

Response of a scleractinian coral, *Stylophora pistillata*, to iron and nitrate enrichment

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Received 4 August 2000; received in revised form 31 January 2001; accepted 12 March 2001

Abstract

The purpose of this study was to determine whether the addition of iron alone or in combination with nitrate affects growth and photosynthesis of the scleractinian coral, *Stylophora pistillata*, and its symbiotic dinoflagellates. For this purpose, we used three series of two tanks for a 3-week enrichment with iron (Fe), nitrate (N) and nitrate + iron (NFe). Two other tanks were kept as a control (C). Stock solutions of FeCl_3 and NaNO_3 were diluted to final concentrations of 6 nM Fe and 2 μM N and continuously pumped from batch tanks into the experimental tanks with a peristaltic pump. Results obtained showed that iron addition induced a significant increase in the areal density of zooxanthellae (ANOVA, $p = 0.0013$; change from $6.3 \pm 0.7 \times 10^5$ in the control to $8.5 \pm 0.6 \times 10^5$ with iron). Maximal gross photosynthetic rates normalized per surface area also significantly increased following iron enrichment (ANOVA, $p = 0.02$; change from 1.23 ± 0.08 for the control colonies to $1.81 \pm 0.24 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ for the iron-enriched colonies). There was, however, no significant difference in the photosynthesis normalized on a per cell basis. Nitrate enrichment alone (2 μM) did not significantly change the zooxanthellae density or the rates of photosynthesis. Nutrient addition (both iron and nitrogen) increased the cell-specific density of the algae (CSD) compared to the control (G -test, $p = 0.3 \times 10^{-9}$), with an increase in the number of doublets and triplets. CSD was equal to 1.70 ± 0.04 in the Fe-enriched colonies, 1.54 ± 0.12 in the N- and NFe-enriched colonies and 1.37 ± 0.02 in the control. Growth rates measured after 3 weeks in colonies enriched with Fe, N and NFe were 23%, 34% and 40% lower

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than those obtained in control colonies (ANOVA, $p = 0.011$). © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Corals; Iron; Nitrate; Calcification; Photosynthesis

1. Introduction

In recent years, it was realized that phytoplankton productivity in oceanic waters is limited by iron availability (Chisholm and Morel, 1991; Timmermans et al., 1998), especially in high-nutrient, low-chlorophyll areas (Coale et al., 1996). Iron is the most essential trace element for algal growth and nitrogen fixation (Falkowski et al., 1998). Twenty-four atoms of iron are indeed required for each molecule of nitrogenase (Eady and Smith, 1979). However, surface water concentrations are low in available iron (de Baar et al., 1995; Gordon et al., 1997), primarily because the major sources of iron for the oceans are from wind-blown terrestrially derived dust (Duce and Tindale, 1991). The latter decreases as one moves away from coastlines into oceanic environments.

Tropical and coral reef waters are considered as oligotrophic “blue deserts” because they contain very low concentrations of dissolved inorganic nutrients (Stambler, 1998). Since reefs are most often far away from terrestrial, fluvial and continental shelf sources, iron may be one limiting nutrient here as well. Studies on the effect of iron enrichment on corals and their associated zooxanthellae (dinoflagellates) are scarce, and rely mostly on toxicological experiments or on field studies where iron concentrations were particularly elevated (Harland and Brown, 1989; Brown et al., 1990, 1991). Only one study, to our knowledge, showed that zooxanthellae (*Symbiodinium microadriaticum*) could be iron limited in the Great Barrier Reef (Entsch et al., 1983). The iron status of other primary producers on coral reefs has also been scarcely investigated (Entsch et al., 1983; Dufour and Berland, 1999). The few results obtained are conflicting because iron requirements greatly differ among phytoplankton species (Sunda and Huntsman, 1995). Entsch et al. (1983) found, on the Great Barrier Reef, that *Phormidium* sp. (a cyanobacterium) was iron-limited, whereas Dufour and Berland (1999) measured no enhancement of picoplankton and nanoplankton growth following iron enrichment in some Tuamotu atolls. Therefore, there is a need to investigate the underlying sources of variability in the response of microalgae to iron exposure. This study was designed to investigate the response of the scleractinian coral, *Stylophora pistillata*, to a 6 nM iron enrichment. For this purpose, the growth and photosynthesis of the algal symbionts as well as the animal calcification were measured in the laboratory, under controlled conditions, in iron-enriched and iron-poor seawater.

We also tested whether the effect of iron can be increased by an addition of nitrate, since their effects are interdependent (Entsch et al., 1983). It has indeed been demonstrated that the growth of free-living phytoplankton may be limited by both nitrogen and iron (Coale et al., 1996). This dual limitation must also be tested in symbiotic algae. Nitrogen is also found in very low concentrations in tropical and reef waters and has long been considered to be one of the major factors limiting primary productivity (Crossland, 1983). Most experimental work on the effect of nitrogen enrichment on

zooxanthellae and corals has centred on nitrogen in its ammonium form. Ammonium enrichment often resulted in an increase in algal density, a decrease in the rate of photosynthesis per algal cell (Hoegh Guldberg and Smith, 1989; Dubinsky et al., 1990; Stimson and Kinzie, 1991) and a decrease in the rate of skeletogenesis (Stambler et al., 1991; Ferrier-Pagès et al., 2000). Very little information, however, exists on the effects of nitrate on coral physiology (Bythell 1990; Marubini and Davies, 1996; Marubini and Atkinson, 1999). Bythell (1990) showed that corals are able to use nitrate at natural environmental concentrations (up to 2 μM) for their nitrogen requirements for tissue growth and production of gametes. However, under certain conditions, nitrate enrichment can also induce an increase in the algal density and a decrease in the rates of calcification (Marubini and Davies, 1996; Marubini and Atkinson, 1999). More studies are, therefore, needed for a better understanding of the effects of the molecular source of nitrogen on coral physiology.

2. Material and methods

Experiments were carried out with nubbins (7 cm long) prepared from different parent colonies of *S. pistillata*. They originated from the surface waters of the Red Sea (Aqaba, Jordan) and were maintained in the aquarium of the Oceanographic Museum (Monaco) for at least 1 year. In culture conditions, corals were fed once a week with artemia and were maintained at an irradiance of 280 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (saturated photosynthesis). Wavelength was provided by metal halide lamps (Philips, HPIT 400 W). The aquaria were supplied with nonfiltered Mediterranean seawater (pumped at 50 m depth and heated to $25 \pm 0.5^\circ\text{C}$). This water had low nutrient ($< 0.4 \mu\text{M}$ ammonium, $< 1 \mu\text{M}$ nitrate, $< 0.2 \mu\text{M}$ phosphorus, $< 4 \text{ nM}$ iron) and chlorophyll concentrations ($0.2\text{--}0.3 \mu\text{g Chl } a \text{ l}^{-1}$). The growth of the nubbins was followed during 4 weeks before the start of the experiment and varied between $0.6 \pm 0.1\% \text{ day}^{-1}$. The amount of zooxanthellae was equal to $6 \pm 0.5 \times 10^5 \text{ cells cm}^{-2}$. At the beginning of the experiment, 24 nubbins were randomly transferred into eight tanks representing four different treatments (two tanks and six nubbins per treatment). Tanks were placed in a water bath fitted with a thermostat and aerated. Nubbins were maintained under the same temperature (25°C) and irradiance ($280 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 12:12 photoperiod) than in culture conditions and were preincubated 1 week in their new tanks. Six tanks (referred as Fe, N, NFe) were then enriched (in duplicate) during 3 weeks with 6 nM iron, 2 μM nitrate and both nutrients at the above concentrations. The two remaining tanks were kept as a control (C). Stock solutions of NaNO_3 and FeCl_3 contained in dark batch tanks were continuously pumped into the experimental tanks with a peristaltic pump. The seawater flow rate was ca. 2 l h^{-1} , with a renewal of 100% of the tanks each hour, in order to keep nutrient concentrations constant. Nitrate concentrations, therefore, varied between 2.2 and 2.9 μM in the batch tanks and between 2 and 2.5 μM in the enriched tanks. All tanks were cleaned every day to avoid algal proliferation.

The buoyant weight of the 24 nubbins was measured weekly using a Mettler AT 261 balance. The skeletal weight was calculated as follows (Jokiel et al., 1978):

$$M_{\text{air}} = M_{\text{water}} / \left[1 - (D_{\text{water}} / D_{\text{object}}) \right],$$

where M_{air} is the weight of the nubbin in air (g); M_{water} , the weight of the nubbin in water; D_{water} and D_{object} , the seawater and skeletal density (2.93 g cm^{-3}). Seawater density was calculated from temperature and salinity (UNESCO 1981).

Growth rates were then calculated as follows:

$$G = n\sqrt{M_f/M_o} - 1.$$

Photosynthesis and respiration were measured at the end of the incubation for the six nubbins incubated in each condition. Each nubbin was placed in a Perspex chamber containing a polarographic oxygen sensor (Ponselle instruments) and immersed in a thermostated water bath (25°C). The chamber was filled with Millipore ($0.45 \mu\text{m}$) filtered seawater, either nutrient-enriched or not. The incubation medium was continuously stirred with a magnetically coupled stirring bar. Nubbins were incubated 60 min under different irradiances (0, 20, 50, 100, 150, 200, 300, 400 500 and $600 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$), and the order in which the subsequent irradiances were studied was at random. Light was provided by a 400 W metal halide lamp (Philips, HPIT), attenuated to the desired intensity with neutral screens. All measurements were performed in the morning. The oxygen sensor was calibrated before each experiment against air-saturated seawater and a saturated solution of sodium dithionite (zero oxygen). Oxygen was recorded every minute on a Li-Cor LI-1000 datalogger. Oxygen flux rates were estimated by measuring the rate of change in oxygen concentration against time (slope). Net photosynthetic rates were calculated, according to the following equation:

$$P_{\text{net}} = (V \times \text{slope}) / M_{\text{air}},$$

where P_{net} is the net photosynthetic rate ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ min}^{-1}$); V , the chamber volume (l); slope, dissolved oxygen variation ($\mu\text{mol O}_2 \text{ l}^{-1} \text{ min}^{-1}$); M_{air} , chlorophyll *a* content (mg), or number of zooxanthellae or surface (cm^2). The following exponential function was fitted to the photosynthesis–irradiance data:

$$P_{\text{net}} = P_{\text{gmax}} \times [1 - \exp(-I/I_k)] - R,$$

where P_{gmax} is maximum gross photosynthetic rate ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ min}^{-1}$); I , irradiance ($\mu\text{mol photon m}^{-2} \text{ s}^{-1}$); I_k , irradiance at which the initial slope intercepts P_{gmax} ($\mu\text{mol photon m}^{-2} \text{ s}^{-1}$); R , respiration rate in the dark ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ min}^{-1}$; it is assigned a positive sign).

At the end of the experiment, all colonies were cut in two pieces (12 pieces/treatment). Surface area was measured using the aluminium foil technique (Marsh, 1970). Four pieces (originating from four different nubbins) were frozen for measurements of protein and chlorophyll concentrations. Chlorophylls *a* and *c*₂ contents were extracted twice in 100% acetone (24 h at 4°C). The extracts were centrifuged at 11,000 rpm during 10 min and the absorbencies were measured at 630, 663 and 750 nm. Chlorophyll concentrations were computed according to the spectrophotometric equations of Jeffrey and Humphrey (1975). Tissues were then solubilized in NaOH 1N at 90°C for 30 min and the protein content was measured as described by Lowry et al. (1951) on five replicates (1 ml) of the extract. The standard curve was established with bovine serum albumin standard and absorbency was measured at 750 nm using a multiscan bichromatic (LabSystem). Four other pieces (originating from four different nubbins) were

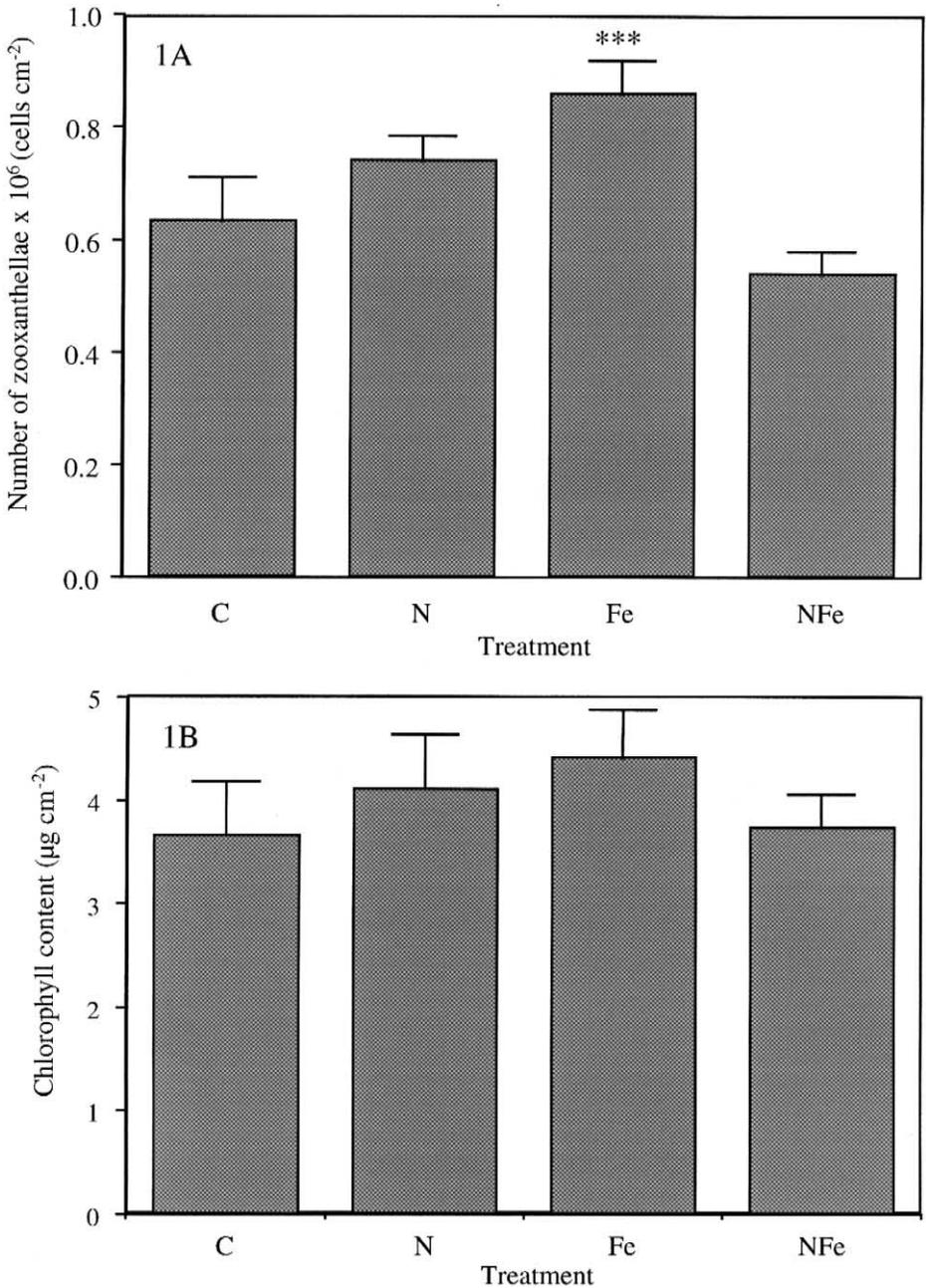


Fig. 1. (A) Areal density of zooxanthellae (cells cm⁻²) in coral colonies sampled from the control (C), nitrate (N), iron (Fe) and nitrate+iron (NFe) treatments. (B) Chlorophyll *a* concentrations (µg cm⁻²) in coral colonies sampled from the same treatments. *** Significantly different from the other treatments. Data are mean and standard deviation of four samples.

used to determine the total number of zooxanthellae using a Neubauer cell. The cell-specific density (CSD) was performed on the four remaining pieces. For this latter parameter, corals were crushed, placed in an Erlenmeyer flask, macerated by agitation on a wrist-action shaker and intact host cells were then counted within 1-h maceration (Muscatine et al., 1998). Host cells containing one or more endosymbiotic algae were recognized as in Muscatine et al. (1998) by the presence of a host cytoplasm and a host cell nucleus (4', 6 diamidino-2-phenylindole staining). Approximately 500 host cells from each colony were observed and ranked according to the number of zooxanthellae that each contained. The mean CSD was calculated according to the following equation:

$$\text{CSD} = \Sigma(r_i \times f_i) / \Sigma f_i,$$

where f_i is the percent distribution of host cells with a given number of algae per cell (r_i).

The likelihood chi-square test (G -test, SYSTAT) was used to compare the zooxanthellae-frequency distribution between the treatments. The cell-specific density of the treatments was tested using t -test. The effect of nutrient enrichment on the content of zooxanthellae and on the rates of photosynthesis, respiration and growth was tested using one-factor ANOVA. When a significant effect was found, means were compared with a Bonferroni/Dunn post hoc test. Statistical analysis was performed using StatView 4.01 (Abacus Concept). Data are reported as mean \pm standard error of the mean.

3. Results

As defined in the material and methods section, the control, nitrogen, iron and nitrogen + iron-enriched tanks will be referred as to C, N, Fe and NFe tanks. The total number of zooxanthellae per animal surface (Fig. 1A) was significantly different between treatments (ANOVA, $p = 0.013$) and was higher in the iron-enriched tank compared to the control ($p = 0.017$) and the NFe tank ($p = 0.0023$). However, there was no difference in the chlorophyll content between treatments (ANOVA, $p = 0.65$), with mean values equal to 3.7 ± 0.5 , 4.1 ± 0.5 , 4.4 ± 0.4 and 3.7 ± 0.3 μg chlorophyll a cm^{-2} (Fig. 1B).

Table 1 shows the average frequency and percent distribution of number of zooxanthellae per animal cell and average cell-specific density (CSD). While host cells

Table 1
Cell specific density measured in the colonies from the four different treatments
Data are mean and standard deviation of four samples.

Number of algae per animal cell	Treatments			
	Control tank	Nitrogen-enriched	Iron-enriched	Nitrogen + iron-enriched
1	69.17 \pm 2.35	57.64 \pm 7.06	51.04 \pm 3.06	59.38 \pm 7.07
2	26.79 \pm 2.77	35.08 \pm 4.73	36.60 \pm 3.45	32.79 \pm 4.73
3	1.53 \pm 0.67	3.07 \pm 1.79	5.37 \pm 1.17	3.45 \pm 1.79
4	2.51 \pm 0.62	3.70 \pm 1.98	5.34 \pm 1.08	3.54 \pm 1.98
5	0.00 \pm 0.00	0.50 \pm 0.62	1.64 \pm 0.56	0.84 \pm 0.62
Mean CSD	1.37 \pm 0.02	1.53 \pm 0.12	1.70 \pm 0.04	1.54 \pm 0.11

Table 2

Photosynthetic rates (P_{\max}^g in $\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$), saturation light level (I_k in $\mu\text{mol m}^{-2} \text{ s}^{-1}$), respiration rates (R in $\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$), measured after 2-week enrichment with nitrate, iron and nitrate + iron

Parameters	Control	Nitrate	Iron	Iron + nitrate
$\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$				
P_{\max}^g	1.23 ± 0.08	1.10 ± 0.13	$1.81 \pm 0.24^{***}$	1.04 ± 0.17
R	-0.50 ± 0.03	$-0.71 \pm 0.08^{***}$	$-0.90 \pm 0.11^{***}$	-0.60 ± 0.21
$10^{-6} \mu\text{mol O}_2 (\text{zoox})^{-1} \text{ h}^{-1}$				
P_{\max}^g	2.05 ± 0.47	1.50 ± 0.43	1.69 ± 0.22	1.68 ± 0.07
R	-0.84 ± 0.23	-0.97 ± 0.24	-0.84 ± 0.11	-0.96 ± 0.22
I_k	280 ± 38	$136 \pm 57^{***}$	397 ± 118	316 ± 97

*** Significantly different. Data are mean and standard deviation of six samples.

containing one zooxanthellae (singlet) predominate in each experimental conditions, the number of zooxanthellae per animal cell and the CSD varied significantly between treatments (G -test, $p < 0.3 \times 10^{-12}$). The average proportion of singlets ranged from 69% in the control to 51–59% in the nutrient-enriched tanks. In contrast, the proportion of doublets (2 zooxanthellae/animal cell) was higher in the nitrate- and iron-enriched tanks (35–37%) than in the control (27%, Table 1). Triplets (3 zooxanthellae/animal cell) were also higher in the iron-enriched (5%) compared to the control tank (1.5%).

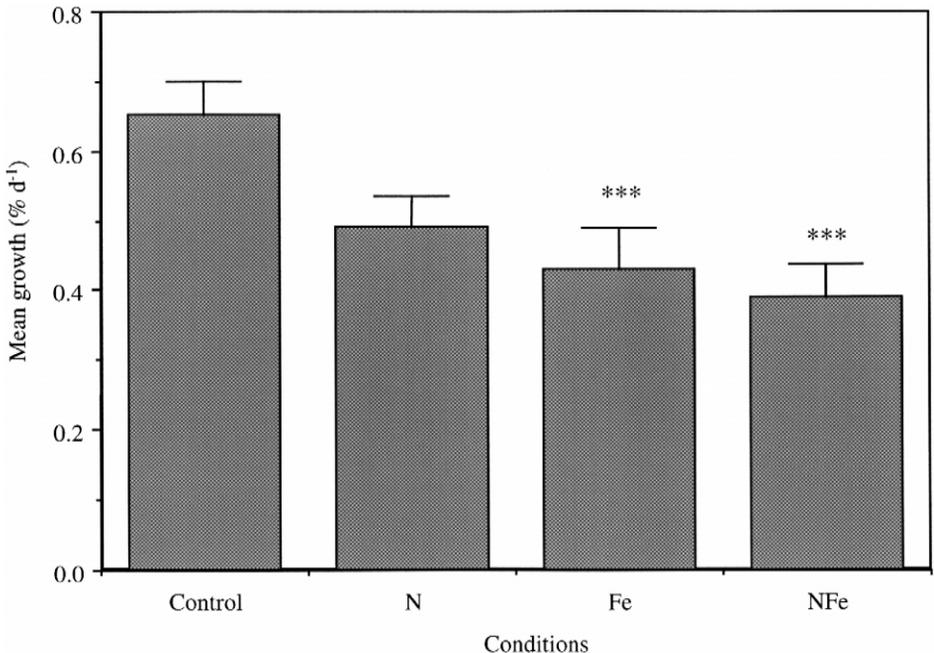


Fig. 2. Increase in growth rates (% day⁻¹) after 3-week incubation in the control (C), nitrate (N), iron (Fe) and nitrate + iron (NFe) tanks. *** Significantly different from the other treatments. Data are mean and standard deviation of six samples.

Photosynthetic parameters obtained after 3-week enrichment with nitrate, iron and both nutrients are summarized in Table 2. The maximal gross photosynthesis (per surface area) was significantly higher in the iron tank compared to the others (ANOVA, $p = 0.02$). Respiration rates were also significantly higher in the nitrate- and iron-enriched tanks compared to the control (ANOVA, $p = 0.01$). However, there was no significant difference between treatments in rates of photosynthesis normalized on a per cell basis (Table 2).

Growth rates, measured with the buoyant weight technique, showed significant differences between treatments (ANOVA, $p = 0.011$). Mean growth rates were equal to 0.65 ± 0.05 , 0.50 ± 0.04 , 0.43 ± 0.06 and $0.39 \pm 0.05\%$ increase day^{-1} in the control, N, Fe and NFe treatments, respectively (Fig. 2). Growth was, therefore, significantly lower in the Fe and NFe tanks when compared to the control tank ($p = 0.008$ and 0.002 , respectively).

4. Discussion

This study investigated for the first time the response of a scleractinian coral, in terms of photosynthesis and calcification, to iron enrichment. Corals used in this study were maintained in nutrient-poor conditions that can be compared to those occurring in mid-ocean water reefs. Iron concentrations were not measured in the experimental tanks; however, concentrations of dissolved iron measured by Sarthou (1996) and Quétel (1991) in the same Mediterranean waters varied between 0.08 and a maximum of 4 nM and are lower than our enrichment. Iron concentrations in most oceans are in any case low, ranging from 1 to 3 nM in the Arabian Sea (Measures and Vink, 1999), the Atlantic Ocean (Timmermans et al., 1999), the Equatorial Pacific Ocean (Rue and Bruland, 1997) and the Mediterranean Sea (Guieu et al., 1991; Sarthou, 1996).

The areal density of zooxanthellae in control corals remained constant during the whole incubation (ca. $6 \pm 0.5 \times 10^5$ cells cm^{-2}). The number of symbionts in corals is likely to be regulated by premitotic or postmitotic controls, such as in situ degradation, expulsion of excess algae (Titlyanov et al., 1996), or nutrient limitation (Muscatine et al., 1998). Growth rate of steady state algal populations is, therefore, low (Falkowski et al., 1993). By keeping this growth rate far from its maximum, coral host ensures a supply of carbon translocated from the zooxanthellae (Benazet-Tambutté et al., 1996). Exposure of corals to small amounts of iron caused a significant increase in the areal density of zooxanthellae as well as in the cell-specific density. An increase in one of these parameters was also observed in some experiments dealing with ammonium enrichment (Dubinsky et al., 1990; Hoegh-Guldberg, 1994; Hoegh-Guldberg and Smith, 1989; Muscatine et al., 1998). These authors suggested that under nutrient enrichment, the host might be unable to control the growth of the symbionts, leading to the breakdown of the balance between the two compartments (Dubinsky et al., 1990). However, regulation processes between the host and the symbionts are complex, since nutrient addition does not always induce an increase in the algal density (Stambler, 1998; Stambler et al., 1994). The response of zooxanthellae to nutrient enrichment may indeed depend on light intensity (Stambler, 1998), on the amount of algae released by

the host (Stimson and Kinzie, 1991), or on the nutritional status (Muller-Parker et al., 1988).

Maximal photosynthesis (normalized to surface area of coral colonies) was also enhanced following iron enrichment, reflecting the increase in algal population density. However, cell-specific photosynthesis did not significantly change between control and iron-enriched colonies (from 2.5 ± 0.47 to 1.69 ± 0.22) and might be due to the self-shading at high cell densities (Hoegh Guldberg and Smith, 1989). Similar results were also obtained in experiments dealing with nitrogen enrichment (Hoegh Guldberg and Smith, 1989; Dubinsky et al., 1990; Marubini and Davies, 1996).

The changes in symbiont density and photosynthesis resulted in a decrease in calcification rates (by 34% compared to the control tank). Some studies have investigated the effect of iron enrichment on the growth of other calcifying organisms such as coccolithophores (Suzuki et al., 1995; Takeda et al., 1995; Zettler et al., 1996; Muggli and Harrison, 1997), and coralline red algae (Matsunaga et al., 1999). Except Takeda et al. (1995), the above studies found that coccolithophores were very well and more adapted to low iron conditions than other algal species. Matsunaga et al. (1999) also showed that coralline algae has a competitive edge over other algae under the extremely low iron levels associated with deforestation in Japanese islands. In corals, the decrease in calcification following nutrient enrichment can be explained by a disruption of the balance between the algal population and host (Falkowski et al., 1993; Dubinsky and Stambler, 1996). In nutrient-poor water, zooxanthellae are nutrient-limited (Muscatine et al., 1989) and most of the fixed carbon is translocated to the animal. Nutrient addition results in an increase growth of the symbionts and, therefore, in a weak translocation of photosynthetic products, leading, in turn, to a lower rate of calcification (Stambler et al., 1991; Dubinsky and Stambler, 1996). It has also been hypothesized that the algae and host may be in competition for inorganic carbon for photosynthesis and calcification, respectively (Stambler et al., 1991; Marubini and Davies, 1996). Despite these observations, the interactions between iron and calcification remain to be understood.

The combined effect of nitrate and iron on the photosynthesis and calcification of *S. pistillata* was also tested. Previous research on nitrate has focused on rates of nitrate depletion by corals (Webb and Wiebe, 1978; Wilkerson and Trench, 1986; Bythell, 1990; Szmant et al., 1990) and produced ambiguous results. Some corals indeed appeared to remove nitrate from seawater (Webb and Wiebe, 1978; Bythell, 1990), whereas others did not (Wilkerson and Trench, 1986). This inconsistency can be explained by changes in the nitrate reductase activity (Crossland and Barnes, 1977; Muscatine et al., 1984). Only few studies have, however, investigated the effect of nitrate on coral physiology (Marubini and Davies, 1996; Marubini and Thake, 1999; Marubini and Atkinson, 1999). In this experiment, nitrate induced a decrease in calcification rate after 2–3 weeks of enrichment (23% compared to the control). These results confirm the decrease in growth rate observed in *Porites porites* and *Montastrea annularis* following a 1 to 20 μM nitrate addition (Marubini and Davies, 1996; Marubini and Thake, 1999). Only one study (Marubini and Atkinson, 1999) did not show any change in coral calcification, maybe due to high ammonium concentrations in seawater that might have inhibited the nitrate reductase activity. Marubini and Davies (1996) suggested that the inhibition of calcification with nitrate could be due to a

competition between photosynthesis and calcification for inorganic carbon. They indeed measured an increase in the zooxanthellae population density and in the rates of photosynthesis following 5 to 20 μM nitrate enrichments. Nitrogen may, therefore, have affected calcification by enhancing the zooxanthellae, which, in turn, have limited the supply of DIC available for calcification (Stambler et al., 1991). An increase in zooxanthellae density and in the rate of photosynthesis followed by a decrease in calcification have also been observed under ammonium enrichment (Hoegh Guldberg and Smith, 1989; Stambler et al., 1991; Ferrier-Pagès et al., 2000). In this study, no significant change in the algal density or in the rates of photosynthesis could be observed between control and nitrate-enriched colonies, probably attributable to the relatively low concentrations of nutrient added. Therefore, the carbon limitation hypothesis does not fit the case of our corals, where calcification was reduced without any change in photosynthesis. It has to be noticed that Marubini and Davies (1996) found the same results for a 1 μM nitrate enrichment and were not able to explain the effect of nitrate on the rate of calcification in this case. The cell-specific density (CSD) was, however, higher in nitrate-enriched corals than in control one. A similar increase in CSD has been observed under other nutrient enrichments such as iron (this study) or ammonium (Muscatine et al., 1998).

Addition of both nitrate and iron induced an increase in the cell-specific density of zooxanthellae, but a maximal decrease in coral growth rate (by 40% compared to control colonies). Iron seems, therefore, to be toxic for the coral host, even if it increases the total number of algae. There seems to be a close relationship between decrease in growth rate and increase in the cell-specific density of zooxanthellae (Muscatine et al., 1998). The shift in the equilibrium between symbionts and host cells may consequently be an effective indicator of coral stress; however, this remains to be confirmed. Corals can draw their metal and nitrogen needs from the heterotrophic nutrition (Sebens et al., 1996) or from high rates of nutrient regeneration in the sediment (Charpy-Roubaud et al., 1996). In subsequent studies, it will be interesting to test the effect of a strong depletion in iron on the photosynthesis and calcification of scleractinian corals. Experimental depletion can be obtained using the exogenous siderophore desferrioxamine B that sequester ambient iron and decrease its availability to the biota (Hutchins et al., 1999). This depletion is likely to occur during strong El Niño events, such as those observed in 1997–1998 (Timmermanns et al., 1999).

Acknowledgements

The present study is part of the O.O.E. program supported by the Centre Scientifique de Monaco and the Council of Europe (Open Partial Agreement on Major Natural and Technological Disasters). Thanks are due to Dr. N. Ounais and the staff of the public aquarium of the oceanographic Museum for providing corals used in these experiments. We also thank the “Service d’Observation en Milieu Littoral, INSU-CNRS”, for providing us the nutrient data and two anonymous reviewers for their helpful comments.

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