Pacific Science (1994), vol. 48, no. 3: 224–233

Carbohydrate, Lipid, and Protein Composition of Zooxanthellae and Animal Fractions of the Coral *Pocillopora damicornis* Exposed to Ammonium Enrichment[1]

Y. ACHITUV,[2] M. BEN-ZION,[3] AND L. MIZRAHI[2]

ABSTRACT: The carbohydrate, lipid, and protein composition of coral tissue and zooxanthellae were compared in Hawaiian *Pocillopora damicornis* (Linnaeus) colonies kept at different ammonium levels. Corals were maintained at two levels of ammonium enrichment (20 μM and 50 μM), in locally drawn seawater with <1 μM ammonium, and in water stripped of ammonium by running over a flume with macroalgae. No significant differences due to the treatment were found in the biochemical composition of the coral tissue. The values from control corals were 900, 275, and 170 $\mu g/cm^2$ for protein, carbohydrates, and lipids, respectively. Under all treatments the carbohydrate levels of zooxanthellae were inconsistent, but did not differ much from the control value of about 650 pg per cell. Lipid content in the control of nonenriched algae remained at ca. 140 pg per cell. However, in the 20-μM treatment algal lipid content increased to about 200 pg per cell during the second and fourth weeks, decreased slightly at 6 weeks, and remained at 164 pg per cell after 8 weeks. In the 50-μM ammonium treatment, there was a decrease to levels of about 40 pg lipids per cell for the entire period. Protein content increased from a control value of 590 pg per cell to ca. 950 pg per cell after 2 and 4 weeks of 20-μM ammonium enrichment and then after 6 weeks dropped back to the control level. At 50-μM ammonium the algal protein content increased after 2 weeks and remained at about 900 pg per cell after 6 and 8 weeks. The preliminary nature of this study is emphasized.

DATA PRESENTED IN this paper are from a workshop on nutrient limitation in the symbiosis between zooxanthellae and reef-building corals, which took place at the Hawaii Institute of Marine Biology during August 1991. The chemical oceanographic context of the workshop was covered by Atkinson (1988), and the overall structure of the various experiments and their relation to the biological aspects of nutrient limitation in zooxanthellate corals are introduced by Stambler et al. (1994).

Literature on phytoplankton indicates that a change in nitrogen supply influences the relative composition of their carbohydrates, lipids, and protein. Planktonic algae have received scientific attention mainly in relation to their nutritional value to other organisms. Parsons et al. (1961) analyzed the chemical composition of 11 phytoplankton species grown in culture under similar chemical and physical conditions. Their results showed that, under uniform conditions, marine phytoplankton have very similar organic composition regardless of their size or the taxon to which they belong. Strickland et al. (1969) compared the composition of phytoplankton cells grown in large tanks where conditions were closer to natural and showed that biochemical composition is not greatly affected by differences in the nutrient concentration of the environment. They did

[1] Manuscript accepted 10 July 1993.
[2] Department of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel.
[3] Department of Chemistry, Bar-Ilan University, Ramat Gan 52900, Israel.

find, however, that differences occur depending on the source of the nitrogen. In the peridinian dinoflagellate *Cachonina niei* (Loeblich) Morrill & Loeblich, a deficiency in nutrient salts leads to an increase in carbohydrate concentration. *Ditylum brightwellii* synthesizes more lipids when nitrogen is supplied in the form of nitrate rather than as ammonium. On the other hand, there are results indicating major changes in the carbohydrate, lipid, and protein content of phytoplankton, grown in large enclosed experimental bags, associated with nutrient limitation (Morris et al. 1983).

Changes in the biomass production and protein and lipid content of two green algae, *Chlorella vulgaris* Beij. (K. & H.) and *Scenedesmus obliquus* (Turp.) Kütz, grown under different nitrogen regimes were studied by Piorreck et al. (1984). They found that high nitrogen levels led to an increase in biomass and protein content. At low nitrogen levels the algae contained a high percentage of total lipids. Harrison et al. (1990), working with three species of marine phytoplankton, *Isochrysis galbana* Parke, *Chaetoceros calcitrans* Ehrenberg, and *Thalassiosira pseudonana* Cleve, grown in batch or semicontinuous culture, found changes in protein, carbohydrate, and lipid concentrations under different nutritional conditions. Under N starvation, the percentage of lipids remained relatively constant, while carbohydrate percentage increased and protein levels decreased. On the other hand, cultures of *Euglena* in nitrogen-deficient conditions accumulated carbohydrates and lipids (Coleman et al. 1988). Shifrin and Chisholm (1981) measured the lipid content of about 30 species of phytoplankton. They found that nitrogen deprivation for 4 to 9 days resulted in a two- to three-fold increase in the lipid content of green algae, whereas both increase and decrease were noted in diatoms, depending on the species.

The effect of salinity and high nutrient concentrations on the marine microalga *Isochrysis galbana* using $NaNO_3$ as a nitrogen source was studied by Fabregas et al. (1986*b*). When nutrient concentration was increased up to 8 mM, the total protein content of the cells increased. At higher nutrient concentrations the total protein content of the cells diminished drastically, probably as a result of toxic effects. The same authors (Fabregas et al. 1986*a*) demonstrated great variability in chemical composition in cultures of *Dunaliella tertiolecta* Butcher. During logarithmic growth, protein concentration was not related to nutrient concentrations, but in the stationary phase, protein cell content reached its maximum value at 16 mM nitrate. Maximum quantities of carbohydrates were also obtained at that nitrate concentration.

In contrast, there is limited information available on the biochemical composition of symbiotic algae. Muscatine et al. (1989) measured the effect of ammonium and phosphate enrichment on the carbohydrate, lipid, and protein content of both zooxanthellae and coral tissue of *Stylophora pistillata* Esper. Analysis of coral tissue revealed no trends with treatment; however, in the zooxanthellae carbohydrate content decreased under ammonium enrichment.

An essential difference exists between free-living algae and symbiotic zooxanthellae in terms of fate of their metabolites. In free-living phytoplankters, most of the excess metabolites are directed toward cell reproduction. In symbiotic zooxanthellae, most of the metabolites are translocated and used by the host (Davis 1984, Muscatine et al. 1984, Achituv and Dubinsky 1990). Muscatine (1990) reviewed the role of zooxanthellae in energy flux in reef corals and concluded that, in general, the in situ growth rate of zooxanthellae in corals appears to be relatively slow and represents a relatively small sink for photosynthetically fixed material. Because of translocation, zooxanthellae concentration in the host's cells remains essentially constant and can be regarded as being in a stationary phase. However, because there is evidence that some zooxanthellae are continually released by the coral, we have to assume that the increase in zooxanthellae numbers has to exceed that required to maintain a constant density in a growing coral, because it also has to account for losses via expulsion. Expulsion of zooxanthellae by *Stylophora pistillata* did not exceed 0.1% of the standing stock of

algae, which in terms of carbon represents 0.01% of the total daily fixed carbon (Høegh-Guldberg et al. 1987). Because most of the metabolites are translocated, it can be assumed that changes in the nutritional conditions of zooxanthellae are also likely to affect the nutritional condition of the coral animal host. Nitrogen content is higher in zooxanthellae from *Porites furcata* Lamarck colonies with resident grunt fish schools than in zooxanthellae from colonies without fish (Meyer and Schultz 1985). Colonies of *Porites furcata* and *Acropora palmata* (Lamarck) with fish schools also showed an increase in coral tissue nitrogen. Nitrogen enrichment by the grunt excretory and fecal material might be responsible for the increase in nitrogen in the zooxanthellae and coral tissue.

A nutrient is limiting when an increase in the flux of that nutrient elicits a metabolic response (Parsons et al. 1984). It has been hypothesized that coral reefs are nutrient-limited and that nutrient enrichment will increase their metabolic response and change the gross productivity (Atkinson 1988, Muscatine et al. 1989). This was one of the hypotheses examined during the Hawaii workshop. Carbohydrate, lipid, and protein content were measured in animal tissue and in zooxanthellae from corals grown in different levels of ammonium enrichment.

MATERIALS AND METHODS

The experimental procedure and treatment of the *Pocillopora damicornis* (Linnaeus) colonies are described by Stambler et al. (1994). After collection, the colonies were incubated in seawater containing 20 μM or 50 μM ammonium, in ambient seawater with <1 μM ammonium, and in nutrient-deprived water, which was run over the macroalga *Gracilaria salicornia* Greville for nutrient stripping. Separation and processing of animal tissue and zooxanthellae are described by Muller-Parker et al. (1994). The number of colonies analyzed for each experimental treatment is given in Table 1. The coral tissue was removed from the skeleton using the Water Pik technique (Johannes and Wiebe 1970) in filtered seawater (glass filter/C), and subsamples from a known volume of the homogenate were taken for cell counts. A subsample of the total coral homogenate was centrifuged, and the algal pellet was resuspended two times in filtered seawater. A subsample of the combined supernatant was used for the determination of carbohydrates, lipids, and proteins of the animal tissue. The algal pellet was resuspended in a known volume of filtered seawater, and the algal cell concentration of this suspension was determined (Muller-Parker et al. 1994). Known volumes (ca. 10 ml) of the algal suspension and of the animal fraction were freeze-dried and used for the analysis of biochemical constituents of the zooxanthellae and coral tissue at a later date at Bar-Ilan University in Israel. Surface area of the corals and algal concentrations were obtained from calculations made by G. Muller-Parker (pers. comm.).

The freeze-dried material was resuspended in distilled water and made up to the original volume. The zooxanthellae samples were sonicated for 5 min, then analyzed for protein concentration using the method of Lowry et al. (1951) with BSA as a protein standard. Lipids were extracted from the samples using chloroform : methanol (2 : 1); then subsamples were transferred to test tubes and evaporated to dryness. Total lipids were analyzed by the microanalytical method of Marsh and Winstein (1966) with palmatic acid as standard. Carbohydrates were analyzed using the anthrone method (Roe 1955) and glucose as standard.

To eliminate the effect of increased algal density in corals exposed to elevated nitrogen concentrations (Muller-Parker et al. 1994), the biochemical constituents of the zooxanthellae are expressed as concentration per cell in contrast to the coral tissue, which is expressed as concentration per surface area of animal tissue.

RESULTS

The biochemical composition (total protein, lipids, and carbohydrates) of isolated

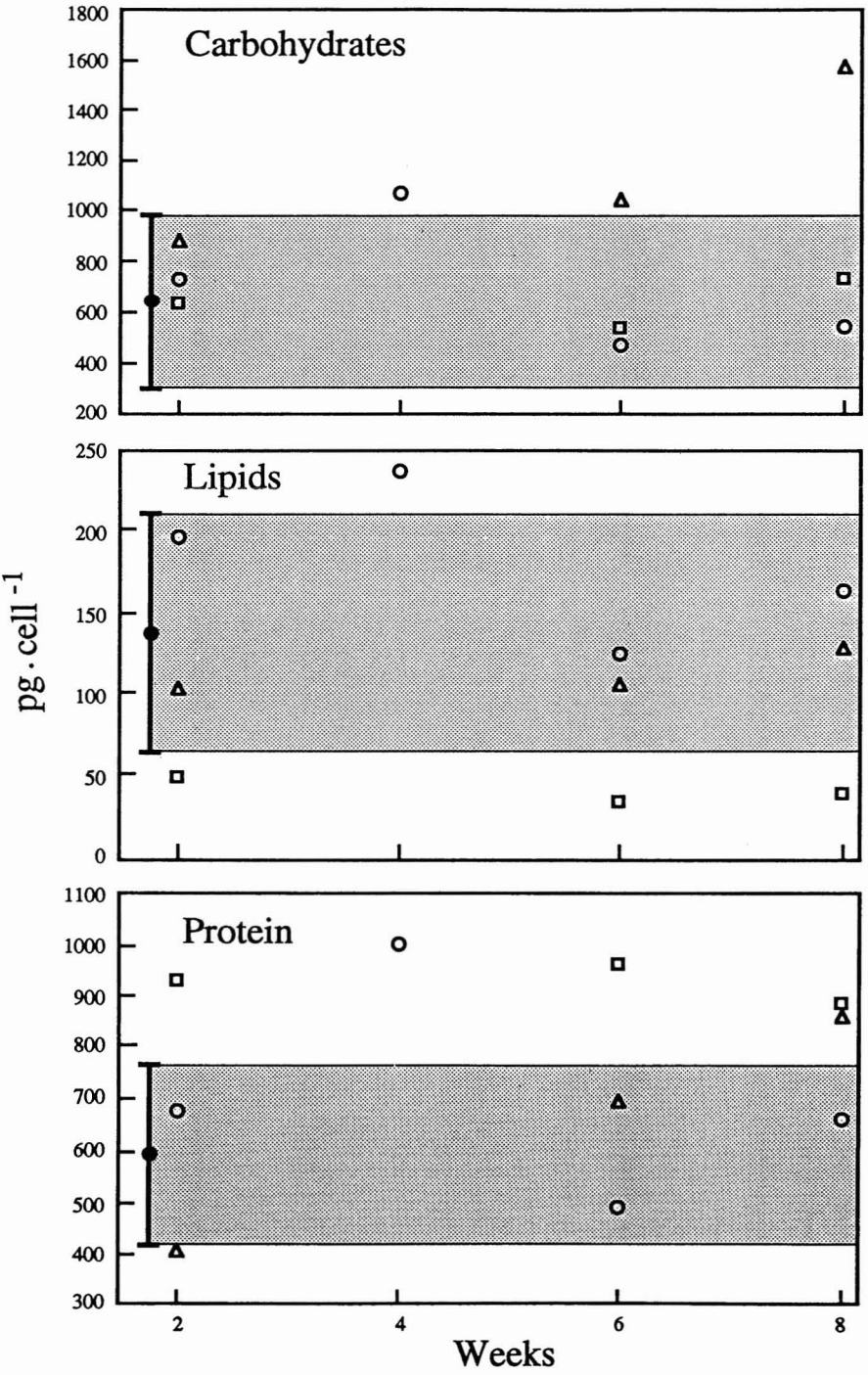

FIGURE 1. Carbohydrate, lipid, and protein content of zooxanthellae from the coral *Pocillophora damicornis*. Filled circle, average control value; its 95% confidence interval is presented as a shaded area; open circle, nitrogen-enriched colonies (20-μM ammonium treatment); open square, nitrogen-enriched colonies (50-μM ammonium treatment); open triangle, nitrogen-stripped colonies. For number of samples see Table 1.

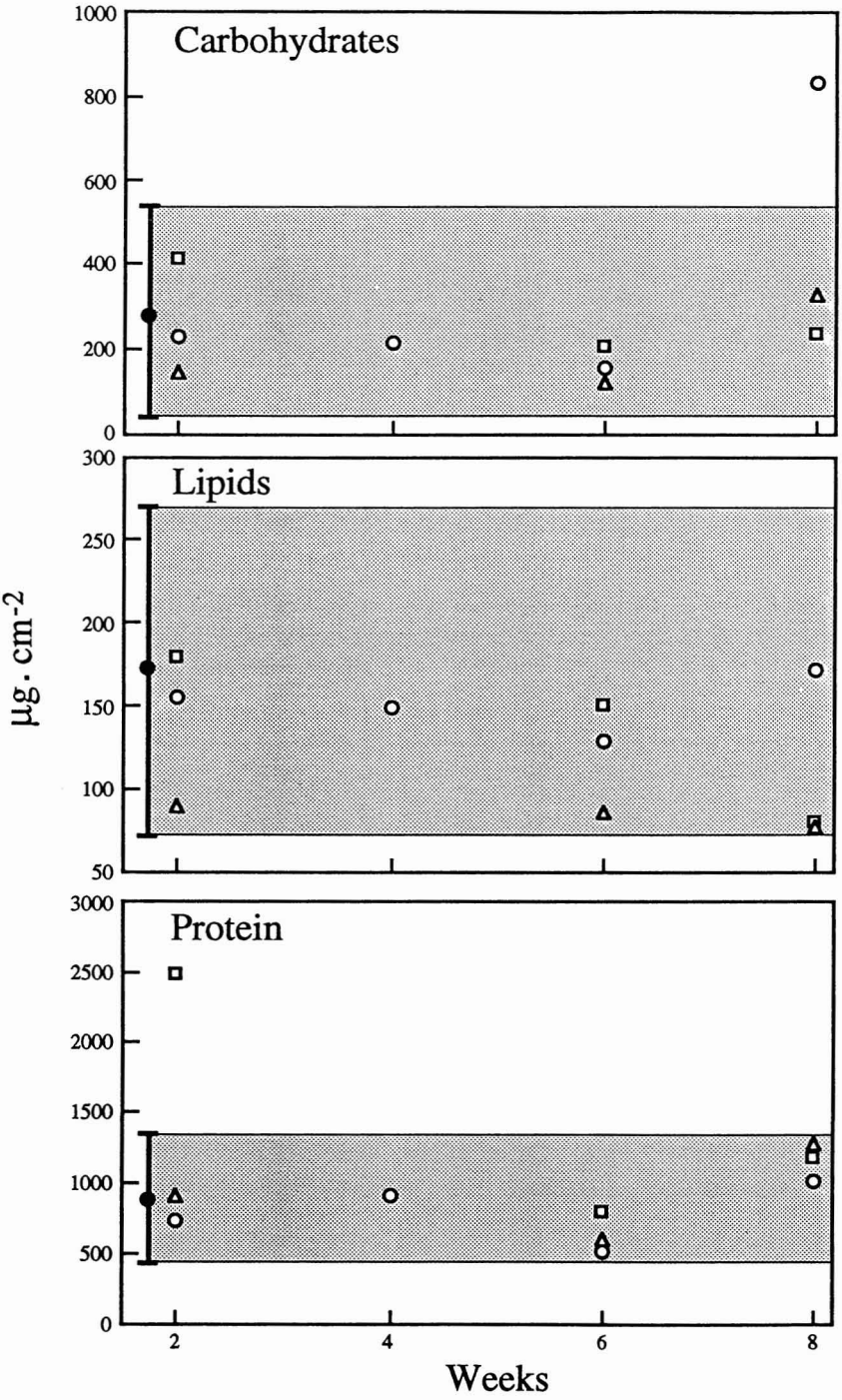

FIGURE 2. Carbohydrate, lipid, and protein content of animal tissue of *Pocillophora damicornis*. For explanation of symbols, see Figure 1.

algae and coral animal tissue at control (ambient seawater) and at three ammonium treatment levels are presented in Figures 1 and 2, respectively, and in Table 1. Because the control corals exposed to ambient seawater were expected to remain unchanged throughout the entire experimental period with respect to biochemical composition, a single, pooled average value was calculated from all time points for each biochemical fraction. This average with its 95% confidence interval is presented as the shaded area in Figures 1 and 2. An examination of the data shows no significant effect of nitrogen enrichment on the biochemical composition of the coral tissue (Table 1, Figure 2).

In the algal cells there was a decrease in lipid content for the 50-μM ammonium enrichment samples (Figure 1, Table 1). At that ammonium concentration, the values remained fairly constant throughout the experimental period. With respect to carbohydrate content, the 50-μM enrichment samples clearly did not differ from the control and again were not so variable. Protein level at that ammonium concentration was constant and always above that found in the control samples. However, the lipid content of zooxanthellae in corals exposed to the 20-μM ammonium treatment was higher than the mean control value, but still within the 95% confidence interval of control corals. At that concentration the changes in lipids, protein, and carbohydrates did not show a constant trend: during the first half of the experiment (i.e., 4 weeks), there was an increase in all three constituents, but during the second 4 weeks there was a decrease in lipid, carbohy-

TABLE 1

PROTEIN, CARBOHYDRATE, AND LIPID COMPOSITION OF ZOOXANTHELLAE AND ANIMAL TISSUE FROM COLONIES OF *Pocillopora damicornis* INCUBATED AT DIFFERENT AMMONIUM LEVELS (VALUES ARE MEANS ± SEM ON *n* DETERMINATIONS)

	ZOOXANTHELLAE (pg CELL^{-1})			ANIMAL TISSUE (μg cm^{-2})		
TREATMENT	PROTEIN	CARBOHYDRATES	LIPIDS	PROTEIN	CARBOHYDRATES	LIPIDS
Stripped						
2 weeks	404 ± 195	881 ± 280	102 ± 82.7	904 ± 249	136 ± 0.64	87 ± 21.3
	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$
6 weeks	694	1,039	106	580	123	87
	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$
8 weeks	863 ± 216	1,547 ± 164	128 ± 52.2	1,265 ± 177	322 ± 126	77 ± 22.6
	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$
Control	594 ± 209	646 ± 399	138 ± 89	902 ± 498	276 ± 267	172 ± 106
	$n = 8$	$n = 8$	$n = 8$	$n = 8$	$n = 8$	$n = 8$
20 μM						
2 weeks	936 ± 435	736 ± 147	197 ± 116	755 ± 257	225 ± 6.7	155 ± 104
	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$
4 weeks	999 ± 597	1,059 ± 196	236 ± 220	923 ± 221	213 ± 10.3	150 ± 13.8
	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$
6 weeks	495 ± 36	473 ± 464	125 ± 58.4	523 ± 196	155 ± 17.1	130 ± 7.7
	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$
8 weeks	669 ± 127	548 ± 322	165 ± 63.1	1,020	831	172
	$n = 2$	$n = 2$	$n = 2$	$n = 1$	$n = 1$	$n = 1$
50 μM						
2 weeks	680 ± 39.4	634 ± 331	47.7 ± 22.6	2,472 ± 2,110	411 ± 341	180 ± 198
	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$
6 weeks	963	544	34.2	804	208	151
	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$
8 weeks	890 ± 98.2	729 ± 180	41.1 ± 7.5	1,175 ± 739	236 ± 91.7	80.4 ± 14.0
	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$

drate, and protein content of zooxanthellae, but the values did not differ from those of zooxanthellae from the control.

In the nitrogen-stripped samples there was a slight and gradual increase in cellular carbohydrates and protein toward the end of the experiment; lipid content remained more or less constant and within the 95% confidence interval of control corals.

DISCUSSION

The results of this investigation show that exposure of the hermatypic coral *Pocillopora damicornis* to nitrogen enrichment did not alter significantly ($P = 0.05$) the biochemical composition of the coral animal tissue. Our coral tissue analyses agree with those of Muscatine et al. (1989), whose values also did not show any clear-cut effect of the various enrichment treatments, including nitrogen enrichment. Muller-Parker et al. (1992) found that the C : N : P ratio of the animal tissue in samples from the very same colonies of *P. damicornis* we studied, and the same experimental treatments, did not change with ammonium enrichment. Although Muller-Parker et al. (1994), also as part of the same experiment, observed an increase in areal animal protein concentration after 8 weeks of exposure to the 20-μM ammonium treatment, we did not detect such an increase.

Our results show that in the zooxanthellae, the amount of protein per algal cell increased in both ammonium concentrations. This change was already noticeable after 2 weeks of ammonium enrichment, and most of the changes occurred within the first 2 weeks of exposure. This agrees with Muller-Parker et al. (1994), who also found that beyond the first 2 weeks continuous ammonium enrichment had little further effect on the algal biomass parameters. The changes in the biochemical composition of zooxanthellae (Table 1) expressed per cell were not caused by differences in cell size. Berner and Izhaki (1994) show that cell diameters of all zooxanthellae isolated from the corals were equal, regardless of nitrogen treatment.

The carbohydrate-to-protein and lipid-to-protein ratios in zooxanthellae and in animal tissue are presented in Table 2. The carbohydrate-to-protein ratio in the zooxanthellae at 20 μM and 50 μM ammonium enrichment did not deviate much from the control value, which was around unity. In the ammonium-stripped corals, this ratio was

TABLE 2

RATIO OF LIPIDS TO PROTEIN AND CARBOHYDRATES TO PROTEIN IN ZOOXANTHELLAE AND ANIMAL TISSUE IN COLONIES OF *Pocillopora damicornis* INCUBATED AT DIFFERENT AMMONIUM LEVELS

	ZOOXANTHELLAE		ANIMAL TISSUE	
TREATMENT	LIPIDS/PROTEIN	CARBOHYDRATES/PROTEIN	LIPIDS/PROTEIN	CARBOHYDRATES/PROTEIN
Stripped				
2 weeks	0.229	2.276	0.097	0.181
6 weeks	0.153	1.498	0.149	0.212
8 weeks	0.161	1.876	0.063	0.246
Control				
	0.242	1.165	0.234	0.317
20 μM				
2 weeks	0.204	0.841	0.193	0.269
4 weeks	0.207	1.363	0.165	0.283
6 weeks	0.248	0.924	0.264	0.299
8 weeks	0.241	0.881	0.169	0.815
50 μM				
2 weeks	0.069	0.919	0.061	0.492
6 weeks	0.036	0.565	0.188	0.258
8 weeks	0.046	0.813	0.090	0.233

higher because of the decrease in carbohydrate content of the algal cells. The ratio between lipids and protein in zooxanthellae from the control samples, and in those from corals exposed to the 20-μM ammonium treatment, was between 0.20 and 0.25. In algae from corals kept in 50 μM ammonium, this ratio dropped from 0.04 to 0.07, which reflects the decrease in lipid content and concomitant increase in protein.

Muller-Parker et al. (1992) showed that zooxanthellae from N-enriched *P. damicornis* had less C and more N per cell than those from the seawater control corals. Analysis of the relative abundance of carbohydrates, lipids, and protein in zooxanthellae from *Stylophora pistillata* (Muscatine et al. 1989) revealed that carbohydrates per cell were significantly lower for N-treated corals than in control colonies. However, we could not demonstrate the same effect in zooxanthellae from *P. damicornis*.

In phytoplankton there is a general predominance of protein over other constituents. However, in the Dinophyceae, to which the zooxanthellae belong, the ratio of protein to carbohydrates is lower than in other algae and also approaches unity (Parsons et al. 1984). In the coral tissue, carbohydrate levels are lower, and therefore the ratio between carbohydrates and protein is rather low. Parsons et al. (1961) emphasized that their results, showing similarity in the chemical composition of various species of free-living phytoplankton cells, were obtained with no nutrient limitation, and the results would have been different had the cells been grown under conditions of nutrient deficiency. Fabregas et al. (1986*a*,*b*) found that under high nitrogen concentrations there was an increase in protein in *Isochrysis galbana* and in both protein and carbohydrates in *Dunaliella tertiolecta*. However, those authors used nitrogen concentrations that were more than two orders of magnitude higher than those used in our experiments. It is not unreasonable to expect that to detect notable changes in the biochemical composition of algae, the colonies should be exposed to much higher concentrations than those used in our experiments.

Berner and Izhaki (1994) show, by analyzing the ultrastructure of zooxanthellae, that in the control corals and in corals grown in nitrogen-stripped seawater the percentage of cell volume of starch and lipid inclusions is higher than in the zooxanthellae from corals grown at elevated nitrogen levels. The storage materials occupied only 15% of the algal cell volume. These findings are not clearly reflected in our results. However, it should be emphasized that our determinations include the total amounts of the biochemical components, of which the storage material comprises only a small portion. Furthermore, the storage bodies found by Berner and Izhaki (1994) may also contain nitrogenous compounds. Nevertheless, as mentioned previously, in the zooxanthellae of nitrogen-stripped and seawater control samples there was an increase in carbohydrate content toward the end of the experimental period. Lipid content of zooxanthellae from the 50-μM ammonium enrichment was higher than that of the control and the nitrogen-stripped zooxanthellae; this reflects the trend found by Berner and Izhaki (1994).

In the N-enriched corals the density of the zooxanthellae increases (Muscatine et al. 1989, Muller-Parker et al. 1994), and this is associated with a relative decrease in lipids in the zooxanthellae. It can be assumed that under these conditions either lipids were not synthesized to the same extent as in the low-nitrogen zooxanthellae or lipids were used for algal proliferation, and thus nitrogen might regulate zooxanthellae density within the coral. Høegh-Guldberg (1994) found that the mitotic index of zooxanthellae from the same corals exposed to ammonium enrichment was two to three times higher than in the control corals. Coleman et al. (1988) suggested that nitrogen deficiency inhibits cell division so that the carbohydrates and lipids produced are divided among fewer cells, increasing the quantity of storage products per cell.

Some of the differences and inconsistencies in our results might be due to the high heterogeneity of the samples. G. Muller-Parker (pers. comm.) reported a high variability in the density of algal cells in the coral

tissue of our control samples, commonly ranging over an order of magnitude.

In conclusion, this initial study on the biochemical composition of coral tissue and zooxanthellae shows that we could not demonstrate major consistent changes in the biochemical composition of coral tissue. In some cases, a general similarity between zooxanthellae and free-living microalgae does exist; nevertheless, differences can be found as well. In free microalgae, considerable variations as a function of species and ecological conditions were shown to exist. It is possible that in different host corals and at different initial nutritional conditions the zooxanthellae will exhibite different responses. This species-specific response might also be reflected in the deviation from results presented by other investigators. Furthermore, because most of the changes, especially under high ammonium concentrations, probably occurred within the first 2 weeks of exposure, the time course and dynamics of change should be studied. Any assumptions drawn from our preliminary measurements must be confirmed by further studies on a larger scale.

ACKNOWLEDGMENTS

We thank the Hawaii Institute of Marine Biology for hospitality during the course of the workshop. The workshop was supported by the U.S.–Israel Binational Scientific Foundation.

LITERATURE CITED

ACHITUV, Y., and Z. DUBINSKY. 1990. Carbon budgets in marine, mutualistic association between microalgae and cnidarians. Pages 36–48 *in* J. Mellinger, ed. Nutrition in wild and domestic animals. Karger, Basel.

ATKINSON, M. J. 1988. Are coral reefs nutrient limited? Proc. 6th Int. Coral Reef Symp., Australia 1:157–166.

BERNER, T., and I. IZHAKI. 1994. Effect of exogenous nitrogen levels on ultrastructure of zooxanthellae from the hermatypic coral *Pocillopora damicornis*. Pac. Sci. 48: 254–262.

COLEMAN, L. W., B. H. ROSEN, and S. D. SCHWARTZBACH. 1988. Environment control of carbohydrate and lipid synthesis in *Euglena*. Plant Cell Physiol. 29:423–432.

DAVIS, P. S. 1984. The role of zooxanthellae in the nutritional energy requirement of *Pocillopora eydouxi*. Coral Reefs 2:181–186.

FABREGAS, J., C. HERRERO, J. ABALDE, R. LIANO, and B. CABEZAS. 1986*a*. Biomass production and biochemical variability of the marine microalga *Dunaliella tertiolecta* (Butcher) with high nutrient concentrations. Aquaculture 53:187–199.

FABREGAS, J., C. HERRERO, B. CABEZAS, and J. ABALDE. 1986*b*. Biomass production and biochemical composition in mass cultures of the marine microalga *Isochrysis galbana* Parke at varying nutrient concentrations. Aquaculture 53:101–113.

HARRISON, P. J., P. A. THOMPSON, and G. A. CALDERWOOD. 1990. Effects of nutrient and light limitation on the biochemical composition of phytoplankton. J. Appl. Phycol. 2:45–56.

HØEGH-GULDBERG, O. 1994. Population dynamics of symbiotic zooxanthellae in the coral *Pocillopora damicornis* exposed to elevated ammonium [$(NH_4)_2SO_4$] concentrations. Pac. Sci. 48:263–272.

HØEGH-GULDBERG, O., L. R. MCCLOSKEY, and L. MUSCATINE. 1987. Expulsion of zooxanthellae from symbiotic cnidarians from the Red Sea. Coral Reefs 5:201–204.

JOHANNES, R. E., and W. J. WIEBE. 1970. A method for determination of coral tissue biomass and composition. Limnol. Oceanogr. 15:822–824.

LOWRY, O. H., N. J. ROSEBOUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 19:265–275.

MARSH, J. B., and D. B. WINSTEIN. 1966. Simple charring method for determination of lipids. J. Lipid Res. 7:574–576.

MEYER, J. L., and E. T. SCHULTZ. 1985. Tissue condition and growth rate of coral

associated with schooling fish. Limnol. Oceanogr. 30:157–166.

MORRIS, R. J., M. J. MCCARTNEY, and G. A. ROBINSON. 1983. Studies of a spring phytoplankton bloom in an enclosed experimental ecosystem. 1. Biochemical changes in relation to the nutrient chemistry of water. J. Exp. Mar. Biol. Ecol. 70:249–262.

MULLER-PARKER, G., C. B. D'ELIA, and C. B. COOK. 1992. Elemental composition of corals exposed to elevated sea water NH_4^+. Page 73 *in* Proc. 7th Int. Coral Reef Symp., Guam.

MULLER-PARKER, G., L. R. MCCLOSKEY, O. HØEGH-GULDBERG, and P. J. MCAULEY. 1994. Effect of ammonium enrichment on animal and algal biomass of the coral *Pocillopora damicornis*. Pac. Sci. 48:273–283.

MUSCATINE, L. 1990. The role of symbiotic algae in carbon and energy fluxes in reef corals. Pages 75–87 *in* Z. Dubinsky, ed. Coral reef ecosystems. Elsevier, Amsterdam.

MUSCATINE, L., P. G. FALKOWSKI, J. W. PORTER, and Z. DUBINSKY. 1984. Fate of photosynthesis fixed in light- and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. Proc. R. Soc. Lond. B Biol. Sci. 222:181–202.

MUSCATINE, L., P. G. FALKOWSKI, Z. DUBINSKY, P. A. COOK, and L. R. MCCLOSKEY. 1989. The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. Proc. R. Soc. Lond. B Biol. Sci. 236:311–324.

PARSONS, T. R., K. STEPHANS, and J. D. STRICKLAND. 1961. On the chemical composition of eleven species of marine phytoplankter. J. Fish. Res. Board Can. 18:1001–1016.

PARSONS, T. R., M. TAKAHASHI, and B. HARGRAVE. 1984. Biological oceanographic processes. Pergamon, Oxford.

PIORRECK, M., K. H. BAASCH, and P. POHL. 1984. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. Phytochemistry (Oxf.) 23:207–216.

ROE, S. H. 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. J. Biol. Chem. 212:334–343.

SHIFRIN, N. S., and S. W. CHISHOLM. 1981. Phytoplankton lipids: Interspecific differences and effects of nitrate, silicate, and light-dark cycles. J. Phycol. 17:374–384.

STAMBLER, N., P. L. JOKIEL, and Z. DUBINSKY. 1994. Nutrient limitation in the symbiotic association between zooxanthellae and reef-building corals: The experimental design. Pac. Sci. 48:219–223.

STRICKLAND, J. D., O. HOLM-HANSEN, R. W. EPPLEY, and R. J. LINN. 1969. The use of a deep tank in plankton ecology. I. Studies of the growth and composition of phytoplankton at low nutrient levels. Limnol. Oceanogr. 14:23–34.