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Zooxanthellae Population Density and Physiological State of the Coral *Stylophora pistillata* During Starvation and Osmotic Shock

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Abstract

Regulation of symbiotic zooxanthellae populations in hermatypic corals may include growth limitation by the host, release of healthy-looking zooxanthellae, and degradation of zooxanthellae. Here we experimentally change the zooxanthellae densities in branches of *Stylophora pistillata* corals through starvation and osmotic shock. We follow the subsequent changes of the zooxanthellae population dynamics. Prolonged starvation (40 days) caused a 50% loss of zooxanthellae, a 70% decrease in photosynthesis (P calculated per polyp), a 3-fold decrease in proliferating zooxanthellae frequency (PZF) and two-three-fold increase in degrading zooxanthellae frequency (DZF). The increase of the numbers of zooxanthellae in various stages of degradation was closely coupled to an increase in the release of degraded zooxanthellae particles (dzp). At the same time, zooxanthellae volume

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increased by 80% but chlorophyll content and the photosynthetic capacity of the zooxanthellae did not change. Polyps subjected to osmotic shock (17‰) expelled about one third of their zooxanthellae along with elements of animal cells. PZF and DZF levels varied slightly. We assume that zooxanthellae densities in corals are a consequence of differential rates of zooxanthellae division and degradation regulated by the coral host. Only under extreme conditions are zooxanthellae population densities regulated by expulsion of healthy zooxanthellae.

Keywords: *Stylophora pistillata, corals*, zooxanthellae, starvation, osmotic shock, feeding, photosynthesis, respiration, chlorophyll

1. Introduction

The rapid and often fatal loss of zooxanthellae in corals is generally a consequence of sudden environmental changes at and above the corals' acclimation capacity. For example, sudden reductions in salinity (Goreau, 1964; Van Woesik et al., 1995) and abnormally high sea water temperatures often lead to coral 'bleaching' (Cook et al., 1988, 1990; Gates et al., 1992; Brown et al., 1995) that results in species specific coral mortality (Goreau, 1964; Jaap, 1979, 1985; Yamazato, 1981; Glynn and D'Croz, 1990). Under experimental conditions, the loss of zooxanthellae in corals, and indeed in other symbiotic cnidarians, can be induced by insufficient zooplankton feeding or removal of their nutrient supply (Muscatine, 1971; Szmant-Froelich, 1981; Steen and Muscatine, 1987), by exposure to constant and bright light (Steele, 1976), high salinity (Hoegh-Guldberg and Smith, 1989), prolonged darkness (Franzisket, 1970; Kevin and Hudson, 1979; Muller-Parker, 1984); and shading (Rogers, 1979; Sandeman, 1988).

Changes in the densities of symbiotic zooxanthellae inevitably lead to changes in the physiological state of the coral. Coral colonies with low densities of zooxanthellae show reduced photosynthetic capacities (Clayton and Lasker, 1984; Titlyanov, 1991), but not when these rates are normalised to numbers of zooxanthellae (Titlyanov, 1991). Corals with low numbers of zooxanthellae also have low dark respiration rates (Leletkin et al., 1996). Consequently, the pathways of the photosynthetic biochemical dark reaction directly depend on zooxanthellae density (Bil' et al., 1992).

Coral hosts appear to regulate zooxanthellae numbers by influencing zooxanthellae growth rates (Trench, 1987; McAuley et al., 1994). Organic nitrogen can be transported from the host to the symbiont as urea, uric acid, and sulphur amino acids, while phosphorus can be supplied as glycerophosphate, adenylic or cytidylic acids (Trench, 1979). The host may regulate the supply of nutrients (mostly nitrogen) or release inhibitors or stimulators that influence growth of symbionts (Muscatine and Pool, 1979; Jacques and Pilson, 1980;

McAuley, 1985). However, there is no direct experimental evidence that hosts regulate zooxanthellae densities.

Under normal physiological conditions the expulsion of zooxanthellae amounts to about 0.1–1% per day of the zooxanthellae stock (Hoegh-Guldberg et al., 1987; Stimson and Kinzie, 1991; Titlyanov et al., 1996). Corals with these low rates of zooxanthellae expulsion are not likely to regulate their symbiont population in this manner because the release rate of zooxanthellae is almost ten times lower than the zooxanthellae growth rate (Muscatine et al., 1985; Hoegh-Guldberg and Smith, 1989; Titlyanov et al., 1996). Thus, the most plausible mechanism of zooxanthellae regulation in corals, under normal physiological conditions, is the digestion of symbionts by host cells. Boschma (1925) was the first to point out the possibility of zooxanthellae digestion by host cells, and recently Titlyanov et al. (1996) confirmed daily zooxanthellae degradation in seven hermatypic corals, including *Stylophora pistillata*. Titlyanov et al. (1996) showed that zooxanthellae degradation is a phased and stable process with maximum activity at night, and degradation rates appear to increase with host starvation.

The main objective of the present work was to experimentally reduce zooxanthellae densities in the coral *S. pistillata* and to follow the recovery process in order to identify underlying mechanism(s) involved with zooxanthellae density regulation. Reduction in zooxanthellae densities was achieved through (1) starvation, inducing a slow reduction, and through (2) osmotic shock, inducing a rapid reduction.

2. Materials and Methods

Biological material

Colonies of *Stylophora pistillata* (Esper, 1797) were collected during spring 1995 from a 2 m depth on the coral reef fringing Sesoko Island (near the Tropical Biosphere Research Centre, University of the Ryukyus), Okinawa, Japan. These colonies were placed in plastic bags and transported to the research station. They were stored overnight in a 12 m^3 semi-open aquarium supplied with seawater (turnover rate: 5% h^{-1}). At 09:00 h the next day branch tips (4 cm lengths) were broken off each colony and placed in experimental aquaria.

Experimental design

From March to June 1995 three experiments were conducted to assess the influence of: (1) starvation, and (2) osmotic shock with starvation, on zooxanthellae densities, on the photosynthetic capacities and other

physiological parameters of *S. pistillata* branches. A control experiment (3) maintained corals in the same environmental conditions as in (1) and (2), but with food and under constant salinity.

Experiment 1. The starvation experiment

Coral branches, each branch taken from one of three different colonies, were placed into six 1.5 l plastic beakers (2 branches of each colony into every beaker) that were supported on perforated bases. The beakers were supplied with filtered seawater (>1 μm GF/B filters) under 80–90% of the incident photosynthetic active radiation (PAR_0), and maintained under constant aeration. The beakers were placed in an outdoor aquarium supplied with running seawater at 24–26°C. The filtered seawater in the beakers was replaced twice daily, at 08:00–09:00 h and at 18:00-19:00 h. The corals were starved for 40 days. Three random branch-tip samples were taken from the beakers on days 1, 5, 10, 15, 30 and 40.

Experiment 2. Osmotic shock with starvation

Coral branches from three colonies, each branch taken from one of three different colonies, were placed into six 1.5 l plastic beakers (2 branches of each colony into every beaker). The beakers were maintained as in experiment 1 (i.e., supplied with filtered seawater (>1 μm GF/B filters) under 80–90% PAR_0, and maintained under constant aeration) and placed in the same outdoor aquarium as in experiment 1. After 18 days the starved corals were given an osmotic shock by replacing the seawater (34‰) in each beaker with diluted seawater (17‰). On the morning (08:00 h) of the 19th day the diluted seawater was replaced with filtered seawater, and starvation of the corals was continued for an additional 16 days. Analyses of corals were made on days 1, 18 (before osmotic shock), 19 (immediately after osmotic shock), and day 34.

Experiment 3. Control

The number of coral branches and beakers were taken as in experiments 1 and 2. The beakers were supplied with filtered seawater (>1 μm GF/B filters) under 80–90% PAR_0, and maintained under constant aeration. The beakers were placed in an outdoor aquarium supplied with running seawater at 24–26°C. On the first day of the experiment (18:00–19:00 h) cultured rotifers (*Branchionus plicatilis* Muller, 160 μm diameter) were added to the beakers, at concentrations of 10–20 rotifers per 1 ml of seawater. The rotifers were cultured at the Hatchery Center of Okinawa Prefecture at Motobu-cho. The rotifers actively swam during each (12 h) exposure period and polyps appeared to consume 1–2 rotifers nightly (Titlyanov et al., 1999). Every morning (at 08:00–09:00 h) and every evening (at 18:00–19:00 h) the seawater in the beakers was replaced by fresh seawater, and every evening rotifers were added with the

water change. The experiment was maintained for 31 days. Coral samples were taken on days 1, 6, 16 and 31.

Estimating release rates of degraded zooxanthellae particles (dzp) and healthy zooxanthellae (hz)

On days 1, 5, 10, 15, 30 and 40 for experiment 1, and days 1, 18, 19, 20, 21, 22, 23, and 34 for experiment 2, and days 1, 6, 16 and 31 for the control, three coral branches from three different colonies were removed from the experimental beakers and individually placed in 0.5 l glass jars and maintained as described in the experimental design of the experiment 1 from 9:00 to 19:00 h and then the samples were again placed in the experimental beakers (Titlyanov et al., 1996). The released zooxanthellae and their remnants (dzp), were collected and counted twice daily by filtering the water from each jar through a Millepore filter (type JH 0.45 μm). The number of individual dzp and healthy-looking zooxanthellae (hz) were counted directly on the filter paper. According to previous data (Titlyanov et al., 1996), corals released single and sometimes double degraded zoohanthellae particles, because dividing cells were at times subjected to degradation. The relative numbers of daily released zooxanthellae (in % to their stock) are presented in Fig. 10. The real numbers of hz released are given in Fig. 11.

Photosynthetic and dark respiration rates

Photosynthesis of zooxanthellae was measured (at 07:00–10:00 h) as the rate of net O_2 production according to Leletkin et al. (1996), with some modifications. Oxygen flux was measured in a respirometer consisting of a cylindrical glass chamber (400 ml volume) and a Clark oxygen electrode coupled to a chart recorder and a magnetic stirrer. The chamber was sealed with a stopper (preventing any gas exchange with the atmosphere). Temperature was maintained at 25°C in a re-circulating water bath. A halogen lamp (150 W) was used for illumination and PAR (photosynethic active radiation) was selected through a thermal filter (with 2% $CuSO_4$ solution). Light intensity was measured with a Li-Cor radiation sensor (Model Li-192 SB). Quantum flux of PAR in the respirometer was 1300 μmol m^{-2} s^{-1}, (photosynthetically saturating irradiance in the coral branches). The O_2 electrode was calibrated before each measurement (Green and Carritt, 1967). The corals were exposed to 30 minutes of light. Oxygen consumption in the dark was comparable to the dark respiration rate (Leletkin et al., 1996). Respiration rates were measured every 30 min following photosynthesis measurements, and 30 min of dark acclimation. The rates of oxygen production (P_{net}^{max}) under

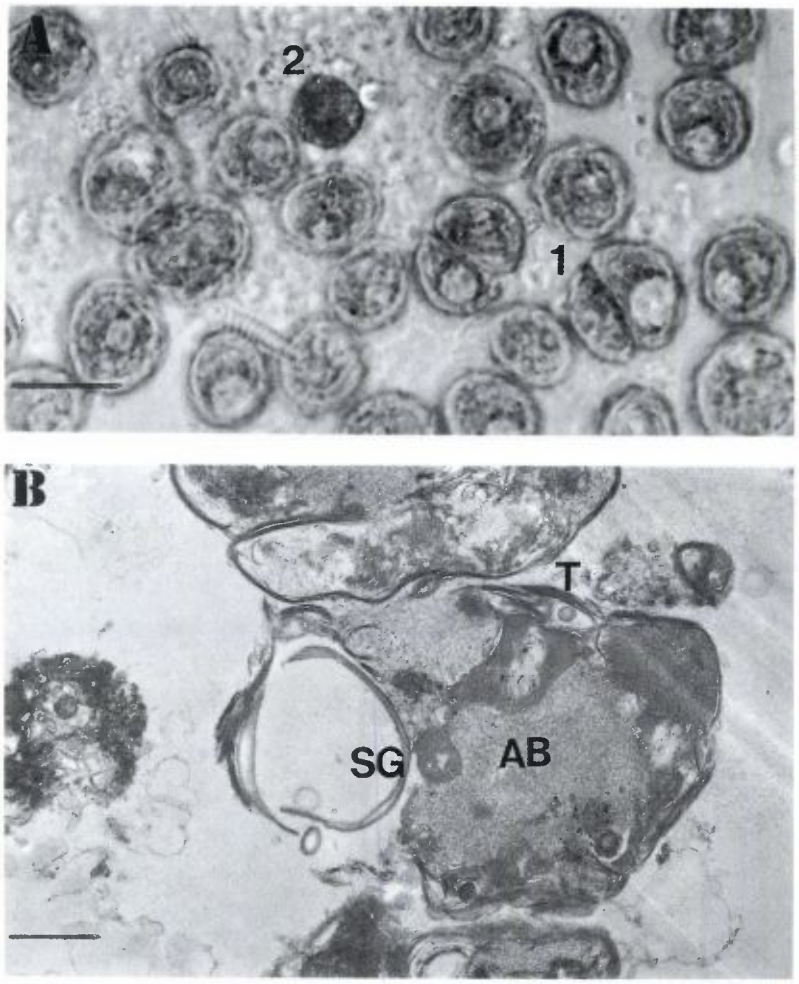

Figure 1. Light and electron micrographs of healthy-looking and degraded zooxanthellae in *Stylophora pistillata*. A – light micrograph of healthy and degraded zooxanthellae in coral tissue under natural conditions, removed from the skeleton at 10.00–11.00 h: (1) dividing zooxanthella; (2) degraded zooxanthella. Bar = 10 μm. B – electron micrograph of degraded zooxanthellae particles released by coral on the 18th day of starvation. AB: 'accumulation body'; T: thylakoids; SG: starch grains. Bar = 1 μm. C – electron micrograph of healthy zooxanthella released by coral on the 18th day of starvation. AB: 'accumulation body'; N: cell nucleus, P: pyrenoid; C: chloroplast; LD: lipid drops. Bar = 1 μm. D – electron micrograph of healthy zooxanthella released by coral the day after osmotic shock. Animal cell remnants are evident on the zooxanthella envelope.

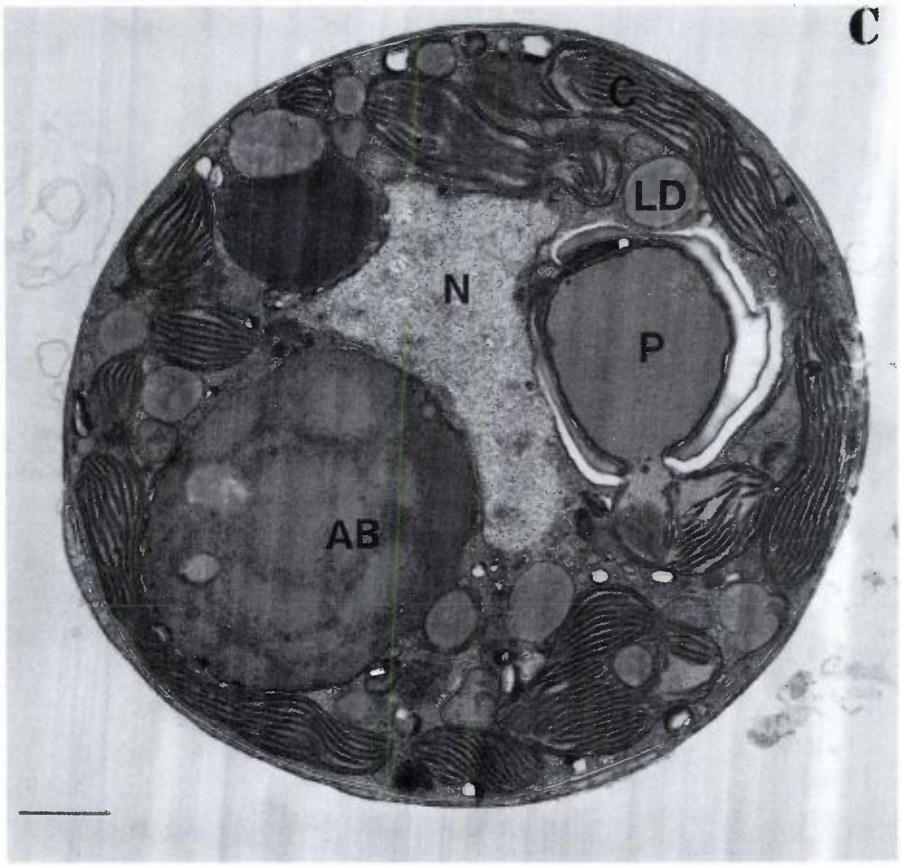

Figure 1. Continued.
Bar = 1 μm. E – electron micrograph of degrading and healthy zooxanthellae released by corals the day after osmotic shock: (1) – zooxanthella in the degradation process, (2) – degraded zooxanthella particle, (3) – healthy zooxanthella with animal tissue on its external membrane. Bar = 10 μm.

saturating light, and oxygen consumption, in the dark (R_d), were calculated per polyp and normalized to 10^6 zooxanthellae. Rates of gross photosynthesis (P^{max}_{gross}) were calculated by summing the P^{max}_{net} and R_d values. Three branches from three different colonies were used in the analysis. The means and standard deviations were calculated using n=3.

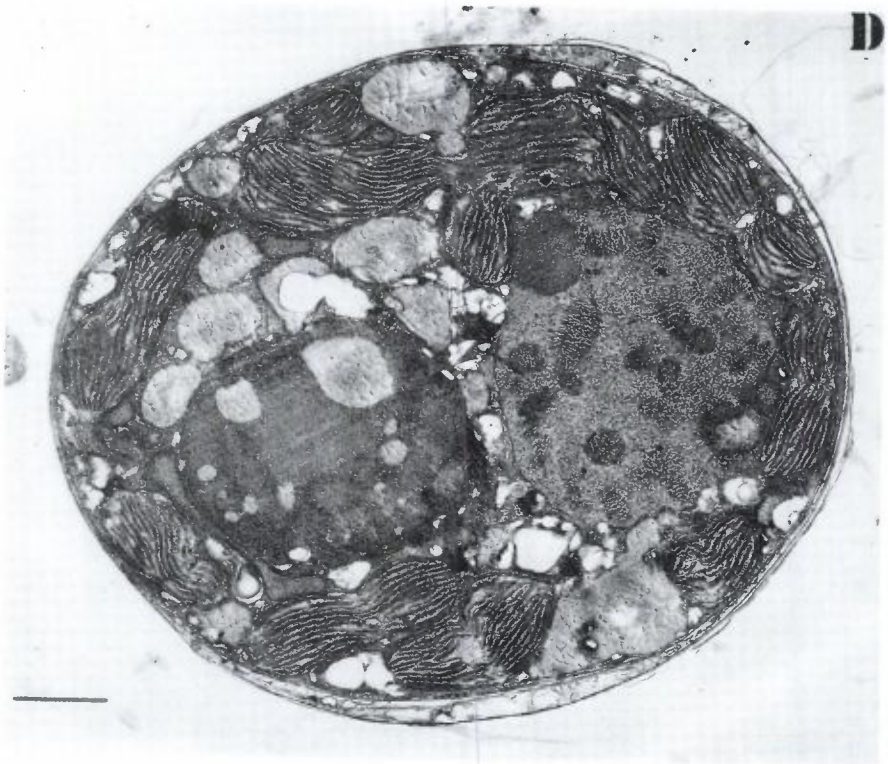

Figure 1. Continued.

Zooxanthellae densities

The densities of zooxanthellae in samples were analysed immediately after measuring rates of photosynthesis and respiration. Coral tissue was removed with a Water-Pik (Johannes and Wiebe, 1970) and the number of algae in the tissue homogenate sample was counted (in10 fields per count) using a hemocytometer. Zooxanthellae density is expressed as number per coral polyp. To make the data in this research comparable with other published results (e.g., normalized to surface area, Falkowski and Dubinsky, 1981), we measured the average number of polyps per cm^2 of skeletal surface area (e.g., the average number of *S. pistillata* polyps per cm^2, at Sesoko Island, was 48±6). Colony surface area was measured using the aluminium foil method (after Marsh, 1970). Three branches from three different colonies were used in the analysis (after measuring rates of photosynthesis and respiration). The means and standard deviations were calculated using n=3.

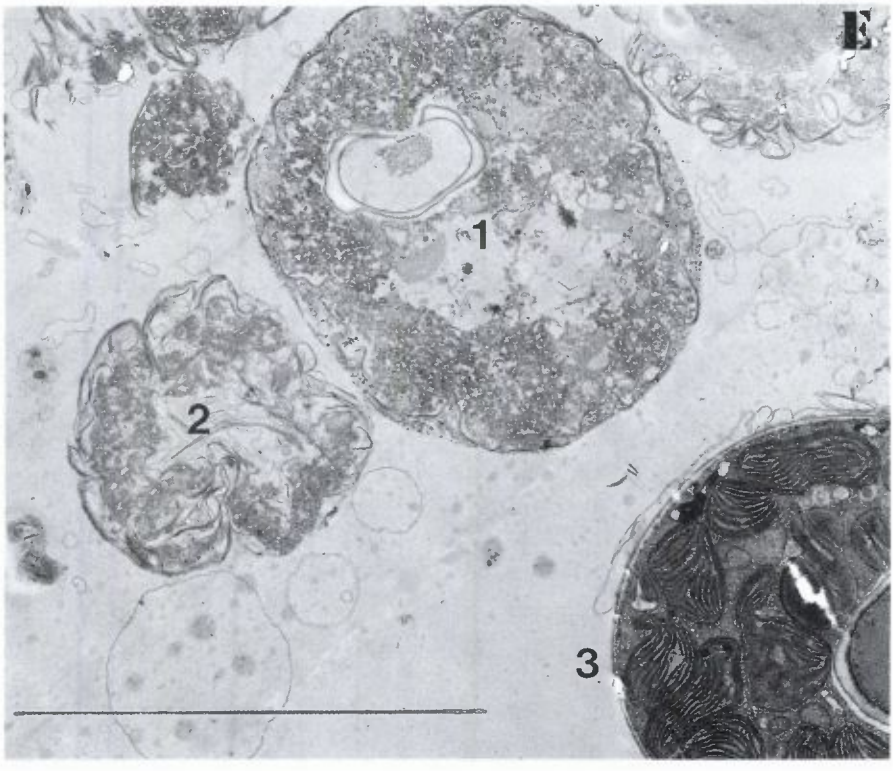

Figure 1. Continued.

Proliferating and degrading zooxanthellae frequency (PZF and DZF)

We measured the proliferating zooxanthellae frequency (PZF) as an indicator of algal division (after Titlyanov et al., 1996). This frequency indicator measures zooxanthellae during cytokinesis or after mitotic division of the nucleus. It differs from the term mitotic index (MI) which measures the frequency of cells undergoing mitotic division of the nucleus (Wilkerson et al., 1983). Therefore, the classification of a proliferating zooxanthellae ranged from the initial appearance of a division furrow in the mother cells, to the formation of their own envelope in the daughter cells.

Tissue homogenates (after a Water Pik) were observed at 400× on a hemocytometer grid. Zooxanthellae were classified as either healthy, dividing or degrading (Fig. 1). Degraded, or degrading zooxanthellae were identified by colour (from orange to dark-brown), size (4–7 m) and irregular shape (Titlyanov et al., 1996). Frequencies were counted between 10:00–11:00 h

when the number of dividing cells amounted to 70–80% of the night maximum (occurring at about 03:00 h) and degraded zooxanthellae numbers were highest (Titlyanov et al., 1996). A total of 500 algae were counted in each homogenate. Three branches from three different colonies were used in the analysis. The means and standard deviations were calculated using n=3.

Chlorophyll concentrations

A known number of zooxanthellae from each sample was filtered under a vacuum (47-mm in diameter AP Millepore filters) and placed in a 90% aqueous solution of acetone and refrigerated for two days. The samples were shaken daily. This method extracts more than 95% of the chlorophyll (unpublished data). The absorbency of acetone extracts was measured at 630 and 663 nm using a Hitachi U-2000 spectrophotometer. Chlorophylls *a* and c_2 of zooxanthellae were determined using the spectrophotometric equations of Jeffrey and Humphrey (1975). Three branches from three different colonies were used in the analysis. The means and standard deviations were calculated using n=3.

Zooxanthellae debris and expelled zooxanthellae (Fig. 1)

The diameters of healthy zooxanthellae were measured with an ocular-micrometer (100 cells for each sample), a light microscope (magnification 400×) and a hemocytometer. Volumes were calculated using the formula for the volume of a sphere. Healthy and degrading zooxanthellae were photographed with a light microscope (×1000). Transmission electron microscopy of healthy and degrading zooxanthellae involved the coral branch tips being: (1) prefixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and 0.5 M NaCl for 2 to 3 h at 4°C; (2) rinsed 3 times with the same buffer saline solution; (3) post fixed in 1% OsO_4 in the same buffer saline solution for 1 h at 0°C; (4) rinsed once with the same buffer saline solution; (5) dehydrated with an alcohol series (50, 70, 90, 99, 100%) for 15 min at each step and at for 1 h at 0°C; (6) embedded with Spurr resin; (7) cut into 70 to 80 nm sections; and (8) stained with uranyl acetate and lead citrate (according to Reynolds, 1963).

Statistical analysis

A "Student´s t-test" was used to analyse the data and to evaluate differences between means. Differences between means with $P<0.05$ was considered significant.

3. Results

Initial state

Branches of *S. pistillata*, taken from a 2 m depth, had the following characteristics: (1) the number of zooxanthellae per polyp ranged from 15×10^3 to 25×10^3, with an average volume of 500–600 mm^3 per cell; (2) zooxanthellae contained from 11 to 16 μg of chlorophylls *a* and c_2 (calculated per 10^6 zooxanthellae); (3) light-saturated gross photosynthesis (P^{max}_{gross}) ranged from 35 to 60 μl O_2 per 10^6 zooxanthellae per h, and gross photosynthesis per polyp per h ranged from 0.7 to 1.4 μl O_2; (4) dark respiration rates (R_d) amounted to one third of the photosynthetic rates, and ranged from 0.3 to 0.5 μl O_2 per polyp per h; (5) of the zooxanthellae in the endoderm, 1–4% were dividing and approximately the same number were degraded cells (at 09:00–10:00 h); (6) coral branches released 2–5% of zooxanthellae stock in a state of dzp and 40 healthy zooxanthellae (hz) per polyp over any 24 h period.

Prolonged starvation experiments (Experiment 1)

Prolonged starvation experiments showed the following physiological changes in *S. pistillata* branches (Figs. 2–11). For the first 18 days, each polyp on average lost about 5000 zooxanthellae, and after the 40 day experiment had lost on average 50% of their zooxanthellae (Fig. 2). The average zooxanthella volume had increased by 1.5 times in comparison with the initial material (Fig. 3), but the chlorophyll concentrations in zooxanthellae had not changed (Fig. 4). By the end of the experiment, the coral's photosynthetic rate (P^{max}_{gross}) per polyp had dropped three-fold (Fig. 6), the photosynthetic capacity of the zooxanthellae and the dark respiration of the coral slightly varied but had not significantly changed (Figs. 5, 7). The experiment showed clear (three-fold) reductions in PZF while the DZF approximately doubled (Figs. 8, 9).

The released degraded zooxanthellae particles (dzp) (Figs. 1A, B) showed an "accumulation body", unpacked thylakoids, starch grains, a pyrenoid starch envelope and other unidentified remnants. The released dzp were surrounded by partly destroyed envelopes. The nuclei (stained with DAPI, as in Titlyanov et al., 1996), pyrenoid protein and lipid drops were lacking. The healthy-looking zooxanthellae that were released from the branches (Fig. 1C) showed no obvious signs of damage to the ultrastructure; all organelles were intact, the cell membrane envelopes were not damaged and there were no conspicuous animal cells attached. During the experiment, on average relative daily number of released degraded zooxanthellae increased 2.5-fold (the relative daily numbers of released dzp were compared at the beginning of the

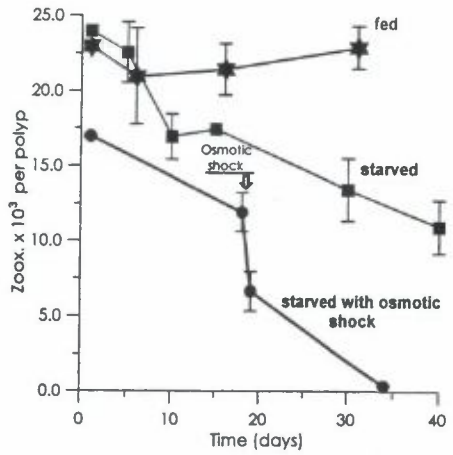

Fig. 2.

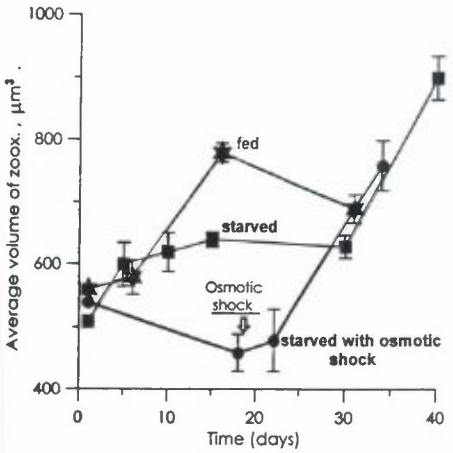

Fig. 3.

Figure 2. Changes in zooxanthellae population density during the experiment. Vertical bars indicate standard deviations. The arrow shows when the salinity was lowered to 17‰ (i.e., osmotic shock). Inscriptions above or under curves as follows: "starved with osmotic shock" is exp. 1; "starved" is exp. 2; "fed" is exp. 3.

Figure 3. Changes in the average volume of the zooxanthellae during the experiments. Other details as in Fig. 2.

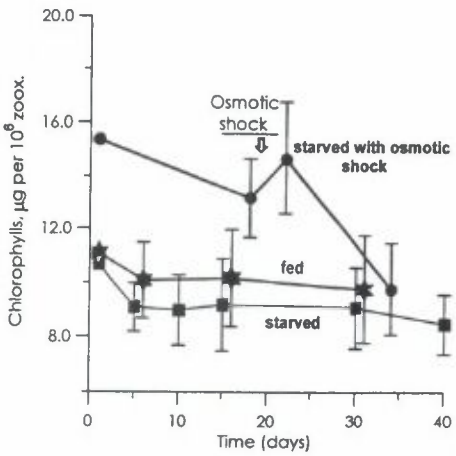

Fig. 4.

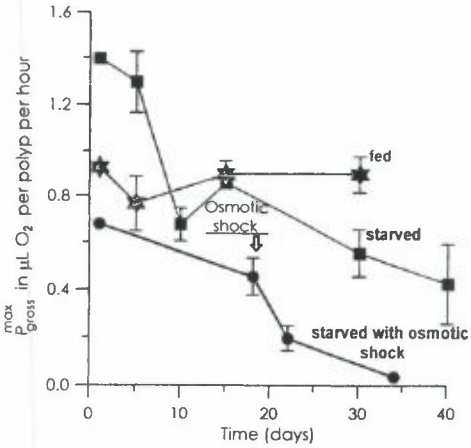

Fig. 5.

Figure 4. Changes in chlorophylls content in zooxanthellae during the experiments. Other details as in Fig. 2.

Figure 5. Change in maximum rate of gross photosynthesis (P^{max}_{gross}), calculated per 10^6 zooxanthellae during the experiments. Other details as in Fig. 2.

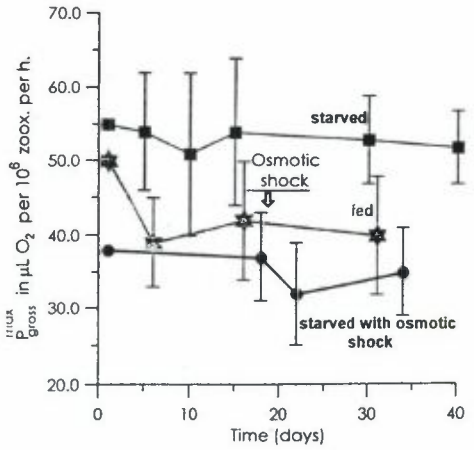

Fig. 6.

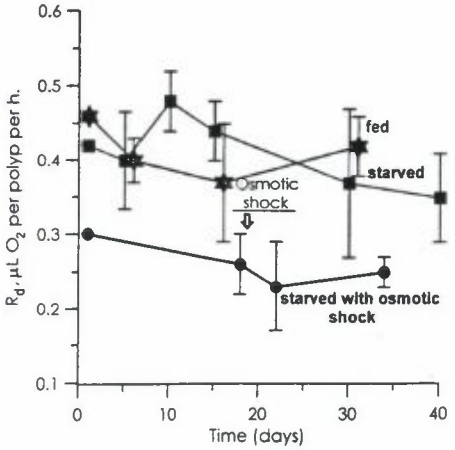

Fig. 7.

Figure 6. Change in maximum rate of gross photosynthesis (P^{max}_{gross}), calculated per average polyp of coral branches during the experiments. Other details as in Fig. 2.

Figure 7. Change in dark respiration rate (R_d) of coral polyps during the experiments. Other details as in Fig. 2.

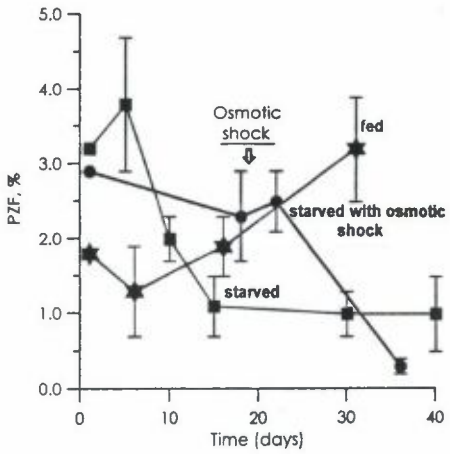

Fig. 8.

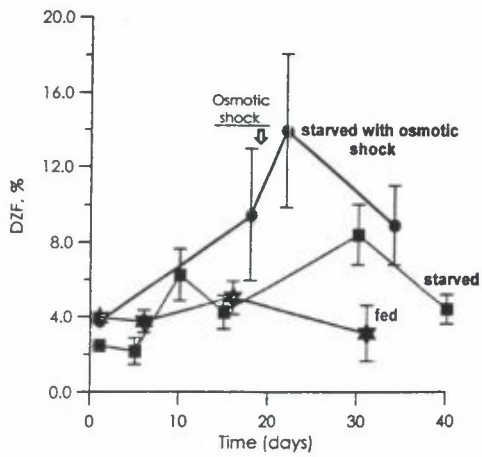

Fig. 9.

Figure 8. Proliferating zooxanthellae frequency (PZF) during the experiments. Other details as in Fig. 2.

Figure 9. Degrading zooxanthellae frequency within the experiments. Other details as in Fig. 2.

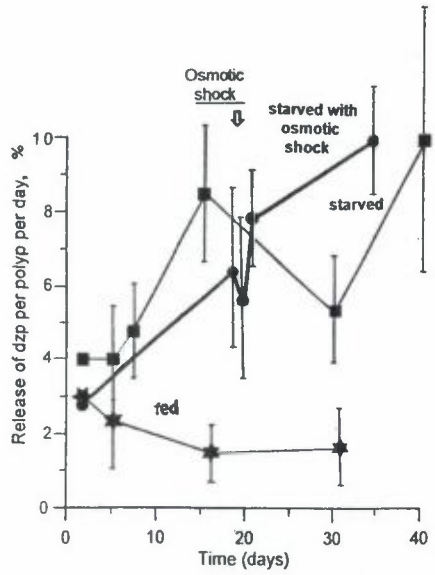

Fig. 10.

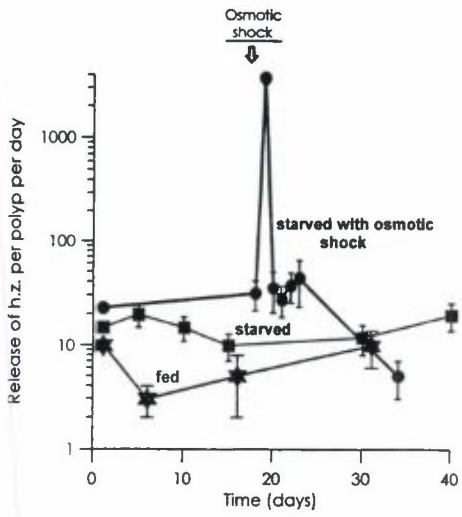

Fig. 11.

Figure 10. Daily release of degraded remnants of zooxanthellae (dzp) by polyps during the experiment in % from the zooxanthellae stock. Other details as in Fig. 2.

Figure 11. Daily release (expulsion) of healthy-looking zooxanthellae (hz) by polyps during the experiments. Other details as in Fig. 2.

experiment and after 40 days of starvation, $P<0.05$) (Fig. 10), at that time as number of released hz only slightly varied (Fig. 11).

Osmotic shock with starvation (Experiment 2)

Changing the salinity from 34 to 17 (i.e., osmotic shock) resulted in the release of, on average, 3000–4000 cells per polyp (Fig. 2; Fig. 11). Zooxanthellae that were released immediately after the osmotic shock appeared quite healthy and had animal cells attached to their envelopes (Figs. 1D, E). The rate of healthy zooxanthellae release decreased by more than an order of magnitude the day after the osmotic shock and then on subsequent 5 days was insignificantly changed. Three days after the shock DZF was not significantly changed in comparison with the level before the shock (Fig. 9), PZF remained virtually the same during the shock and after (Fig. 8). The released dzp were in different stages of degradation, ranging in size from 4 to 8 μm in diameter (Fig. 1E). Relative numbers of released dzp during the pre-shock, shock and post-shock days varied, but not significantly changed (Fig. 10). Post-shock

photosynthesis, calculated per zooxanthella, varied insignificantly (Fig. 5), but the rate of photosynthesis per polyp was reduced to half (Fig. 6), while polyp respiration rates was not changed (Fig. 7).

Starvation of the coral branches for another 16 days after the osmotic shock reduced the number of zooxanthellae to approximately 3% of the initial numbers – 500 zooxanthellae per polyp (Fig. 2). The DZF level remained high (Fig. 9) and the PZF dropped to 0.3% (Fig. 8). The zooxanthellae volume increased significantly ($P<0.05$) (Fig. 3) and the zooxanthellae lost chlorophyll (Fig. 4), but there was no detectable change in their photosynthetic capacity (Fig. 5). Photosynthesis of coral branches continued to drop in parallel with reductions in zooxanthellae densities (Figs. 2, 6). Respiration rates did not noticeably change (Fig. 7).

Feeding experiment (Experiment 3, control)

The control experiment, where *S. pistillata* colonies were fed with rotifers, showed three main changes: (1) there were detectable increases in zooxanthellae volume, (2) the PZF level increased by 50%, and (3) the number of dzp released, per day, was reduced by 50% (Figs. 3, 8, 10) the number of hz released did not change.

4. Discussion

Zooxanthellae population densities in corals (Szmant-Froelich and Pilson, 1984) and in sea anemones (Clayton and Lasker, 1984; Cook et al., 1988) decrease under starvation. We show that starvation led to a gradual loss of zooxanthellae in the coral *S. pistillata*. This was achieved by two means, firstly by increasing zooxanthellae degradation, and secondly by decreasing zooxanthellae division. It was also shown by us in previous experiments (without feeding control with rotifers) (Titlyanov et al., 1996). In the sea anemone *Aiptasia pallida* Cook et al. (1988) also showed that mitotic index decreased from 8% to 2% in the first 20 days of starvation. Healthy zooxanthellae were released during starvation, but this process did not play an essential role in regulating zooxanthellae population density. In our experiments during the first week of starvation, PZF levels did not decrease and DZF levels did not increase. Zooxanthellae did not lose their photosynthetic abilities and chlorophylls and did not reduce their sizes. In our opinion, everything points to the fact that coral polyps under short-term starvation continue to supply zooxanthellae with essential biogenic elements from its own reserves supporting their division, growth and photosynthetic function. The fact that organic nitrogen and phosphorus can be transported from

the host to the symbionts under normal physiological conditions is well-known (Trench, 1979).

It was shown that host cells are capable to destroy zooxanthellae by subjecting them to digestion (zooxanthellae degradation process). Zooxanthellae under degradation lost DNA, protein of pyrenoids and lipid drops. However, it is not clear if polyps are using these substances in their metabolism (Titlyanov et al., 1996). Other studies have shown active symbiont digestion, for example, in the sea anemone *Phyllactis flosculifera* (Steele and Goreau, 1977), in the marine hydroid *Myrionema ambionense* (Fitt and Cook, 1990), and in the polyp stage of the jellyfish *Cassiopeia xamachana* (Colley and Trench, 1985). The intensity of zooxanthellae degradation by polyp changed in dependence on conditions of maintenance of the coral. It was increased under starvation (the present study) or with increasing light intensity (Titlyanov, unpubl. data) and decreased by providing the corals with an *ad lib* supply of zooplankton (Titlyanov et al., 1999) or when light intensity was reduced (Titlyanov, unpubl. data). According to the described above, we can assume that the host is capable of regulating the level of symbiont degradation.

The debatable question is: do hosts regulate the rate of the division of symbionts? Some authors assume the possibility of such regulation. McAuley and Cook (1994) suggested that under starvation the hydroid *Myrionema ambionense* reduced the nitrogen supply to its symbiotic zooxanthellae, in effect restricting cell division. Rees (1991) also suggested that hosts regulate the supply of nutrients to their symbionts. Our 40-day starvation experiments in the coral *S. pistillata* led to a three-fold decrease in the proliferating zooxanthellae frequency (PZF). At the same time no change in the rate of symbiont division within the first week of the starvation was found. This suggests that a starving polyp is capable of regulating the zooxanthellae division intensity only during the first days of starvation by supplying essential biogenic elements to the zooxanthellae from its own reserves.

A decrease in salinity to 17‰ induced an immediate and short-term expulsion of healthy-looking zooxanthellae. The polyps expelled the zooxanthellae together with elements of their own cells. This expulsion has been classified as a type of necrosis or "a pinching off" of the host cell (Glider, 1983; Gates et al., 1992). Brown et al. (1995) reported a similar response of the corals *Favites abdita* and *Goniopora pandoraensis* to high sea surface temperatures. Similarly, Steen and Muscatine (1987) described exocytosis of zooxanthellae when the anemone *Aiptasia pulchella* was subjected to extremely low temperatures (4°C), but in this case the algal cells were released without host cell elements. In our experiments the released zooxanthellae showed no apparent damage of their internal structure, suggesting that the osmotic shock primarily affected the animal components of the coral, but not

the zooxanthellae. Polyps returned to their normal zooxanthellae release rates the day after the osmotic shock (i.e., 20–40 zooxanthellae per polyp per day). Released zooxanthellae appeared healthy throughout the experiments, and only immediately after the osmotic shock did the zooxanthellae cells support animal cells on their envelopes. Normal daily zooxanthellae expulsion is therefore most likely via exocytosis (Steen and Muscatine, 1987).

5. Conclusion

- Regulation of zooxanthellae densities in colonies of *S. pistillata* under starvation occurs by increasing the rate of zooxanthellae degradation and by decreasing the rate of zooxanthellae division. The release of healthy zooxanthellae from corals takes place, but does not play any essential role in the regulation of the symbiotic algal population density.
- Under osmotic shock *S. pistillata* corals expelled healthy-looking and morphologically unchanged zooxanthellae, together with elements of their own animal cells. The shock appeared to mainly influence the animal component of the polyp. Zooxanthellae degradation and division are not the mechanisms regulating the symbiont population densities under osmotic shock.
- On the basis of the results obtained we suggest that without animal food the polyp continues to supply zooxanthellae with nutrients from its own reserves. As a result, the zooxanthellae divide, grow and remain functionally active under starvation.
- Zooxanthellae densities in corals are a consequence of differential rates of zooxanthellae division and degradation, and only under extreme conditions are zooxanthellae population densities regulated by expulsion of healthy zooxanthellae.

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