D-4.1.1 Effects of environmental stresses on the Zooxanthellae (Final report)

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Abstract Effects of main environmental factors, temperature, irradiation, salinity and inorganic nitrogen concentration on the growth or photosynthetic activities of two strains of zooxanthellae cultures were investigated.

The algal growth rates were reduced greatly when the irradiation reduced from 6 to 3 $\mu E/m^2/s$. The algae had maximum growth rate at 32℃ but the photosynthetic activities were h igher at 28℃ than at 32℃. The optimum salinity for these algae were 30 but they had comparable activities at 25 as much as at 35. Temperature and irradiation influenced the algal tolerance against low salinity.

Effects of nitrogen enrichment on the growth rate and photosynthetic activities of two strains of zooxanthellae culture were investigated. A low concentration DIN enrichment (2 μM/day as $NH4^+$) increased their growth rates and cellular chlorophyll a contents but it had no effect on their photosynthetic activity. Cellular chlorophyll a contents increased as the increasing DIN concentration up to 20 mM/day but photosynthesis per cell did not increase over 5 μM/day DIN enrichment.

Key Words Coral, Zooxanthellae, Environmental stress, Eutrophication

Introduction

There are many environmental stresses to corals such as the elevation of seawater temperature by the El Nino, reduction of irradiation by solid suspensions or low salinity by the land off water, changes of inorganic nutrients concentrations by the eutrophication of the seawater. The purpose of this study was to define the influences of these environmental factors on the coral activities. As a coral is a complex of a cnidarian animal and a symbiotic dinoflagellate; zooxanthellae, it is needed to determine the effects of the factors on each organisms. In this study, we investigated the responses of algal biotic activities against the considered environmental stresses with cultured symbiotic algae isolated from hermatypic corals in vitro to exclude the influences of host animals.

Materials and Methods

Maintenance of dinoflagellate cultures

The symbiotic algae were isolated from the Hawaiian hermatypic coral *Pocillopora damicornis* and *Montipora verrcosa* and kindly offered by Dr. Kinzie. They were cultured in 20ml of ESM medium at 24 ℃ , with illumination provided by cool-white fluorescent tube at 20 $\mu E/m^2/s$ on a 14hL:10hD cycle.

Effects of temperature, light, and salinity

Growth experiments were set up using the algae precultured for 2 weeks at 28 ℃, with

illumination at 10 $\mu E/m^2/s$. The algae were inoculated to 24-well multiwell plate and incubated under various temperature (24-36 ℃), light (6-24 $\mu E/m^2/s$) and salinity (5-35) conditions. The cell numbers in wells were checked daily under an inverted microscope and the cells were harvested at the middle of exponential growth period with a scraper.

Growth rates were determined by the increase of cell numbers counted with a hemacytometer. The photosynthetic activities were determined from the cellular fluorescence capacity (FRI)using photosystem 2 inhibiter DCMU.[1] The cellular chlorophyll a contents were determined from the chlorophyll a concentration in the algal cell suspension[2] and the cell density determined with a hemacytometer. The percentages of motile cell were determined under inverted microscope. All experiments were done at the middle of the light period.

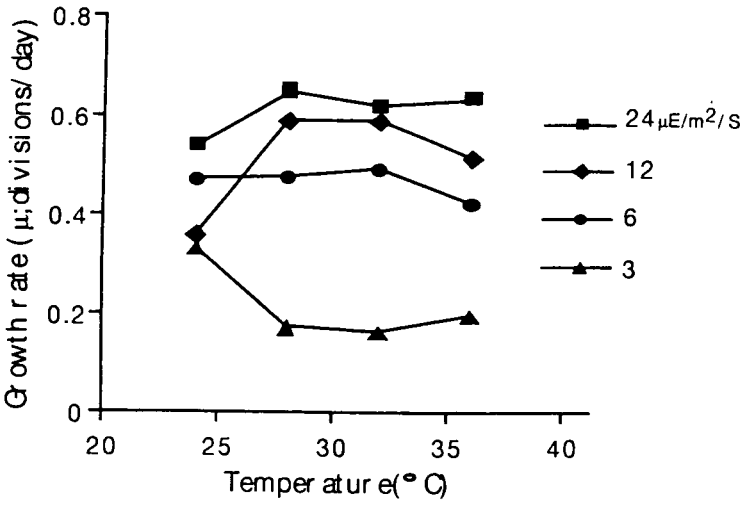

Fig.1 Effects of temperature on growth of symbiotic algae strain M in various light intensity.

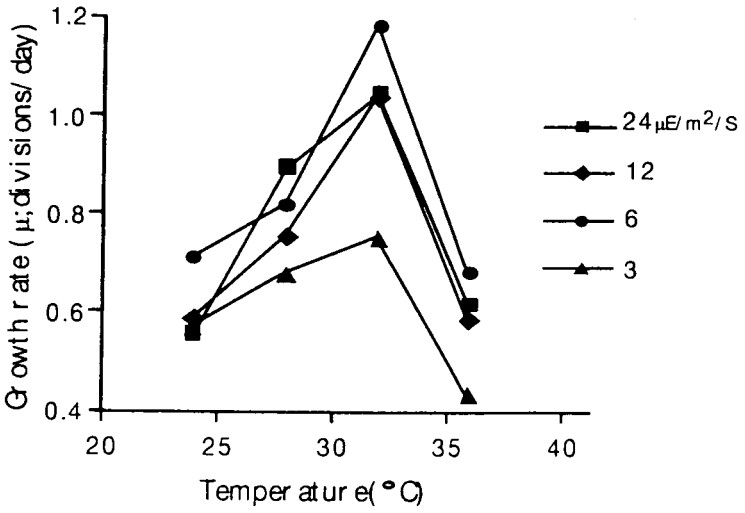

Fig.2 Effects of temperature on growth of symbiotic algae strain P in various light intensity.

Effects of inorganic nitrogen concentration

The following four kinds of inorganic nitrogen sources were enriched to aged sea water at concentration of 2-20 μM. 1)modified ESM medium ; as converted the nitrate salt into ammonium salt. 2) ammonium salt only, 3) ammonium and phosphorous salt (N:P=14:1), 4) ammonium and phosphorous salt (N:P=14:1) and vitamins-trace metal mixture of the Guillard f/2 medium. The inorganic nitrogen and phosphorous concentration of aged seawater were 0.2 μM and 0.12 μM, respectively.

The algae precultured in aged seawater twice were inoculated to filter sterilized media at the initial density of $4x10^6$ cells/ml and incubated at 28 ℃, 20 $\mu E/m^2/s$ on a 12hL:12hD cycle under semi-continuous culture condition (dilution rate = 0.1/day). After 7-8days when the cell density became stationary, the algal cells were harvested and the cell density, cellular chlorophyll a content, photosynthetic activity (FRI) were determined as described before. In some experiments, the photosynthesis were also determined using the oxygen probe (YSI model 5300).

Photosynthetic activity of the symbiotic algae in corals

12 coral samples were collected at Urasoko-bay, Okinawa pre. in March, 1997(see Table 1). The corals were maintained for 2-3days under running seawater. The symbiotic algae were collected by homogenizing the coral tissues and were washed three times with filtered sea water by centrifugation. The photosynthetic activities (FRI) of the symbiotic algae were determined as described before.

Results

The effects of temperature, light and salinity

On strain P, the light intensity were saturated over 6 $\mu E/m^2/s$ at 24-36 ℃ and it grew fastest at 32 ℃ but the growth rates declined quickly at 36 ℃. (Fig. 1) On strain M, the light intensity did not saturated at 24 $\mu E/m^2/s$ and its growth rate were affected more by light intensity than by temperature. The growth rate was declined much by the reduction of light intensity from 6 to 3 $\mu E/m^2/s$. (Fig. 2)

The photosynthetic activities (FRI) were maximum at 28℃ but declined at 32℃ in both algal strains(Fig. 3, 4). The cellular chlorophyll a contents tended to increase in high temperature. The percentages of motile cells were high at 28, 32 ℃ and low at 24 and 36 ℃.

Low salinity of 20-15 did not affect much on the growth and photosynthetic activities of the algae under moderate temperature conditions but the algae harmed by the low salinity at 36 ℃(Fig. 5, 6). The light intensity were also influenced the resistance to the low salinity under high temperature 36 ℃(Fig. 7).

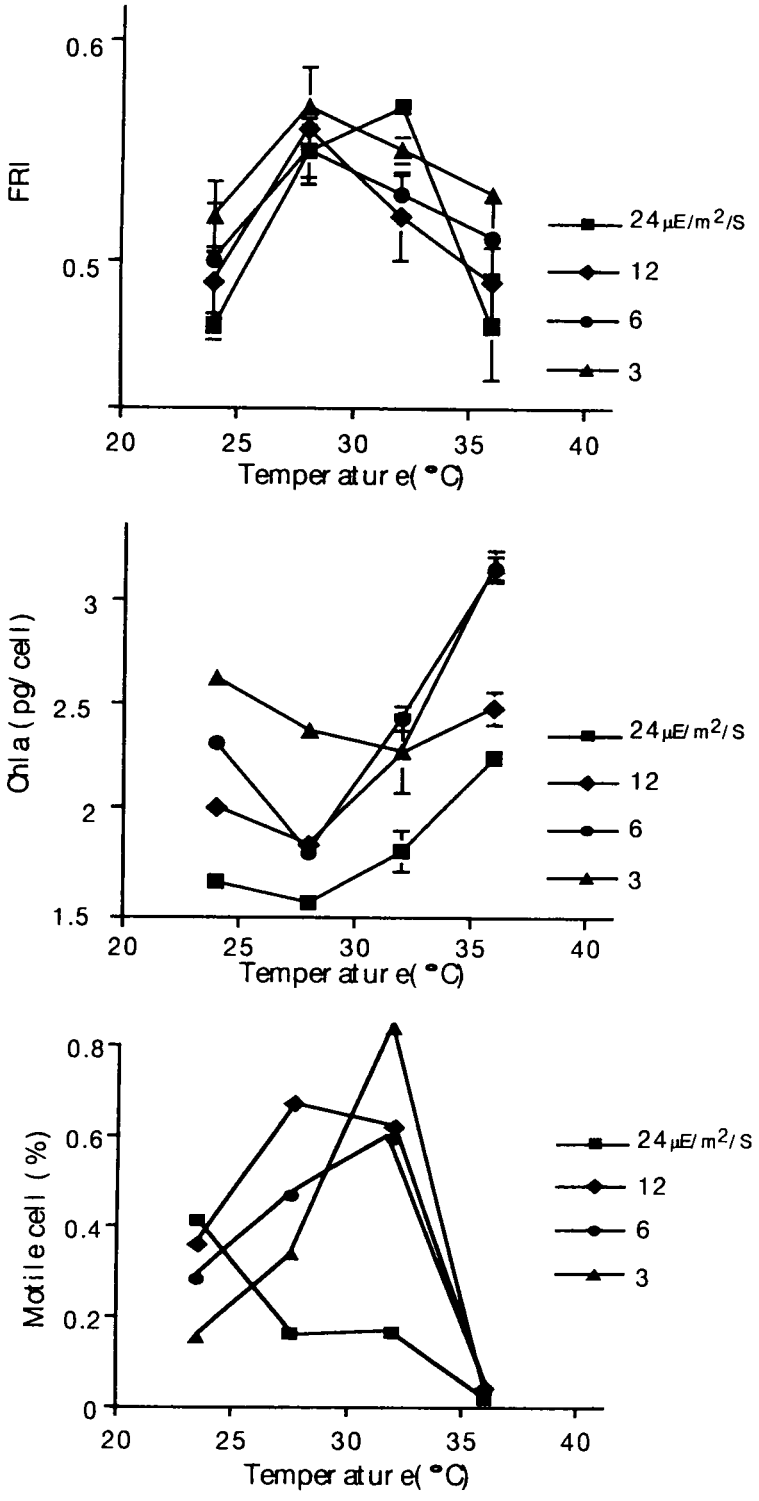

Fig.3 Effects of temperature on the photosynthetic activity (FRI), cellular chlorophyll a contents, and cell motility of symbiotic algae strain P in various light intensity.

The effects of inorganic nitrogen concentration

When the diluted modified ESM medium were used as the inorganic nitrogen source, the cell density and cellular chlorophyll a contents were increased relatively to the enriched nitrogen concentration 0-20 μM/day(Fig. 8). But the photosynthesis did not increase over 5mM/day and photosynthetic activities (FRI) declined at 20 μM/day .

On the other hands, when the ammonium was used solely as the inorgnic nitrogen source,

the reduction of photosynthetic activity (FRI) was clear even in the 5mM/day enrichment, although the cellular chlorophyll a concentration was increasing as much as the ESM enrichment (Fig. 9). The addition of phosphorous did not retrieve the reduced photosynthetic activity but the vitamin and trace metal mixture did.

The photosynthetic activity (FRI) of the symbiotic algae in corals

The values of FRI of symbiotic algae in corals were about 0.4-0.6 which corresponded with the values of the cultured algae(Table. 1). Differences were observed between coral species as *Galaxea fascicularis* ; 0.42-0.52 (average 0.50) , *Heliofungia actiniformis* ; 0.38-0.44 (0.50), *Goniopora* spp. ; 0.54-0.63 (0.58). The diel change was not shown in *Galaxea fascicularis* from 8:50 to 17:50. The difference within *Galaxea fascicularis* samples collected from a good (3/11) and a bad (3/12) environment was not clear.

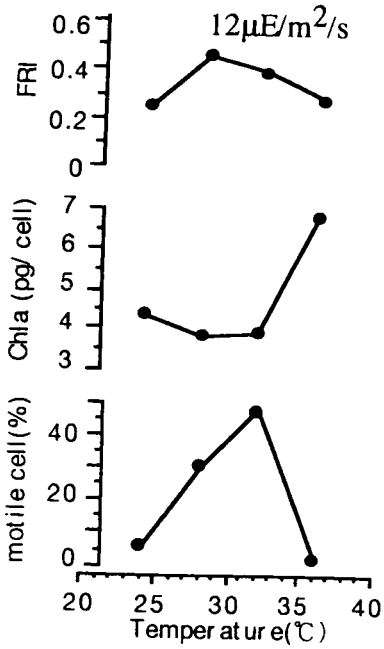

Fig.4 Effects of temperature on photosynthetic activity (FRI), cellular chlorophyll a contents, and cell motility of symbiotic algae strain M.

Discussion

It is observed widely that the elevation of seawater temperature causes the 'bleaching' of corals, a phenomenon that the symbiotic algae are expelled from the coral tissues and the coral become colorless, and the threshold temperature of bleaching seems to be around 30 ℃ from field observations and laboratory experiments[3]. In the present experiments, the two symbiotic algae isolated from hermatypic corals grew well at 32 ℃ but the photosynthetic activity became declining in thist emperature. This results suggest that the algae suffered a stress of high temperature physiologically and the efficiency of photosynthesis would be declining at 32℃.

As DCMU inhibits the reaction of photosystem II (PS2), the FRI value shows the

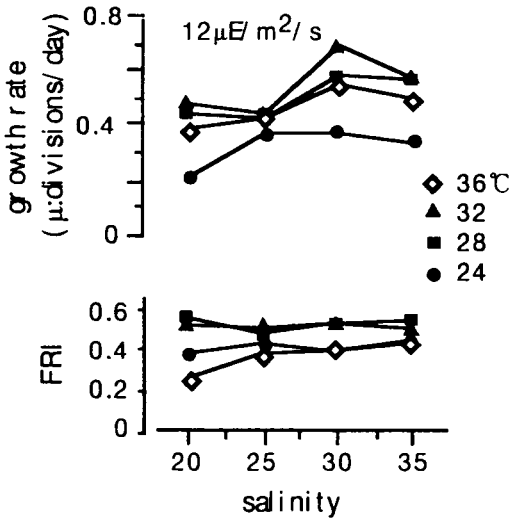

Fig.5 Effects of salinity on growth and photosynthetic activity (FRI) of symbiotic algae strain M in various temperature.

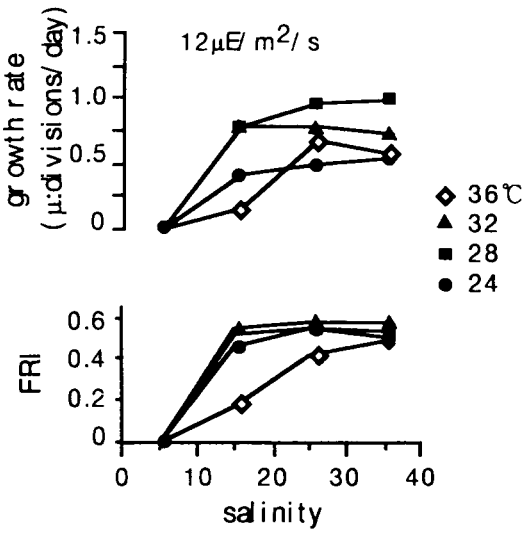

Fig.6 Effects of salinity on growth and photosynthetic activity (FRI) of symbiotic algae strain P in various temperature.

quantum yield of PS2 fluorescence. The mechanism of photosynthesis inhibition by high temperature or UV irradiation is known to be because of the reduction of the electron transport in photosystem. Absorption of charged energy under hyperoxic condition leads to increasing flux of highly reactive hydroxyl radicals that act on the PS2 or Rubisco and inactivate or give damages to the PS2 [4]. It is also shown that the inhibition of photosynthesis by high temperature or UV irradiation were due to the same mechanism in the cultured zooxanthellae[5][6].

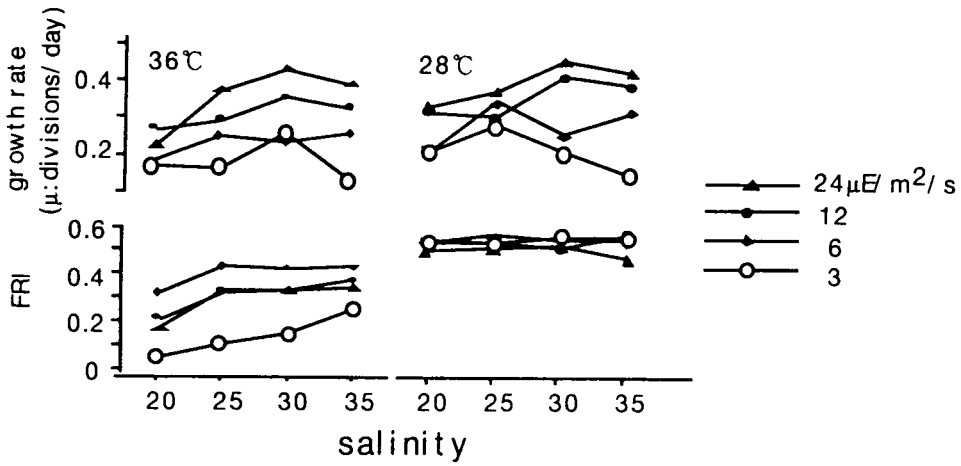

Fig.7 Interactive effects of salinity , temperature and light intensity on the growth and photosynthetic activity (FRI) of symbiotic algae strain M.

Usually symbiotic algae in animal tissues are non-motile and its growth rate were estimated as 0.04-0.08/day , which is very low comparing with the cultured cells. These facts suggest that there must be some growth inhibiting mechanism of the symbiont[7]. As in the cultured symbiotic algae, most cells lose their motility at stationary phase when the growth were inhibited by the nutrient limitation[8], the non-motile phase might be a stage that the algae are under some inhibitive condition. However, 32 ℃ is not an inhibitive condition for

Table 1 Photosynthetic activity (FRI) of symbiotic algae in corals

sampling date/site	coral spieces	sample name	date and time of measurement 3/12 13:00~16:00	3/13 8:50	10:30	12:50	14:30	17:50	average ± s.d.
3/11	*Galaxea fascicularis*	Ga1	0.42	0.49		0.50		0.50	0.50±0.04 n=7 (Ga1+Ga2)
		Ga2		0.50		0.54		0.54	
	Heliofungia actiniformis	He1	0.38				0.44		0.40±0.03 n=3 (He1+He2)
		He2	0.39						
3/12	*Galaxea fascicularis*	Ga3	0.51			0.52	0.51		0.52±0.01 n=5 (Ga3~Ga5)
		Ga4				0.51			
		Ga5				0.54			
	Goniopora spp.	Go1	0.55		0.63		0.57	0.62	0.59±0.04 n=4 (Go1)
		Go2			0.59				
		Go3			0.58				
		Go4			0.56				0.59±0.04 n=8 (Go1~Go5)
		Go5			0.54				

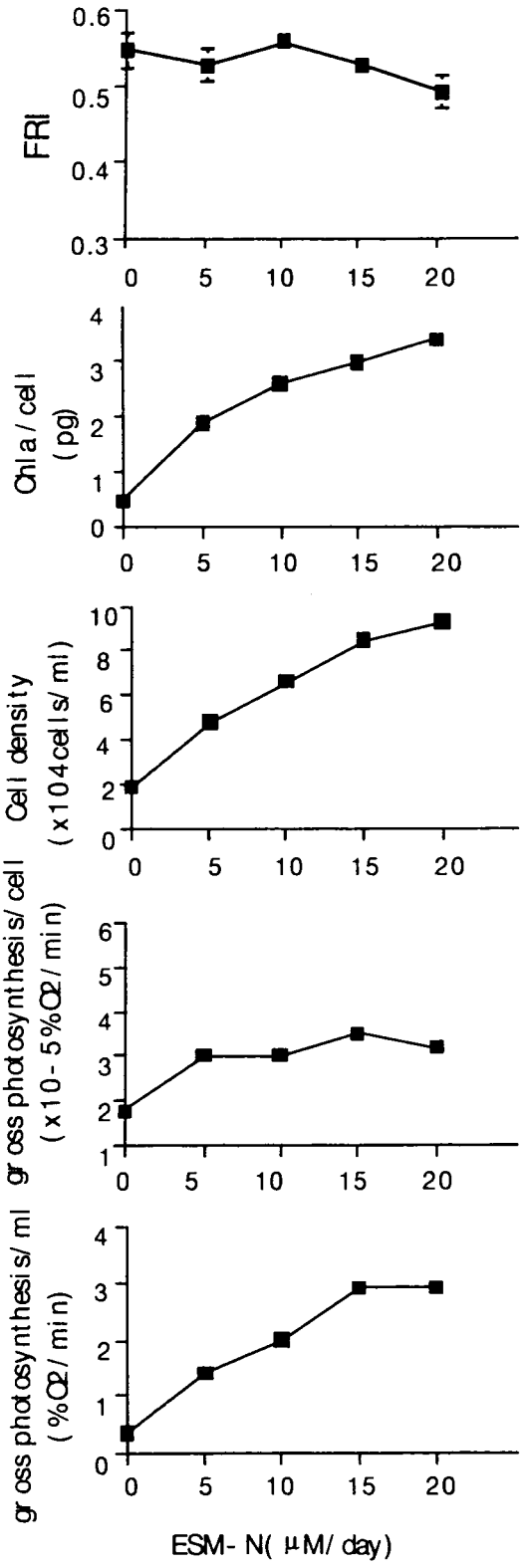

Fig.8 Effects of inorganic nitrogen concentration of modified ESM medium on symbiotic algae strain P.

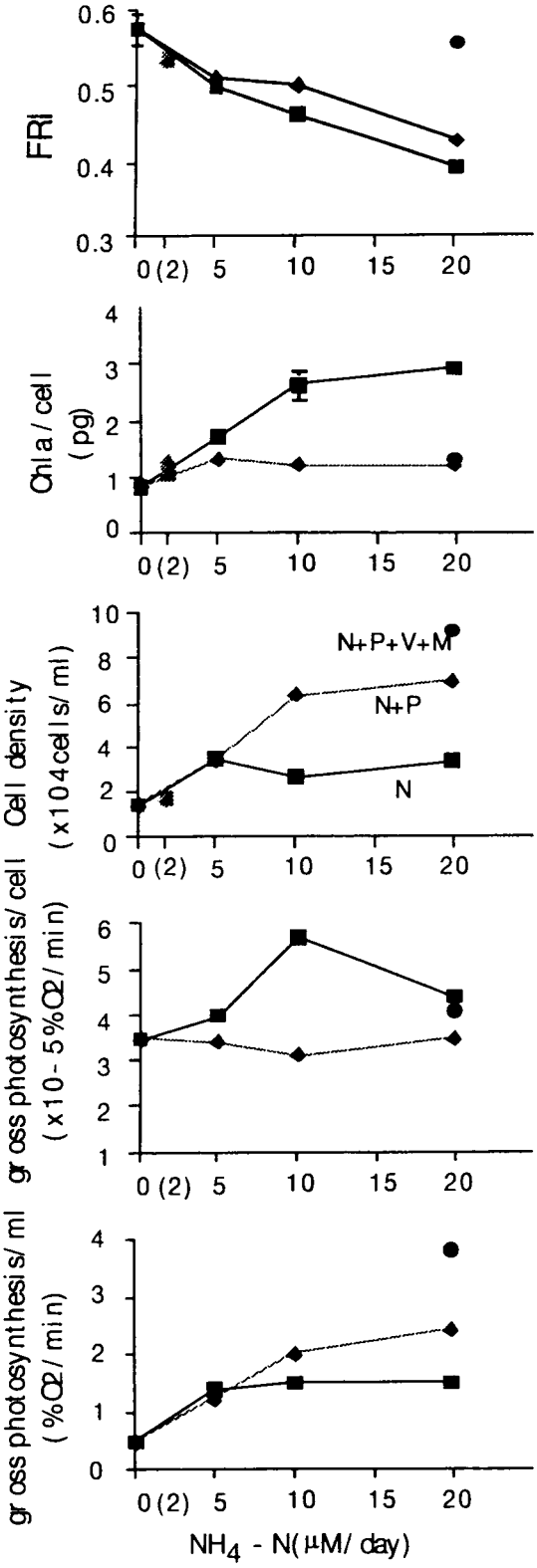

Fig.9 Effects of inorganic nitrogen concentration of definite enrichment on symbiotic algae strain P.

the algae because most cells were motile at the temperature. Considering from this, it seems fallacious that "Breaching" occurs when the water temperature exceeds the algal tolerance range.

In the coral, the animal offers bicarbonate or nitrogen to the symbiotic algae and the organic carbons synthesized by algae are transported to the animal; the relationship seems mutualistic symbioses[9)10)]. On the other hands, they also have antagonistic relationships as a competition for nitrogen which is a limiting nutrient for both organisms. Moreover, the animal must guard themselves from the toxic effects of molecular oxygen producedby algal photosynthesis[11)]. Recently it is shown that the animal controls the symbiotic algal density by means of digestion[12)] or expelling[13)]. The "Breaching" may occur when the animal could not control the symbiotic system due to the excess algal growth[14)] or too much active oxygen production by algal photosynthesis[15)] caused by high temperature.

As corals grow well in oligotrophic seawater when the inorganic nitrogen concentration is under 1 μM, it is thought that the animal could capture zooplankton and recycle the nitrogen nutrient inside the symbiotic system so that corals have a very high productivity. But the symbiotic algae can uptake inorganic nitrogen directly from seawater, and many researchers reported that the enrichment of nitrogen to the seawater caused the increase of algal cell density and cellular pigment contents in the coral[16)17)18)19)]. As thealgae uses the uptake nitrogen for their cell divisions, the excess organic carbon which havebeen transported to animal and used for mucus excretion or calcification etc. will become limited[10)]. The eutrophication (N-enrichment) of seawater would be good for symbiotic algae but bad for corals because it breaks the nutrient balance of the symbiosis.

When the photosynthetic activity of symbiotic algae inside corals ismeasured by oxygen production, the results should be influenced much by contaminated animal tissues respiration. On the other hand, there are many limitation to use ^{14}C method now. The PS2 activity measurement using DCMU have some promoting point ; as it measures the fluorescence of chlorophyll a, it can avoid the interference of animal tissue and is easy to measure. However, the FRI value just suggests the condition of the algal photosystem and cannot be compared with other methods. In this study, we measured the FRI value of symbiotic algae cultured under various conditions and showed that it declined under stresses such as high temperature or imbalanced excess nitrogen. These results suggested that FRI value could be a indicator of algal condition. The FRI values were also measured on the symbiotic algae inside the corals and the values were corresponding with those of cultured algae. This result imply that this method could be used in the field work. When this method is used for the comparisonof symbiotic algal conditions in the field corals. The species specific variations or diel and annual variations should be examined carefully.

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