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Induced bleaching of *Stylophora pistillata* by darkness stress and its subsequent recovery

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Abstract. Bleaching, the visible effect of the loss of zooxanthellae by corals has been observed in the last few decades with increasing frequency in most of the world's reefs. Since it usually leads to coral colony death and whole reef destruction, it has been the subject of several studies in recent years. In the present article we describe a method for causing complete, but reversible, bleaching in the common Red Sea coral, *Stylophora pistillata* by darkness stress. This treatment resulted in the reduction of the density of the zooxanthellae, the endosymbiotic algae living within the coral's cells. After 8 days in the dark the coral began to show visible bleaching, which a week later progressed to 70%, and by the 44th day we obtained complete bleaching. At that time no zooxanthellae or chlorophyll were detected in the coral tissue. Following the transferal of the corals to darkness, at first the algal cells exhibited a photoacclimative response, accumulating chlorophyll to levels above the initial values. Fragments that were 70% bleached showed full recovery 30 days after having been returned to light. In the course of recovery, cellular chlorophyll gradually decreased to control – and initial concentrations. From the recovery rate we calculated the maximal doubling rate in the log phase of zooxanthellae repopulating *S. pistillata* to be 2.5 days.

Key words: Coral, bleaching, recovery, chlorophyll.

Introduction

Coral reefs are built by hermatypic corals, in which there is a mutualistic symbiotic relationship between the coral host and single celled microalgae, the zooxanthellae, harbored within the endodermal cells of the coral. The coral affords protection to the algae and provides them with essential nutrients and CO_2 from its metabolic processes. In return, the algae supply the coral with high–energy photosynthate translocated to the animal host. On shallow, well illuminated reefs this nutritional source covers as much as 95% of all metabolic needs of the coral (Falkowski et al 1990). Under high light the energy suffices for maintenance, calcification, growth and reproduction whereas in deep lying, shaded reefs, supplementary predation on zooplankton is needed to satisfy these needs (Dubinsky et al. 1986).

The algae-coral association fine-tunes to ambient light and nutrient regimes by photoacclimation of the zooxanthellae and adjustment of their density, as well as changes in shape and orientation of the entire colony. The photoacclimation is evident from as much as 5 fold changes in the chlorophyll content of the symbionts (Falkowski & Dubinsky 1981; Dubinsky et al. 1984), whereas eutrophication brings about proliferation of the zooxanthellae and reduction of their contribution to the coral (Dubinsky et al. 1990). In branching species the response of the holobiont to high light results in profuse growth in all directions, whereas in the shade or dim light it fans out growing horizontally (Fricke and Schuhmacher 1983). The constant density of symbionts is kept in equilibrium with the growth rate of the colony, as the balance between their division rates, colony expansion and expulsion of excess cells is maintained. It has been found that hermatypic corals release about 0.1%-1% of the algal cells to the water every day, which is usually less then the 0.5%-10% of algae produced in the coral tissue during the same time (Stimson and Kinzie 1991; Hoegh-Guldberg 1994; Titlyanov 1996). In a few cases there are reports of digestion of some of the zooxanthellae by corals (Fabricius et al. 1996). These finely tuned equilibria are maintained on one hand by the translocation of most photoassimilated carbon to the host coral, and on the other hand by the paucity of the key nutrients, nitrogen and phosphorus needed for new zooxanthellar production. A typical doubling rate for the symbionts of *S. pistillata* in nature was 70 and 100 days for high and low light corals, respectively (Falkowski et al 1984).

This equilibrium can be upset if the coral is under stress; if the stress is strong enough the process of bleaching can be triggered, in which case the coral becomes colorless as the white color of the skeleton shows through the thin and transparent tissue (Hoegh-

Guldberg & Smith 1989). The loss of color can occur in two ways: **The coral losing its algae (bleaching);** it has been found that under heat stress the zooxanthellae are expelled from the coral tissue at rates about 1000 higher than in normal conditions (Hoegh-Guldberg & Smith 1989; Ruth et al. 1992). This phenomenon has been described by Glynn (1983) from direct microscopic observation. **The algal cells losing chlorophyll pigments (paling**) – when exposed to a combined heat and high light stress for 7 hours a reduction of ~x 7 in cellular chlorophyll levels has been measured (Salih et al.1998). Chlorophyll content per area of bleached *Agaricia tenuifolia* has been found to be 50 times less than normal (Lovelock et al. 1996).

The converse process, namely the out-of-control proliferation of the zooxanthellae, takes place whenever corals are exposed to anthropogenic (Dubinsky et al 1990) or rarely natural (Genin et al. 1995) eutrophication. This phenomenon, whereby zooxanthellar density increased fivefold in two weeks is detrimental to the colony, since under such conditions nutrients are used up by the algae in their multiplication severely curtailing the life-supporting translocation of photosynthate to the animal (Dubinsky and Jokiel 1994; Dubinsky and Berman-Frank 2001).

Bleaching events stop skeleton growth of the coral (Glynn 1983; Glynn and D'Croz 1990) and usually cause colony death and entire reef collapse (Brown & Suharsono 1990).

The chances of recovery following bleaching depend on the severity of the causative stress, its nature, intensity and duration, and on the conditions after the event. It has been suggested that in some cases it is possible that bleaching and subsequent recovery are the coral's way to achieve quick genetic change in the zooxanthellar community to populations better suited to withstand stress and react to environmental change (Buddemeier & Fautin 1993).

The recovery process can be achieved in two ways: fast reproduction of the "best" algal clone within the coral or by acquisition of a new clone from the water in the immediate environment (Goreau 1991; Rowan and Knowlton 1995).

Stylophora pistillata is the most abundant reef building coral in the Gulf of Eilat (Northern Red Sea) in both numbers and area coverage (Loya 1976). It is an "r" strategist (Loya 1972) which outcompetes all other species in the struggle to monopolize areas becoming available following natural or man-made disturbances. Since it also easily recovers from experimental handling and breakage into fragments it has been a favored model organism in the study of coral biology (e.g. Loya 2000).

Darkness Stress

Due to its dependence on the photosynthesis of the algal symbionts, darkness is a powerful stressor of zooxanthellate corals (Rogers 1979). By placing the coral in a light-tight container that blocks all light without affecting any other parameter such as temperature, pH or flow regime we easily cause darkness stress. Goreau (1959) found that when *Manicina areolata* was maintained in darkness for 10 days to two months it bleached.

Bleaching in darkness is caused by the loss of zooxanthellar cells, as found by Kevin & Hudson (1979) in a study of the cold water coral *Plesiastrea urvillei*.

Materials and Methods

Three colonies of *Stylophora pistillata* were collected from the reef near the Interuniversity Institute, Gulf of Eilat (Aqaba), Israel. Corals of 15-cm diameter were collected from artificial objects at a depth of 2.5-3.5 m. The corals were allowed to acclimate for one month in our main aquarium system under irradiance of 500 µmol quanta $m^{-2}s^{-1}$. Then the corals were divided into 3-cm-long fragments and glued with Super Glue Gel (Scotch, 3M) to plastic tips (Fig. 1).

Figure 1: *Stylophora pistillata* fragments during acclimation in our main aquarium system.

The fragments were left undisturbed for a period of 4 weeks for recovery and acclimation. Only fragments that showed a beginning of tissue growth (onto the plastic tip) were taken for the experiment.
The experiment was performed in a closed system at Bar-Ilan University.

Lighting was obtained from 3 fluorescent lamps of type T5 (10,000K, ATI), which delivered 500µmol quanta $m^{-2}s^{-1}$ at the water surface.

The Darkness Experiment

The experiment was conducted in two plastic containers, 10 L each, with openings for water exchange. The openings were connected by a "T" fitting preventing light from penetrating. A black plastic sheet assuring darkness covered one container and the other container was left uncovered as the control. The two containers were connected to the main water supply system. The light regime of the system was a 12 hours light and 12 hours dark photoperiod.

In each container were placed 30 fragments (a total of 60 fragments). Once every two weeks, 3 fragments from each container were removed and the following were performed: Determining algal areal density and measuring chlorophyll concentration per area and cell. Sixteen days after the beginning of the darkness treatment fragments showed 70% bleaching. At that time 9 fragments from the darkness treatment were transferred for recovery to the illuminated control tank. We monitored their recovery process over six weeks, by taking the same measurements described above for three fragments every two weeks.

We used standard methods for quantifying chlorophyll density in *Stylophora pistillata* and other coral species (Dubinsky et al. 1990; Titlyanov et al. 2000; Nordemar et al. 2003). The procedure requires sacrificing the sample using the Water-Pik method (Johannes and Wiebe 1970; Falkowski and Dubinsky 1981; Hoegh-Guldberg and Smith 1989; Edmunds and Gates 2002) to remove the live animal tissue with the zooxanthellae from the coral skeleton. The resulting homogenate was filtered through a 25-mm Ø glass fiber paper filter (Whatman GFC). The zooxanthellae collected on the filter were extracted in 10 ml of 90% acetone/water by grinding the filter with a glass/PTF homogenizer. The resulting slurry was filtered again through the same type of filter. Chlorophyll concentration was quantified by measuring the optical absorption at 665 nm and 755 nm with a Varian DMS 100 spectrophotometer according to the Jeffrey and Humphrey (1975) equations, and normalized to sample area.

The fragment area was determined using the standard aluminum foil weight method (Falkowski and Dubinsky 1981; Hoegh-Guldberg and Smith 1989; Edmunds and Gates 2002).

Results & Discussion

The difference between the control and the dark treatment was statistically significant, as already by the 7th day areal chlorophyll decreased by 70% (t-test, n=3, $p<0.05$). By the 44th day when the experiment was terminated, the dark treated fragments had totally bleached, and looked completely white; as the zooxanthellae were lost, the coral tissue became transparent and the white skeleton was revealed (Figure 2).

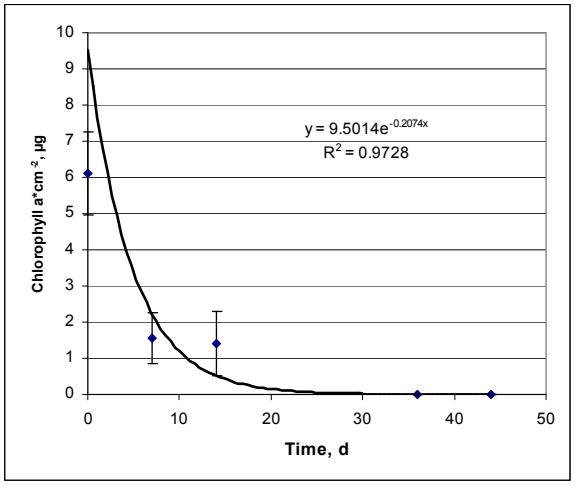

Figure 2: *Areal chlorophyll of bleached* S. pistillata *fragments in darkness (n=3).*

From the plot of chl *a* per zooxanthella cell (figure 3) we can learn that in the first 30 days the dominant factor in the bleaching in darkness was the loss of zooxanthellae, whose numbers began declining immediately; by the end of the experiment less than 1% of the symbionts remained.

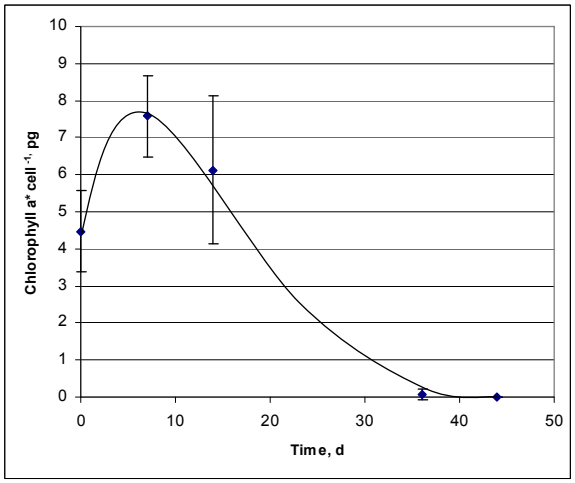

Figure 3: *Chlorophyll per zooxanthella cell of bleached* S. pistillata *fragments (n=3).*

The plot of chlorophyll *a* per zooxanthella (figure 3) shows that during the first 10 days after the corals were placed in full darkness the zooxanthellae were actually gaining chlorophyll, which is in agreement with the photoacclimation studies on *S. pistillata* (Falkowski & Dubinsky 1981; Dubinsky et al. 1983).

The recovery of the coral's chlorophyll concentration is shown in figure 4.

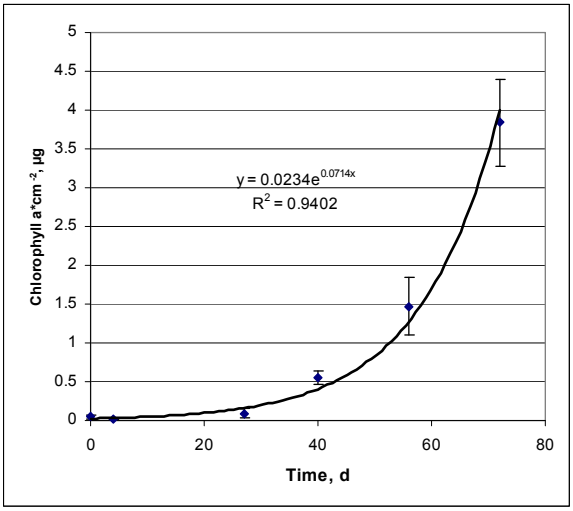

Figure 4: *Chlorophyll concentration in* S. pistillata *fragments recovering from darkness bleaching*

In the first 20 days the chlorophyll concentration in the coral tissue did not change much. In the plot following the changes in the chlorophyll in the zooxanthella cells (figure 5) we can see that in the first 10 days the chlorophyll content per cell was decreasing. We suggest that again it could be explained as a photoacclimation of the zooxanthellae upon being suddenly transferred from darkness to bright light.

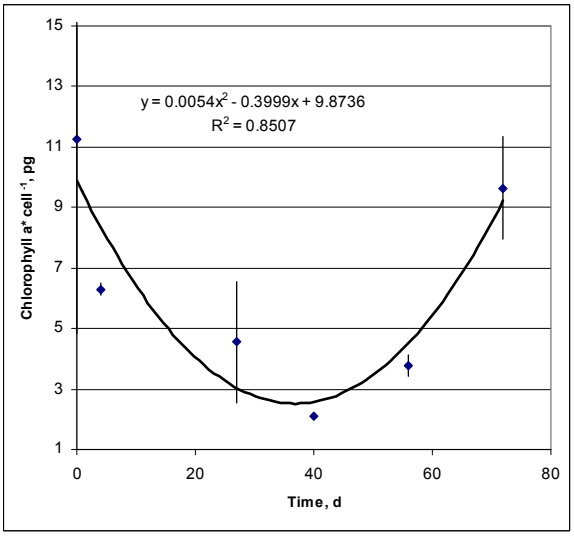

Figure 5: *Chlorophyll per zooxanthella in* S. pistillata *fragments recovering from darkness.*

After 20 days the chlorophyll and the algal population were being restored rapidly, until they reached initial and control levels at about the 70th day.

We followed the increase in zooxanthella density per cm^2 and calculated their rate of doubling and found that the fastest doubling time (after the initial lag period) was about 2.5 days (figure 6).

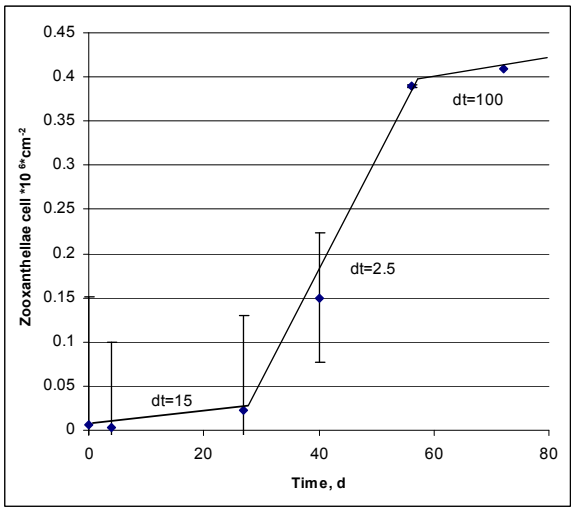

Figure 6: *Zooxanthella doubling rate in* S. pistillata *fragments recovering from darkness*

That is probably the maximal potential doubling rate of the zooxanthellae in the coral tissue when they are not constrained by their own population. Doubling times in steady state (presumably at their carrying capacity in the tissue) were determined as 70 and 100 days in high and low light *S. pistillata* colonies, respectively (Falkowski et al 1984).

Conclusions

The study showed that in the common Red Sea coral *S. pistillata* darkness leads to bleaching. However, presumably a few zooxanthella cells remained, and upon illumination, these rapidly repopulated the bleached coral tissue. It is especially noteworthy that the bleached corals survived in total darkness for nearly two months, and that the doubling rate of the zooxanthellae during the recovery phase was as short as 2.5 days, whereas in nature it was reported to be 70-100 days. We interpret it as representing a population's reaction to finding itself way below its carrying capacity, thereby realizing its maximal potential growth rate.

On the technical level the study illustrates the power of the described photographic method as a tool in coral research.

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