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Effects of Light Intensity and Ammonium Enrichment on the Hermatypic Coral *Stylophora pistillata* and its Zooxanthellae

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Abstract

The response of zooxanthellate corals to ammonium enrichment depends on light intensity. In these corals the growth of both algal symbionts and the host animal is controlled by nitrogen and carbon fluxes and their ratios. The combined effects of light intensity [100% (HL), 50% (ML), 10% (LL), and less than 1% (D) of sunlight] and ammonium concentration (<1 and 20 μM) on the symbiotic coral *Stylophora pistillata* were examined. Algal density depended on light intensity; being lowest (4.60×10^5 cells/cm^2) for colonies maintained under <1% of sunlight, and highest (1.97×10^6 cells/cm^2) under high light (HL). Under low light intensity (LL), the algal population density also increased from 8.89×10^5 to 1.95×10^6 cells/cm^2 as a response to ammonium enrichment, whereas under other light intensities there was no such response. Chlorophyll concentration per algal cell increased as light intensity decreased, concomitantly with structural change in the chloroplast. There was an increase in surface density of thylakoid per cell without change in surface density of thylakoids per chloroplast. Contrary to changes in light intensity, ammonium concentration did not cause pigment changes or any ultrastructural changes in the algae. There was no clear effect on the metabolism of the colonies, since both respiration and maximal photosynthesis remained nearly constant under all treatments.

Keywords: Coral, zooxanthellae, light, enrichment

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1. Introduction

Global distribution of coral reefs is controlled by water temperature, and nutrient concentration, limiting reefs to the coastal regions of tropical oceans between 30°N and 30°S. Within this belt of oligotrophic "blue deserts", the local and regional distribution of reefs are determined by salinity and sedimentation, whereas the depth limit of reefs and the bathymetric zonation of coral species within reefs is governed by light (Achituv and Dubinsky, 1990).

The intensity of the underwater light decreases exponentially with depth, roughly following the Beer-Lambert's law. Underwater light is attenuated by the water itself, by dissolved and suspended matter and, most importantly, by phytoplankton. Within the reef, light is absorbed primarily by the coral canopy. Because of this bathymetric decay of light and the dependence of the algae on light for photosynthesis, hermatypic corals (Schuhmacher and Zibrowius, 1985) are generally limited to the euphotic zone, which is usually set at 1% of sea subsurface light level (Wells, 1957; Dustan, 1982). The maximal depth of the reef depends on the attenuation of light in any given locality, and may extend as deep as ~100 meters.

Corals may be considered, in part, primary producers due to the photosynthetic activity of their symbiotic algae, the zooxanthellae. Zooxanthellae, like all phytoplankton and algae, are capable of photoacclimation, responding to changes of irradiance by cellular changes which facilitate light harvesting capability (Falkowski, 1980, 1994 reviews) such as increased chlorophylls and peridinin (Porter et al., 1984; Dubinsky et al., 1984). In addition, ultrastructural modifications in response to light intensity take place, which include changes in chloroplast volume, and number and length of thylakoids in the chloroplast (Dubinsky et al., 1984; Berner et al., 1987; Anderson et al., 1988; Lesser and Shick., 1990; Bar et al., 1995; Ambariyanto and Hoegh-Guldberg, 1996; Mueller-Parker et al., 1996). Photoacclimation also includes changes in the respiration of the zooxanthellae, their light utilization efficiency, and the light saturated rate of photosynthesis (Porter et al., 1984). The increase in chlorophyll per algal cell may increase the light absorptivity of the coral up to five fold, from 20% to nearly 100%. This darkening of low-light corals usually occurs with no change in the density of the zooxanthellae (Falkowski and Dubinsky, 1981; Falkowski et al., 1984), although Titlyanov (1991) did find light related changes in algal density.

Light not only drives the photosynthetic accumulation of organic carbon, but also may affect some metabolic pathways. For example, there are changes in the carbohydrate:protein:lipid and C:N:P ratios in corals growing under different irradiance levels (Gattuso et al., 1993).

The mutualism between the corals and the zooxanthellae, is defined by translocation of photosynthate components from the algae to the host and by nutrients moving from the host to the algae. Shallow water corals exposed to high light intensity may obtain up to 100% of their respiratory demands from algal photosynthesis as compared to 40% in shade adapted ones (Muscatine et al., 1984; Falkowski et al., 1984; Edmunds and Davies, 1986, 1988).

Coral reefs are found in oligotrophic waters, which are poor in nutrients such as nitrogen, phosphate, and possibly iron. In spite of this coral reefs exhibit high gross primary productivity rates (Odum and Odum, 1955; Crossland et al., 1991). Corals are adapted to nutrient-poor environments and can take up, retain, and recycle both dissolved inorganic and organic nutrients (Muscatine and Porter, 1977; Rahav et al., 1989).

The sources of nutrients in the waters surrounding coral reefs are sediments, nitrogen fixation or, in some nearshore reefs and atolls, runoff and groundwater input (D'Elia and Wiebe, 1990), and "endo-upwelling" in volcanic, warm core reef systems (Rougerie and Wauthy, 1988). Nutrient uptake by the coral colony is accomplished by both zooxanthellae and the coral. The animal acquires nutrients by predation on, and subsequent digestion of zooplankton (Erez, 1990) and probably from dissolved organic nutrients as well. Zooxanthellae take up dissolved inorganic nutrients from sea water (Rahav et al., 1989). In addition nutrients resulting from animal metabolism and prey digestion by the host animal are shared with the algae (Muscatine et al., 1989).

The coral *Stylophora pistillata* from the Red Sea responded to enrichment with ammonium or ammonium + phosphate, mostly by increasing algal density (Muscatine et al., 1989; Dubinsky et al., 1990; Dubinsky and Stambler, 1996). An increase in algal density in response to ammonium enrichment was also found in colonies of *Seriatopora hystrix* and *Stylophora pistillata* from the Great Barrier Reef (Hoegh-Guldberg and Smith, 1989) and *Pocillopora damicornis* from Hawaii (Stambler et al., 1991). Higher chloroplast volume and increased surface density of thylakoids were found in zooxanthellae from *P. damicornis* exposed to very high nitrogen concentrations (50 µmole) (Berner and Izchaki, 1994).

The photosynthetic rates of nutrient enriched colonies of *Stylophora pistillata* increased compared to unenriched controls (Dubinsky et al., 1990). However, there was a considerable decrease in photosynthesis per cell as zooxanthellae density increased. The decrease in the contribution of each alga to the energy budget of the colony (Dubinsky et al., 1990) may have led to the observed decrease in the growth rate of the colony under nutrient enrichment conditions (Stambler et al., 1991). Under increasing densities of algae resulting from nutrient enrichment, the algae may become CO_2 limited and may compete

with the animal for carbon for calcification (Dubinsky et al., 1990; Lesser et al., 1994).

In the present study, the combined effects of light intensity and ammonium enrichment on the algal symbionts and their animal host, as well as on the animal-algae relationship and the physiology of the coral colony were examined.

2. Methods and Materials

Collection and incubation of colonies

The experiments were conducted during March and August 1990 at the Interuniversity Institute for Marine Sciences of Eilat (The H. Steinitz Marine Biology Laboratory) Israel.

High-light adapted colonies of *Stylophora pistillata* (Falkowski and Dubinsky, 1981) of similar size (~15 cm diameter) were collected from a depth of 2 m. The colonies were placed in two Plexiglas aquaria, each partitioned into five chambers (28.5 cm × 19.3 cm × 20 cm). Each chamber was supplied with unfiltered running sea water from the laboratory system at a flow rate of 400 ml min^{-1}. Before the beginning of the experiment, colonies were acclimated for one week in these aquaria under low light levels. Based on preliminary data it was assumed that there are no differences between colonies collected at different times of the year.

Two of the chambers were exposed to full solar radiation (High light, HL). Two were covered with black plastic netting, reducing radiation to about 50% of full sunlight (Medium light, ML). Two were covered with black plastic transmitting about 10% of sunlight (Low light, LL). Two chambers were covered with 3 layers of black plastic which reduced light to less than 1% of full sunlight (Dark, D). The light intensity was measured by LI-190SA cosine Quantum sensor connected to a Li-Cor LI-1000 Datalogger.

One of each pair of chambers, at each light intensity, was supplied with 8 mM $(NH_4)_2\ SO_4$ at a rate of 1 ml per min. The nutrient-enhanced water was delivered by a peristaltic pump. The final ammonium concentration was 20 μM (Ammonium, A). Ammonium concentration of 20 μM is much higher than natural (ambient) levels (<1 μM), but was chosen because it stimulates an upper limit of response (in preliminary experiments higher ammonium concentrations cause death of the corals). The ammonium concentration in the unenriched seawater control chambers were below the limits of detection (<1 μM). Flow rates were monitored frequently. Chambers were cleaned as necessary, to minimize the growth of algae on the aquarium walls and on the corals.

Four colonies of *Stylophora pistillata* were incubated in each chamber for 35 days. All measurements were conducted at the end of the incubation.

Photosynthesis and dark respiration

These colonies were placed in a 630 ml, double walled, cylindrical Plexiglas chamber for photosynthesis and dark respiration measurements. The chamber was filled with freshly filtered seawater. Temperature was kept at 22°C±0.1°C using a thermostat water bath (Braun Melsingen). Oxygen production and consumption were measured by a Clark type polarographic oxygen electrode (YSI 5331) (Dubinsky et al., 1987). For measurements of photosynthesis light was provided by a slide projector with a quartz halogen lamp, attenuated to the desired intensity using perforated metal screens. Irradiance, between 400 to 700 nm, was measured as μmole quanta m^{-2} sec^{-1} using a LI-190SA cosine Quantum sensor connected to a Li-Cor LI-1000 Datalogger. Respiration was measured as oxygen uptake during the initial dark period. Parameters of the P vs E curves: α, P_{max} and compensation light levels; P_{max}, maximal photosynthesis rate, E_c, compensation intensity and E_k, saturating intensity; were calculated using an inverse quadratic equation (Ben-Zion and Dubinsky, 1988).

Biomass parameters

Tissue homogenate was prepared by removing all tissue with a WaterPik (Johannes and Wiebe, 1970). The volume of the homogenate was measured, and sub-samples were taken for determination of the following: 1. zooxanthellae density, from direct counting on a hemacytometer; 2. chlorophyll a concentration determined spectrophotometrically on a Beckman DU-6 spectrophotometer, using 90% acetone as a solvent (Jeffrey and Humphrey, 1975); and 3. protein analyses according to Lowry et al. (1951).

Surface area of colonies was measured on a Delta-T area meter after the tissue-free skeleton was broken up, to avoid overlapping of colony branches. Total surface area was calculated from the projected area multiplied by π, assuming a sub-cylindrical geometry of the branches (Falkowski and Dubinsky, 1981).

Transmission electron microscopy

Samples of tissue homogenate (2 ml) were fixed in Karnovsky's solution in 0.1 M (pH 7.2) phosphate buffer. After 2 h post fixation in 2% osmium tetroxide the samples were block-stained with uranyl acetate, followed by serial

dehydration in ethanol and propylene oxide, and then embedded in Spurr's low viscosity medium (Spurr, 1969). Sections were cut with a diamond knife and observed on a Jeol TEM 1200×, operating at 80 kV.

To calculate the relative volume in percents of chloroplast to total cell volume a transparency of short lines was superimposed on the TEM micrographs that crossed the centre of the cell (Freere and Weibel, 1967). Surface density of thylakoids per chloroplast and per cell (in μ^2/μ^3) was determined using the equation:

$$S = \frac{4 \times L}{P \times L}$$

where

S = surface density of thylakoids (in μ^2/μ^3)
P = number of times the lines hits the chloroplast or the cell
I = number of times the lines cuts the thylakoids
L = length of one line (Freere and Weibel, 1967).

Statistical analysis

Data were analyzed using the SAS software (SAS 1987). Analysis of Variance (ANOVA) was performed using PROC GLM (General Linear Models Procedure), in order to compare between different treatment groups. Multiple comparisons of means were performed using the Waller-Duncan, Duncan and T\LSO tests ($p<0.05$) (SAS 1987). The statistic tests Waller, Duncan and T\LSD show how close the treatments were. Treatments with the same letter are not significantly different. Treatments with different letter are significantly different. In all of the paper the term significant is based on the results of these tests.

3. Results and Discussion

Effect of light intensity

Zooxanthellate corals grow under a wide range of irradiance levels. There are coral species growing in shallow water, exposed to more than 1,500 µmole quanta m^{-2} sec^{-1}, while zooxanthellate deep water corals such as *Leptoseris fragilis* are exposed to less than 10 µmole quanta m^{-2} sec^{-1}. Previous studies of the Red Sea coral *Stylophora pistillata*, growing at light levels ranging from 0.3% to 90% of the subsurface light intensity, showed a constant algal density

over this range of 1.6–1.7×10^6 cells per cm^2 (Falkowski and Dubinsky, 1981; Dubinsky et al., 1984). In the present study it was found that the density of algal population in *Stylophora pistillata* were within this range, 1.60–1.97×10^6 cells per cm^2, under HL and ML, but decreased in the low light (LL) and dark (D, 0.46×10^6 cells per cm^2) treatments (Fig 1). The trend for decreasing algal density in the unenriched hermatypic coral *S. pistillata* with decreasing light intensity was significant (Waller, Duncan test). Decreased algal density with decreased light intensity was also found for *Pocillopora verrucosa* (Titlyanov et al., 1980). Growing the coral *L. fragilis* in very low light (at 160 m depth) caused degradation of the algae (Kaiser et al., 1993).

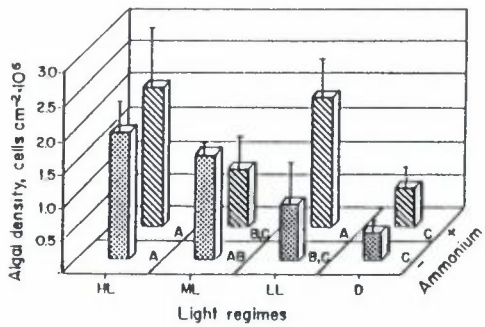

Figure 1. Effect of light intensity and ammonium enrichment on cell density in the coral *Stylophora pistillata* (n=4 for each treatment, mean±SD). The letters A, B, C represent the result of the statistic ANOVA tests Waller, Duncan and T\LSD. Treatments with the same letter are not significantly different ($p>0.05$).

In general, corals lose their algae in the dark (Yonge and Nicholls, 1931; Kevin and Hudson, 1979). The decrease in zooxanthellae densities under extremely low light, like that found in nature on the undersides of coral colonies growing under very low light (Dubinsky and Jokiel, 1994), may be the result of the following scenario: coral colony growth, albeit slow, is supported by the translocation of photosynthate from zooxanthellae growing on the upper face of the colony and is supplemented by hetrotrophic feeding by coral polyps. The zooxanthellae retain some of the products of their photosynthetic activity to support their own respiration and cell division, presumably sufficient to keep up with coral growth. On the lower, deeply shaded face of the colony, whose orientation diminishes the chance of capturing much prey, animal tissue survives on organic compounds transported or diffused from the upper, more illuminated surface that is also more likely to capture zooplankton. The zooxanthellae in the lower part of the colony are slowly "diluted" by the

growth of the host, as their division rates decline in the deepening shade cast by the growing colony. Under these conditions the zooxanthellae may leave the host (Yonge and Nicholls, 1931) in search of higher light conditions, or alternatively, may be expelled by the animal, because they become an additional drain on the energy sources available to the colony, rather than a partner contributing to the economy of the associations (Lewin, 1987). Based on currently available information it is not possible to choose between these two hypotheses of what causes the release of zooxanthellae to the sea water.

The most obvious photoacclimative response of algae to changes in light intensity is the adjustment in chlorophyll concentration to optimize light harvesting. Algae growing under low light have more chlorophyll per cell as compared to algae growing under high light levels (Dubinsky et al., 1984). Without ammonium enrichment, the lowest chlorophyll-a per cell was measured in the Dark (D) treatment, and highest in the low light (LL) treatment (Fig. 2). This increase in chlorophyll concentration increases the ability of the algal cell to absorb light. As light intensity diminishes, chlorophyll concentrations rise, peaking at some low irradiance level. Below this critical light level, chlorophyll concentrations decline. In the dark, the chlorophyll has no function and is degraded (Post et al., 1984) and little or no de novo synthesis occurs, resulting in a net decrease in chlorophyll concentration per algal cell (Fig. 2). In this experiment the photoacclimation did not involve changes in the chlorophyll a to chlorophyll c ratio which ranged from 4.4 to 6.1 and did not differ significantly among treatments (Fig. 4).

The effect of light intensity on cellular chlorophyll concentration was reflected in the ultrastructure of the algae. The relative volume of the chloroplast was significantly different, 43% in LL treatments, compared to 34% in HL algae, with and without ammonium enrichment (Table 1). The surface density of thylakoid per cell was significantly higher in the low light, regardless of ammonium concentration. The surface densities of thylakoids per chloroplast were similar in all cases, a result of the difference in chloroplast volume.

The increase in chloroplast volume and surface density of thylakoids increases the ability of the light-limited cell to intercept photons. Even though there was an increase in the thylakoid surface density per cell, there was no change in the thylakoid surface density per chloroplast, because of a concomitant increase in the chloroplast volume. Our results were in agreement to those published for zooxanthellae in other marine coelenterates (Dubinsky et al., 1984; Berner et al., 1987; Anderson et al., 1988). The increase in chloroplast volume, in this case, enabled the packaging of more thylakoids, and therefore, higher efficiency of light utilization. This increase in surface

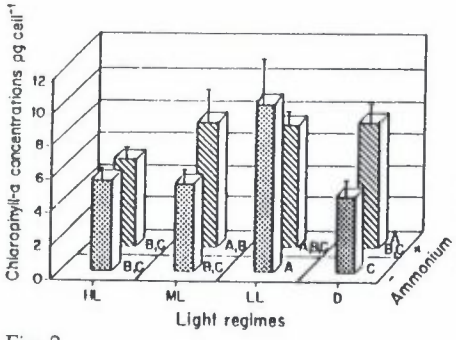

Fig. 2

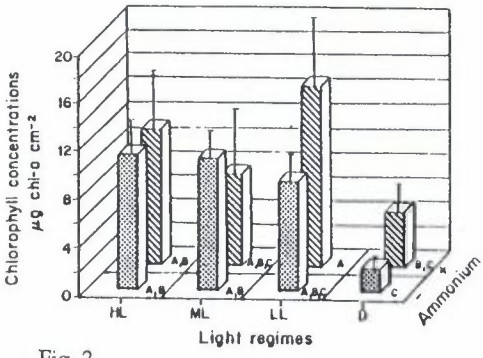

Fig. 3.

Figure 2. Effect of light intensity and ammonium enrichment on chlorophyll a concentration per algal cell in the coral *Stylophora pistillata* (n=4 for each treatment, mean±SD). The letters A, B, C represent the result of the statistic ANOVA tests Waller, Duncan and T\LSD. Treatments with the same letter are not significantly different (p>0.05).

Figure 3. Effect of light intensity and ammonium enrichment on chlorophyll a concentration per unit colony surface area in the coral *Stylophora pistillata* (n=4 for each treatment, mean±SD). The letters A, B, C represent the result of the statistic ANOVA tests Waller, Duncan and T\LSD. Treatments with the same letter are not significantly different (p>0.05).

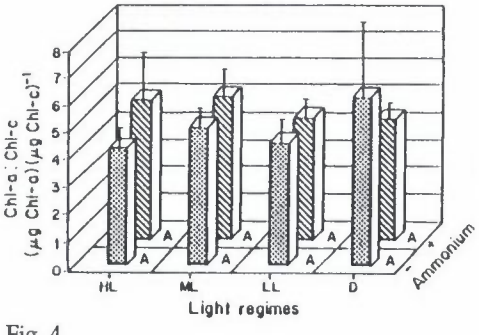

Fig. 4.

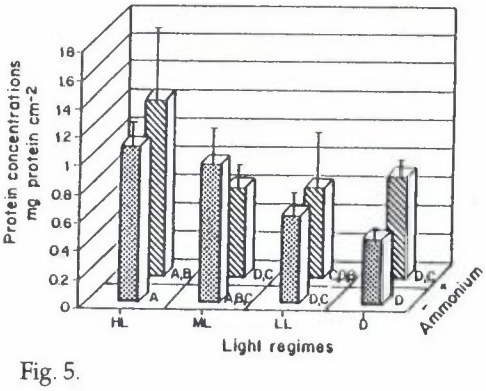

Fig. 5.

Figure 4. Effect of light intensity and ammonium enrichment on chlorophyll a to chlorophyll c ratio in the coral *Stylophora pistillata* (n=4 for each treatment, mean±SD). The letters A, B, C represent the result of the statistic ANOVA tests Waller, Duncan and T\LSD. Treatments with the same letter are not significantly different (p>0.05).

Figure 5. Effect of light intensity and ammonium enrichment on protein per cm^{-2} in the coral *Stylophora pistillata* (n=4 for each treatment, mean±SD). The letters A, B, C represent the result of the statistic ANOVA tests Waller, Duncan and T\LSD. Treatments with the same letter are not significantly different (p>0.05).

Table 1. Effect of light intensity and ammonium enrichment on ultrastructure of zooxanthellae in the coral *Stylophora pistillata*.

	HL	LL	HL+ ammon.	LL+ ammon.
Chloroplast, %	34.63 (B)	42.67 (A)	34.38 (B)	43.46 (A)
Surface density of thylakoids per cell (μ^2/μ^3)	1.53 (B)	2.02 (A)	1.57 (B)	2.15 (A)
Surface density of thylakoids per chloroplast (μ^2/μ^3)	4.54 (A)	4.75 (A)	4.66 (A)	4.86 (A)

The letters A, B, C represent the result of the statistic tests ANOVA by Waller, Duncan and T\LSO. Treatment with the same letter are not significantly different ($p>0.05$).

density is, probably, limited to the point where self shading will restrict light absorption.

Metabolism of the coral symbiosis, respiration and photosynthesis were measured. Respiration rate was calculated per cm^2 colony surface area, per algal cell, and per chlorophyll concentration. The lowest respiration rate per algal cell that was found in colonies kept in the dark but was not significantly different from the respiration rate of the other colonies. There were no significant effects of light intensity, or of ammonium concentration, on respiration rate per area, or per unit chlorophyll (Waller test, Table 2). In other studies, respiration rate of *S. pistillata*, per unit colony area and per algal cell was also the lowest for colonies kept in the low light and the highest in high light (Porter et al., 1984; Hoegh-Guldberg and Smith, 1989).

There were some differences in the parameters of photosynthesis as a response to different light intensities. The compensation intensity (E_c) decreased with lowered incubation irradiance (Table 3). Decreases in compensation intensity like this are one of the most common responses of algae (Richardson et al., 1983) and corals (Chalker et al., 1983; Porter et al., 1984; Barnes and Chalker, 1990) to decreased light. Although it was expected that the corals exposed to low light would have lower maximal rates of photosynthesis (Chalker et al., 1983; Porter et al., 1984; Barnes and Chalker, 1990), there was no clear relationship between irradiance and the initial linear portion of the curve P_{max}, the saturation "constant" (Table 5). However, in corals, one would not expect to find light saturation since the elevated light will increasingly expose more algae to light.

It was clear that colonies kept in the dark would not survive as well as those kept in light even when enriched with ammonium and supplied with zooplankton (Muscatine et al., 1989).

Table 2. Effect of light intensity and ammonium enrichment on respiration rate of the coral *Stylophora pistillata*.

Treatment	Respiration per algal cell µmole O_2 cell^{-1} min^{-1} × 10^{-9}	Respiration per cm^2 µmole O_2 cm^{-2} min^{-1} × 10^{-3}	Respiration per chl µmole O_2 mole chl^{-1} min^{-1} × 10^{-1}
HL	−8.05 ± 5.71 A	−1.32 ± 4.86 A	−1.30 ± 9.17 A
ML	−4.37 ± 3.66 A	−3.47 ± 1.70 A	−3.79 ± 4.05 A
LL	−11.60 ± 4.49 A	−9.96 ± 3.86 A	−1.11 ± 5.82 A
D	−4.23 ± 1.08 A	−1.88 ± 6.46 A	−2.06 ± 8.21 A
HL + ammonium enrichment	−13.30 ± 3.55 A	−2.09 ± 4.20 A	−2.19 ± 8.24 A
ML + ammonium enrichment	−5.42 ± 1.08 A	−3.25 ± 1.10 A	−1.21 ± 4.21 A
LL + ammonium enrichment	−7.26 ± 6.32 A	−1.03 ± 3.79 A	−1.48 ± 9.65 A
D + ammonium enrichment	−6.95 ± 1.22 A	−1.38 ± 1.37 A	−2.43 ± 2.45 A

The letter A represent the result of the statistic ANOVA tests Waller, Duncan and T\LSD. Treatments with the same letter are not significantly different ($p<0.05$).

Table 3. Effect of light intensity and ammonium enrichment on E_c, compensation intensity and E_k, saturating intensity.

Treatment	E_c (µmole quanta m^{-2} s^{-1})	E_k (µmole quanta m^{-2} s^{-1})
HL	337 ± 50 B	513 ± 131 A
ML	102 ± 35 C	105 ± 64 B, C, D
LL	126 ± 41 C	272 ± 156 B, C
D	107 ± 59 C	148 ± 99 B, C, D
HL + ammonium enrichment	544 ± 284 A	82 ± 59 C
ML + ammonium enrichment	113 ± 36 C	112 ± 79 B, C, D
LL + ammonium enrichment	119 ± 7 C	314 ± 101 B
D + ammonium enrichment	47 ± 9 C	54 ± 34 D

The letters A, B, C, D represent the result of the statistic ANOVA tests Waller, Duncan and T\LSD. Treatments with the same letter are not significantly different ($p<0.05$).

Table 4. Effect of light intensity and ammonium enrichment on P_{max}, maximal photosynthesis rate, of the coral *Stylophora pistillata*.

Treatment	P_{max} per algal cell μmole O_2 cell^{-1} min^{-1} $\times 10^{-9}$	P_{max} per cm^2 μmole O_2 cm^{-2} min^{-1} $\times 10^{-3}$	P_{max} per chl-a μmole O_2 mole chl^{-1} min^{-1}
HL	8.8 ± 7.5 A	33.4 ± 2.33 A	3.20 ± 1.13 A,B
ML	19.5 ± 7.3 B	5.21 ± 1.88 C	0.84 ± 0.78 B
LL	32.6 ± 8.9 A	2.82 ± 6.65 B	3.07 ± 1.26 A,B
D	8.0 ± 2.1 B	3.82 ± 1.93 C	4.01 ± 0.88 A
HL + ammonium enrichment	13.5 ± 2.2 A	6.56 ± 8.95 B	2.25 ± 0.76 A,B
ML + ammonium enrichment	8.9 ± 2.9 B	23.20 ± 1.21 C	1.72 ± 0.87 A,B
LL + ammonium enrichment	20.8 ± 13.0 B	32.71 ± 9.46 A, B	2.57 ± 1.71 A,B
D + ammonium enrichment	10.3 ± 5.0 B	4.42 ± 0.87 C	3.56 ± 3.00 A,B

The letters A, B, C represent the result of the statistic ANOVA tests Waller, Duncan and T\LSD. Treatments with the same letter are not significantly different ($p<0.05$).

Table 5. Effect of light intensity and ammonium enrichment on α, the initial slop of the photosynthesis irradiance relationship, of the coral *Stylophora pistillata*.

Treatment	α per algal cell μmole O_2 cell^{-1} min^{-1} (μmole quanta m^{-2} s^{-1})$^{-1}$ $\times 10^{-10}$	α per cm^2 μmole O_2 cm^{-2} min^{-1} (μmole quanta m^{-2} s^{-1})$^{-1}$ $\times 10^{-5}$	α per chl μmole O_2 mole chl^{-1} min^{-1} (μmole quanta m^{-2} s^{-1})$^{-1}$ $\times 10^{-2}$
HL	1.46 ± 0.35 A	7.73 ± 2.44 B	1.78 ± 1.06 A
ML	0.501 ± 1.59 A	5.72 ± 2.11 B	1.42 ± 1.66 A
LL	2.19 ± 1.92 A	19.8 ± 17.4 B	2.24 ± 2.10 A
D	45.8 ± 64.7 A	2,360.0 ± 3,340.1 A	3.88 ± 1.48 A
HL + ammonium enrichment	2.57 ± 1.57 A	40.2 ± 19.3 B	3.84 ± 1.93 A
ML + ammonium enrichment	1.70 ± 1.76 A	13.9 ± 12.8 B	3.54 ± 5.31 A
LL + ammonium enrichment	0.91 ± 0.93 A	12.0 ± 5.76 B	1.14 ± 1.18 A
D + ammonium enrichment	6.31 ± 7.08 A	21.4 ± 21.3 B	17.1 ± 17.2 A

The letters A, B represent the result of the statistic ANOVA tests Waller, Duncan and T\LSD. Treatments with the same letter are not significantly different ($p<0.05$).

Effect of ammonium enrichment

N is usually a limiting factor in oligotrophic water, and when it becomes available to the zooxanthellae, it is used for algal growth (Dubinsky and Jokiel, 1994). As a result, there is an increase in algal population density with increased N availability. An increase in algal population was found for *S. pistillata* and *P. damicornis* colonies (Muscatine et al., 1989; Dubinsky et al., 1990; Hoegh-Guldberg and Smith, 1989; Stambler et al., 1991), but was not found for small branch tips of *P. damicornis* and *Montipora verrucosa* (Stambler et al., 1994). Enrichment with ammonium caused a significant increase in algal density only for the LL treatment (Waller, Duncan test). Algal densities were significantly higher for ammonium enriched colonies than non enriched colonies only for ML treatment (Fig. 1). An increase in algal density represents a breakdown of the balance between the host and the algae (Dubinsky et al., 1990) and results in decreased growth rate of the coral (Stambler et al., 1991).

Chlorophyll concentration in the coral colony is the product of zooxanthellae density and the chlorophyll per cell. Corals kept in the LL treatment, and enriched with ammonium, had the highest concentration of chlorophyll-a per area (14.8 $\mu g\ cm^{-2}$, Fig. 3), whereas colonies in both dark treatments had the lowest (2–4 $\mu g\ cm^{-2}$). Ammonium enrichment did not increase significantly chlorophyll concentration in the D, LL and ML treatments. Increase in chlorophyll concentration as a response to ammonium enrichment was found for *S. pistillata* and *P. damicornis* (Dubinsky et al., 1990; Stambler et al., 1991). The increase in chlorophyll per surface area unit of coral colony is mainly due to increase in algal density, while a packing effect in high algal density causes a secondary increase of chlorophyll per cell as well. Since there was significant increase in algal cells the chlorophyll per cm^2 did not change as well.

The fact that ammonium does not affect the ability of absorbing light is supported by the ultrastructural results. The TEM morphometric analysis did not show any difference in chloroplast volume or surface density of thylakoid between cells exposed to nitrogen enrichment as compared to those that were not exposed. The same results for chloroplast volume were found in *P. damicornis*, but not for surface density, which showed significant increase with nitrogen elevation (Berner and Izchaky, 1994). Our morphometric results were consistent with those of the chlorophyll results. However, the different results between those of *S. pistillata* and *P. damicornis* could be due to the fact that the zooxanthellae were from different species or the distinct effect of the animal on the physiology of the algae. Changes in the chlorophyll were mainly a response to light and the ambient ammonium concentration in this experiment did not affect chloroplast or thylakoids densities in the algal cells

(Table 1, see also Berner and Izchaky, 1994; Ambariyanto and Hoegh-Guldberg, 1996; Mueller-Parker et al., 1996).

Protein concentration responded to both irradiance level and nutrient enrichment. Unenriched corals in the dark had the lowest protein concentration 0.46 mg cm^{-2}, which increased to 1.1 mg cm^{-2} in HL ammonium enriched colonies. In general, ammonium enriched colonies had higher protein concentrations as compared to unenriched ones at parallel light levels, although these differences were not significant (Fig. 5). The protein per cm^2 was the highest for corals incubated at high light intensity, and the lowest for corals in the dark treatments (Fig. 5).

Rate of both respiration and photosynthesis are the main indicators of conditions effecting the coral algal symbiosis. The compensation intensity for photosynthesis (E_c) was found to be the highest, 337–544 μmole quanta m^{-2} s^{-1}, for HL colonies that were kept with and without ammonium enrichment (Table 3). The lowest E_c value, was observed in colonies kept in the dark, 47 μmole quanta m^{-2} s^{-1} (Table 3), and ammonium enriched. There was no significant effect of the ammonium concentration on the compensation intensity, except in the case of HL.

The level of irradiance of onset of light saturation, E_k, at which the initial linear portion of the curve intersects P_{max}, was highest for HL colonies and lowest in the dark. There was no clear trend in E_k in response to the nutrient enrichment (Table 3). There were no significant differences in α (the initial slope of the photosynthesis irradiance relationship) among treatments, when photosynthesis was normalized to chlorophyll or to algae. Even though colonies differed in their P_{max} there was no clear relationship either with light or with nutrient treatment (Table 4). The colonies kept in the dark had the lowest α values per cm^2 compared to all other treatments (Table 5).

No change in gross photosynthesis as a response to starvation was found for the coral *Astrangia dana* even though in this case there was an increase in respiration rate as a response to feeding (Szmant and Pilson, 1984). Other work has shown an increase in algal cell and photosynthesis rate per unit area in response to ammonium enrichment (100 μM) and a decrease per algae cell (Dubinsky et al., 1990). In the experiment reported here ammonium enrichment (20 μM) did not change the cell density (Fig. 1) and therefore no increase in the respiration per cm^2 was expected.

Carbon is used both as a structural element and as the main energy source, while nitrogen is needed for growth, as a component of protein and nucleic acids. The main source of carbon to the algae is photosynthesis, which provides also a significant but variable fraction of the metabolic needs of the animal host. Predation is another source of carbon for the animal, and the importance of predation depends on coral species and on the magnitude of the light-driven

translocation flux. However, predation and prey digestion are major sources of nutrients other than carbon which are directly available to the animal host (in corals as well as sea anemones) and subsequently, to the zooxanthellae (Muscatine et al., 1989; Cook et al., 1992).

Nutrients are available also to the algae from the sea, as dissolved inorganic compounds. Since reefs reside in oligotrophic waters, animal metabolism and zooplankton digestion by the host are the main source of nutrients for the zooxanthellae.

The population density of algae is controlled by nitrogen and carbon limitation. The coral host keeps the algal growth rate far from its maximum, as compared to growth rates in culture, and thereby ensures a supply of carbon translocated from the zooxanthellae (Benazet-Tambutte, et al., 1996). Additions of ammonium cause an increase in algal populations, and decreased translocation of carbon (Dubinsky et al., 1990; McCloskey et al., in preparation) to the host (Falkowski et al., 1993). Under low light intensity the rate of photosynthesis is low, and it may limit the carbon flux in the cell (Dubinsky and Jokiel, 1994), causing a decrease in algal density. The carbon availability, at high light, is as much as the algae can utilize, and under these conditions nitrogen may be the limiting factor. Under low light conditions the carbon and not nitrogen limits algal growth. Both the coral host and the zooxanthellae are impacted by carbon and nitrogen availability.

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