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Differences in the Symbiotic Interrelation in Dark and Light Coloured Colonies of the Hydrocoral *Millepora dichotoma*

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Abstract

The occurrence of dark and light coloured colonies of the hydrocoral *Millepora dichotoma* at the same depth and irradiance levels is reported. The 3.73 fold increase in pigmentation in the dark coloured colonies is the product of both 2.48 times higher algal density and 1.53 times higher cellular chlorophyll levels in these colonies. The high algal density in the dark *M. dichotoma* causes some shade adaptation. This adaptation is reflected in the increase in cellular chlorophyll and in the far lower (×2.9) saturated photosynthetic rate in the dark colonies. Calcification rate was also much lower in the dark coloured colonies. This was attributed to carbon deficiency in the much denser population of zooxanthellae.

Keywords: *Millepora*, hydrocoral, zooxanthellae, symbiosis

1. Introduction

Hermatypic corals, like numerous other invertebrates form mutualistic associations with endozoic microalgae, belonging to the Dinoflagellata, usually known as zooxanthellae (for reviews see Taylor, 1973; Trench, 1981). Such associations are dominant in many coral-reef ecosystems which are widespread in the nutrient poor and well illuminated, coastal water of the tropical oceans (Muscatine and Porter, 1977). There is general agreement that the algae benefit from the phosphorus and nitrogen in the body fluids of the host while

supplying it with various energy-rich products of photosynthesis. The algae mediate the acceleration of skeletal calcium carbonate deposition in corals, known as light enhanced calcification (Goreau and Goreau, 1959). This phenomenon, whose precise nature has not yet been completely understood, in addition to the light dependence of algal photosynthetic process, limits the distribution of hermatypic corals to the upper layers of the tropical seas (Stoddart, 1969).

Falkowski et al. (1984) compared light and shade adapted colonies of the coral *Stylophora pistillata*. These colonies differed in many ultrastructural, biochemical and physiological characteristics of the zooxanthellae and their functional relations with the animal host. The shade adapted colonies looked almost black in colour (e.g. Fig. 1 in Falkowski and Dubinsky, 1981) absorbed nearly all of the incident irradiance upon them whereas the whitish, high light adapted colonies, absorbed less than one half of the incident irradiance (Dubinsky et al., 1984). It was found that this difference in colour and the resulting absorption properties were solely due to light and shade adaptation of the zooxanthellae. While areal concentration of the algal cells remained nearly constant, the chlorophyll content of the cells was about 4 times higher in the shade-adapted colonies than in the high light adapted ones (Falkowski and Dubinsky, 1981, Dubinsky et al., 1984). Similar, but less pronounced differences were reported also for zooxanthellae from the octocorallian *Litophyton arboreum* (Berner et al., 1987) where differences in colour were found between shaded and illuminated parts of the same colony.

The photoadaptive changes in the symbiotic algae result in many changes: increase in the thylakoid number and density; light utilization efficiency and reduced light saturated photosynthetic rates; reduced respiration; and reduced growth rates in the shade adapted algae as compared to their high light adapted counterparts (Porter et al., 1984; Dubinsky et al., 1984). These changes in the symbiotic algae are similar to those reported in numerous studies on free living phytoplankton (see reviews by Falkowski, 1980a,b and Richardson et al., 1983).

According to Porter et al. (1987) irradiance affects the cellular chlorophyll levels in the zooxanthellae, usually without bringing about changes in their areal concentration. He also showed a decrease in algal concentration with depth. Since irradiance usually decreases with depth, in most studies the effects of these two factors on the zooxanthellae are not separated.

In shallow reefs in the Gulf of Eilat (Aqaba), we observed that both light and dark coloured colonies of the hydrocoral *Millepora dichotoma* (Fig. 1)

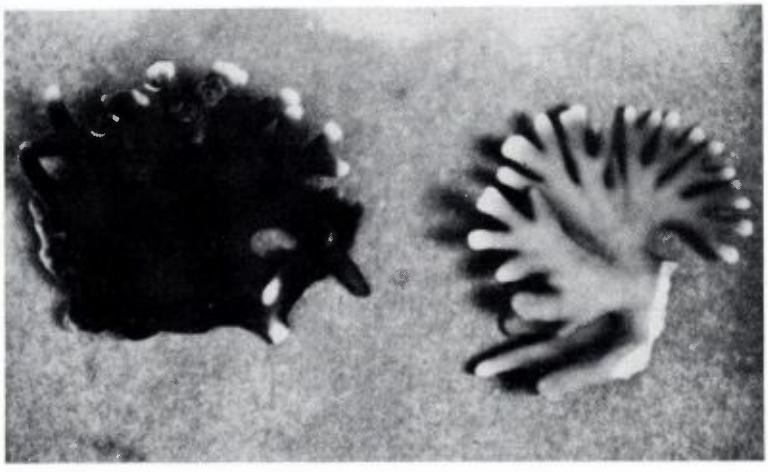

Figure 1. Samples of dark coloured and light coloured colonies of *Millepora dichotoma*.

occur at the same depth and irradiance levels. Our purpose was to compare the two types of colonies regarding algal densities, cellular chlorophyll levels and the relationships between irradiance and photosynthesis and between rates of photosynthesis and calcification.

2. Materials and Methods

Samples of dark and light colonies were collected from coral knolls at depths between 0.5–1.5 m, in the Gulf of Eilat, near to the Interuniversity Marine Institute. The samples were transferred underwater to the laboratory and were kept in aerated sea water until used, usually within 1 hr after collection.

For determination of the chlorophyll absorbtion spectrum, samples of hydrocoral slurry were prepared with a Water Pik (Johannes and Wiebe, 1970). Chlorophyll was extracted in 90% acetone according to the procedure described by Dubinsky et al., (1984). In order to compare the carotenoid content of the algae the level of chlorophyll absorption in both samples was brought to the same level at the 665 nm peak by dilution of the extract with 90% acetone.

Chlorophyll concentration in the 90% acetone extract was determined on a Beckman DU-6 spectrophotometer, and was calculated following the equations of Jeffrey and Humphrey (1975).

The surface area of the selected pieces of dark and light colonies was determined planimetrically. This was done by tracing the outline of the shadow casted by the sample when placed on an overhead projector. These traces were cut out and the areas were determined by comparing them gravimetrically to known areas of the same type of paper. Based on the average eccentricity of the elliptical cross section of *M. dichotoma*, the planar projection was multiplied by 3 to obtain the surface area. From the volume of the slurry, the algal concentration, and the area from which it was removed, the areal concentration of the algae was calculated. The diameter of the algae was measured under the microscope using a calibrated eyepiece at $\times 400$ magnification.

Respiration and photosynthesis of freshly isolated zooxanthellae were measured in a small thermostated cuvette (15 ml vol). Temperature was regulated by a digital, Lauda RTE 9DD controlled temperature circulator (0.01° C). Oxygen was measured by a Clark type oxygen electrode (YSI 5331). The electrode was connected via a multi-gain amplifier to a recorder set at 10 mv (full scale). From the recorder traces rates of oxygen uptake or evolution were calculated (Dubinsky et al., 1987). Irradiance was measured as photon flux, with a Li-Cor-LI-185B light meter and a LI-190SB quantum sensor in the algal suspension, as described by Dubinsky et al. (1987).

The deposition of skeletal material was estimated using ^{14}C as a tracer (Muscatine et al., 1983, 1984; Dubinsky et al., 1983). Whole colonies were incubated in 800 ml jars covered by various thicknesses of black plastic netting which transmitted 10,37,16,8 and 0 percent of full irradiance. To each jar 50μCi NaH $^{14}CO_3$ (Amersham) were added. The jars were incubated underwater, in the sea for 4 hr, after which samples of branches of 1 cm length from the uppermost part of the colony were used. The uppermost 0.5 cm tips were discarded. The colonies were rinsed thoroughly in sea water and sun dried. The tissue was removed by boiling in two changes of hot Solueve (Packard) which were pooled and triplicate subsamples were counted on an automatic liquid scintillation counter (Packard MR 300) and used for another study. Weighed (ca. 400 mg) triplicate samples of the skeleton were then dissolved in concentrated HCl in a closed system (Dubinsky et al., 1983) and the $^{14}CO_2$ trapped in Carbosorb (Packard) and counted in a Permaflour V. on the same counter.

The area was measured planimetrically as described above. The areal concentration of algae and of chlorophyll a in subsamples of the colonies used for these experiments was determined.

3. Results

The absorption of light by 90% acetone extracts of zooxanthellae from light and dark *Millepora* colonies are shown in Fig. 2. The absorption of carotenoids at 434 nm, in extracts from the light hydrocoral is about 30% higher than in the dark one, for identical concentration of chlorophyll a.

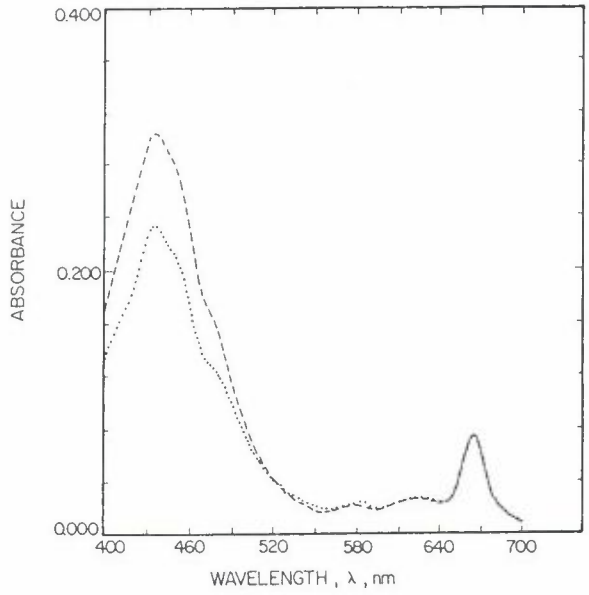

Figure 2. Light absorption spectrum of *Symbiodinium* sp. from dark coloured (dotted line) and light coloured (broken line) *Millepora dichotoma*.

Table 1 presents the chlorophyll content of the algae, the density of zooxanthellae, areal chlorophyll a concentration and the diameter of zooxanthellae in both populations of *M. dichotoma*. Data from extremely dark and light *M. dichotoma* selected from the same depth were used for the calculation of ratios of these parameters in the dark and light colonies. The density of zooxanthellae and the chlorophyll content in algae from the dark hydrocoral, respectively is 2.48 and 1.53 times higher than in the light hydrocoral. as a result, the amount of chlorophyll per cm^2 is 3.73 times higher in the dark coral (Table 1). No significant difference in diameter of algae from the two hydrocorals ($p < 1.15$, t test) was found.

Table 1. Characteristics of zooxanthellae in light and dark coloured colonies of *Millepora dichotoma*.

	Light	Dark	Dark/ Light
pg Chl*a*· cell^{-1}	5.05 ± 1.10	7.77 ± 0.48	1.53
cells ·10^5· cm^{-2} coral	$3.9 \pm 0.9 \times 10^5$	$9.6 \pm 1.7 \times 10^5$	2.48
μChl*a*· cm^{-2} coral	2.1 ± 0.4	7.8 ± 1.0	3.73
diameter μ	11.67 ± 1.34	10.96 ± 1.35	0.94

The effect of light intensity on photosynthesis (P vs I curve) is shown in Figs. 3 and 4. These curves present the much higher light saturated photosynthesis rates of the light coloured colonies than that of the dark ones. Results of carbon incorporation into the skeleton shown drastic differences between the two coral types (Fig. 5 and 6). The rate of carbon incorporation is more effected by irradiance in the light hydrocoral as compared to the dark one. The same striking differences were found when skeletal carbon incorporation is related to tissue ^{14}C incorporation.

4. Discussion

The comparison of areal chlorophyll a concentrations in light and dark-coloured colonies of *M. dichotoma* shows that the dark colonies have 3.73 times more pigment than the light ones. This ratio is very similar to the ratio of 3.9 found for colonies of the hermatypic coral *Stylophora pistillata*, from the Red Sea, adapted to extremely low- and high-light environments (Falkowski and Dubinsky, 1981; Dubinsky et al., 1983, 1984).

In the *S. pistillata* studies where colonies were selected from different irradiance levels but similar depths, virtually all of the areal differences in chlorophyll a concentration was due to a corresponding change in cellular pigment concentration in the zooxanthellae. The situation in the work of Wyman et al. (in press) is more complicated since cellular chlorophyll levels invariably change with depth, but as it was pointed out by Porter et al. (1987) algal densities decrease with depth. Therefore, the ratios of areal chlorophyll levels from deep and shallow colonies do not show a consistent pattern, as areal chlorophyll concentration is a product of both cellular chlorophyll and algal density.

In the case of *M. dichotoma* the nearly four-fold difference in areal chlorophyll is the product of a 2.48 difference in zooxanthellae density and of the

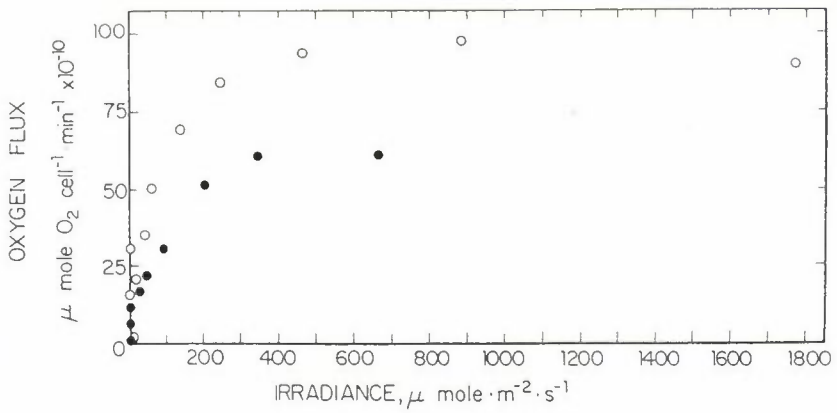

Figure 3. Effect of light intensity on photosynthesis in *Symbiodinium* sp. isolated from light and dark colored *Millepora dichotoma*, values are related to 1 cell. o — gross photosynthesis, light algae; • — gross photosynthesis, dark algae.

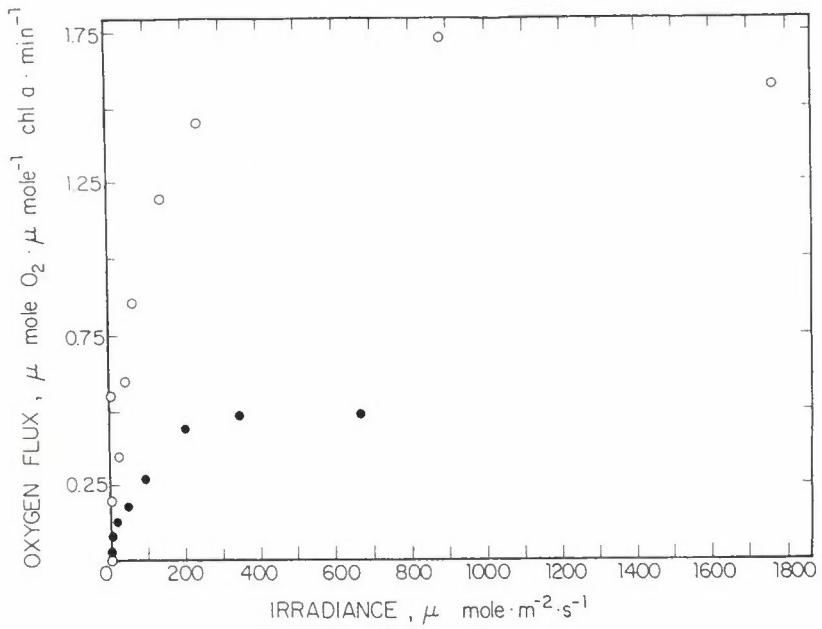

Figure 4. Effect of light intensity on photosynthesis in *Symbiodinium* sp. isolated from light and dark coloured *Millepora dichotoma*, values are related to chlorophyll a. o — gross photosynthesis in light algae; • — gross photosynthesis in dark algae.

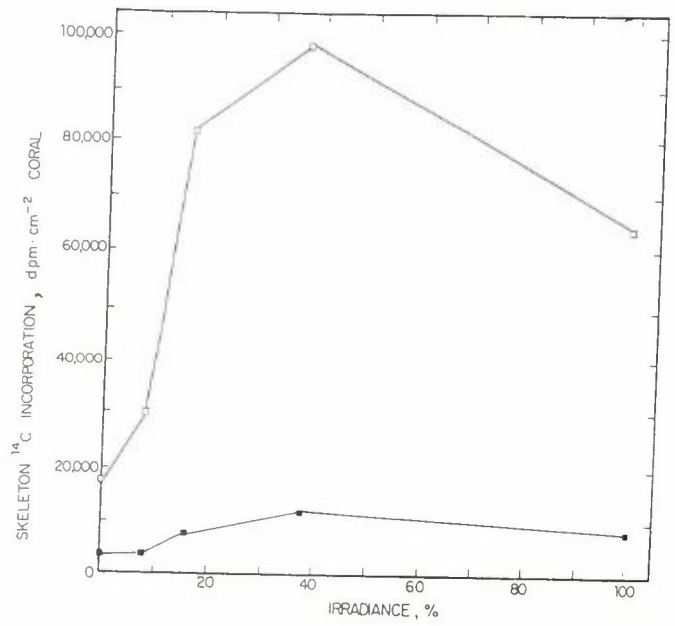

Figure 5. Carbon incorporation into skeleton of the dark and light coloured *Millepora dichotoma*, related to the surface area of the coral. □ — light coloured colonies; ■ — dark colonies.

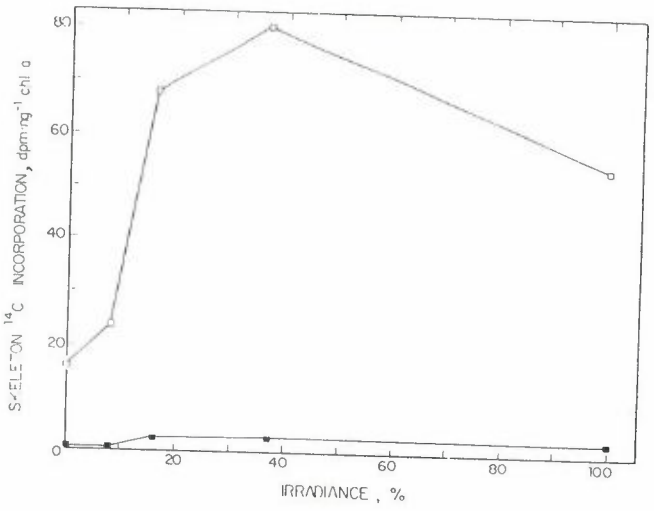

Figure 6. Carbon incorporation into the skeleton of dark and light coloured *Millepora dichotoma*, related to chlorophyll a. □ — light coloured colonies; ■ — dark colonies.

1.53 increase in cellular chlorophyll concentration. One is tempted to hypothesize that since in the case of *M. dichotoma* both colonies abound at similar irradiance levels, the increase in cellular chlorophyll in the dark coloured colonies results from the mutual shading by the increased algal density in these colonies. Taking into account the dimension and density of the algae in the dark and light coloured colonies (Table 1) it can be seen that in the dark colonies the algae can cover 89% of the surface area in a monolayer. This is nearly the maximum coverage by densely packed circles on a surface (90.6%). In the light coloured colonies the algae can cover only 42% of the surface in a monolayer. Since algae are not packed in a monolayer in the densely populated hydrocoral cells, considerable mutual shading must result. This causes some shade adaptation of the zooxanthellae, as can be clearly seen from the moderate 1.53 fold increase in cellular chlorophyll. This assumption agrees with Crossland and Barnes (1977) who claimed that there is mutual shading in algae within coral branches similar to that found in dense suspensions of zooxanthellae isolates. The fewer algae in the light-coloured colonies do not experience such mutual shading and are therefore somewhat more high-light adapted. This assumption is supported by the higher carotenoid to chlorophyll ratio in algae from the light coloured *Millepora*(Fig. 2), similar to what was shown in corals exposed to different light intensities (Zvalinskii et al., 1980).

When the cellular chlorophyll levels of light and dark *M. dichotoma* and *S. pistillata* are compared they range between 5.05–7.77 pg Chl*a*· cell^{-1} in *M. dichotoma* against 2.2–8.3 pg Chl*a*· cell^{-1} for *S. pistillata* (Falkowski and Dubinsky, 1981). Transmission electron micrographs of zooxanthellae from light and dark *M. dichotoma* do not show detectable differences in thylakoid density and arrangement. Such differences were easily discernible between zooxanthellae adapted to extremely low and high irradiance levels in both *S. pistillata* (Dubinsky et al., 1983,1984) and *Litophyton arboreum* (Berner et al., 1987).

Comparison of photosynthetic performance of both *Millepora* types revealed considerable differences. Light saturated photosynthetic rates (Pmax) in the light coloured colonies were far higher than those measured in the dark coloured ones. This agrees with other data which show that Pmax values per chlorophyll are always inversely correlated with cellular chlorophyll levels (Dubinsky et al., 1986; Falkowski et al., 1985; Porter et al., 1984; Post et al., 1985).

Figure 7 shows the effect of photoadaptation on light saturated photosynthetic rates in a number of studies on zooxanthellae and free living phytoplankton. The results are presented as the ratio between Pmax in the light adapted algae and Pmax in the shade adapted ones against the ratio of cellular chlorophyll concentration. It is clear from our data that the Pmax ratio for *M. dichotoma* is considerably higher than the rest. The calculated Pmax ratio corresponding to the chlorophyll ratio in *M. dichotoma* should have been 1.55 while actually it is 2.9. We speculate that this discrepancy may be due to the differences in algal densities causing CO_2 limitation in the dark coloured colonies. The much higher densities in the dark coloured colonies result in carbon limitation, which may lower photosynthetic activity in these colonies.

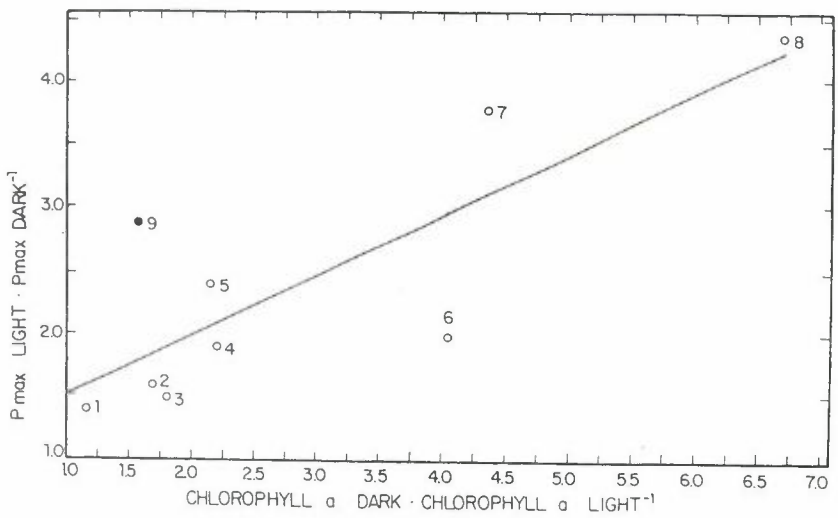

Figure 7. The effect of photoadaptation. Ratios of light saturated photosynthesis, and cell chlorophyll a concentration in free living and symbiotic microalgae. Sources: (1) Porter (in press); (2) Porter (in press); (3) Porter et al. (1984); (4) Post et al. (1984); (5) Post et al. (1984); (6) Herzig and Dubinsky (1986); (7) Herzig and Dubinski (1986); (8) Herzig and Dubinsky (1986); (9) Present study.

Our results (Figs. 5,6) indicate that the calcification rate is considerably below that expected from the moderate shade adaptation in the dark coloured hydrocoral. In both colony types calcification rates increased with irradiance, a trend which is in agreement with the light enhanced calcification theory

(Goreau and Goreau, 1959). However, the ratio of calcification to photosynthesis was much higher in the light coloured colonies. One might speculate that the relative low calcification rate in the dark coloured *Millepora* is result of the high density of algae in these colonies. It is probable that the algae experience carbon deficiency and as a result show reduced rates of photosynthesis which then limits, by as yet unknown mechanism, the calcification by the hydrocoral.

We have described physiological differences between the dark and light coloured *M. dichotoma*. However, unlike other hermatypic corals and some soft corals where dark and light colour is related to irradiance level, this is not the case in *M. dichotoma* where the cause of these differences is still enigmatic.

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