STRESS-INDUCED EXPULSION OF ZOOXANTHELLAE IN TWO SCLERACTINIAN CORALS

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Dave:
A first-rate study! Good theoretical framework, careful observations, and excellent discussion of results.
John

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ABSTRACT

When subjected to stress, scleractinian corals have the ability to expel part or all of their complement of endosymbiotic zooxanthellae. In order to investigate this ability, samples of Porites porites and Colpophyllia natans from different depths were stressed under a variety of conditions. Expulsion of zooxanthellae was quantified by isolating zooxanthellae from the experimental corals; photosynthetic pigments were later extracted from these cells. The following trends were observed:

1. Although the absolute density of zooxanthellae decreases with depth, the number of algal cells per coral polyp remains relatively constant.
2. When subjected to stresses found primarily in surface waters, deep-water coral populations are less well adapted to expel zooxanthellae than their shallow water counterparts.
3. Zooxanthellae with low chlorophyll *a* content are preferentially expelled from stressed corals.

These results compare favorably with published research dealing with different species of coral.

INTRODUCTION

"Zooxanthellae" is a vernacular term used to describe the endosymbiotic algae found in Protozoa, Porifera, Mollusca, Platyhelminthes, and Cnidaria. A diverse array of algal species may be found as symbionts in these classes of organisms. The zooxanthellae of Cnidarians have been well characterized as belonging to the order Dinophyceae, the yellow-brown dinoflagellates. Muscatine (1974) has narrowed this classification to the species Gymnodinium microadriaticum, although there is some doubt about the universality of this species (Lewis, 1977). The magnitude of this symbiotic interaction is impressive; zooxanthellae may account for 50% of the protein Nitrogen found in some coelenterates (Muscatine, 1974).

Ecologically and geologically, this symbiosis is most important in the reef-building corals. Goreau (1961) and others have unequivocally shown that calcification by hermatypic corals is directly correlated to the presence of zooxanthellae and sunlight. In the absence of zooxanthellae or photosynthesis, calcareous reef-building could not take place.

Muscatine (1974) has shown that zooxanthellae are functionally dependent on the metabolic products of coral when they exist as endosymbionts. The algae actively takes up uric acid, succinate, and pyruvate (among other chemicals) produced by the host. In addition, the endosymbionts may aid coral in taking up nutrients from seawater. It has been suggested that the zooxanthellae-cnidaria symbiosis allows tight nutrient cycling to be maintained in the reef ecosystem. This may account for the high productivity of the reef in a relatively nutrient-poor environment.

There has been a great deal of controversy concerning the value of zooxanthellae to the coral

host. Goreau (1960, 1961) postulated that the association ranged from total dependence on zooxanthellae for nutrition (in some zooanthids), to nutritional independence in the Scleractinia (which were specialized as carnivores). This view was contradicted by the finding that corals can utilize labeled photosynthate from endosymbiotic zooxanthellae (Muscatine and Cernichari, 1969). In 1970, Johannes and others calculated that scleractinian corals could derive only a fraction of their nutritional needs from carnivory of available zooplankton. In addition, it was shown by Muscatine (1974) that endosymbiotic zooxanthellae release up to 50% of their photosynthate to the host. The modern view is that reef building corals rely on both carnivory and zooxanthellar primary production to meet their nutritional needs. Indeed, it has been calculated that energy requirements of the entire reef could be met by zooxanthellar photosynthesis.

It can be seen that zooxanthellae interact with their hosts by regulating calcium deposition, by taking up nutrients and wastes from the host, and by translating organic carbon to the host. There are, however, conditions under which the endosymbionts might impose stress on the host. This could conceivably occur when the number of zooxanthellae contained within the host reached a certain threshold, at which point the host was no longer able to maintain the population of algae. Some authors feel that space rapidly becomes a limiting resource within the coral polyp (Drew, 1972). Taylor (1969) asserts that this happens frequently and continually in nature, and that the coral hosts periodically expel zooxanthellae so as to keep the population at manageable levels.

Another similar situation in which zooxanthellae might become a stress is if the metabolism of the coral host decreased for some reason. Yonge and Nicholls (1931) showed that there were several possible experimental manipulations of coral which would reduce metabolism, and resulted in the expulsion of some or all of the coral's complement of zooxanthellae. These stresses included starvation, elevated temperature, and prolonged exposure to darkness. Goreau (1964) and Jaap (1979) have recorded natural disturbances (altered salinity and elevated temperature) which resulted in the bleaching of reef corals through the expulsion of zooxanthellae. In every case, there were few long term effects on the viability of the corals involved, with the exception of the most severe stresses. Expulsion of zooxanthellae appears to be a transient and reversible occurrence; most corals regained their full complement of endosymbionts in four to ten weeks following the disturbance. There appears to be some adaptive value in the ability to expel zooxanthellae under stress, since this ability is found in a wide variety of corals with many different morphologies.

The purpose of this study was to quantify the effects of several stresses on two species of scleractinian corals: Porites porites, a branching coral with small polyps, and Colpophyllia natans, a coral characterized by large polyps and intratentacular budding. These corals were chosen because of their differences in morphology, their wide depth range (Goreau and Wells, 1967), and the fact that they are relatively unstudied.

The stresses studied were altered salinity, shade, and elevated temperature. In each case, the manipulations

were designed so as to approximate naturally occurring stresses. Shading of a coral could be caused by one coral overgrowing another; salinity stress and temperature stress may be caused by meteorological and tidal conditions (Goreau, 1964; Jaap, 1979).

In an attempt to ascertain whether or not there are variations in a species' ability to expel zooxanthellae, corals from two different depths were compared in the study. It was hypothesized that deeper coral populations would be less well adapted to expel zooxanthellae under stresses which normally occur near the surface.

Finally, the results were analyzed so as to shed light on other coral adaptations in response to depth. These findings were compared to the work of other researchers dealing with different species of coral.

MATERIALS AND METHODS

The study site was Mooring 1 at Discovery Bay Marine Lab, Discovery Bay, Jamaica. Small samples of Colpophyllia natans and Porites porites were obtained at depths of twenty feet and sixty feet with the aid of SCUBA. With the exception of the shade stress study, all experimental corals were maintained and manipulated in the wet lab under running seawater.

Controls:

Two samples each of P. porites and C. natans were collected from 20' and 60' for analysis as controls. Zooxanthellae and chlorophyll densities were determined immediately after collection, using a standard method described below. Polyp density for these corals was also determined by counting the number of polyps within a standard area mask, and by averaging three replicates for each sample.

EXPERIMENTAL APOSYMBIONTS:

A method suggested by Goreau (1964) was employed in an attempt to obtain experimental aposymbiotic individuals. Coral samples were exposed to fresh water for four hours in order to induce expulsion of zooxanthellae. Necrosis was observed in both species after five hours of exposure to fresh water; It was hoped that this severe sub-lethal stress would produce nearly aposymbiotic individuals.

TEMPERATURE STRESS:

An attempt was made to simulate the type of sub-lethal temperature stress which might occur during a period of combined low tides, calm, and high midday temperature

(as described by Jaap, 1979). Jaap reports that the optimum temperature for most corals is 25-29°C, while the maximum tolerable temperature ranges from 34-36° C. Ambient temperature in the wet lab was 26° C; stress was induced by adjusting the water temperature to 32° for six hours per day on three consecutive days. When not being stressed, corals were maintained under running seawater.

SALINITY STRESS:

Salinity stress may occur naturally when heavy rainfall is accompanied by low surface mixing. Goreau (1964) mentions reports of persistent freshwater to a depth of 2.5 meters following Hurricane Flora in 1963. This type of stress was simulated by exposing corals to 50% seawater for 48 hours. Fresh water and seawater were continuously run into the experimental vessel at equal flow rates (360 ml/minute).

SHADE STRESS:

The effects of shade on coral were studied in situ at 60'. Because of heavy surge in the study area, shade structures could not be maintained at the 20' depth. Corals of each species were shaded by surrounding them with a mesh ring and covering them with a piece of masonite. Samples were collected and analyzed after being shaded for six days.

ISOLATION OF ZOOXANTHELLAE AND EXTRACTION OF CHLOROPHYLL:

Zooxanthellae were extracted from the coral using the Method of Dustan (1979), modified by Ian Sandeman

and myself. A mask was prepared by cutting a 16 mm circle (area = 2 cm^2) from a sheet of flexible polyethylene. This mask was affixed to the coral to be studied in two different locations, and all coral tissue within the mask removed with a Water-Pik. [The medium used for the extraction was 0.45 µm Millipore Filtered seawater (MFSW)]. The resulting suspension was homogenized for 10 seconds in a spinning blade Virtis apparatus in order to break up mucus released during removal of coral tissue. The homogenate was centrifuged at 2,200 RPM for five minutes, and the pellet rehomogenized in MFSW in a loose-fitting tissue grinder. Washing was repeated four times with centrifugation at 1,500 RPM. The final pellet consisted almost entirely of intact zooxanthellae. This pellet was resuspended in 10 ml MFSW, and 0.5 ml removed for cell counting. The remainder was repelleted for extraction of pigments.

Cells were counted on a standard hemocytometer. Three replicates of five 0.1 mm^3 fields were averaged; to arrive at a final calculation of zooxanthellae per square centimeter of coral surface, this number was multiplied by 5,000.

Pigments were extracted in 10 ml 90% acetone, buffered with $MgCO_3$, for one hour. Spectroscopy was performed on a Bausch and Lomb Mini Spec 20. Absorbance readings were taken at 630 and 663 nm, and the chlorophyll *a* content determined from the following equation:

$$\mu g \text{ Chl } a = 13.31 A_{663} - 0.27 A_{630} \quad \text{(Jeffrey and Haxo, 1968).}$$

This data was then correlated with the surface

area of the coral, and the number of zooxanthellae isolated.

Time limitations prevented performing replicates of the experimental trials; only the controls were run in duplicate. For this reason, data from the experimental manipulations were frequently pooled in order to reduce the effects of natural variation and systematic error. This operation allowed more confidence to be placed in the observed trends, and permitted the application of statistical tests.

RESULTS AND DISCUSSION

The control data and results of the experimental manipulations are tabulated on the following page (Table 1). The entire body of experimental measurements and values is compiled in the appendix, but most of this information is superfluous and is provided solely for the edification of the reader.

The extractions of zooxanthellae and pigments were accomplished with few problems. Occasionally, a zooxanthellae prep from Porites ~~porites~~ appeared "dirty," that is, contaminated with a large amount of coral cell material. Although corals supposedly have few pigments of their own, it appeared that the chlorophyll *a* absorption in these samples was inordinately high, and for this reason they were not considered valid.

The freshwater treatment to induce experimental aposymbiosis was partially successful, but did not induce greater expulsion than the other experimental manipulations. (See Table 1 (B)). Apparently the stress was applied too rapidly, and the corals did not have a chance to expel zooxanthellae maximally before mortality ensued. The experimental aposymbionts could not be used as an indicator of maximum zooxanthellar expulsion; they were included with the other manipulations when results were pooled for comparison.

The most severe stresses, as evidenced by zooxanthellae expulsion, were the salinity stress and the shade stress. Reduced salinity resulted in greatly reduced zooxanthellae numbers, especially in the 20' samples; the stress was severe enough to kill the 60' Colpophyllia sample. At 60', shade seemed to have

Table 1. Control data and results of experimental manipulations.

Coral type and Depth		EXPERIMENTAL GROUP: Controls (average of two values ± one S.D.)	Experimental Aposymbiotic Corals	Temperature Stressed Corals	Salinity Stressed Corals	Shade Stressed Corals
(A) Micrograms of Chlorophyll *a* per square centimeter of coral surface:						
C. natans	20'	7.8 ± 0.70	5.1 μg/cm²	5.3 μg/cm²	trace	—
	60'	5.8 ± 0.07	4.1	7.2	DIED	2.5 μg/cm²
P. porites	20'	7.9 (one value)	4.6	7.7	4.0 μg/cm²	—
	60'	5.2 ± 1.06	—	10.2	6.4	4.1
(B) Number of zooxanthellae per square centimeter of coral surface:						
C. natans	20'	$1.62(\pm 0.33)\times10^5$	0.65×10^5 cells/cm²	0.50×10^5 cells/cm²	$<0.10\times10^5$ cells/cm²	—
	60'	$0.90(\pm 0)\times10^5$	0.53×10^5	0.88×10^5	DIED	0.45×10^5 cells/cm²
P. porites	20'	$1.58(\pm 0.45)\times10^5$	0.55×10^5	0.68×10^5	0.53×10^5	—
	60'	$0.95(\pm 0.07)\times10^5$	1.10×10^5	1.27×10^5	0.80×10^5	0.59×10^5 cells/cm²
(C) Number of zooxanthellae per coral polyp:						
C. natans	20'	$7.01(\pm 0.62)\times10^4$	2.82×10^4	2.17×10^4	$<0.5\times10^4$	—
	60'	$9.00(\pm 0)\times10^4$	5.30×10^4	8.80×10^4	DIED	4.50×10^4
P. porites	20'	$4.70(\pm 1.88)\times10^3$	1.59×10^3	1.97×10^3	1.54×10^3	—
	60'	$4.17(\pm 0.01)\times10^3$	4.80×10^3	5.52×10^3	2.83×10^3	2.57×10^3
(D) Micrograms of Chlorophyll *a* per zooxanthellae cell:						
C. natans	20'	$4.95(\pm 0.63)\times10^{-5}$	7.8×10^{-5}	10.6×10^{-5}	—	—
	60'	$6.05(\pm 0.49)\times10^{-5}$	7.7×10^{-5}	8.2×10^{-5}	DIED	5.5×10^{-5}
P. porites	20'	4.20×10^{-5} (one value)	8.3×10^{-5}	11.3×10^{-5}	7.5×10^{-5}	—
	60'	$5.40(\pm 0.71)\times10^{-5}$	—	8.0×10^{-5}	8.0×10^{-5}	6.9×10^{-5}

the most profound effect (see Table 1 ⑬).

Both coral species exhibited a decrease in polyp density with increasing depth (Table 2). This trend

Table 2. Polyp density as a function of depth.

	20'	60'
P. porites	$34.5 \pm 4.2/cm^2$	$22.8 \pm 1.8/cm^2$
C. natans	2.3 ± 0.28	1.0 ± 0.1

has also been observed in Montastrea annularis (Dustan, 1979) and Montastrea cavernosa (O'Kane et al., 1980). It does not appear that polyp size increases with depth; rather, the interpolypary space seems to become more important.

The number of zooxanthellae per square centimeter of coral surface clearly decreases with depth (Table 1⑬ controls). But, because of decreasing polyp density, the number of zooxanthellae per polyp remains surprisingly constant (Table 3). No significant differences were noted

Table 3. The number of zooxanthellae cells per polyp as a function of depth (controls).

	20'	60'	
P. porites	$4.70(\pm 1.89) \times 10^3$	$4.17(\pm 0.01) \times 10^3$	$t = 0.39$ with 2 d.f. (not significant)
C. natans	$7.01(\pm 0.62) \times 10^4$	$9.00(\pm 0) \times 10^4$	$t = 2.76$ with 2 d.f. (not significant)

using Student's T-test for either species at the two different depths. An interesting sidelight here is that the chlorophyll *a* content of the zooxanthellae endosymbionts appears to increase with depth (Table 1⑪ controls). Given the small

sample size, this is a rather dubious trend. Nonetheless, it seems feasible that the zooxanthellae should exhibit some sort of adaptation with depth in response to decreased light levels. Increasing the amount of photosynthetic pigment would be the simplest adaptation (Drew, 1972).

As hypothesized, coral populations in deeper water appear to be less well adapted to expel zooxanthellae under stress. The expulsive capability of each coral species is presented as a function of depth in Table 4.

Table 4. Ability to expel zooxanthellae as a function of depth.

		control zooxanthellae/cm^2	post stress (pooled) zooxanthellae/cm^2	Mean % expulsion
C. natans	20'	$1.62(\pm 0.33)\times 10^5$	$0.42(\pm 0.28)\times 10^5$	74 %
	60'	$0.90(\pm 0)\times 10^5$	$0.62(\pm 0.23)\times 10^5$	32
P. porites	20'	$1.58(\pm 0.45)\times 10^5$	$0.58(\pm 0.08)\times 10^5$	63 %
	60'	$0.95(\pm 0.07)\times 10^5$	$0.94(\pm 0.30)\times 10^5$	1

Apparently, the deeper populations of coral are not well adapted to deal with stresses which are not frequently encountered. The only stress studied which might naturally occur at 60' is the shade stress; both shaded corals efficiently expelled zooxanthellae at 60' (Table 1 (B)).

Perhaps the most important finding of this study appears in Table 5 (following page). This table compares the mean amount of chlorophyll a in control zooxanthellae populations with that found in animals which have expelled some zooxanthellae. ~~In both species of coral,~~ In both species of coral, the zooxanthellae remaining after

Table 5. Comparison of chlorophyll a concentrations of control zooxanthellae and zooxanthellae remaining after expulsion (Data pooled and averaged ± one S.D.).

	Pre-expulsion CONTROL	Post-expulsion EXPERIMENTAL	significance
P. porites	$5.0(\pm 0.85)\times10^{-5}$	$8.3(\pm 1.53)\times10^{-5}$	$t=2.37$ with 7 df $p<0.05$, significant
C. natans	$5.5(\pm 0.79)\times10^{-5}$	$8.0(\pm 1.81)\times10^{-5}$	$t=3.31$ with 7 df $p<0.02$, significant

some expulsion has taken place have significantly higher chlorophyll a concentrations than the original population. This strongly suggests that the corals are somewhat selective about which zooxanthellae are expelled; it is possible that senescent or less productive zooxanthellae (containing less chlorophyll a) are preferentially expelled.

This last finding would support the work of Taylor (1969). Taylor feels that the ratio of animal to algal cells is determined by the metabolic rate of the host. Assuming that zooxanthellae are either wholly or partially dependent upon the excreted products of the host's metabolism, this appears to be a valid hypothesis. Through natural division, zooxanthellae would increase their numbers to the maximum permissible levels. At this point, Taylor says, the animal would expel some zooxanthellae in the form of "degenerative vegetative cysts." These are collections of senescent zooxanthellae which have become less productive. Taylor asserts that this process is merely accelerated by stresses which reduce the coral metabolic rate, resulting in the observed mass expulsion of zooxanthellae. The mean

amount of chlorophyll *a* in the remaining cells would become greater following an expulsion episode. This is exactly what is suggested by Table 5.

The adaptive significance of the ability to expel zooxanthellae is still somewhat unclear, although it may simply be a response to the zooxanthellae themselves becoming a stress to the coral. Goreau feels that scleractinians can continue carnivorous feeding after expulsion of zooxanthellae; thus the only detrimental effect would be a decrease in the calcification rate. The ability to harbor and expel zooxanthellae is found in such a broad variety of species that it must confer fitness in some way. This is certainly an area which deserves further study.

The results of this study would be strengthened by performing a greater number of replicates in each experimental group. In addition, it would be helpful to obtain aposymbiotic individuals to act as comparisons for the stressed individuals.

Acknowlegement:

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Compiled Data (APPENDIX)

* indicates dirty prep, chlorophyll data spurious.

			A_{630}	A_{663}	μg Chl a/ml	μg Chl a/cm^2	polyps/cm^2	zooxanthellae cells/cm^2	zooxanthellae cells/polyp	zooxanthellae μg Chl a/cell
Controls:										
2/24	C. natans	20'	0.140	0.250	3.29	0.83 $\times 10^1$ (corrected)	2.5	1.86×10^5	7.44×10^4	4.5×10^{-5}
↓	C. natans	60'	0.100	0.175	2.30	0.58 $\times 10^1$	1.0	0.90×10^5	9.00×10^4	6.4×10^{-5}
	P. porites	20'	0.130	0.240	3.16	0.79 $\times 10$	31.5	1.90×10^5	6.03×10^3	4.2×10^{-5}
	P. porites	60'	0.075	0.135	1.78	0.44 $\times 10$	21.5	0.90×10^5	4.18×10^3	4.9×10^{-5}
2/27	C. natans	20'	0.120	0.220	2.90	0.73 $\times 10$	2.1	1.38×10^5	6.57×10^4	5.4×10^{-5}
↓	C. natans	60'	0.100	0.175	2.30	0.57 $\times 10$	1.0	0.90×10^5	9.00×10^4	5.7×10^{-5}
	P. porites	20' *	0.210 *	0.350	—	—	37.5	1.26×10^5	3.36×10^3	—
	P. porites	60'	0.100	0.180	2.37	0.59 $\times 10$	24.0	1.00×10^5	4.16×10^3	5.9×10^{-5}
Expt'l Aposymbiotic:							(control averages)			
2/24	C. natans	20'	0.080	0.155	2.04	0.51 $\times 10$	2.3	0.65×10^5	2.82×10^4	7.8×10^{-5}
2/25	C. natans	60'	0.070	0.125	1.64	0.41 $\times 10$	1.0	0.53×10^5	5.30×10^4	7.7×10^{-5}
2/24	P. porites	20'	0.075	0.140	1.84	0.46 $\times 10$	34.5	0.55×10^5	1.59×10^3	8.3×10^{-5}
2/25	P. porites	60' *	0.190 *	0.330	—	—	23.0	1.10×10^5	4.80×10^3	—
3/1	C. natans (natural aposymbiotic)	45'	0.060	0.075	0.98	0.25 $\times 10$	2.0	0.45×10^5	2.25×10^4	5.5×10^{-5}
Temperature Stress:										
2/28	C. natans	20'	0.100	0.160	2.10	0.53 $\times 10$	2.3	0.50×10^5	2.17×10^4	10.6×10^{-5}
↓	C. natans	60'	0.130	0.220	2.89	0.72 $\times 10$	1.0	0.88×10^5	8.8×10^4	8.2×10^{-5}
	P. porites	20	0.130	0.235	3.09	0.77 $\times 10$	34.5	0.68×10^5	1.97×10^3	11.3×10^{-5}
	P. porites	60'	0.190	0.310	4.07	1.02 $\times 10$	23.0	1.27×10^5	5.52×10^3	8.0×10^{-5}
Salinity Stress:										
2/28	C. natans	20'	~0	~0	trace	trace	2.3	$<0.10 \times 10^5$	$<0.5 \times 10^4$	—
↓	C. natans	60'	DIED	—	—	—	1.0	—	—	—
	P. porites	20'	0.060	0.120	1.58	0.40 $\times 10$	34.5	0.53×10^5	1.54×10^3	7.5×10^{-5}
	P. porites	60'	0.100	0.195	2.57	0.64 $\times 10$	23.0	0.80×10^5	2.83×10^3	8.0×10^{-5}
Shade stress:										
3/1	C. natans	60'	0.060	0.075	0.98	0.25 $\times 10$	1.0	0.45×10^5	4.50×10^4	5.5×10^{-5}
↓	P. porites	60'	0.075	0.125	1.64	0.41 $\times 10$	23.0	0.59×10^5	2.57×10^3	6.9×10^{-5}

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