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Rhythmicity in Division and Degradation of Zooxanthellae in the Hermatypic Coral *Stylophora pistillata*

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Abstract

Three types of rhythmic changes in division and degradation of symbiotic dinoflagellates-zooxanthellae were found in the scleractinian coral *Stylophora pistillata* from the fringing reef of Sesoko Island, Okinawa, Japan. The first type of phased change in proliferating zooxanthellae frequency (PZF) and degrading zooxanthellae frequency (DZF) indices is a diurnal cycle with a period of 24 h. The second type of the phased change in PZF and DZF levels is rhythmicity with a period of 3 days. The rhythmicity of these changes was disrupted by changes in the weather conditions (from rainy to sunny days and conversely) and by the sharp changes in irradiation during the experiment. The third type of rhythmic change in the levels of zooxanthellae division and degradation is a rhythmicity with a period of 6–7 days. Sharp changes in daily light intensity did not disrupt 6-days cycles. These changes in the PZF and DZF levels of the second and third types of rhythmicity occurred in an opposite trend. Amplitudes of changes in both processes are depended on the light intensity. Bright light (95% PAR_0) stimulates both the division and degradation of zooxanthellae. We suggest that all three types of rhythmic changes in zooxanthellae division and degradation are the reactions that regulate algal population density in symbiotic corals.

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1. Introduction

Symbiotic dinoflagellates-zooxanthellae, which are living intracellularly in marine invertebrates including reef-building corals, display daily rhythmicity in the frequency of division of their own cells (Patton and Burris, 1983; Smith and Hoegh-Guldberg, 1987; Cook et al., 1988; Hoegh-Guldberg and Smith, 1989; Fitt and Cook, 1990; Hoegh-Guldberg, 1994; Titlyanov et al., 1996a, 2000a; Smith and Muscatine, 1999; Fitt, 2000). Patton and Burris (1983) found a peak of zooxanthellae division in *Stylophora pistillata* at night hours. According to Hoegh-Guldberg (1994), cell division of zooxanthellae in tissue of the coral *Pocillopora damicornis* was phased with a peak of dividing cells occurring within 00:00 and 06:00 h. In the tissue of *Seriatopora hystrix*, time of division was found between 04:00 and 06:00 h (Hoegh-Guldberg and Smith, 1989).

Degradation of zooxanthellae (Fitt and Cook, 1990; Titlyanov et al., 1996a) exhibited diurnal rhythms. Fitt and Cook (1990) found a peak of accumulation of dead or moribund zooxanthellae in tissue of the hydroid *Myrionema ambionense* at midnight. Fitt (2000) reported that changes in a number of unhealthy looking zooxanthellae (in a phase of digestion or degradation) seen in digestive cells of *Myrionema ambionense* also followed a diel periodicity. As was previously shown (Titlyanov et al., 1996a) for the scleractinian coral *Stylophora pistillata*, peaks of zooxanthellae degradation (digestion) were detected at the second part of the nighttime (03:00–04:00 h) and duration of this process was amounted to about 16 h. The extrusion of degraded zooxanthellae remnants took place from 14:00 to 18:00 h. Zooxanthellae in some cnidarians displayed diel rhythmicity in expulsion of healthy looking zooxanthellae (e.g., Reimer, 1971; Hoegh-Guldberg et al., 1987).

The mechanisms evoking changes in diel rhythmic division and degradation of algal symbionts are not clear. It was shown that light and feeding are two environmental factors that are responsible for diurnal rhythmic changes in division of zooxanthellae and host cells in cnidarians (Fitt, 2000). Nitrogen pulses are known to affect the timing and amount of algal division (Doyle and Poore, 1974). At the same time, continuous addition of dissolved nitrogen in high concentrations to the coral *Pocillopora damicornis* caused a damping of diel peaks in zooxanthellae mitotic index (Hoegh-Guldberg, 1994). It was also shown that diel periodic changes in division and degradation of zooxanthellae are mechanisms, which regulate symbiont population in hermatypic corals (Titlyanov et al., 1996a, 2001c).

Although diurnal phased changes in division and degradation of symbiotic algae in cnidarians are well known and relatively well studied, a long-term periodicity with a period of more than 24 h in these organisms was only mentioned (Titlyanov et al., 1996a, 2000a, 2001b). Changes in PZF of the coral *Stylophora pistillata* from the fringing reef of Sesoko Island were phased with a 3-days period (Titlyanov et al., 2001b, 2002), while changes in mitotic index of zooxanthellae in the coral *S. pistillata* from the Gulf of Eilat, were phased with a period of time around 10 days (Titlyanov et al., 2000a). It was shown (Titlyanov et al., 1996a) that the corals *S. pistillata*, *Seriatopora caliendrum* and *Porites horizontalata* had not only diurnal rhythms in the release of degradated zooxanthellae particles but the rhythmicity with a 3-days time-period.

In the present study, we continued the investigation of the rhythmic long-term changes in the intensity of zooxanthellae division and degradation in the hermatypic coral *S. pistillata*. The study was focused on two main tasks: 1) to confirm the existence of long-term cycles in the division and degradation of zooxanthellae (or to display new one) by daily measurements of maximum PZF and DZF indices within four weeks, and 2) to study the dependence of these (zooxanthellae division and degradation) cycles upon light intensity in nature and experiments.

2. Materials and Methods

Collection site and biological specimens

Coral colonies of *Stylophora pistillata* Esper, 1797 were collected in September, 1997 from the fringing reef of Sesoko Island (Tropical Biosphere Research Center), University of the Ryukyus, Okinawa, Japan. All samples were collected from the unshaded sites at a depth of 2 m. Samples were stored in 12-m^3 outdoor aquarium supplied with running seawater until the beginning of the experiments. The experiments were conducted from September, 1997 to February, 1998. At that time, the temperature of seawater did not exceed 27°C and incident surface photosynthetic active radiation (PAR_0) was not more than 1900 μmol m^{-2} s^{-1}. These conditions allowed to avoid the negative effects of high temperatures and high light intensities on zooxanthellae (Müller-Parker, 1984) and to exclude coral bleaching (Brown, 1997; Titlyanov et al., 2001c).

Experimental design

Five colonies of *S. pistillata* (close in size, morphology and color) were used in the experiments. External coral branches of about 5 cm length were detached

from the colonies, fixed with cement to ceramic tile pieces in their natural orientation to light and placed into the same aquarium. In October 1997, the temperature in the aquarium was 25–27°C during the day time and 23–24°C at night. In February 1998, the temperature was 22–24°C during the day and 20–22°C at night. Nutrients and zooplankton concentrations in the aquarium were similar to natural seawater which was pumped directly from a depth of 2 m of the fringing reef without filtration and settling (Titlyanov et al., 2001a). Seawater in the aquarium was intensively aerated; water exchange was approximately 30% per hour. The aquarium system was partly shaded by black plastic mesh to reduce light intensity to 30% PAR_0. In open part of the aquarium, light intensity was 80–95% PAR_0.

After maintenance of the coral branches in 95% and 30% PAR_0 for no less than 2 months, the samples were used in further experiments for measuring PZF and DZF. Every day during the experiment, we registered weather conditions and measured PAR_0 at midday within 12:00–14:00 h.

Analytical procedures

Coral tissue was removed from the coral branch and homogenized with a Water-Pik (Johannes and Wiebe, 1970). Tissue homogenate of each sample was observed freshly with optical microscopy at 400× on a hemocytometer grid. A total of 500 to 1000 cells was counted in each homogenate sample and the percentage of zooxanthellae dividing was classified as proliferating zooxanthellae frequency (PZF) and the percentage of zooxanthellae degrading was classified as degrading zooxanthellae frequency (DZF) according to Titlyanov et al. (1996a). Cells were classified as dividing if they showed the initial appearance of a division furrow in the mother cells, to the formation of cell envelopes in daughter cells. Degrading cells were identified by color (from orange to dark brown), size (4–7 μm) and irregular shape. The means and standard deviations were calculated on a basis of three tissue homogenates extracted from three branches. The frequencies were counted between 09:00 and 10:00 h at time when the number of dividing zooxanthellae in *S. pistillata* amounted to approximately 80% of maximum and the number of degradated zooxanthellae was the highest (Titlyanov et al., 1996a).

Light intensity was measured on the water surface in the open part of aquarium and near the tops of coral branches in both the open and shaded parts of the aquarium with a LI-COR radiation sensor (Model LI-192 SB). The measurements were performed three times a day: at 9–10 h, 13–14 h and 17–18 h. Relative light intensity near the corals was calculated from these measurements and represented in percents of PAR_0. Daily average relative light intensity was calculated on the basis of these three measurements.

Table 1. Summary of results for correlation analysis between PZF and DZF values in the scleractinian coral *Stylophora pistillata* under the different light conditions

Source of correlation	Light treatment (PAR_0)	Correlation relationship
PZF	95% and 30%	$r = 0.72^*$:$PZF_{95\%PAR}=0.93+0.98\times PZF_{30\%PAR}$
DZF	95% and 30%	$r = 0.82^*$:$DZF_{95\%PAR}=0.57+0.51\times DZF_{30\%PAR}$
PZF×DZF	95%	$r = -0.76^*$:$PZF_{95\%PAR}=5.46-1.19\times DZF_{95\%PAR}$
PZF×DZF	30%	$r = -0.65^*$:$PZF_{30\%PAR}=3.51-0.46\times DZF_{30\%PAR}$

* Significant at the level of $p<0.05$.

Statistical analysis

Differences among mean values were tested for significance by the t-test for dependent samples. Probability for type I errors was set to a=0.05. The influence of internal rhythmicity, weather conditions and PAR_0 level on PZF and DZF was assessed by multivariate analysis of variance (MANOVA) hypothesis tests. Portion of the influence of factors was analysed with the Snedekor function (Snedekor, 1961). The significance of values was evaluated by the Fisher test at the p=0.05 level. The periodicity of the rhythm was analysed with the Spectral Fourier test (SFT) using Time Series/Forecasting module. Computations were done using "Statistica" program for Windows version 5.0 (Stat. Soft. Inc., Cary, NC, USA).

3. Results

Time Series analysis of daily measurements of PZF and DZF levels in the coral branches of *S. pistillata* was conducted from 16 to 22 December, 1997 at 30% PAR_0 and from 20 to 26 January, 1998 at 95% PAR_0 (Fig. 1). Mean values for the both PZF and DZF levels were significantly (t-test: $p<0.05$) different between the 95% and 30% PAR_0 treatments during a day. Correlation analysis demonstrated that PZF and DZF levels were positively correlated with photon irradiance (PZF: $p<0.05$; DZF: $p<0.05$, Table 1). There was a strong negative correlation ($p<0.05$) between both PZF and DZF values at the same light conditions, independently on the irradiance treatment (Table 1). These results suggest a significant ($p<0.05$) simultaneous increase in the frequency of cell division and reduction in the algal degradation, and vice versa.

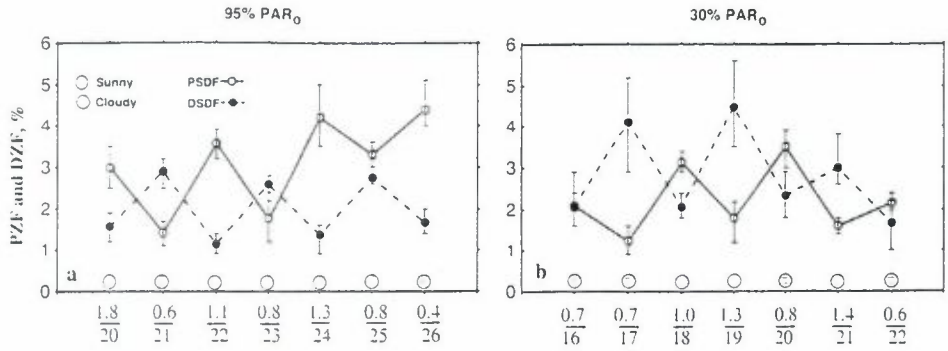

Figure 1. Representative daily measurements of the average means of PZF and DZF in the coral *Stylophora pistillata* (one of five experiments): a - samples acclimated to 95% PAR_0, the measurements were performed from 20.01.98 to 26.01.98; b - samples acclimated to 30% PAR_0, the measurements were carried out from 16.12.1997 to 22.12.1997. Numerals on axis of abscissas: above the line - an average means of irradiance (PAR_0) at midday in μmol m^{-2} s^{-1} 10^{-3}, under the line - dates of analysis. Symbols above the axis of abscissas: open circles - weather was sunny most of the day, PAR_0 was from 900 to 1700 μmol m^{-2} s^{-1} at midday; striped circles - weather was cloudy most of the day, PAR_0 was from 400 to 800 μmol m^{-2} s^{-1} at midday; closed circles - weather was rainy most of the day, PAR_0 was from 300 μmol m^{-2} s^{-1} and lower at midday.

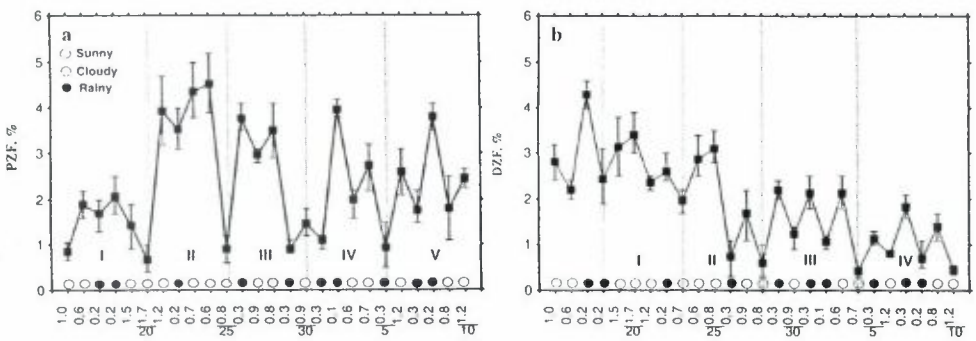

Figure 2. Representative daily changes in the average means of PZF (a) and DZF (b) in the coral *Stylophora pistillata*, acclimated to 95% PAR_0 from 15.11.1997 to 10.12.1997 (one of two experiments). Numerals on axis: under the line - days in November and December, 1997. The rest of indices are as in Fig. 1.

MANOVA showed significant ($p<0.01$) effects of 3-days rhythmicity and photon irradiance on the PZF values. Snedekor's test indicated that 3-days rhythmicity and PAR_0 level provided 52% ($p=0.00000$) and 18% ($p=0.0007$) of

PZF variation, respectively. The DZF level exhibited significant (MANOVA: $p<0.01$) effects of 3-days rhythmicity and photon irradiance that provided 63% (Snedekor's test: $p=0.00000$) and 18% (Snedekor's test: $p=0.00006$) of DZF variation, respectively.

Daily measurements of PZF and DZF levels in *S. pistillata* within a period of 4 weeks at 95% PAR_0 were conducted from 14.11.1997 to 10.12.1997. At that period the weather conditions changed sharply: continuous rainy weather alternated by sunny weather for 1–2 days (Fig. 2). Significant (SFT: $p<0.05$) rhythmic long-term changes in zooxanthellae division and degradation with a period of 6–7 days were observed (Fig. 2). Periodic changes in DZF usually showed an opposite trend to the changes in PZF. In this experiment, saw-edged curve of rhythmic changes in the PZF level with a period of 3 days was broken sometimes after the sharp change-over in the weather conditions. It was found in the first (I) and second (II) 6-days cycles of rhythmic changes the next day after the weather was changed from a rainy to sunny one or vice verse (Fig. 2a). Regularity of rhythmic changes in the DZF level with a period of 3 days was also broken under the sharp changes in the weather conditions.

Experiments with the transference of corals from high to dim light and the reverse were conducted from 05.02.1998 to 16.02.1998. At this period, cloudy weather prevailed. The transfer of coral branches from high to dim light and vice verse showed that sharp and significant changes in the light intensity affected the division and degradation of zooxanthellae on the following day of the experiment (Fig. 3). Transfer from 95% to 30% PAR_0 resulted in an increase in the PZF level and a decrease in the DZF level in *S. pistillata* (Fig. 3a). Transfer from 30% to 95% PAR_0 resulted in an opposite change: PZF decreased and DZF increased (Fig. 3c). On the consecutive (the 5th and 6th) days of the experiment, the PZF and DZF levels varied insignificantly ($p>0.05$). In control, when coral branches were subjected to 95% and 30% PAR_0, the saw-edged character of changes in the PZF and DZF levels was observed from day to day (Fig. 3b, d). The PZF and DZF levels exhibited significant (MANOVA: $p<0.05$) effects of 3-days rhythmicity and photon irradiance that provided 80-86% (Snedekor's test: $p<0.05$) and 64–85% (Snedekor's test: $p<0.05$) of PZF and DZF variations, respectively.

4. Discussion

Our previous (Titlyanov et al., 1996a, 2000a, 2001b, 2002) and the present studies show that division and degradation of symbiotic algae (zooxanthellae) of the coral *Stylophora pistillata* are phased and have at least three different types of periodicity. The first type of periodic changes are daily changes in frequency of the division and degradation of symbionts.

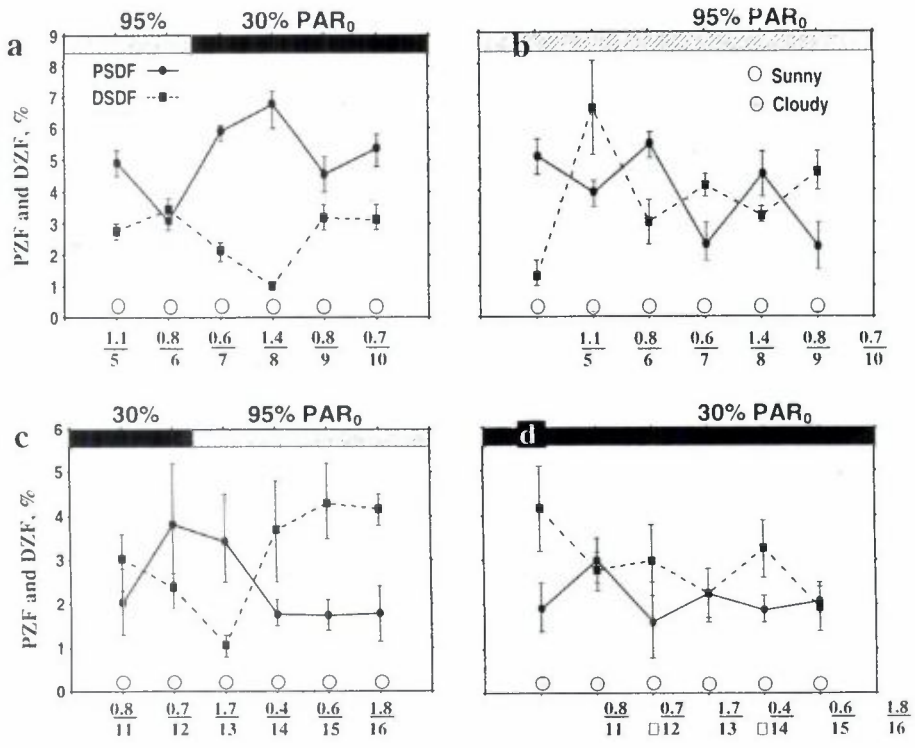

Figure 3. The representative measurements of the average means of PZF and DZF in *Stylophora pistillata* under changes in light regime: a - from 95% to 30% PAR_0 (one of three experiments); b - under maintenance in 95% PAR_0 (one of five experiments); c - under changes in light regime from 30% to 95% PAR_0 (one of three experiments); d - under maintenance in 30% PAR_0 (one of five experiments). Numerals on axis of abscissas: under the line - days in February, 1998. The rest of indices are as in Fig. 1.

In *S. pistillata*, these processes took place in all endodermal cells containing zooxanthellae (Titlyanov et al., 1996a). Analogous daily rhythms of the cell division with a maximum appearance of paired cells were also found in the marine free-living dinoflagellates *Gonyaulax polyedra* (Sweeney and Hastings, 1958). The authors showed that rhythm of the cell division persisted in continuous dim light for a few days with a progressive reduction in the amplitude. The period of the cell division rhythm in free-living dinoflagellates was 24 hours in light-dark regime and in continuous dim light. Consequently, daily rhythms of the cell division both in free-living dinoflagellates (Sweeney and Hastings, 1958) and in marine macroalgae

(Makarov et al., 1995; Titlyanov et al., 1996b; Lüning et al., 1997) are related to a basic metabolic circadian rhythm, which is regulated by the endogenous biological clock (Chisholm, 1981). Unfortunately, insufficient study of the processes of symbiotic division and degradation in corals so far does not allow to identify the daily rhythms of changes in the PZF and DZF levels as circadian rhythms.

Previously it was shown that daily rhythmic change in the frequencies of zooxanthellae division and degradation in *S. pistillata* is the main mechanism of regulation of symbiont population density in the coral tissue (Titlyanov et al., 1996a, 1999, 2000b, 2001b, 2002). It provides constancy in a steady state of the symbionts and during acclimation to the changed environmental conditions, especially to light intensity and zooplankton supply. In a case of relatively stable zooxanthellae density (steady state) during week or more, the ratio of the average diel maximum PZF value to the average maximum DZF value (within the period) is close to 1 (Titlyanov et al., 1996a). During acclimation of corals to low light (after transfer the corals from 90% to 30% PAR_0), the ratio PZF to DZF reached to 2 or higher the next day after the transfer (by increasing a diel PZF level and decreasing diel DZF level) and was higher than 1 during 40–60 days up to the complete photo-acclimation of corals to low light (Titlyanov et al., 2001a). With the reduction of the number of zooxanthellae, a decrease in the ratio PZF to DZF up to 0.2 and lower occurs. This phenomenon was found, for example, under the starvation of hermatypic corals (Titlyanov et al., 2000b).

The second type of rhythmic changes in the PZF and DZF indices in *S. pistillata* with a period of 3 days was presented by oscillation of the PZF and DZF levels from high to low and than again to high level (saw-edged curve). Furthermore, if the PZF level was increased from day to day, the DZF level was decreased. A similar regularity with a period of a few (mostly 3) days was shown in the pattern of extrusion of dividing symbionts in *Aiptasia tagetes* (Steele, 1976). The opposite changes in the intensities of the processes of zooxanthellae division and degradation also depend on the process of regulation of the symbiont density in the host tissue. A daily increase in zooxanthellae population density through an increase in the rate of cell division may evoke the digestion of excessive zooxanthellae the next day. However, this periodic daily oscillation of PZF and DZF occurred only at insignificant changes of the light conditions from day to day (Fig. 1). Sharp and significant changes of the coral exposure to other light intensities were able to break the saw-edged curve of daily shifts in zooxanthellae division and degradation. Disruption of the saw-edged curve of the PZF and DZF oscillation took place the next day after sharp changes in daily light intensities (Fig. 2) and also after transfer of the corals from bright to dim light or vice verse (Fig. 3).

The third type of rhythmic changes in the levels of zooxanthellae division and degradation is a long-term rhythm with a period of 6–7 days (Fig. 2). Similar rhythmicity with a period of 10 days (but only for zooxanthellae division) was observed in previous study (Titlyanov et al., 2000a). Maximum in the PZF level coincided with the minimum in the DZF level in the 6–7-days cycles. Thereby, we suggest that zooxanthellae density is decreased in the first part of the cycle when corals showed an increase in the DZF level and decrease in the PZF level, but recovered again in the second part of the cycle with a reduction in the DZF level and an increase in the PZF level.

It is well known that in nature, physiological processes of plant cells are often phased with a period longer than day-night: seasonal or annual cycles (Lüning and Kadel, 1993; Sweeney, 1987), monthly, tidal, lunar and semilunar rhythms (Sweeney, 1987; Jokiel et al., 1985). Seasonal rhythms of growth of marine algal cells were synchronized by daylength cycles of the natural year (Lüning and Kadel, 1993). Periodic changed factors such as solar activity (period of 27 d), lunar light (lunar month 29.5 d), spring tide (period of 29 d); even cyclic monthly variations in the geomagnetic field can be a trigger in the monthly biological cycles (Jokiel et al., 1985; Sweeney, 1987). There are also semilunar rhythms (with a period of 14.8 d) in marine plants and animals for those inhabiting the intertidal zone (Sweeney, 1987). This rhythm depends on the low tide ('neap tides'). Sometimes, rhythms, which are shorter than 'semilunar' ones, were detected. Thus, Ziegler-Page and Kingsbury (1968) and Ziegler-Page and Sweeney (1968) found that the interval between the formation of gametangia in the green alga *Halicystis parvula* was usually 4–5 days when the growth conditions were optimal. Unfortunately, the authors did not suggest any mechanisms for regulation of this rhythmic process.

In our speculative supposition, rhythmic changes in the division and degradation of zooxanthellae in the scleractinian coral *S. pistillata* with a period of 3 and 6–7 or 10 days are determined not by the external factors but according the internal causes – by effects of the animal component (coral polyp) on the vital functions of algal symbionts. It is known that coral is able to regulate the number of zooxanthellae, killing and digesting the part of them and also capable to regulate the intensity of the zooxanthellae photosynthesis, deflux of photosynthetic products and cell division (reviews of McAuley, 1994; Titlyanov, 1999).

As it was shown in this study and in previous papers (Titlyanov et al., 2000a, 2001b), at least two detected rhythmic changes in PZF and DZF in *S. pistillata* depended on the intensity of incident light. Amplitude of daily phased changes in the PZF and DZF values markedly decreased with the reduction in light intensity and under conditions maintenance of corals in the lowered light intensity. Sometimes, the phased diel changes in PZF were not displayed under the low light intensities in the habitats (Titlyanov et al., 2000a). In the

present study, the peaks in 3-days cycle of rhythmic changes in zooxanthellae division and degradation were significantly higher in coral branches acclimated to 95% PAR_0 compared with those acclimated to 30% PAR_0. At the same time, the transfer of corals from dim (30% PAR_0) to high (95% PAR_0) light led to a temporary drop in the zooxanthellae division and to a temporary increase in the zooxanthellae degradation during the following days, especially the day after transfer. The decrease in the light intensity led to the converse processes. In our opinion, the decreased levels of zooxanthellae division and degradation in corals acclimated to low light indicate the relatively lower metabolic activity of shaded corals compared to those exposed to high light. It is confirmed by the decrease in the rate of dark respiration and the level of excretion of organic substances in corals during the acclimation to low light (Crossland, 1987; Titlyanov et al., 2001a). At the same time, a temperate increase in the level of symbiont division and a decrease in the level of their degradation during the transfer of corals from bright to dim light is a mechanism of regulation of zooxanthellae density in the animal tissue.

In conclusion, we show that zooxanthellae of the scleractinian coral *Stylophora pistillata* exhibited not only daily rhythms of the cell division and degradation, but also two other rhythmicities of these processes: diurnal oscillation of the PZF and DZF levels from high to low level on the consecutive days (3-days period) and a long-term rhythmicity with a period of 6–7 days. Changes in the PZF and DZF levels occurred in an opposite trend. Amplitudes of changes in both processes are depended on the light intensity. Bright light (95% PAR_0) stimulates both the division and degradation of zooxanthellae. Sharp change-over in the light regime from high to low irradiation temporally stimulates cell division of symbionts and depresses their digestion (degradation). We suggest that all three types of rhythmic changes in zooxanthellae division and degradation are the reactions that regulate algal population density in symbiotic corals.

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