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# Co-variation between autotrophy and heterotrophy in the Mediterranean coral *Cladocora caespitosa*

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## SUMMARY

**This study quantifies the relative contributions of autotrophy and heterotrophy to the energy budget of the temperate scleractinian species *Cladocora caespitosa* Linnaeus 1767. Colonies were incubated under different light and feeding regimes, and changes in carbon acquisition through photosynthesis and feeding were measured during a 2-month time period. This approach allowed us to quantify the rate at which adjustments to physiology occurred, as well as the magnitude of up- or downregulation of both feeding modes. In addition, we explored how shifts in carbon acquisition mode influenced tissue biomass (protein content), energy stores (lipid content) and colony growth (calcification). Increases in feeding capacity during prolonged exposure to darkness were correlated with decreases in photosynthetic capacity. However, feeding effort did not decrease when photosynthesis was high. In fact, feeding was maximal under high light conditions when food was not available. During starvation, colonies used their lipid stores to partially meet their metabolic requirements. Colonies kept in low light used nutrients from feeding to supplement calcification, whereas those kept at high light converted carbon from feeding into tissue biomass. This work provides the first estimates of rates of adjustment of heterotrophic feeding capacity in a Mediterranean scleractinian coral. For the study species, flexibility in carbon acquisition through heterotrophy was approximately equal to flexibility in photosynthesis both in magnitude and in the rate at which physiological adjustments occurred. The ability to alter feeding effort rapidly and strongly may explain the wide depth distribution of *C. caespitosa*, and its ability to survive in turbid coastal waters where light is often limited.**

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/213/14/2399/DC1

Key words: energy balance, heterotrophic feeding, lipid stores, scleractinian coral, photoacclimation, photosynthesis.

## INTRODUCTION

Nutritional symbioses are widespread in aquatic and terrestrial ecosystems, and involve a diversity of different host and symbiont taxa (Saffo, 1992). Many insects that subsist on poor-quality food sources form symbioses with bacteria that supply essential nutrients (Douglas, 1998). Cyanobacteria are symbiotic with terrestrial plants, lichens and marine invertebrates, supplying carbon to non-photosynthetic partners or providing fixed nitrogen to the host in low nutrient environments (Usher et al., 2007). Although in many cases the precise role of the different partners of such symbioses has not been quantified, in general the formation of symbiosis influences the habitat range and inter-species relationships of the host organism (Saffo, 1992). One of the most widely recognised nutritional symbioses is that between corals and photosynthetic dinoflagellates from the genus *Symbiodinium* (‘zooxanthellae’). This symbiosis augments the carbon supply to the coral, while the symbionts benefit from nutrients provided by the host (Yellowlees et al., 2008). However, corals also obtain carbon through heterotrophic feeding on a variety of sources. Corals prey actively upon zooplankton (Ferrier-Pagès et al., 2003; Palardy et al., 2005), and consume bacteria, pico- and nano-plankton (Bak et al., 1998; Houlbrèque et al., 2004). In addition, corals are known to feed upon suspended particulate matter (Anthony, 1999; Mills et al., 2004) and to take up dissolved organic matter directly from the water column (Trench, 1974; Ferrier, 1991).

The significance of heterotrophy to the energy budget of corals has long been debated. Early work on tropical corals indicated that feeding contributed little carbon but was an important source of other limiting nutrients (Odum and Odum, 1955; Johannes et al., 1970; Muscatine and Porter, 1977). However, recent work indicates that when feeding on all possible food sources is taken into account, heterotrophy contributes up to 70% of daily carbon requirements (Houlbrèque and Ferrier-Pagès, 2008). Moreover, there is growing evidence that corals upregulate heterotrophic feeding when photosynthesis is suppressed, either due to decreased water clarity (Anthony and Fabricius, 2000) or when symbionts are lost from coral tissue (Grottoli et al., 2006). Therefore, it is now clear that heterotrophy can be a compensatory mode of carbon acquisition for corals. This compensatory mode is particularly important in temperate symbioses. Indeed, temperate symbiotic corals are thought to be more heterotrophic than tropical corals (FitzGerald and Szmant, 1988) probably because the former live in habitats with low light levels, cold temperatures, high nutrient concentrations and with strong seasonal fluctuations in environmental conditions. In addition, for many temperate corals, the symbiosis seems to be facultative and varies with environmental conditions depending upon the trophic requirements of symbiont and host. The primary aim of

this study was to quantify, in a temperate coral, co-variation in photosynthetic and heterotrophic carbon acquisition in response to changes in light and food availability. Specifically, we hypothesised that heterotrophic feeding capacity would increase over time when corals were shifted into darkness, and would decrease over time when corals were shifted into higher light intensity.

Quantifying the relationship between autotrophy and heterotrophy is complicated by the variability of carbon acquisition displayed by both corals and symbionts. Several studies on tropical corals have demonstrated that not only does feeding capacity vary between species (Anthony, 1999; Palardy et al., 2006; Houlbrèque et al., 2004) but also within species, depending upon prey availability (Ferrier-Pagès et al., 2003), depth (Palardy et al., 2008), sedimentation regime (Anthony, 2000) or the density of symbionts within tissue (Grottoli et al., 2006). Similarly, symbiont photosynthetic activity varies because of light-induced modifications of the photosynthetic apparatus through processes of 'photoacclimation' (Falkowski and Raven, 1997): such variation affects total photosynthetic carbon acquisition (Anthony and Hoegh-Guldberg, 2003; Hoogenboom et al., 2006). In temperate systems, which are characterised by strong seasonal variation in environmental conditions (Rodolfo-Metalpa et al., 2008a), an even stronger flexibility of nutritional modes may be expected.

In this study, we measured the relative contributions of autotrophy and heterotrophy to the carbon budget of the temperate scleractinian Mediterranean coral *Cladocora caespitosa* Linnaeus 1767. This coral has a wide habitat range from relatively dark conditions, down to 40 m depth or in very turbid water, to well-lit shallow-water habitats (Peirano et al., 1999). However, calcification rates of colonies of this species do not change with light intensity (Rodolfo-Metalpa et al., 2008a), suggesting an efficient regulation of nutritional modes. Therefore, *C. caespitosa* is an ideal model organism with which to investigate the extent to which heterotrophy compensates for reduced photosynthetic capacity. To explore this further, we quantified the change in carbon acquisition over time through both photosynthesis and feeding, for colonies exposed to different light and feeding regimes. This allowed us to measure the rate at which physiological adjustments occurred, as well as the magnitude of up- or downregulation of both feeding modes in response to different conditions. In addition, we explored how shifts in carbon acquisition mode influenced tissue biomass (protein content), energy stores (lipid content), colony growth (calcification rate) and symbiont densities. These latter analyses aimed to resolve which component of biomass (lipid or protein) is respired to meet maintenance metabolic costs when corals are starved, and to quantify how calcification rate depends upon light and food availability.

## Theoretical framework

### Autotrophy

The relationship between light and photosynthesis is generally characterised using one of several empirical equations that describe the rate of photosynthesis using three parameters (Jassby and Platt, 1976): the maximum rate of photosynthesis ($P_{max}$, μmol $O_2$ $cm^{-2}$ $h^{-1}$), the 'subsaturation' irradiance at which photosynthesis is a percentage of maximum ($E_K$, μmol photons $m^{-2}$ $s^{-1}$) and the rate of respiration in darkness ($R_D$, μmol $O_2$ $cm^{-2}$ $h^{-1}$). In this study, we used the rectangular–hyperbola equation that expresses the net rate of photosynthesis ($P_N$, μmol $O_2$ $cm^{-2}$ $h^{-1}$) as:

$$P_N = \frac{P_{max} E}{\left(E_K + E\right)} - R_D \,, \qquad (1)$$

where $E$ is light intensity (μmol photons $m^{-2}$ $s^{-1}$).

Owing to photoacclimation, the parameters of Eqn 1 ($P_{max}$, $E_K$ and $R_D$) vary according to the light intensity under which organisms grow (Chalker et al., 1983; Falkowski, 1984). The magnitude of such variation in the photosynthesis irradiance response, and the rate at which shifts in photophysiology occur, differs between taxa (Falkowski, 1984; Cullen and Lewis, 1988; Anthony and Hoegh-Guldberg, 2003). Moreover, there can be differences in the rates at which organisms acclimate to low compared with high light (Cullen and Lewis, 1988), and differences in photoacclimation of $P_{max}$ compared with $E_K$ (Anthony and Hoegh-Guldberg, 2003). The hydrodynamic environment surrounding corals also affects photosynthesis, and could potentially influence photoacclimation dynamics: changes in flow rates affect rates of photosynthetic gas exchange by altering the diffusive boundary layer adjacent to coral tissue (Patterson, 1992).

To quantify photoacclimation in *Cladocora caespitosa*, we applied a model formulated by Anthony and Hoegh-Gulberg (Anthony and Hoegh-Gulberg, 2003). This model is based on the principle that the value of a particular photosynthesis parameter ($P_{max}$, $E_K$ or $R_D$) reaches a steady state when growth irradiance is constant and, after a shift in growth irradiance, the parameter changes at a rate proportional to the difference between the current value and the new steady-state value (Anthony and Hoegh-Guldberg, 2003). That is, the parameter value changes rapidly immediately after a shift in the light environment but the rate of change slows as the value approaches steady state. From these principles, the value of particular parameter ($Y$) at a particular time after a shift in the light environment ($t$) is:

$$Y(t) = Y_S + (Y_0 - Y_S)\exp^{-\varepsilon t}, \qquad (2)$$

where $Y_S$ is the steady-state value of the parameter under the new light environment, $Y_0$ is the initial value and ε describes the rate at which the parameter changes over time (i.e. the 'acclimation' rate).

### Heterotrophy

There are no existing data quantifying the rate of change of heterotrophic feeding capacity for corals following a shift in food availability. Based on general foraging principles, when food availability is approximately constant, coral colonies can be expected to capture and consume prey at rates that optimise energy and/or nutrient gain from feeding (MacArthur and Pianka, 1966). Following a change in food availability, a coral may alter its feeding rate by adjusting its ability to capture prey and/or the speed at which prey is digested. Mechanistically, increasing capture success requires higher tentacle mobility, greater nematocyst density and/or increased mucus production (Johannes and Tepley, 1974; Lewis and Price, 1975). Similarly, handling prey more quickly requires greater tentacle mobility and/or a potential increase in digestion rate (Shimeta and Koehl, 1997). Day-to-day changes in these mechanisms will result from synthesis and degradation of proteins (e.g. for tentacle retraction muscles), processes that follow first-order kinetics (Srividhya and Schnell, 2006). In the absence of formal theory, we here assume that, because the mechanisms by which feeding rate change follow first order kinetics, changes in feeding rate ($dH/dt$) will be proportional to the difference between current, $H$, and expected, $H_S$, feeding rates:

$$\frac{dH}{dt} = \varepsilon_H \left[ H_S - H \right], \qquad (3)$$

where $\varepsilon_H$ is a coefficient describing the rate at which feeding changes over time. Eqn 3 is a differential equation that is solvable by integration to obtain feeding rate, $H$, as a function of time after a

change in food availability, $t$, using feeding rate prior to the change in food availability, $H_0$, as an initial condition:

$$H(t) = H_S + (H_0 - H_S)\exp^{-\epsilon_H t}. \quad (4)$$

Note that Eqn 4 has the same functional form as Eqn 2 because both represent the dynamics of first-order processes.

## MATERIALS AND METHODS

### Study species and experimental treatments

Colonies of *Cladocora caespitosa* were collected from the Gulf of La Spezia (Italy, 44°04′N, 9°52′E) and maintained in culture aquaria at a temperature of 18°C and light intensity of 50 μmol photons $m^{-2}$ $s^{-1}$. These temperature and light levels approximate field conditions during spring and are the most appropriate to guarantee long-term maintenance of this coral in culture. From these colonies, 16 large (4–6 polyps) and 256 small (1 or 2 polyps) nubbins were created 2 weeks before the experiment and allowed to heal under the culture conditions. Subsequently, experimental treatments with two light-intensities (0 and 200 μmol photons $m^{-2}$ $s^{-1}$) and two feeding (fed and unfed) levels were established in eight glass aquaria (18 l volume) with two replicate tanks for each light and feeding combination (Fig. 1). Each tank was equipped with a small pump to ensure adequate water mixing, consistent temperature within tanks and equivalent flow regimes between tanks. The seawater supply for the experimental aquaria came from the Mediterranean Sea and was filtered and stored in a reservoir with a 1-day turnover prior to use. This water contains very few plankton and typically has background concentrations of total organic carbon (i.e. from bacteria and dissolved organic matter) between 50 and 100 μmol $l^{-1}$ (Ferrier-Pagès et al., 1998). The 'fed' treatments were provided with *Artemia salina* nauplii twice weekly, directly into the experimental aquaria at a feeding density of ~1000 cells $l^{-1}$. Capture of nauplii, and loss of nauplii caused by the flow-through of seawater, meant that food remained present in aquaria for ~2 h. Light intensities of 0 and 200 μmol photons $m^{-2}$ $s^{-1}$ were chosen because the greatest difference in photosynthesis curve parameters has been observed for colonies acclimated to light levels over this range (Anthony and Hoegh-Guldberg, 2003; Rodolfo-Metalpa et al., 2008b). Therefore, monitoring photophysiology at these light levels provides a good indication of the overall scope for photoacclimation. Light intensities within aquaria were set to the required level, either using a black cupboard or using metal halide lamps and neutral-density shade screens, and were measured using a LI-COR data logger (LI-1000) with a spherical quantum sensor. Temperature was monitored daily and was maintained at 18±1.5°C using thermostat-regulated aquarium heaters (Visy-Therm, 300 W). For each of the large nubbins, photosynthesis-irradiance curves and feeding rates were measured weekly as indicators of autotrophic and heterotrophic capacity, respectively. For these colonies, calcification was also measured as a proxy for colony growth. Finally, six small nubbins were sampled each week from each tank (12 replicates per treatment), frozen at –20°C and later used for determination of protein and lipid content, as well as symbiont density.

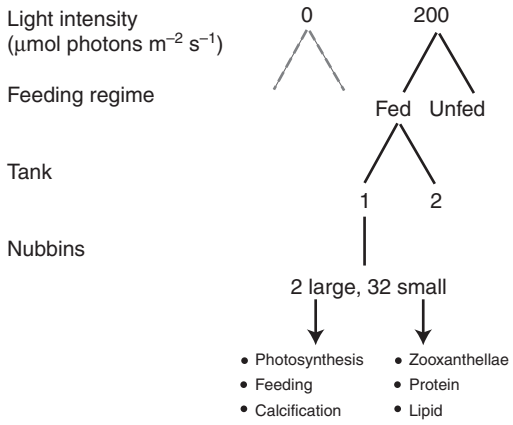


Fig. 1. Experimental design – colonies of *Cladocora caespitosa* were divided between two light and feeding treatments and maintained over a period of 8 weeks.

### 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 (Strathkelvin 928 oxygen meter and computer interface). Electrodes were calibrated at the incubation temperature 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 incubations and the chambers were stirred 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, light intensity was increased incrementally in eight steps up to a maximum of 450 μmol photons $m^{-2}$ $s^{-1}$ and photosynthesis rates were measured during a 15 min incubation at each light intensity. Photosynthesis–irradiance data were then used to quantify the value of the parameters $P_{max}$, $E_K$ and $R_D$ for each colony at each sampling time.

### Measurement of feeding rates

Grazing rates were measured by incubating colonies in seawater to which a fixed density of *Artemia salina* nauplii was added, and measuring the decrease in nauplii abundance over time. Feeding chambers had a volume of 0.9 l and were equipped with small motors that generated a unidirectional current of 3 cm $s^{-1}$. This flow rate was sufficient to recirculate the nauplii without causing the colonies to retract their tentacles. Controls containing prey but no coral were implemented to account for natural mortality of nauplii. Prior to each feeding trial, colonies were placed in the chambers and allowed to acclimate for 30 min or until their tentacles had expanded. Nauplii were then added to bring the total concentration of nauplii within the chamber to 100 $l^{-1}$. An initial 50 ml sample was taken from each chamber and the abundance of nauplii determined using a counting chamber. Subsequent 50 ml samples were counted every 15 min during each incubation (45 min) and grazing rate determined by regressing nauplii abundance against incubation time.

### Calcification

Calcification rates were measured using the total alkalinity (TA) technique (Smith and Kinsey, 1978). For these assays, colonies were incubated in glass beakers containing 100 ml of 0.45 μm filtered seawater for a period of 4 h. Beakers were covered with clear film, mixed using a magnetic stirring bar and incubations were performed in a temperature-regulated water bath (18°C) under the same irradiance at which colonies were grown (0 or 200 μmol photons $m^{-2}$ $s^{-1}$). At the end of the incubations the incubation water was poured into 50 ml plastic tubes and refrigerated in darkness prior to analysis of total alkalinity (TA). Seawater samples were taken prior to each of the incubations and the difference in TA between

these 'control' and the coral-incubation samples was used to estimate calcification rates (Tentori and Allemand, 2006). 'Blank' incubations (i.e. seawater incubated without a coral colony) were also conducted, analysed for TA, and the resulting rates subtracted from the measurements for each colony.

### Tissue properties

Protein biomass was estimated using a bicinchoninic acid protein assay (Uptima, Interchim, Montluçon, Allier, France). For each sample, protein was extracted by incubation in a sodium hydroxide solution (1 N) maintained in a water-bath for 30 min at 90°C. Subsequently, samples were transferred into 96-well microplates, and incubated with a dye reagent (Uptima Reagents, Interchim) for 30 min at 60°C. Protein standards across a concentration range from 0 to 2000 μg ml$^{-1}$ were also prepared using bovine serum albumin (BSA, Interchim), transferred to microplates and incubated as above. Samples and standards were homogenized for 30 s at 400 r.p.m. on a microplate shaker. Absorbance was measured at 560 nm, and sample protein content determined using the GENESIS program (Kontron Instruments, Bletchley, Buckinghamshire, UK) with reference to the standards.

Lipid content was quantified using a modification of the method developed by Bligh and Dyer (Bligh and Dyer, 1959). Frozen nubbins were ground into a fine powder using a mortar and pestle and mixed with a solution of dichloromethane, methanol and distilled water (3.0 ml, 1.5 ml and 1.2 ml, respectively). Samples were sonicated for 10 min, incubated at 40°C for 1 h and subsequently filtered through Whatman GF/C filters to remove skeleton fragments from solution. Subsequently, 1.5 ml of both dichloromethane and methanol were added to the filtrate and the solution centrifuged at 2000 r.p.m. for 10 min to separate the phases. The upper (methanol and water) phase was removed and the lower (lipid containing) layer was transferred into cleaned, pre-combusted and pre-weighed glass vials (4 ml). The solution was evaporated under nitrogen, and the remaining amount of lipid determined by weight.

Zooxanthellae abundance was determined by removing coral tissue using an airpick, and homogenising the tissue in 5 ml of 0.45 μm filtered seawater using a Potter tissue grinder. Symbiont densities in samples of volume 100–300 μl of this tissue slurry were determined using Histolab 5.2.3 image analysis software (Microvision, Every, France). Finally, the surface area of all nubbins was calculated using the method described by Rodolfo-Metalpa et al. (Rodolfo-Metalpa et al., 2006) and all measurements were normalised to surface area.

### Data analysis

#### Feeding and photoacclimation kinetics

The parameters of the photosynthesis-irradiance (PE) relationship for each sampled colony, at each sampling time, were determined by fitting Eqn 1 to photosynthesis data. These non-linear regressions were performed using the 'nls' routine in the statistics platform R (R Development Core Team, 2008). In all cases, $R^2$ values for the fitted curves were greater than 0.9. Subsequently, photoacclimation kinetics were quantified by fitting Eqn 2 to the data describing the variation in each photosynthesis parameter over time (i.e. the $P_{max}$, $E_K$ and $R_D$ estimates from the fitted PE curves) again using the 'nls' routine. Similarly, adjustment of heterotrophic feeding capacity over time was quantified by fitting Eqn 4 to measurements of feeding rate for corals from each treatment at each time point.

These analyses parameterise the rates of, and scope for, adjustment of autotrophic and heterotrophic capacity over time for colonies from the different light treatments. To determine whether food availability during the experiment had a strong effect on these adjustments, we used Akaike weights (*wAIC*). This information theoretic approach determines the support for different candidate models taking into account the number of parameters (Burnham and Anderson, 2002). Overall, *wAIC* represents the probability that a model is the best among the set of models under consideration; more precisely, it is an estimate of the probability that the model would be chosen as the best model again, if the study were repeated. For these analyses, we compared two 'candidate models' being: (1) that the acclimation response within each light treatment was the same for fed and unfed colonies; and (2) that the acclimation response within each light treatment was different for fed compared with unfed colonies. The more complex model (model 2) has twice the number of parameters of the simpler model because the data are divided into two treatments and two regressions are fitted. When errors are normally distributed, *wAIC* values can be calculated from the reduction in residual sums of squares (i.e. deviations between the model and the data) that is obtained by including additional parameters into the model (Burnham and Anderson, 2002).

#### Autotrophy *versus* heterotrophy: daily carbon acquisition

Correlation analyses (Pearson's $R$) were used to investigate whether autotrophic capacity depends on heterotrophic capacity. These analyses determine whether, during the experimental period, the observed shifts in the capacity for autotrophic and heterotrophic carbon acquisition are related to, or independent of, each other. In other words, these correlations compare total daily (potential) autotrophy and heterotrophy, given our quantification of photosynthetic and feeding behaviour, and under a simulated exposure of all colonies to the same light and feeding regime. Total daily carbon acquisition through autotrophy was calculated using the fitted photosynthesis versus irradiance parameters. To do this, we assumed a sinusoidal daily irradiance cycle with a maximum irradiance of 475 μmol photons m$^{-2}$ s$^{-1}$ and a 12 h day length divided into time intervals of 0.1 h. The irradiance mentioned above was selected because it corresponds to approximately the same total daily irradiance level as a constant light intensity of 200 μmol photons m$^{-2}$ s$^{-1}$ (supplementary material Fig. S1). Based on the sinusoidal irradiance curve, we calculated rates of photosynthesis over the diurnal cycle and approximated potential daily (integrated) carbon acquisition, $P_{day}$, by summing up photosynthesis over the daylight period, $P_N$, and subtracting respiration in the dark, $R_{day}$, calculated overnight. Oxygen fluxes were converted to carbon equivalents based on molar weights, as $P_N$=mol $O_2$ produced × 12/PQ and $R_{day}$=mol $O_2$ consumed × 12 × RQ (Anthony and Fabricius, 2000), where PQ and RQ are photosynthetic and respiratory quotients (1.1 and 0.8, respectively) (Muscatine et al., 1981). Potential carbon acquisition from feeding, $H_{day}$, was calculated based on the measured feeding rates, assuming that colonies feed actively for 3 h per day and acquire 0.15 μg C prey$^{-1}$ (Ribes et al., 1998). Finally, we calculated the proportional contribution that heterotrophy makes to meeting daily maintenance costs of the experimental colonies, or CHAR (Grottoli et al., 2006). CHAR measures the percentage of daily maintenance metabolism that can be met by feeding.

#### Calcification and tissue properties

We did not have an *a priori* expectation of how calcification rates, lipid content and protein biomass would vary over time for colonies in the experimental treatments. Therefore, as a first approximation, we used linear regression to characterise variation in these data. Regressions were implemented in R ('lm' routine) (R Development

Core Team, 2008) and *wAIC* values were again calculated for the simpler (no feeding effects) and more complex models (different responses of fed and unfed corals).

## RESULTS

Photosynthetic and feeding capacities varied strongly in response to changing light and feeding regimes (Fig. 2). Corals transferred into darkness showed a consistent decline in both the maximum rate of photosynthesis ($P_{max}$, Fig. 2A) and the sub-saturation irradiance ($E_K$, Fig. 2B). Although values of these parameters had approximately halved by the end of the experiment (Table 1), both reached a steady state, suggesting that a stable and photosynthetically viable symbiosis can persist even after prolonged periods of darkness. Corals transferred into higher light intensity (high light) showed a general increase in $P_{max}$ and $E_K$ (Fig. 2D,E), although the response was more variable than that observed for colonies incubated in darkness. Food provision during the photoacclimation period did not have a strong effect on the capacity of colonies to adjust their photophysiology (Fig. 2, Table 1). The data did not indicate a different photoacclimation response for fed compared with unfed corals, with *wAIC* values for the more simple models above 60% in all cases (supplementary material Table S1). There was some evidence that photoacclimation to high light occurred more rapidly than did photoacclimation to darkness (Table 1). However, high variability in the response of the high light corals lead to high uncertainty about the value of the rate parameter ε (Table 1). For the corals in dark conditions, rates of photoacclimation of $P_{max}$ were not different from rates of photoacclimation of $E_K$ (Table 1). Finally, rates of respiration ($R_D$) were relatively consistent over time, and between treatments, taking an average value of 0.7 μmol $O_2\,cm^{-2}\,h^{-1}$ (~157 μg C $cm^{-2}\,d^{-1}$ total daily respiration, $R_{day}$). Although respiration generally decreased for the corals kept in the dark, and increased for the high light corals, these trends were not statistically significant (data not shown).

During the first 4 weeks of the experiment, rates of feeding increased over time for colonies from all treatments (Fig. 2C,F). The magnitude of this increase ranged between twofold for colonies incubated in darkness [initial feeding rate 179±37 (s.e.m.) compared with a final rate of 338±46 *Artemia* $cm^{-2}\,h^{-1}$] and approximately fivefold for unfed colonies incubated under high light conditions [initial feeding rate 138±29 (s.e.m.) compared with a final rate of 773±59 *Artemia* $cm^{-2}\,h^{-1}$]. In general, the corals incubated in darkness adjusted their feeding capacity more rapidly than those kept under high light ($\varepsilon_H$, Table 1). Contrary to our expectations, the corals in the high light treatment upregulated their feeding capacity to a higher level than those grown in darkness [see values of *y(S)*, Table 1]. In addition, the extent to which the high light corals increased their feeding rates was strongly dependent upon food

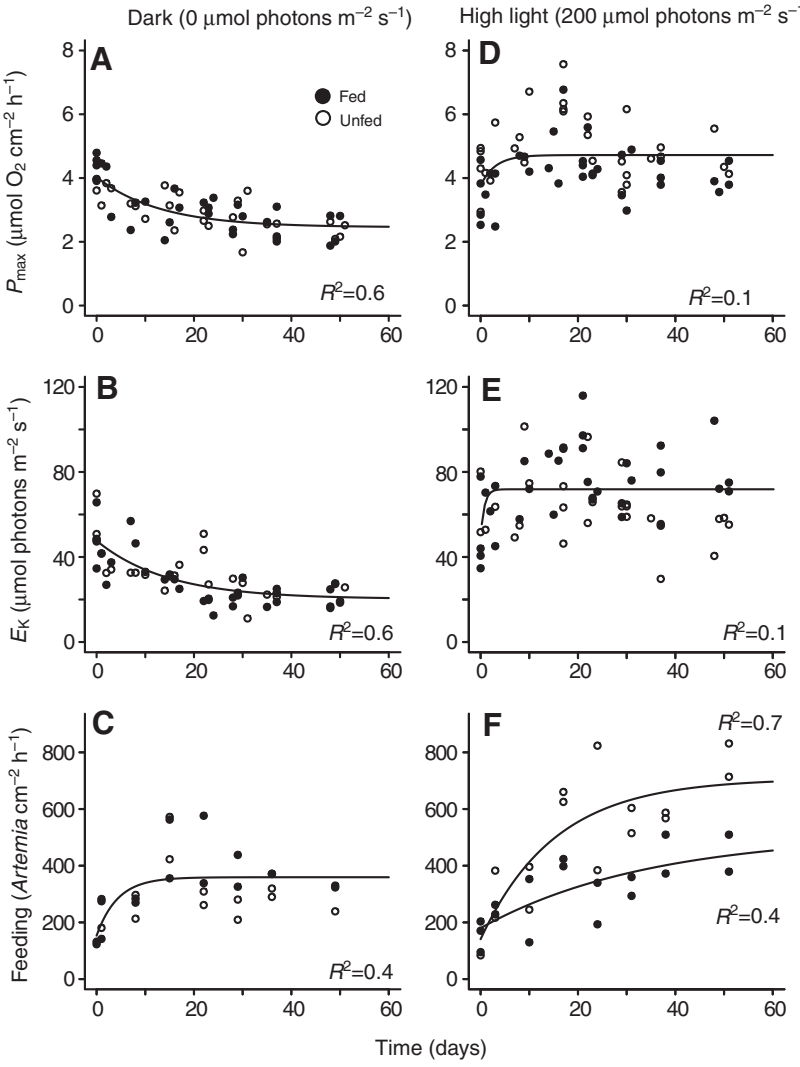


Fig. 2. Variation in photosynthetic and feeding capacity for *Cladocora caespitosa* incubated in darkness (A–C) and high light (D–F). Variation in maximum rate of photosynthesis (A,D), subsaturation irradiance (B,E) and grazing rate (C,F) are shown. Filled circles represent colonies that were supplied with food during the experiment and open circles are for starved colonies. Parameters for the fitted curves are given in Table 1.

Table 1. Parameter estimates of models characterising variation in carbon acquisition over time

| Parameter | Treatment | Equation | Initial $Y_0$ or $H_0$ | Steady-state $Y_S$ or $H_S$ | Rate ε or $\varepsilon_H$ |
|---|---|---|---|---|---|
| $P_{max}$ | Dark | 2 | 4.03 (0.16) | 2.46 (0.18) | 0.08 (0.03) |
| | Light | 2 | 3.77 (0.33) | 4.72 (0.15) | 0.33 (0.40) |
| $E_K$ | Dark | 2 | 48 (2.6) | 20 (3.4) | 0.07 (0.03) |
| | Light | 2 | 54 (7.2) | 71 (2.6) | 1.3 (2.6) |
| $H$ | Dark | 5 | 153 (54) | 359 (24) | 0.23 (0.21) |
| | Light, fed | 5 | 179 (43) | 512 (238) | 0.03 (0.04) |
| | Light, unfed | 5 | 141 (103) | 708 (110) | 0.07 (0.04) |

Standard errors are in parentheses. $P_{max}$ is maximum rate of photosynthesis (μmol $O_2$ $cm^{-2}$ $h^{-1}$), $E_K$ subsaturation irradiance (μmol photons$^{-2}$ $s^{-1}$) and $H$ is feeding rate (*Artemia* $cm^{-2}$ $h^{-1}$). 'Treatment' refers to fed or unfed colonies incubated in light or darkness. $Y_0$ and $H_0$ are the initial parameter values, $Y_S$ and $H_S$ are the steady-state ('acclimated') parameter values, and ε and $\varepsilon_H$ are the rates at which the parameters approach the steady-state value. All parameters were estimated by fitting Eqn 2 or Eqn 5 to data shown in Fig. 3, standard errors are given in parentheses. Data for fed versus unfed colonies were analysed separately when the model selection procedure indicated this was appropriate (supplementary material Table S1).

availability during acclimation: the model that allowed for different feeding–acclimation responses depending upon food provision received 84% support (supplementary material Table S1). At the experimental feeding density of 1000 *Artemia* $l^{-1}$, the measured adjustments in feeding activity resulted in rates of heterotrophy that were sufficient to meet daily metabolic costs. For the corals incubated in darkness, CHAR (at the stated prey density) increased from an initial value of 37±5% (s.e.m.) to a final value of 100±16% and 104±23% for the fed and unfed colonies, respectively. This increase was even more marked for the corals grown under high light: for these colonies potential CHAR increased to 160±16% (s.e.m.) for the unfed colonies and 100±6% for the fed colonies. Overall, although feeding rates increased over time for all colonies within all treatments (initial c.f. steady-state values in Table 2), the greatest increase was observed for the unfed colonies grown under high light conditions.

The relationship between the capacities for carbon acquisition through autotrophy compared with heterotrophy differed between the two experimental light levels (Fig. 3). For the colonies maintained in darkness, autotrophy was negatively correlated with heterotrophy (Pearson's $R$=–0.43, $P$<0.01). That is, when the capacity for photosynthesis was low, the capacity to capture food, when available, was high (Fig. 3A). Data were pooled for this correlation analysis because food availability during the acclimation experiment did not affect either photosynthesis parameters or feeding rates (Fig. 2). For the corals acclimated to high light, there was considerable within-colony variation in daily photosynthesis and heterotrophy (190–500 μg C $cm^{-2}$ $d^{-1}$ for photosynthesis and 50–350 μg C $cm^{-2}$ $d^{-1}$ for heterotrophy, Fig. 3B). For this analysis, the relationship between autotrophy versus heterotrophy was analysed separately for the fed compared with unfed colonies because, under high light conditions, the feeding capacity of these two treatments varied differently over time (Fig. 2F). In both cases, carbon acquisition through photosynthesis was not significantly correlated with carbon acquisition through feeding (Pearson's $R$=–0.04, $P$=0.84 for fed colonies and Pearson's $R$=0.23, $P$=0.21 for unfed colonies). Overall, although heterotrophy appeared to compensate for reduced photosynthesis for the corals maintained in darkness, the same trend was not observed for the colonies grown under high light levels.

Light treatment and feeding regime both influenced tissue composition, but different dynamics were observed for protein compared with lipid content (Fig. 4). For the corals incubated in darkness, protein content remained approximately constant over the experimental period but lipid content decreased (Table 2) at a rate that was independent of feeding regime (the simpler model received 86% support, supplementary material Table S1). Neither time during the experiment nor food availability had a significant effect on lipid levels for the high light corals (Fig. 4D, Table 2). Conversely, protein content increased significantly for the high light corals, but only for colonies that were fed during the course of the experiment (Table 2). In summary, colonies maintained in darkness appeared to use their lipid stores to supplement their daily carbon requirements and tissue biomass only increased over time when colonies received both light and food.

Symbiont densities decreased over time for the colonies maintained in darkness, declining to less than half the initial value by the end of the experimental period (Fig. 4C). There was no evidence that food availability during dark-acclimation influenced symbiont densities (supplementary material Table S1). Congruent with the high variation in the values of the photosynthesis-irradiance parameters, we observed a threefold range of variation in symbiont

Table 2. Regression analyses of variation in tissue composition and calcification rate during incubation of fed and unfed colonies of *Cladocora caespitosa* at two light intensities

| Parameter | Treatment | Intercept | Slope | $P$ (slope) |
|---|---|---|---|---|
| Lipid content (mg $cm^{-2}$) | **Dark** | **3.2 (0.14)** | **–0.02 (0.01)** | **<0.001** |
| | Light | 3.5 (0.21) | –0.004 (0.008) | 0.6 |
| Protein content (mg $cm^{-2}$) | Dark | 1.7 (0.07) | –0.001 (0.002) | 0.6 |
| | **Light, fed** | **1.7 (0.09)** | **0.01 (0.003)** | **<0.01** |
| | Light, unfed | 1.8 (0.08) | –0.004 (0.003) | 0.1 |
| Symbiont density ($10^6$ cells $cm^{-2}$) | **Dark** | **3.1 (0.17)** | **–0.04 (0.01)** | **<0.001** |
| | Light | 3.5 (0.25) | –0.0008 (0.0009) | 0.4 |
| Calcification (nmol $cm^{-2}$ $h^{-1}$) | Dark, fed | 171 (22) | –0.5 (0.7) | 0.5 |
| | **Dark, unfed** | **169 (17)** | **–2.7 (0.6)** | **<0.001** |
| | Light | 257 (18) | 0.9 (0.7) | 0.2 |

Standard errors of regression coefficients are given in parentheses and statistically significant results are in bold. Data are presented in Figs 4 and 5. Data for fed *versus* unfed colonies were analysed separately when the model selection procedure indicated this was appropriate (supplementary material Table S1).

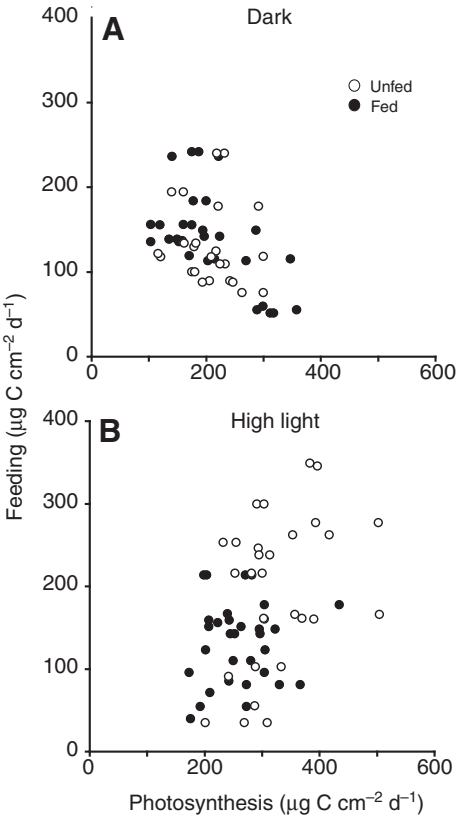


Fig. 3. Relationship between carbon acquisition through photosynthesis (*x*-axis) and feeding (*y*-axis) for colonies of *Cladocora caespitosa* incubated in darkness (top panel) or high light (bottom panel). Points depict carbon acquisition through each mode calculated for experimental colonies based on (1) measured $P_{max}$, $E_K$ and $R_D$ for experimental colonies using a 10 h sinusoidal daylight cycle with a maximum of 475 μmol photons $m^{-2} s^{-1}$ and (2), assuming colonies feed at the measured rates for 3 h each day and obtain 0.15 μg C $prey^{-1}$ (Ribes et al., 1998). Filled and open circles are for fed and unfed colonies, respectively.

density for the corals incubated under high light. Nevertheless, for these corals, symbiont numbers were not affected by time during the experiment or feeding regime (Fig. 4F, Table 2).

For the corals incubated in darkness, calcification rates decreased over the course of the experiment but only when food was not provided (Fig. 5A, Table 2). When food was available, colonies incubated in darkness maintained an approximately consistent calcification rate throughout the course of the experiment [initial value of 161±14 nmol $cm^{-2} h^{-1}$ (s.e.m.) compared with a final value of 157±43 nmol $cm^{-2} h^{-1}$] compared with an approximately fourfold decrease for the unfed colonies (final value of 42±4 nmol $cm^{-2} h^{-1}$). For this analysis, there was strong support for differences in calcification dynamics over time for fed compared with unfed colonies: the *wAIC* value for the more complex model was 84% (supplementary material Table S1). In the high light treatment, there was a trend of increasing calcification over time (Fig. 5B), but this effect was not statistically significant (Table 2). In contrast to the results for the colonies incubated in darkness, food availability during the experiment did not strongly influence how calcification rates changed over time for the high light treatment (the simpler model received 76% support, supplementary material Table S1).

## DISCUSSION

By simultaneously quantifying co-variation in photosynthesis, heterotrophic feeding, calcification and tissue composition, this study allows a deeper understanding of the dynamics of nutritional symbioses. Specifically, our results demonstrated that, in terms of daily carbon acquisition, the degree of flexibility in heterotrophic feeding for *Cladocora caespitosa* was equivalent to the capacity for photoacclimation. The nutritional function of heterotrophy depended upon the light intensity under which colonies were grown. For corals grown in darkness, heterotrophy functioned as an alternative source of carbon (feeding rates increased to compensate for the lack of photosynthesis). However, despite a prolonged incubation in darkness under an entirely heterotrophic mode of feeding, colonies maintained a viable photosynthetic symbiosis throughout the experimental period. That is, they maintained a significant population of symbionts that retained their photosynthetic function. For colonies grown at high light intensities, heterotrophy functioned as an important source of nutrients: maximum feeding effort was observed in colonies kept under high light without a regular source of food, and tissue growth occurred only at high light when food was provided. Finally, when both heterotrophic and photosynthetic carbon acquisition were restricted, colonies used their lipid stores to meet metabolic maintenance costs, while protein levels remained approximately constant.

### Nutritional flexibility: photosynthetic versus heterotrophic carbon acquisition

For colonies of *Cladocora caespitosa*, a shift in the nutritional function of heterotrophy was apparent between different light treatments. Previous studies on tropical corals have suggested two contrasting roles for heterotrophy: that it primarily contributes nutrients to the symbiosis (Johannes et al., 1970; Davies, 1984) or that it can act as a compensatory mode of carbon acquisition (Anthony and Fabricius, 2000; Grottoli et al., 2006; Palardy et al., 2008). For *Cladocora caespitosa*, increases in feeding capacity during prolonged exposure to darkness were correlated with decreases in photosynthetic capacity. This result is congruent with observations that corals rely more strongly on heterotrophic carbon as depth increases, photosynthesis is restricted and light becomes limiting (Muscatine et al., 1989; Palardy et al., 2008). That heterotrophy can indeed compensate for reduced photosynthesis was supported by our result that provision of food to colonies kept in darkness was sufficient to maintain calcification rates at approximately the same level throughout the experimental period. Although calcification is generally thought to be fuelled by photosynthesis (Barnes and Chalker, 1990), our findings for the corals kept in the dark are consistent with other work showing feeding enhancement of calcification rate in tropical (Houlbrèque et al., 2003) and temperate corals (Miller, 1995; Rodolfo-Metalpa et al., 2008c). Although this trend was not observed for the high light corals (see 'Effects of food availability on colony energetics' below), these experimental results indicate that a shift to using heterotrophic carbon to fuel calcification in low-light/dark habitats underlies field observations that colony growth rates are not correlated with depth–light intensity (Peirano et al., 1999; Rodolfo-Metalpa et al., 2008a).

In contrast to the compensatory role of heterotrophy for carbon acquisition in the dark, in high light conditions, food mainly provides a source of nitrogen and phosphorus, which enables corals to increase their tissue growth. The concept that multiple factors limit coral growth is not new (Muscatine and Porter, 1977). Indeed, it is generally thought that carbon is limiting under low light, whereas

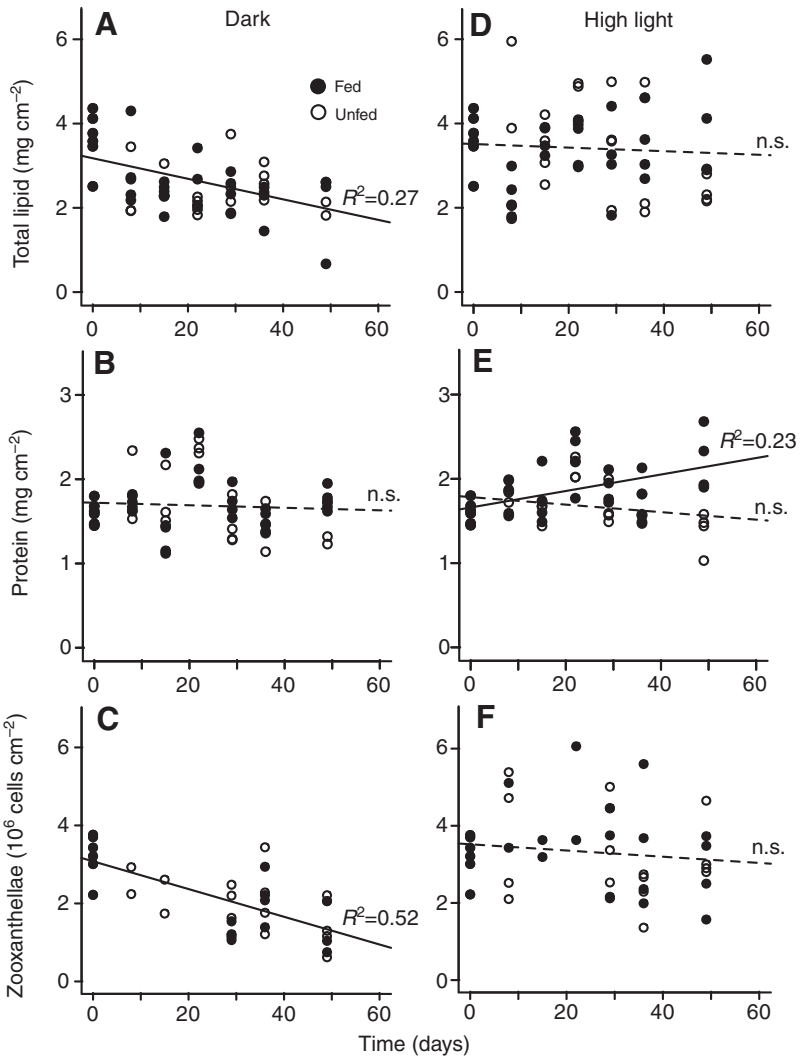


Fig. 4. Variation in tissue quality for colonies of *Cladocora caespitosa* incubated in darkness (A–C) or high light (D–F). Panels describe variation in lipid content (A,D), protein content (B,E) and zooxanthellae density (C,F). Filled circles represent colonies that were supplied with food during the experiment, whereas open circles represent starved colonies. Parameters of the regression lines are given in Table 2, and regressions were fit through all data points.

nitrogen and/or phosphorus availability limits carbon assimilation into biomass in high light (Falkowski et al., 1984; Dubinsky and Jokiel, 1994; Stambler, 1998). Several of our findings support this conclusion. First, the increase in feeding capacity during acclimation to high light demonstrates that, when light is not limiting, heterotrophic feeding contributes nutrients other than carbon to the symbiosis. Second, colonies were only able to increase their tissue biomass when both light and food were provided, indicating that nutrients acquired through heterotrophy drive the conversion of carbon to tissue. In other words, colonies of *Cladocora caespitosa* can grow new biomass only when both light (for energy) and food (for nutrients) are available.

Consistent with the general perception that temperate corals are more heterotrophic than tropical corals (FitzGerald and Szmant, 1988), this study indicates that, at the prey abundances used in our feeding trials, *Cladocora caespitosa* is potentially able to meet its carbon requirements by feeding for less than 2 h per day. For logistical reasons, our feeding trials required high prey concentrations. However, natural zooplankton abundances in the Mediterranean are ~12-fold lower than the experimental levels, rarely exceeding 80 individuals l$^{-1}$ (Calbet et al., 2001). Consequently, it is not possible to directly extrapolate our findings to predict heterotrophic carbon acquisition under field conditions. Nevertheless, the fact that colonies retain the capacity to capture large numbers of prey when available provides strong evidence about the importance of heterotrophy for this species. Furthermore, our results demonstrate that *C. caespitosa* is able to adjust its feeding effort at approximately the same rate at which variations in photophysiology occur. At present there are no comparable data on rates of adjustment of heterotrophic capacity in other coral species. Nevertheless, the ability to rapidly alter feeding effort in response to ambient light and food availability may explain the wide depth distribution of this species (Schiller, 1993; Peirano et al., 2005).

The photoacclimatory changes observed here for *Cladocora caespitosa* are generally consistent with those observed for other symbiotic corals and anemones: the maximum rate of photosynthesis and subsaturation irradiance increased during acclimation to high light and decreased during acclimation to darkness (Chalker et al., 1983; Harland and Davies, 1994; Anthony and Hoegh-Guldberg, 2003). Moreover, the rates at which photoacclimation occurred were of a similar magnitude to those reported for the tropical coral *Turbinaria mesenterina* (Anthony and Hoegh-Guldberg, 2003). However, compared with other corals, *C. caespitosa* had a limited capacity to adjust to high light intensities. This was indicated by the small, although rapid, changes in photophysiology during high light acclimation following a shift in light intensity of 50–200 μmol photons m$^{-2}$ s$^{-1}$. Overall, these results are in general agreement with a previous study on this species that showed smaller differences in

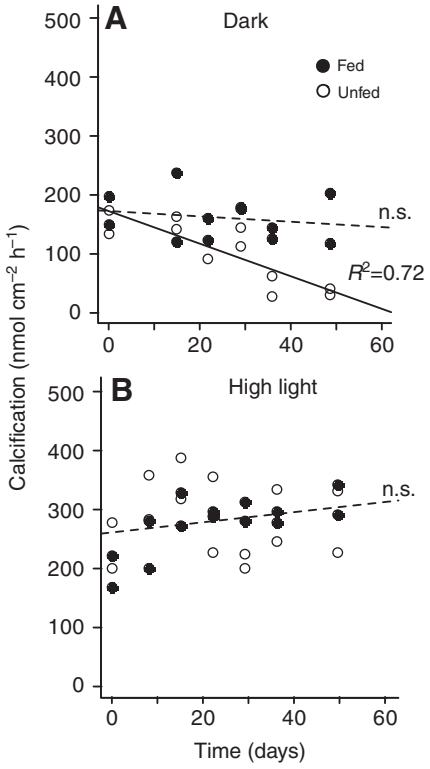


Fig. 5. Variation in calcification rates for colonies of *Cladocora caespitosa* incubated in darkness (A) or high light (B). Filled points represent colonies that were supplied with food during the experiment, whereas unfilled circles represent starved colonies. Parameters of the regression lines are given in Table 2.

photophysiology between 80 and 250 µmol photons m$^{-2}$ s$^{-1}$ than between 30 and 80 µmol photons m$^{-2}$ s$^{-1}$ (Rodolfo-Metalpa et al., 2008b). These findings indicate that *C. caespitosa* has a greater capacity to acclimatise to low-light than to high light conditions, in accordance with the dark turbid environments in which this species is naturally abundant. In such habitats, the measured sub-saturation irradiance values ($E_K$) demonstrate that the symbiosis receives enough light for saturated photosynthesis during most of the day (Schiller, 1993) in summer as well in winter (Peirano, 2007). Collectively, these studies suggest that, despite strong seasonal fluctuations of light intensities in temperate environments, temperate corals have a limited capacity for photoacclimation to high light levels compared with tropical corals (Chalker et al., 1983; Mass et al., 2007; Frade et al., 2008).

### Tissue composition and symbiont dynamics during starvation

Contrary to the limited high light acclimation observed for *Cladocora caespitosa*, this study shows that symbiosis with an intact capacity for photosynthesis can persist during prolonged periods of darkness. Regression analyses of the change in symbiont density over time (Table 2) indicated that zooxanthellae density would reach zero after ~80 days in darkness. Although the present experiment was terminated after 50 days, a similar experiment performed in our laboratory showed continuation of symbiosis in darkness for up to 90 days (R. R.-M., unpublished). Indeed, there have been several observations of the continuance of a suppressed but viable symbiont population in corals incubated at very low light intensities. In another temperate coral species, symbiosis with an intact capacity for calcification persisted up to 48 days in darkness (Kevin and Hudson, 1979). Similar results were also observed for four tropical coral species for which symbionts remained present after 120 days of incubation at light intensities less than 1% of surface irradiances (Titlyanov et al., 2002). These findings suggest that persistence in very low light conditions is a general phenomenon of coral–zooxanthellae symbioses.

The ability of the coral–zooxanthellae symbiosis to survive in the absence of photosynthetic carbon acquisition raises the issue of how both corals and symbionts cope with starvation. Conceptually, there are several possible strategies that could be employed. First, both the coral host and the symbionts may use their lipid stores to meet metabolic costs (Anthony et al., 2009). Although we did observe a decrease in lipid content for corals incubated in darkness, 71 µg lipid cm$^{-2}$ d$^{-1}$ would need to be catabolised to meet the measured respiratory demands of 157 µg C cm$^{-2}$ d$^{-1}$, based on a lipid-to-carbon conversion of 0.45 µg lipid µg C$^{-1}$ (Anthony et al., 2009). Therefore, at the observed rate of 20 µg lipid cm$^{-2}$ d$^{-1}$ (0.02 mg lipid cm$^{-2}$ d$^{-1}$, Table 2), lipid catabolism was insufficient to meet maintenance costs. Second, coral colonies take-up dissolved organic carbon (DOC) and microplankton present in the seawater (Ferrier, 1991; Houlbrèque et al., 2004). *C. caespitosa* might also rely on bacteria as source of carbon. Herndl and Velimirov (Herndl and Velimirov, 1985) found a large bacterial population within the coelenteron of four temperate corals, including *C. caespitosa*, suggesting that they are able to farm and feed on bacteria. Later, Schiller and Herndl (Schiller and Herndl, 1989) also measured a high microbial biomass in the interstitial space formed between individual polyps. Although estimates of DOC and bacteria uptake are not available for *C. caespitosa*, this source has been shown to contribute up to 6% of daily photosynthetic carbon fixation in other coral species (Houlbrèque et al., 2004). Therefore, consumption of farmed bacteria, and increased uptake of dissolved and micro-particulate carbon available in seawater, potentially made a large contribution to the energy budget of colonies grown in darkness.

### Effects of food availability on colony energetics

Collectively, the results of this study indicate that feeding plays a strong role in the energy budget of *Cladocora caespitosa*: colonies can persist and calcify in the absence of photosynthetic carbon, and feeding rates remain high even when light, and photosynthesis, are high. Nevertheless, food provision during the experiment did not affect photosynthesis-irradiance relationships or symbiont densities under any treatment, or calcification rates for corals grown under high light. These findings are consistent with some previous work on temperate and tropical corals: food availability did not affect symbiont density or light-calcification rates in the temperate coral *Plesiastrea urvillei* (Kevin and Hudson, 1979). Similarly, supply of particulate organic matter did not strongly affect the photosynthesis–irradiance response of two tropical coral species during a 3-month experiment (Anthony and Fabricius, 2000). However, other studies indicate that food availability enhances both tissue lipid and protein content (Anthony and Fabricius, 2000; Ferrier-Pagès et al., 2003; Houlbrèque et al., 2003) and the capacity for photoacclimation (Titlyanov et al., 2001). Previous research has also shown that food availability increases calcification rates for colonies of *C. caespitosa* exposed to low and intermediate light levels (50 and 120 µmol photons m$^{-2}$ s$^{-1}$) (Rodolfo-Metalpa et al., 2008c). At present, it is not clear why, as observed in this study, nutrition from feeding promotes skeletal growth in dark habitats versus tissue growth in high light habitats. A likely explanation is that an abundance of photosynthetically acquired carbon removes the need for high light

corals to use heterotrophic carbon for skeletal growth. Taken in concert with previous research, this work illuminates a light-driven shift in the fate of heterotrophically acquired carbon in coral symbioses. At low and intermediate light levels, nutrients acquired through heterotrophy are used for calcification but at high light levels energy from feeding is converted to biomass and potentially used for colony growth through polyp budding.

An alternative explanation for the observed feeding effects on symbiont and host properties is that food provision may not have been sufficient to influence photoacclimation or symbiont density. Our results indicated that the corals incubated in darkness needed to feed for ~3 h each day to obtain enough carbon for daily metabolic costs. However, under our experimental feeding regime, food was only available for 2 h twice a week. Over the course of each week, fed colonies were therefore able to acquire, at the most, ~20% of their total carbon requirements. This effectively low level of feeding may explain why feeding capacity increased over time for all of the experimental treatments, and why photosynthesis properties, feeding rates and tissue biomass did not differ between the dark fed and unfed treatments. Nevertheless, food provision did influence calcification under dark conditions, indicating that heterotrophic carbon was used by the coral host to maintain relatively high calcification rates. Although further research is required to clarify this observation, these findings suggest that maintaining growth of the coral skeleton takes priority over other potential energy allocation strategies.

Contrary to our expectations, respiration rates did not vary significantly over time for colonies of *Cladocora caespitosa* in any of the experimental treatments. The literature indicates general trends of decreasing rates of respiration for corals in low light (Chalker et al., 1983; Leletkin et al., 1996; Rodolfo-Metalpa et al., 2008b) and higher respiration for fed compared with unfed corals (Kevin and Hudson, 1979; Titlyanov et al., 2001; Borell et al., 2008). Nevertheless, there are many exceptions to these trends, with several studies also demonstrating a lack of sensitivity of coral respiration rate to light intensity (Stambler, 1998; Anthony and Fabricius, 2000), nutrient enrichment (Stambler, 1998) and/or food provisioning (Anthony and Fabricius, 2000; Borell et al., 2008). There is currently no clear explanation for such species- and experiment-specific effects on respiration rate. However, in the present study, as in many other physiology studies (Sears, 2005; Millidine et al., 2006), respiration was used as a proxy for metabolic rates. In fact, measurements of oxygen consumption represent maintenance metabolism plus metabolism associated with growth and reproduction (Kiorboe and Mohlenberg, 1987; McCauley et al., 1990). For organisms of equal size, basal metabolic costs can be expected to be constant per unit tissue mass because they represent the fundamental costs of maintaining tissue (McCauley et al., 1990). Indeed, in this study, there was no trend of variation in respiration within or between treatments when data were normalised to protein content. Therefore, we suggest that previously observed variation in coral respiration rates may reflect changes in tissue mass, colony growth rates (Leletkin et al., 1996) or a stress response, rather than changes in basal metabolic costs.

## CONCLUSIONS

Although nutritional symbioses are common in nature, there is little evidence quantifying how different feeding modes interact and are regulated, or how a shift in the mode of energy acquisition influences growth of the holobiont. For scleractinian corals, photosynthesis is generally regarded as the primary mode of carbon acquisition (Barnes and Chalker, 1990; Hoogenboom et al., 2006; Mass et al., 2007). Nevertheless, this study demonstrates that for a temperate scleractinian species, the scope for carbon acquisition through heterotrophy can be equal to that through photosynthesis, both in magnitude and in the rate at which physiological adjustments occur. This work also confirms that feeding mode is regulated according to a light-driven shift in the limiting factor for coral growth: when carbon is limiting, feeding compensates for decreased photosynthesis; but when photosynthesis is high, feeding effort is increased to supply nutrients for conversion of carbon into biomass. When colonies are found in very low-light habitats, carbon acquired through heterotrophic feeding is used for skeletal growth (calcification). However, if both heterotrophic carbon and photosynthetic carbon are restricted, colonies metabolise lipids, not proteins, in order to meet basic maintenance costs. Finally, for *Cladocora caespitosa,* a stable and photosynthetically viable symbiosis can persist for up to 90 days in darkness. The ability of this species to strongly upregulate its heterotrophic capacity, and to maintain an active symbiosis even under suboptimal conditions, may contribute to its wide depth distribution (Peirano et al., 1999; Peirano et al., 2005), and its ability to tolerate a broad range of environmental conditions (Rodofo-Metalpa et al., 2006; Rodofo-Metalpa et al., 2008b).

## LIST OF ABBREVIATIONS

| | |
|---|---|
| CHAR | contribution of heterotrophy to animal respiration |
| $E$ | light intensity |
| $E_K$ | sub-saturation irradiance |
| $H_{day}$ | daily carbon acquisition through heterotrophy |
| $H_S$ | steady state (acclimated) heterotrophic feeding rate |
| $H(t)$ | heterotrophic feeding rate as a function of time during acclimation |
| $H_0$ | initial heterotrophic feeding rate, prior to a change in conditions |
| $P_{day}$ | net carbon acquisition through photosynthesis (i.e. $P$–$R_{day}$) |
| PE | Photosynthesis *versus* irradiance relationship |
| $P_{max}$ | maximum (light-saturated) rate of photosynthesis |
| $P_N$ | net rate of photosynthesis integrated over a 12 h daylight period |
| PQ | photosynthetic quotient |
| $R_D$ | dark respiration |
| $R_{day}$ | dark respiration integrated over the night |
| RQ | respiratory quotient |
| $t$ | time |
| TA | total alkalinity |
| $wAIC$ | Akaike weight |
| $Y$ | parameter that changes according to first-order kinetics |
| $Y_0$ | initial value of $Y$, prior to a shift in conditions |
| $Y_S$ | steady-state value of $Y$, after acclimation to new conditions |
| $\varepsilon$ | rate at which the parameter $Y$ changes during acclimation |
| $\varepsilon_H$ | rate of heterotrophic feeding rate changes during acclimation |

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