkar et al. 2006; Rodolfo-Metalpa et al. 2008a) species, although such differences are typically of a smaller magnitude compared to seasonal variation.

Few studies have quantified the point at which a decline in symbiont density begins to have negative impacts for corals. It has recently been shown that photosynthetic carbon acquisition increases non-linearly with increasing chlorophyll content (or symbiont density, e.g., Anthony et al. 2009). As symbiont densities increase, processes such as self-shading of zooxanthellae (e.g., Jones and Yellowlees 1997), or limited availability of dissolved inorganic carbon (Dennison and Barnes 1988; Muscatine et al. 1989a, b), reduce rates of photosynthesis per cell. This indicates that, over a certain range of chlorophyll (or zooxanthella) concentrations, changing symbiont density per unit tissue biomass does not necessarily influence the amount of photosynthetically generated carbon that is available for coral growth and reproduction. On the other hand, work by Hoegh-Guldberg and Smith (1989) indicated that carbon acquisition by the coral host does increase linearly with the size of the symbiont population. This lack of consensus about the functional relationship between symbiont density and coral energy acquisition means that it is unclear when seasonal and/or bleaching-related changes in zooxanthella numbers begin to affect coral energetics.

A robust quantification of the relationship between coral symbiont density and net photosynthesis requires the analysis of colonies sampled from the same environmental conditions but exhibiting a wide range of zooxanthella densities. The Mediterranean scleractinian coral, *Cladocora caespitosa*, occurs naturally with varying densities of symbionts within polyps of the same colony (Schiller 1993), thereby providing an opportunity to explore the relationship between variation in zooxanthella population density and net photosynthetic carbon gain of the polyp. This approach was preferred over experimentally bleaching colonies (by exposure to high light intensity and/or temperature) because the latter directly affect photosynthetic activity and baseline metabolic rates (e.g., Jones et al. 1998; Howe and Marshall 2001; Anthony and Hoegh-Guldberg 2003) such that it may no longer be possible to isolate the effect of symbiont density alone.

The primary aim of this study was to determine the optimal zooxanthella density for polyps of *Cladocora caespitosa*, and to evaluate how strongly net photosynthetic carbon acquisition varies in response to natural variation in symbiont numbers. In addition, the analytical approach taken in this work provided a mechanism to quantify the symbiont-related component of coral colony respiration. Inability to separate coral and algal contributions to total coral respiration has complicated studies of coral energetics: such contributions are typically apportioned on the basis of animal compared with symbiont biomass (Muscatine et al. 1981; Verde and McCloskey 1996). Overall, the analyses presented in this study quantify how zooxanthellae respiration, coral respiration, and zooxanthellae photosynthesis depend on symbiont density and identify the symbiont density which maximizes net photosynthetic carbon gain.

Materials and methods

Net photosynthetic carbon gain

The daily photosynthetic carbon budget, $P_{\mathrm{day}}(z)$, was modeled as an explicit function of symbiont density (z) and was calculated as the ratio of net daily photosynthesis, $P_{\mathrm{t}}(z)$, to total colony respiration overnight, $R_{\mathrm{t}}(z)$. Hereafter, the term 'carbon acquisition' is used to refer to the net generation of carbon through photosynthesis.

$$P_{\mathrm{day}}(z) = P_{\mathrm{t}}(z)\ [R_{\mathrm{t}}(z)]^{-1} \tag{1}$$

From Eq. 1, the optimal zooxanthella density is given by the cell concentration, z, that maximizes the value of $P_{\mathrm{day}}(z)$. This is based on the concept that conditions that allow maximal energy acquisition also maximize organism growth, reproduction, and survival (e.g., Kooijman 2000).

Assuming constant biomass-specific rates of animal and zooxanthella respiration, total colony respiration rate can be expected to increase linearly with symbiont density. Therefore, $R_{\mathrm{t}}(z)$ was modeled as:

$$R_{\mathrm{t}}(z) = (24 - t)\ (a + r\,z) \tag{2}$$

where a is respiration of the coral tissue, r is symbiont respiration (see Table 1 for details), t is the length of the daylight period (see below), and $(24 - t)$ denotes the overnight product of (hourly) respiration rate. Using this approach (after Hoegh-Guldberg and Smith 1989), the animal and symbiont contributions to total colony respiration can be apportioned by regressing measured rates of respiration on symbiont density: the intercept quantifies the animal component, a, and the slope quantifies respiration per zooxanthella, r. Light-enhanced respiration (e.g., Kuhl et al. 1995) is indirectly accounted for in these equations because measurements of net photosynthesis implicitly include any effects on respiration.

Previous studies have shown that coral photosynthesis is not solely limited by zooxanthella density (e.g., Dennison and Barnes 1988; Muscatine et al. 1989b). Instead, there is an asymptotically increasing relationship between rates of (maximum) photosynthesis and chlorophyll concentration, a proxy for zooxanthella density (Anthony et al. 2009). Therefore, in this study we modeled $P_{\mathrm{t}}(z)$ as:

Table 1 Results of regression analyses and parameter estimates (with SE in parentheses) for the relationships between zooxanthella density with rate of photosynthesis, rate of respiration, and total chlorophyll content (i.e. sum of chlorophyll *a* and *c2*)

Analysis	Regression equation	Parameter and units	Estimate	*p* value
Photosynthesis as a function of zooxanthella density	$P_t = P_x \times \tanh[z(z_{sat})^{-1}]$	P_t = maximum rate of photosynthesis (μmol O_2 (mg protein)$^{-1}$ h^{-1})	Variable	–
		P_x = asymptotic value of P_{max} (μmol O_2 (mg protein)$^{-1}$ h^{-1})	1.2 (0.2)	$p < 0.001$
		z = zooxanthella density (10^6 cells (mg protein)$^{-1}$)	Variable	–
		z_{sat} = sub-saturation density of zooxanthellae (10^6 cells (mg protein)$^{-1}$)	1.8 (0.5)	$p < 0.01$
Respiration as a function of zooxanthella density	$R_t = a + r \times z$	R_t = rate of respiration (μmol O_2 (mg protein)$^{-1}$ h^{-1})	Variable	–
		a = rate of animal respiration (μmol O_2 (mg protein)$^{-1}$ h^{-1})	0.1 (0.02)	$p < 0.001$
		r = respiration per zooxanthella (μmol O_2 10^{-6} cells h^{-1})	0.2 (0.03)	$p < 0.001$
Total chlorophyll per zooxanthella	$C = c \times z$	C = total chlorophyll (μg Chl (mg protein)$^{-1}$)	Variable	–
		c = chlorophyll per zooxanthella (μg Chl 10^{-6} cells)	3.3 (0.3)	$p < 0.001$

$$P_t(z) = t \times P_x \times \tanh\left[z(z_{sat})^{-1}\right] \quad (3)$$

where P_x is the asymptotic rate of maximum photosynthesis, z_{sat} is the zooxanthella density at which $P_t(z)$ is 75% of its asymptotic value, and t is the length of time over which photosynthesis is occurring at its maximal rate. In this study, t was set equal to 10 h to approximate the amount of time for which photosynthesis is saturated during a 12-h diurnal cycle (e.g., Hoegh-Guldberg and Smith 1989). Because carbon acquisition is here determined as the ratio of net daily photosynthesis to night respiration, the results are not sensitive to the value of t (see Electronic Supplementary Material, ESM). The model does not include the sub-saturation irradiance parameter (E_k, e.g., Jassby and Platt 1976) that quantifies how rapidly photosynthesis approaches its maximum rate as light intensity increases. This parameter was omitted because variation in E_k has only a small effect on calculations of $P_{day}(z)$ (see ESM).

Experimental setting

Colonies of *Cladocora caespitosa* used in this study were collected from Fiascherino Bay, in the Gulf of La Spezia (Italy) and maintained in culture aquaria at a temperature of 18°C and at a light intensity of 50 μmol photons m^{-2} s^{-1}. These colonies naturally presented patches of polyps with different symbiont densities (as observed by Schiller 1993; Kruzic and Bencovic 2008) and were cut into pieces selecting patches of polyps with the same color (e.g., light or dark polyps). Sixteen small experimental colonies (3–4 polyps) were created with the specific aim of generating nubbins with a broad range of symbiont densities (spanning between 1×10^5 and 2.6×10^6 cells mg^{-1} protein, or between 2×10^5 and 3.7×10^6 cells cm^{-2}). The expected asymptotic relationship between net photosynthesis and symbiont density implies that changes in symbiont density have the strongest influence on rates of photosynthesis at low zooxanthellae numbers. Therefore, multiple nubbins with low symbiont densities were required in order to accurately quantify this relationship. Additional nubbins were created to isolate the zooxanthellae for measurements of symbiont respiration rates (freshly isolated zooxanthellae, FIZ).

Photosynthesis and respiration

Rates of photosynthesis and respiration were measured using a set of three temperature-controlled respirometry chambers (50 ml volume) coupled with a Strathkelvin oxygen electrode system (Clark-type electrodes connected to a Strathkelvin 928 oxygen meter and a computer). Electrodes were calibrated immediately prior to respirometry measurements using N_2- and air-bubbled seawater as 0 and 100% oxygen saturation values, respectively. Temperature was maintained at 18°C during all of the incubations, and chambers were stirred continuously using magnetic stirrers. A metal halide lamp mounted on a sliding platform was used as a light source. For each colony, respiration (oxygen consumption) was measured during a half-hour incubation in darkness. Subsequently, colonies were exposed to an intermediate irradiance of 125 μmol photons m^{-2} s^{-1} for a period of 15 min, after which maximum photosynthesis rate was measured during a half-hour incubation at 400 μmol photons m^{-2} s^{-1}. Following the photosynthesis measurements, colonies were separated into two sections (one for protein analysis and one for zooxanthellae and chlorophyll analysis) and placed in a freezer at −20°C.

In addition to the measurements of colony respiration, rates of respiration of freshly isolated zooxanthellae (FIZ) were measured. This additional set of measurements was conducted as an independent validation of the estimates of symbiont-specific respiration obtained from the regression model (Eq. 2). To do this, symbionts were extracted into

100 ml of 0.45 μm filtered seawater using a Water-Pik, and the tissue centrifuged at 2,000 rpm for 10 min to pellet the zooxanthellae. The supernatant was discarded, and the symbionts were resuspended in filtered seawater. Respiration rates were then measured in small respirometry chambers (5 ml volume) using the same technique described earlier. Following respiration measurements, a 1 ml sub-sample of the symbiont suspension was retained for symbiont density determination. A total of six replicate measurements were made (three from each of two extractions) for comparison with the respiration rates estimated using the regression technique.

Zooxanthellae density, chlorophyll content, and protein biomass

To estimate zooxanthella density and chlorophyll concentration, tissue was removed from the skeleton of one sub-sample from each colony using an air-pick and collected in a beaker with 7 ml of 0.45 μm filtered seawater. The tissue slurry was homogenized using a Potter tissue grinder, and a 1 ml sub-sample was taken for zooxanthellae counts. Symbiont densities in samples of volume 100–300 μl were counted using an inverted microscope (Leica, Wetzlar, Germany) and the Histolab 5.2.3 image analysis software (Microvision, Every, France). The same counting procedure was used to determine the abundance of FIZ in the aliquots used for symbiont respiration measurements. The remaining tissue slurry for each colony was centrifuged at 8,000*g* for 10 min. Subsequently, the supernatant was removed, and the zooxanthellae resuspended in 5 ml of acetone for extraction of chlorophylls *a* and c2. Samples were kept in darkness at 4°C for a period of 24 h to insure total extraction. Chlorophyll content was determined using a spectrophotometry method. Samples were centrifuged for 15 min at 11,000*g* before the absorbance was measured at three wavelengths (750, 663, 630 nm). The wavelength of 750 nm was used to control for sample turbidity, and chlorophyll content was calculated based on A663 and A630 using the equations of Jeffery and Humphrey (1975).

Protein biomass was estimated using a bicinchoninic acid protein assay (Uptima, Interchim). For each sample, total protein was extracted by incubation in a sodium hydroxide solution (1N) maintained in a water bath for 30 min at 90°C. Subsequently, samples were diluted by a factor of 2, transferred into 96-well microplates, and incubated with a dye reagent (Uptima Reagents, Interchim) for 30 min at 60°C. Protein standards of concentration 0, 50, 100, 200, 500, 1,000, 1,500, and 2,000 μg ml^{-1} were also prepared using bovine serum albumin (BSA, Interchim), transferred to microplates and incubated as earlier. Samples and standards were homogenized for 30 s at 400 rpm on a microplate shaker. Finally, absorbance was measured at 560 nm, and the protein content of samples was determined by reference to the calibration samples using the GENESIS program (Kontron Instruments).

The surface area of all colony sub-samples was calculated using the method described by Rodolfo-Metalpa et al. (2006a). Briefly, polyp surface area was measured as the surface area of a cylinder with dimensions equal to the diameter and height of each polyp. The average of two polyp diameter measurements was used for these calculations. Protein, chlorophyll content, and zooxanthella density of each sub-sample were normalized to surface area, and these values used to calculate whole-nubbin values. Finally, all data were normalized to total protein content.

Statistical analyses

Statistical analyses were implemented using the software package R (R Development Core Team 2008). Parameter estimates for the relationships between zooxanthella density and rates of photosynthesis, $P_t(z)$, and respiration, $R_t(z)$, were determined using standard least-squares regression techniques. Similarly, a regression analysis was used to confirm linearity of the relationship between zooxanthella density and chlorophyll concentration. The statistical significance level (α) was set at 0.05 for all analyses.

A Monte Carlo simulation technique was used to account for variation in parameter estimates of the fitted relationships between rates of photosynthesis and symbiont density ($P_t(z)$, Eq. 3) and between rates of colony respiration and symbiont density ($R_t(z)$, Eq. 2). To do this, calculations of $P_{day}(z)$ were repeated 1,000 times at zooxanthella densities ranging between 0 and 5×10^6 cells (mg protein)$^{-1}$. For each set of calculations, parameter estimates were drawn at random from multivariate normal distributions generated from the variance–covariance matrix of the best-fit parameters of Eqs. 2 and 3. This approach was also used to generate a 95% confidence interval around the optimal zooxanthella density.

Results

Rates of photosynthesis increased asymptotically with increasing symbiont density (Fig. 1a), indicating that at high zooxanthella concentrations, individual symbionts contribute proportionally less carbon to the symbiosis. On the other hand, respiration rates increased linearly with symbiont numbers (Fig. 1b). Moreover, symbionts within *C. caespitosa* contributed strongly to total colony respiration, with rates of oxygen consumption estimated to be approximately 0.2 μmol O_2 10^{-6} cells h^{-1} (parameter *r* in Table 1, slope of Fig. 1b). All of the parameters of the equations describing variation in both photosynthesis and

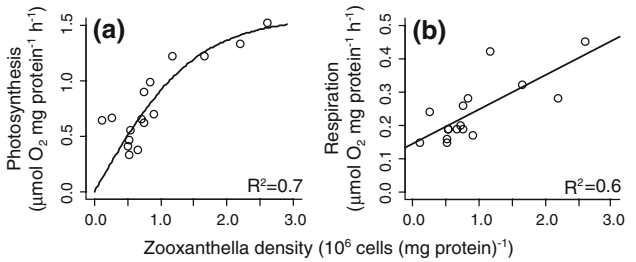

Fig. 1 Relationship between zooxanthella density and **a** rate of maximum (net) photosynthesis, $P_t(z)$, and **b** rate of respiration, $R_t(z)$. *Points* represent values measured for individual nubbins. *Lines* represent fitted regressions of Eq. 3 (photosynthesis) and Eq. 2 (respiration)

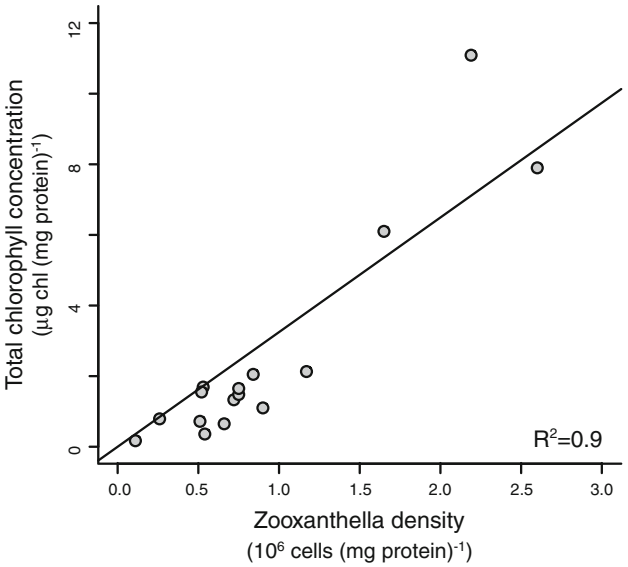

Fig. 2 Linear relationship between zooxanthella density and chlorophyll concentration. Values of both variables are normalized to protein content, *points* represent values for individual nubbins

respiration (Eqs. 3, 2, respectively) were significantly different than zero (Table 1), and the equations provided an adequate fit to the data (Fig. 1). Measured rates of respiration of freshly isolated zooxanthellae ranged between 0.09–0.28 μmol O_2 10^{-6} cells h^{-1}, in good agreement with the value estimated using the regression technique (see Table 2).

Total chlorophyll concentration was linearly related to zooxanthella density (Fig. 2; Table 1). There was a degree of bias in the fitted regression line through these data, primarily due to one datum with higher than expected chlorophyll content that had strong leverage on the line of best fit. Nevertheless, the overall linearity of the relationship between symbiont density and chlorophyll content indicates that the shape of the relationship between rate of photosynthesis and symbiont density was not driven by variation in chlorophyll concentration. Finally, the mean protein content of colonies analyzed in this study was 2.1 ± 0.1 mg cm^{-2} (mean ± SE).

Daily energy acquisition initially increased as zooxanthella density increased from zero, reached a maximum (optimum) at 1.75 × 10^6 cells (mg protein)$^{-1}$ and subsequently declined (Fig. 3). However, the 95% confidence

Table 2 Summary of literature estimates of cell-specific rates of respiration of symbiotic dinoflagellates, including details of the host organism and method used

Host	Respiration rate (μmol O_2 10^{-6} cells h^{-1})	Method	References
Aiptasia pulchella (Anemone)	0.04	Biomass	Muller-Parker (1984)
Aiptasia pulchella (Anemone)	0.22	Measured, FIZ	Muller-Parker (1984)
Aiptasia pulchella (Anemone)	0.005	Measured, culture	Steen (1987)
Anthopleura elegantissima (Anemone)	0.001	Biomass	Verde and McCloskey (1996)
Astrangia danae (Coral)	≈0	Regression	Jacques et al. (1983)
Cassiopeia xamachana (Jellyfish)	0.11	Measured, culture	Iglesias-Prieto and Trench (1994)
Cladocora caespitosa (Coral)	0.2	Regression	This study
Cladocora caespitosa (Coral)	0.17	Measured, FIZ	This study
Montastrea annularis (Coral)	≈0.05–0.3	Measured, FIZ	Dustan (1982)
Montipora verrucosa (Coral)	0.03	Measured, culture	Iglesias-Prieto and Trench (1994)
Myrionema amboinense (Hydroid)	0.26–0.36	Measured, FIZ	Fitt and Cook (2001)
Seriatopora hystrix (Coral)	≈0	Regression	Hoegh-Guldberg and Smith (1989)
Stylophora pistillata (Coral)	7.3–230	Multiple	Leletkin et al. (1996)
Zoanthus sociatus (Zoanthid)	0.12	Measured, culture	Iglesias-Prieto and Trench (1994)

Values have been converted from original units of measurement to units of μmol O_2 10^{-6} cells h^{-1}. 'Biomass' indicates that symbiont respiration was estimated based on the ratio of host versus symbiont biomass, 'Measured' indicates measurements made using oxygen respirometry, 'FIZ' and 'culture' refer to Freshly Isolated Zooxanthellae and cultured zooxanthellae, respectively, 'Regression' indicates the same technique used in this study

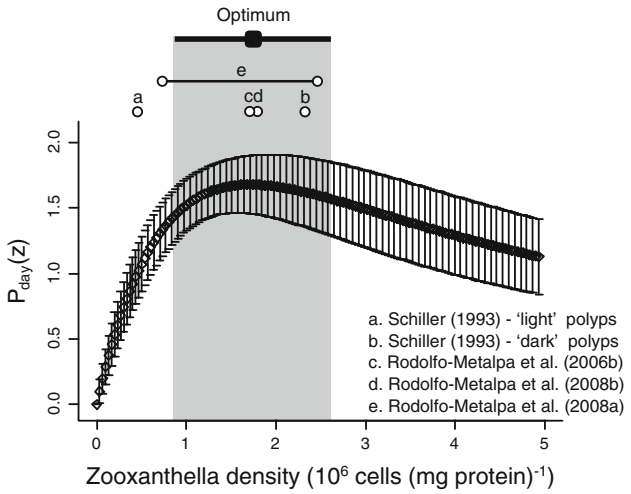

Fig. 3 Optimal zooxanthella density for *Cladocora caespitosa* based on ratio of daily photosynthesis to respiration. *Solid line* is the PR ratio ($P_{day}(z)$, Eq. 1) calculated based on the best-fit parameters of the relationships in Fig. 1 and *bars* show standard deviation of model prediction (generated by Monte Carlo simulation). *Open circles* represent zooxanthella densities for *C. caespitosa* observed in field and laboratory studies. *Horizontal bar* and *shaded area* indicate the 95% confidence interval of the optimal zooxanthella density (*closed square*)

interval around the optimal cell density was broad (Fig. 3), ranging from 0.9×10^6 to 2.7×10^6 cells (mg protein)$^{-1}$ (i.e., from approximately 1.8×10^6 to 5.5×10^6 cells cm^{-2}). At densities close to the optimum, the slope of the relationship between symbiont numbers and energy acquisition was relatively flat. Therefore, a shift in symbiont density away from the optimum value had only a small effect on energy balance (Fig. 3), and this translated into greater uncertainty around the optimum. Independent field and laboratory data describing variation in zooxanthella density for *Cladocora caespitosa* generally fell within the 95% confidence interval for the optimal density (Fig. 3, open points). From the available field data, only 'pale' polyps isolated from field colonies (Schiller 1993) displayed symbiont densities well outside of the optimal range.

Discussion

Using a novel analysis based on established techniques for measuring carbon acquisition by coral colonies (oxygen respirometry; e.g., Muscatine et al. 1981), this study has identified the optimal symbiont density for a temperate scleractinian coral species. Increasing symbiont density enhanced carbon acquisition over the range of cell densities between 0 and 0.9×10^6 cells (mg protein)$^{-1}$. However, the asymptotic relationship between symbiont density and rates of photosynthesis meant that further growth in symbiont numbers above 0.9×10^6 cells (mg protein)$^{-1}$ (approximately 1.8×10^6 cells cm^{-2}) did not substantially increase net photosynthetic carbon acquisition. In fact, the results of this work indicate that the daily net photosynthesis is approximately equivalent across a broad range of cell densities (between 1.8×10^6 and 5.5×10^6 cells cm^{-2}). This range corresponds to the range observed under field conditions over an annual cycle (approximately 1.5–5.1×10^6 cells cm^{-2}, Rodolfo-Metalpa et al. 2008a).

The functional relationship between rates of photosynthesis and zooxanthella density reported in this study is in agreement with data for two tropical coral species (*Montipora monasteriata* and *Acropora intermedia*, Anthony et al. 2009). However, these results are inconsistent with the linear relationships reported for *Seriatopora hystrix* (Hoegh-Guldberg and Smith 1989) and another temperate coral species (*Astrangia danae*, Jacques et al. 1983). One explanation for this inconsistency is that symbiont type may determine how photosynthesis per symbiont varies with symbiont density: recent studies have identified clear effects of symbiont identity on coral growth and susceptibility to disease (Little et al. 2004; Stat et al. 2008). However, *M. monasteriata*, *A. intermedia*, and *S. hystrix* are all associated predominantly with clade C symbionts (Loh et al. 2001; LaJeunesse et al. 2004), *A. danae* with clade B (Rowan and Powers 1991) and *C. caespitosa* with clade 'A temperate' (Visram et al. 2006). Therefore, clade differences do not explain species differences in the functional response between photosynthesis and zooxanthella density. Although physiological differences at the subclade level cannot be ruled out (e.g., Sampayo et al. 2008), these results suggest that traits of the coral host are important drivers of the relationship between colony photosynthesis and symbiont density.

The optimal density determined in this study is close to the mean value of zooxanthellae concentrations previously observed in field and laboratory studies. For colonies of *Cladocora caespitosa* grown under similar laboratory conditions to those analyzed in this study, symbiont densities were close to the optimal value determined here (Rodolfo-Metalpa et al. 2006b, 2008b; based on a protein content of 2.1 mg protein cm^{-2}, see "Results"), independently of the light level at which the corals were maintained. Similarly, when normalized to protein content per unit surface area, a field-based estimate of symbiont numbers for 'dark' polyps sampled from colonies of *C. caespitosa* in the Adriatic Sea also lay within the optimal band (Schiller 1993). In contrast, symbiont densities within 'pale' polyps isolated from otherwise healthy colonies (Schiller 1993) were well below the optimal range. At present, the precise cause of this natural paling is not known. Studies suggest that such 'patchy' bleaching of *C. caespitosa* is caused by anomalously high seawater temperatures (e.g., Schiller 1993; Kruzic and Bencovic 2008). However, zooxanthellae in symbiosis with

this coral species are resistant to short-term increases in seawater temperatures (Rodolfo-Metalpa et al. 2006a). Moreover, long-term temperature increases generally cause tissue necrosis for *C. caespitosa* rather than a loss of symbionts per unit biomass (Rodolfo-Metalpa et al. 2006b). Further field investigations are required to identify the cause of polyp-scale bleaching in this species. Nevertheless, natural seasonal and depth-related variation in zooxanthella concentration observed over a period of 18 months in the northwestern Mediterranean Sea (Rodolfo-Metalpa et al. 2008a) did not deviate strongly from the model calculation of the optimal density. The model calculations do not take into account potential seasonal fluctuations in protein content. Protein levels of Mediterranean cnidarians are generally highest following winter, during the same time period when symbiont densities per unit surface area are maximal (Rossi and Tsounis 2007; Rodolfo-Metalpa et al. 2008a). Based upon this trend, if seasonal variation in protein biomass were taken into account in the analyses presented in Fig. 3, the range of values depicting the results of Rodolfo-Metalpa et al. (2008a) would shrink closer to the optimal value predicted from the model. Therefore, our results are conservative as to the agreement between model calculations and field observations. Overall, these comparisons indicate that seasonal fluctuations in symbiont densities have only a small influence on energy acquisition for this species.

The results of this study highlight functional differences between tropical and temperate Mediterranean corals. Temperate corals are generally thought to obtain a greater proportion of their daily carbon requirements through heterotrophy (FitzGerald and Szmant 1988). A weaker dependence on photosynthesis could lead to greater flexibility in the regulation of the symbiont population, and this may explain the relative insensitivity of photosynthetic carbon acquisition to variation in zooxanthella density observed here. Enhanced heterotrophic feeding also yields additional nutrients (i.e., nitrogen and phosphorus) particularly in the turbid and sediment-enriched coastal biomes that *Cladocora caespitosa* inhabits (Peirano et al. 2005). This nutrient supply might also induce a lack of zooxanthellae regulation, independent of the host. In contrast, tropical corals live in habitats with substantially higher light intensities and are more strongly limited by nutrient availability (Muscatine et al. 1989b). Strong nutrient limitation is likely to substantially lower the maximum size of the symbiont population. Consequently, under natural conditions, it might not be possible for tropical corals to achieve zooxanthella population densities equivalent to those observed in this study. Correspondingly, a strong plateau in the relationship between daily photosynthesis and symbiont density may not be observed (e.g., Hoegh-Guldberg and Smith 1989). For temperate corals, higher nutrient availability is likely to relax the constraint on maximum symbiont density. This allows these corals to increase symbiont numbers in order to maximize light capture, even to the extent where the yield of photosynthate per symbiont is strongly reduced.

Symbionts within *C. caespitosa* contributed strongly to total colony respiration. Previous estimates of the respiration rate of symbiotic dinoflagellates vary widely, ranging from 0 to 230 μmol O_2 10^{-6} cells h^{-1} (Table 2). One factor that is likely to contribute to this lack of consistency is that several different methods have been used, including measurements of freshly isolated symbionts, measurements of cultured zooxanthellae, or a regression technique similar to that used here. Estimates of symbiont respiration based on an equivalent methodology to that used in this study range from not being statistically different from zero (Jacques et al. 1983; Hoegh-Guldberg and Smith 1989) to between 80 and 130 μmol O_2 10^{-6} cells h^{-1} (Leletkin et al. 1996). However, reported respiration rates of freshly isolated and cultured zooxanthellae are generally consistent with the values both measured and estimated in this study (range 0.005–0.36 μmol O_2 10^{-6} cells h^{-1}, Table 2). Although care must be taken in generalizing the rates of respiration measured with different techniques, and for different symbionts and host taxa, the results of this study are generally consistent with several studies that demonstrate a significant symbiont-related component to the respiration of coral colonies (Smith and Muscatine 1986; Leletkin et al. 1996; Titlyanov et al. 1999).

There are two important caveats to the conclusions of this study. First, energy acquisition was measured using oxygen flux as a proxy for carbon acquisition. Experimental evidence confirms that oxygen release and carbon fixation during photosynthesis are linearly related (Gatusso and Jaubert 1990; Carpenter and Williams 2007). Therefore, converting calculations of energy balance to represent carbon acquisition more directly (i.e., by multiplying by a constant representing the photosynthetic quotient, Muscatine et al. 1981) would have no effect on the shape of the relationship between P:R ratio and symbiont density, and therefore would have no effect on the optimal symbiont density determined here.

Second, this study assumes that a constant proportion of carbon fixed by symbionts is translocated to the coral host, irrespective of symbiont density. It is generally accepted that up to 95% of carbon fixed by zooxanthellae is translocated to coral tissue (Muscatine et al. 1981; Steen and Muscatine 1984). If translocation per zooxanthella is independent of symbiont density, then multiplying calculations of energy acquisition by a constant representing percentage carbon translocation would have no effect on the optimal density. However, if the proportion translocated is strongly dependent upon cell density, this would affect the relationship between symbiont concentration and energy acquisition. At present, we have no evidence as to how strongly percentage carbon translocation varies with

zooxanthella density for *C. caespitosa*. The most likely scenario is that carbon translocation is reduced when symbiont densities are low, because a higher proportion of carbon acquired by symbionts is used for growth of the symbiont population rather than being translocated to the coral host (e.g., Jones and Yellowlees 1997; Davy and Cook 2001). For the results presented here, the net effect of such dynamics would be to reduce energy acquisition at low zooxanthella densities and shift the optimal density toward higher values. Nevertheless, a study of symbiotic clams showed that symbiont density and photosynthate translocation were linearly related, and therefore that translocation per symbiont was a constant independent of density (Leggat et al. 2003). The good agreement between the optimal range of symbiont densities and observations of symbiont densities under field conditions also suggests that the percentage translocation per symbiont does not vary strongly with zooxanthella density.

The asymptotic relationship between photosynthesis and symbiont density observed in this study, combined with a linear increase in rates of respiration as symbiont numbers increase, means that colony energy acquisition decreases at very high zooxanthella densities. In other words, increasing symbiont density does not lead to increased energy acquisition for the temperate coral species considered here. In fact, for *Cladocora caespitosa*, a broad range of symbiont densities provides approximately equivalent potential for carbon acquisition. Because the aim of this study was to quantify effects of symbiont density on photosynthetic carbon acquisition, heterotrophic feeding has not been incorporated. Nevertheless, even without including potential effects of feeding on the relationship between photosynthesis and symbiont density, model estimates of the optimal range of cell densities corresponded well with independent observations of symbiont concentrations obtained from field and laboratory investigations. Overall, this study demonstrates that over a broad range of symbiont densities, natural fluctuations in symbiont numbers do not necessarily have a strong effect on energy acquisition.

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