D-4.2.1 Measurement of physiological intensity of Scleractinian corals and its application to field studies

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Abstracts Since 1970's, catastrophic events such as sedimentation, crown-of-thorns starfish plague and coral bleaching severely affected coral reef ecosystems in Okinawa, Japan. Therefore it is important to assess the health of Scleractinian corals. RNA/DNA ratio is known as an index metabolic intensity of larval fishes, copepod, oyster and so on and has been used to measure nutritional condition. In the present study, we tested whether RNA/DNA ratio can be used as an index of the nutritional status of corals. For the extraction and quantification of nucleic acids of corals, we used the Clemmesen's method with slight modification. We extracted nucleic acids from *Galaxea fascicularis* with this technique, and confirmed the purity of the nucleic acids using agarose gel electrophoresis. We found that RNA/DNA ratio was positively correlated with tissue dry weight/DNA content in *Galaxea fascicularis* and *Montipora digitata*. Tissue dry weight/DNA is considered to reflect nutritional condition of corals, therefore it is also considered RNA/DNA ratio can be used as an nutritional index of corals. We investigated the effect of continuous darkness on RNA/DNA ratio of *G. fascicularis*. After rearing under continuous darkness for 4 days, RNA/DNA ratio, tissue dry weight/DNA and number of zooxanthellae in the tissue of the corals were statistically lower as compared with the corals reared in an aquarium receiving full sunlight. Under continuous darkness, it is considered that the protein synthesis of the corals is restrained.

Key Words Coral, Nutritional Condition, RNA/DNA ratio, Tissue dry Weight/DNA

1. Introduction

Coral reef ecosystem in Okinawa has been degraded drastically since Okinawa reverted to Japan. From 1970's to 1980's, most of the coral reefs were severely damaged by crown-of-thorns starfish *Acanthaster planci*. Since 1980's, coral bleaching events had been observed. Sedimentation derived from fields for agriculture has also drastically degraded the coral reefs. With these catastrophic events, about 90% of the coral reefs in Okinawa is assumed to be degraded. To conserve the ecosystem, not only rehabilitation but also assessment of the health of the coral reefs is of quite importance.

The RNA/DNA ratio is an index of metabolic intensity of cell's insame animals and has been used to measure nutritional condition of larval fishes, copepod, oyster and so on. For example, Buckley[1),2)] demonstrated the relations between food availability and larval RNA/DNA ratio, and between RNA/DNA ratio and growth rate of larval fish. Clemmesen[3)] developed a highly sensitive fluorimetric method, allowing the determination of the RNA/DNA ratio of individual larvae. Shimizu *et al.*[4)] analyzed RNA/DNA ratio of wild Japanese sardine larvae and showed larvae in offshore waters of the Kuroshio current were in a poor nutritional condition compared with those in inshore waters. But RNA/DNA ratio studies on corals have not been

reported yet.

In the present study, first we tested whether Clemmesen's method (extraction and quantification of nucleic acids) [3] could be applied to scleractinian corals. Secondly, to evaluate the validity of the RNA/DNA ratio as an nutritional index of corals, we reared the coral *Galaxea fascicularis* under full sunlight and continuous darkness, and measured RNA/DNA ratio, number of zooxanthellae and tissue dry weight.

2. Materials and Methods

(1) Test of Clemmesen's method

• Sample Collection

Colonies of *G. fascicularis* and *Montipora digitata* were collected in Sesoko Island and Ishigaki Island. For *G. fascicularis*, the analysis was carried out on each polyps, because the polyps were relatively large. For M. digitata, the analysis was carried out on each branch beca use of the smallness of the polyps.

• Chemicals

Ethidium bromide (EB) and salmon sperm DNA were obtained from Life Technologies (USA). yeast RNA, sodium N-lauroyl sarcosinate, phenol, chloroform, isoamylalcohol and proteinase K were purchase from Wako Pure Chemical Industries (Japan).

• Elimination of zooxanthellae from coral tissue

Most of scleractinian corals have zooxanthellae in there own endoderm cells. Therefore the content of nucleic acids in zooxanthellae must be determined before quantifying those in corals. To eliminate zooxanthellae from coral tissue, the following procedure was carried out. The tissue of *G. fascicularis* was homogenized with Tris buffer (0.05 M Tris, 0.1 M NaCl, 0.01 M EDTA, pH 8.0), added proteinase K (final concentration 0.2mg/ml) and sodium N-lauroyl sarcosinate (final concentration 0.5%) to the homogenate, mixed for 5 min, incubated at 37 ℃ for 60min and centrifuged for 5min (3000 × g). We observed the supernatant and the deposit of the homogenate with an microscope, and confirmed that the intact cells of zooxanthellae were contained only in the deposit. Then, we extracted nucleic acids from the supernatant and depos its separately with the method as follows, and evaluated the purity of the nucleic acids with agarose gel electrophoresis using 1% agarose in TBE buffer. Sty I digested lambda phage DNA fragments were run in parallel on the gels as a molecular marker. The gels were stained with E B solutions, and photographs of the gels were taken under UV rays with a Polaroid camera.

• Extraction and purification of nucleic acids

Based on the extraction and purification method described by Clemmesen[3], the following procedure was carried out. Coral tissue was blasted from the skeleton using small, forceful, intermittent jets of the Tris buffer delivered rapidly by a Water Pik (New Deluxe model, Teledyne)[5]. The blasted tissue was collected by centrifugation, homogenized, added proteinase K (final concentration 0.2mg/ml) and sodium N-lauroyl sarcosinate (final concentration 0.5%), mixed for 5 min, and incubated at 37℃ for 60min. For purification of the nucleic acid solutions, the sample were extracted twice with Phenol/Chloroform/Isoamylalcohol (25:24:1).

• Fluorescence assay

Fluorescence assay procedure was based on the method described by Clemmesen[3]. First the fluorescence of DNA and RNA was measured using 0.15ml of the sample after adding 1.00ml of the Tris buffer and 0.05ml of EB solution (1.0mg/ml). Secondly an aliquot of the sample was treated with RNase incubated at 37℃ for 30min and measured fluorimetrically using EB. Measurements were carried out with an spectrofluorophotometer exciting at 365nm, and reading the emission at 590nm. The fluorescence due to RNA was calculated as the difference between total fluorescence (RNA and DNA) and fluorescence after RNase treatment (only DNA).

• Tissue dry weight

To compare the RNA/DNA ratio with tissue dry weight, replicate aliquots (n=3) of the hom

ogenate were dried at 60℃ for 24 hours and weighed by an electrical balance.

(2) Effect of continuous darkness on RNA/DNA ratio of corals

This experiment was performed on the coral *G. fascicularis*. One colony of *G. fascicularis* which had about 100 polyps was collected from Urasoko Bay in Ishigaki Island. After collection, the colony was transported to a seawater aquarium at Yaeyama Station of Japan Sea-Farming Association. The polyps of the colony were taken apart, and attached to plastic plates one by one. These polyps had been kept in aquarium receiving a continuous supply of seawater for three months, and then these polyps were used in the experiment.

After three months, the polyps (n=6) were placed in an aquarium illuminated by sunlight. Another polyps (n=6) were placed in another aquarium covered with a black plastic sheet not

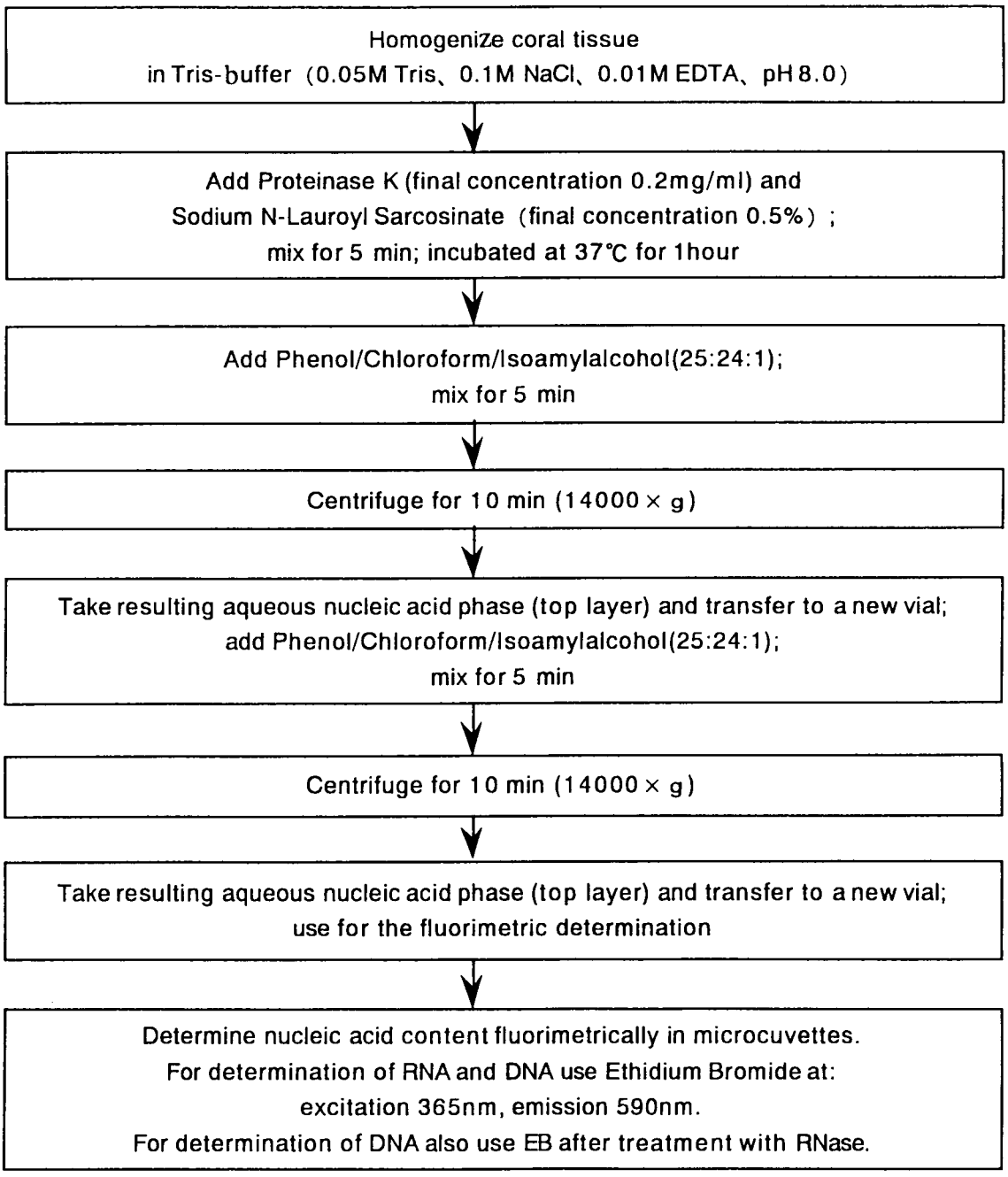

Fig. 1. Flowchart of the analytical procedure

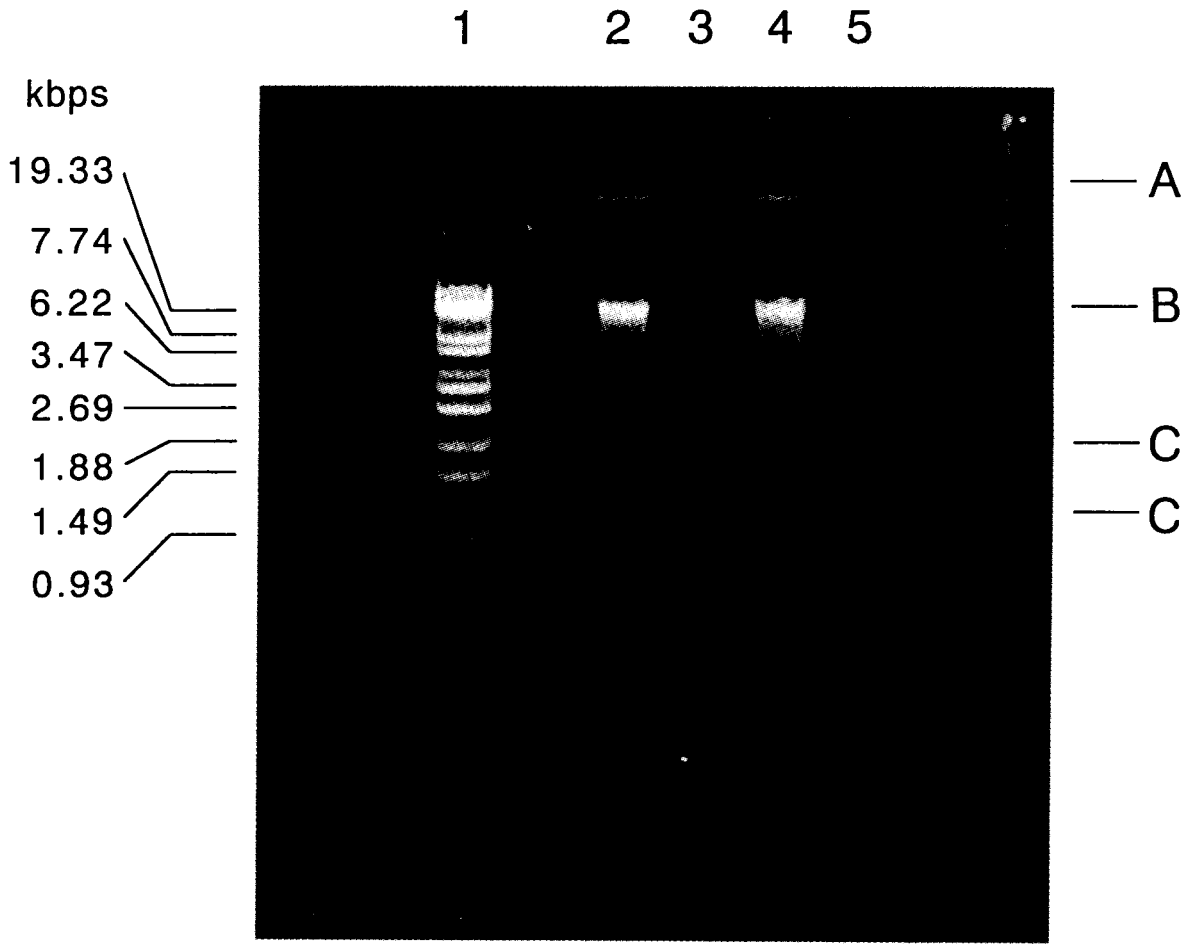

Fig. 2. Agarose gel electrophoresis of purified nucleic acids extracted from *Galaxea fascicularis*. (A), (B) and (C) indicate the start slots of the samples, DNA bands and RNA bands, respectvely.

1: the molecular weight standard (Lambda phage DNA/ Sty I digest)
2: the nucleic acids extracted from the supernatant of the homogenate of the coral
3: the nucleic acids extracted from the deposits of the homogenate of the coral
4: same sample as Lane 2 treated with RNase
5: same sample as Lane 3 treated with RNase

to receive sunlight. Both aquaria had received continuous supply of seawater throughout the experiment. The experiment was terminated at the end of four days at which time all polyps were killed and processed for measurements of nucleic acids content, number of zooxanthellae and tissue dry weight. These measurements were performed on each individual polyps.

Nucleic acids and tissue dry weight were measured with the method mentioned above. Number of zooxanthellae was determined using a haemocytometer (Thoma, Kayagaki).

3. Results

(1) Test of Clemmesen's method

• Extraction and purification of nucleic acids

With the analytical procedure based on the Clemmesen's method, high molecular weight of DNA and defined RNA subunits are extracted from the supernatant of the homogenate as shown by agarose gel electrophoresis (Fig. 2 Lane 2). On the other hand, any bands of nucleic acids were not observed in the Lane 3 (the deposit of the supernatant). With the spectrofluorophotometer, we could measure nucleic acids content in the supernatant, but not in the deposit. The effect of RNase treatment was shown in Lane 4 where RNA bands were disappeared.

• RNA/DNA ratio and tissue dry weight

RNA/DNA ratio of *G. fascicularis* was positively correlated ($p<0.001$) with tissue dry weight/DNA content (Fig. 3). Similar correlation ($p<0.001$) was also observed on *M. digitata* (Fig. 4).

(2) Effect of continuous darkness on the RNA/DNA ratio of corals

After rearing under continuous darkness for four days, number of zooxanthellae per 1 μg tissue (dry weight) of *G. fascicularis* was

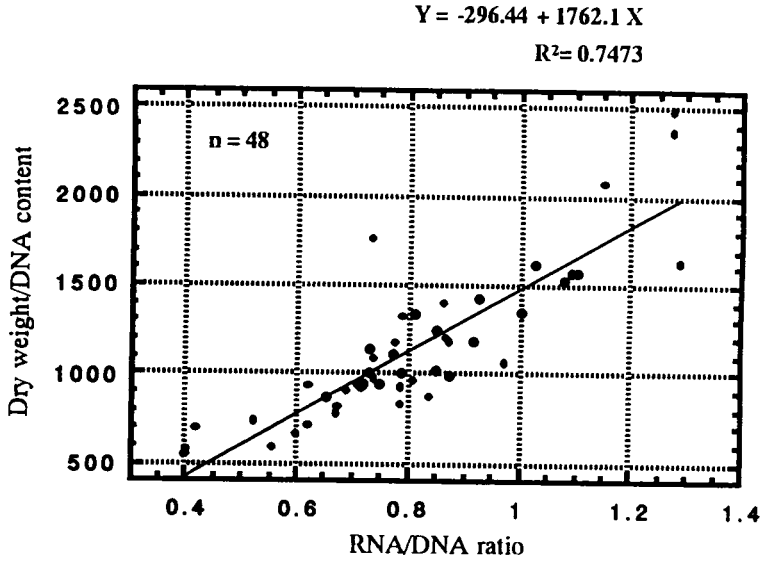

Fig. 3. Tissue dry weight/DNA content of the coral *Galaxea fascicularis* in relation to RNA/DNA ratios.

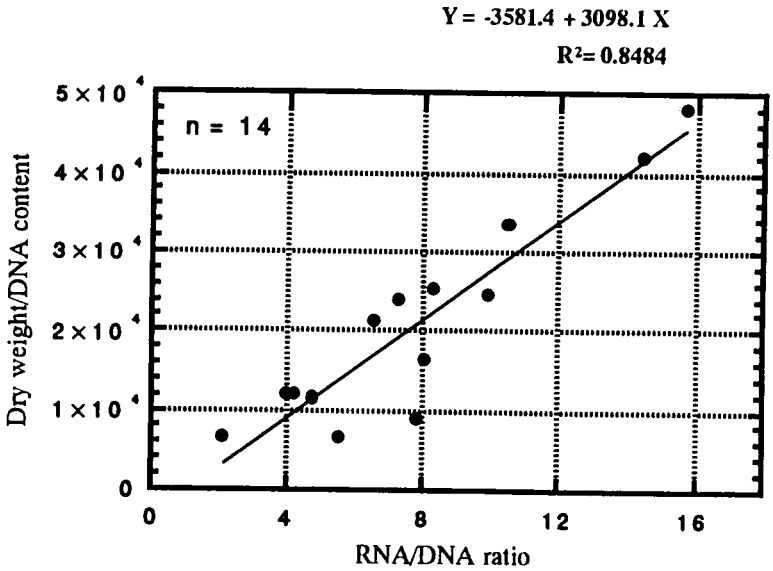

Fig. 4. Tissue dry weight/DNA content of the coral *Montipora digitata* in relation to RNA/DNA ratios.

statistically lower ($p<0.001$) as compared with the polyps reared in the aquarium receiving full sunlight (Fig. 5).

RNA/DNA ratio of *G. fascicularis* reared under dark condition was statistically lower ($p<0.005$) than the coral under sunlight (Fig. 6).

Tissue dry weight/DNA content of *G. fascicularis* reared under dark condition was also statistically lower ($p<0.01$) than the coral under sunlight (Fig. 7). But RNA/DNA ratio and tissue dry weight/DNA were not correlated statistically.

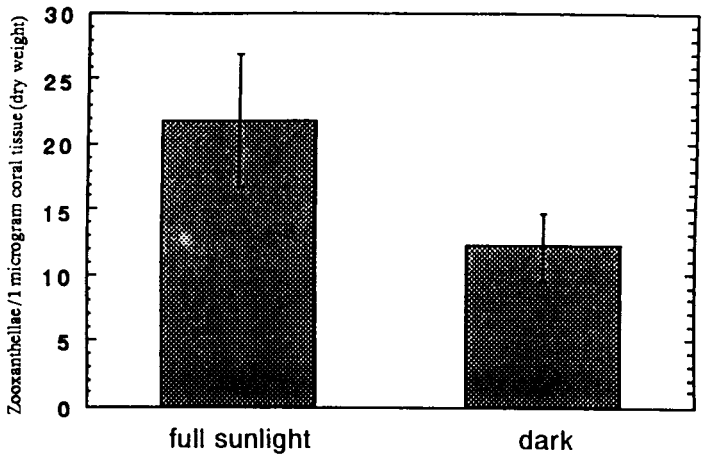

Fig. 5. Number of zooxanthellae/ 1 microgram coral tissue(dry weight) of *Galaxea fascicularis* maintained for four days under full sunlight and continuous darkness. Vertical bars indicate standard deviation of the means(n=6).

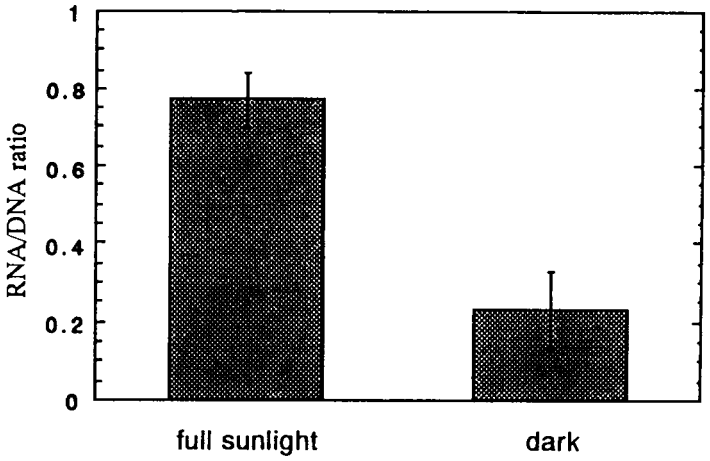

Fig. 6. RNA/DNA ratios of *Galaxea fascicularis* maintained for four days under full sunlight and continuous darkness. Vertical bars indicate standard deviation of the means(n=6).

4. Discussion

(1) Test of Clemmesen's method

• Extraction and purification of nucleic acids

The result of the electrophoresis (Fig. 2) showed that nucleic acids content in zooxanthellae were very small and negligible. Consequently, it is not necessary to eliminate zooxanthellae from coral tissue when quantifying DNA/RNA ratio of corals.

The purification procedure for nucleic acids resulted in high molecular weight of DNA and defined RNA subunits, and was highly sensitive so that only one polyp of *G. fascicularis* could be analyzed. Therefore it is proved that Clemmesen's method can be applied to scleractinian corals.

• RNA/DNA ratio and tissue dry weight

Tissue dry weight/DNA is considered to reflect nutritional condition of corals. The statistically high correlation between RNA/DNA ratio and tissue dry weight/DNA proved that RNA /DNA ratio can be used as an nutritional index of corals.

The correlation was observed in two species which are remote in phylogeny (*G. fascicularis* belongs to the Family Oculinidae, and *M. digitata* to the Family Acroporidae). Therefore it is assumed that the correlation may be observed in other corals.

(2) Effect of continuous darkness on RNA/DNA ratio of corals

Carbon fixed by zooxanthellae is translocated to corals[6),7),8)], but zooxanthellae cannot

photosynthesize under dark condition. In this experiment, continuous darkness resulted in decreases of zooxanthellae, RNA/DNA ratio and tissue dry weight/DNA. It is assumed that protein synthesis of corals was restrained, which leaded to deterioration of nutritional condition. But RNA/DNA ratio and tissue dry weight/DNA were not correlated statistically. It may be due to small numbers or replicates of this experiment.

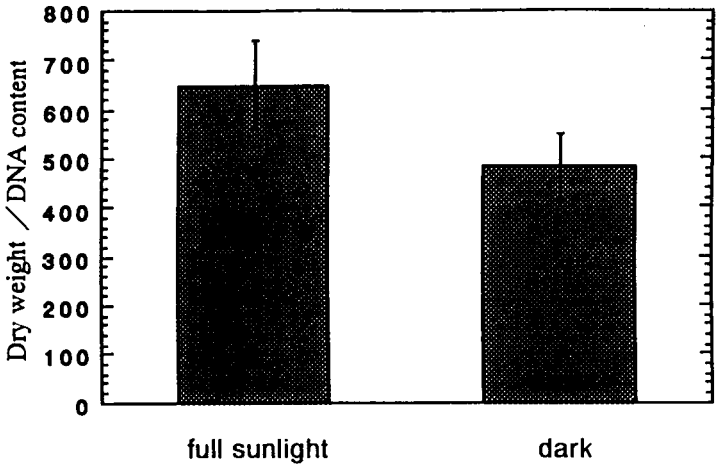

Fig. 7. Tissue dry weight/DNA content of *Galaxea fascicularis* maintained for four days under full sunlight and continuous darkness. Vertical bars indicate standard deviation of the means(n=6).

Fitt *et al.*[9] analyzed colonies of Montastrea annularis which suffered bleaching in various degree, and observed that zooxanthellae density positively correlated with protein, lipid and ash-free dry weight per unit surface area. Since 1995 coral bleaching events had occurred in Okinawa at relatively low level, and we could not collect bleached corals sufficiently. Consequently we could not investigated relationship between RNA/DNA ratio and coral bleaching. This subject remains to be answered.

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