Final Report:

Lipid content in relation to decreased light in *Montipora verrucosa* and *Porites compressa*

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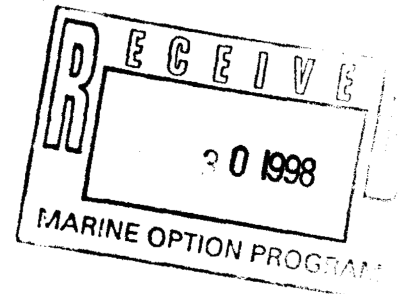

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In June 1997 I proposed to do my Marine Option Program Certificate project with Dr. E.S. Reese, Ethology, Department of Zoology, at University of Hawaii at Manoa. The proposed project title was **Feeding Rates and nutritional values in the coral-feeding butterflyfish *Chaetodon multicinctus*.** The project was designed to conduct research with Dr. Reese and his graduate student Javier Mendez looking at the behavior of the obligate corallivore *Chaetodon multicinctus*. Dr. Reese is head of a study which goal is to find out if the behavior of the butterfly fish changes with a changing environment, and if you therefore can use these fish as indicator-species to detect environmental degradation of coral reefs. The short term goal in the part I was to participate in, was to look at what nutritional values (in terms of lipids) the coral polyp supply the fish with, and to investigate if *C. multicinctus* feeding rates would change if corals were subjected to stress in terms of run-off or pollution. I worked on the project for nine months, and ended up doing it differently from planned. This is a short review of what I did and where that took me.

I started working with Dr. Reese and Mendez in late May of 1997. My first assignment was to assist J. Mendez in marking territories for *C.multicinctus*. This was done three days a week on the outer sloop of the barrier reef in Kaneohe Bay. Using SCUBA we descended onto the reef-slope (50-60 feet) and observed the fish-community looking for pairs of *C. multicinctus*. Once we identified a pair each, we would hover above our fish-pair following them throughout their territory and putting down a marker (a nail with specific colored flagging tape) every time the fish changed direction. We would stay with each pair for an hour, and by the end of the hour we could identify a marked territory for the pair. As we followed neighboring pairs one after another, we did end up with a large area divided into smaller territories marked by different colors of flagging tape. I participated in this project as a diver for two months.

The next step in the project was to determine whether it was possible to get the tentacles removed from a coral polyp. The plan was to find a way of doing this so that we could compare the nutritional values of the tentacles compared to entire polyp as the butterflyfish only feeds on extended tentacles. I attempted this by trying to relax the coral polyp with magnesium chloride to prevent it from retract its extended tentacles. The MgCl was hypothesized to substitute the sodium chloride of the seawater, and thereby restrict the ionic flow in the coral polyp. I managed to do this successfully, but did not succeed in removing the tentacles from the polyp.

At this point, Javier Mendez and Dr. Reese were busy fulfilling other duties, and I was left to design the next step of the project; determining lipid content in light deprived corals compared to corals subjected to ambient light. Enclosed is a report on that experiment and the results. The study took approximately six months to complete and I feel that I learned a great deal in that process.

Looking back at the proposal written at the start of this project, I realize that the final outcome is different from what I originally proposed. However, I feel that I have learned a great deal during the nine months with Dr. Reese and I would like to shortly discuss that. First of all, working with Dr. Reese and his laboratory exposed me to a new perspective of research: there are so many additional questions attached to each question answered that you can not anticipate where your research will finally take you. I also learned that patients and enthusiasm are essential characteristics for every successful researcher, and that research takes time – much more time than you think. A very valuable lesson with this project was because research takes longer than you anticipate, it is very easy to over-commit, and that is dangerous as lots of time is vital for obtaining good results. Besides these very important experiences, I learned a great deal about coral reef systems, chemistry and

biochemistry, experimental design and statistics. All together, this project might be one of my most valuable resources I'm bringing with me to graduate school.

Lipid content in relation to decreased light in *Montipora verrucosa* and *Porites compressa*

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Abstract

Declining lipid content in corals has been suggested as a measurement of the amount of stress the coral is subjected to. It has been hypothesized that decreased sunlight could be a stress that would be reflected in the decreased amount of lipid in the coral tissue. In this experiment this hypothesis was tested on the Hawaiian corals *Montipora verrucosa* and *Porites compressa*. Three colonies of each species were cut in half, and one half put under ambient light and the other under 50% light reduction for seven days. Colonies were tested both in outdoor tanks and on the reef flat.

Shading had a significant effect on *Porites compressa* in the tank as it showed an increase in lipid content in the shaded part. The trend was similar in the reef flat colony of *P. compressa* and in the colonies of *Montipora verrucosa*, but not significantly different. This suggests that shading might serve as a relief in corals living in high radiation environments. Analyses also showed great variability in lipid content both within colonies, and between colonies for both species.

Introduction

Coral reefs are subjected to different environmental stress such as run-off and siltation from land, sewage and agriculture (Hourigan et al., 1988; Jokiel and Coles, 1974) as well as other contaminants that can affect the reef-building scleractinian corals and the biota around these reefs. Stress on coral is defined as to halt or restrict its growth and reproduction (Hourigan et al., 1988), but the amount of stress the coral is subjected to might be difficult to measure as pollution on coral reefs can occur in constant chronic levels over long periods of time. In response to this, Dr. E.S. Reese (1995) at University of Hawai'i at Manoa has developed a method to use butterflyfishes of the family Chaetodontidae as indicators to detect change on coral reefs.

The technique is designed to work as an early warning of stress on the reef by detecting low-level and sub-lethal changes in the habitat (Crosby and Reese, 1996). The chaetodontids (Perciforms) are a circumtropical family which includes 114 species, with 90 of these in the genus Chaetodon (Motta, 1988). Twenty species in the family occur in Hawaii, and these are planktivores, corallivores or benthic omnivores. In Hawaii, the studies have focused on two species in the coral-feeding guild: *Chaetodon. multicinctus* and *C. ornatissimus*. Other species of corallivores have been studied at Enewetak Atoll, Marshall Islands, and on Australia's Great Barrier Reef.

These two species of corallivores are generalists, common inhabitants on the reef, territorial and permanent inhabitants of their home reefs (Reese, 1993). These characteristics make them valuable indicators of the health of the corals on the reef, as they may respond to declines in coral quality or abundance by spatial and behavioral adjustments that can be measured (Mendez, 1990).

Since shallow water corals contain relatively large amounts of lipid throughout their tissues (Stimson, 1987), it is likely that the coral feeding butterflyfish utilize the lipids, as lipid content has been shown to affect prey choice of the butterflyfish (Hourigan et al., 1988, Tricas, 1989).

The high lipid content in the coral is suggested as an energy reserve for the coral used in reproduction as well as for daily losses of lipid in the form of mucus. The lipid is transferred from the zooxanthellae that fixate the carbon (Kellog and Patton, 1983), to the coral. Research strongly suggests that lipid production, being dependent on the photosynthesis of the zooxanthellae (Crossland et al., 1980), is thereby dependent on the amount of light in the water. Several studies (Stimson, 1987; Harriott, 1993; Ward, 1995) have suggested that

decreased amounts of lipid in coral tissue could be an indication of stress, and decreased light could account as a stress. Davies (1991) proposes that shallow water corals have to use stored lipids for metabolism on cloudy days. Experiments conducted by Stimson (1987) and Harland et al. (1992) show that shaded corals had lower lipid levels than unshaded corals.

This study was designed to test, if indeed, the lipid values decreased with a decrease in sunlight as would be the case with low level run-off or pollution. If that was the case, the next study would then be to confirm that the coral feeding Chaetodontidaes did modify their feeding behavior to make up for the decrease in nutritional value (lipids).

Material and Methods

The experiment was conducted at Hawaii Institute of Marine Biology (HIMB), Coconut Island in Kaneohe Bay, Oahu, Hawaii. Three coral colonies each of *M. verrucosa* and *P. compressa* were collected on the reef flat of Coconut Island on the morning of July 2, 1997. Coral colonies were immediately put into buckets of seawater after collection. Each colony was then cut into two similar halves using an electric rock saw, and then returned to seawater.

Two colonies of each species were put into an outside tank with constant flow of seawater. The tank measured .5 m in depth and 3.5 m in length. Half of the tank (1.75 m) was covered with a shading cloth blocking 50% of ambient light and half of each colony was put in this end of the tank, whereas the other half was put in the non-shaded part. Corals were left there for seven days (July 2-July 9).

The third colony of each species was put back on the reef flat under similar conditions as in the tank. Two cages were constructed from chicken wire with two open ends (Fig. 1). One cage was wrapped in the same kind of shading cloth used for the tank, and the other had

no shading cloth. Cages were put onto the reef flat in the same area were the colonies were collected (1 m. depth at intermediate tide). One half of each colony was then put into each cage, and left there for the same amount of time as in the tank.

During the seven days of the experiment, air and water temperatures as well as Quanta umol/cm2/minute was recorded on an hourly basis 24 hours a day. The mean of these can be found in Table 1. Quanta measures micromoles (umol) of photosynthetically active radiation (PAR) per unit area (cm2) and time (seconds). This measurement do not include ultraviolet nor infrared light. Corals reach saturation around 300 PAR (Cox, 1998).

On July 9, 1997 the sampling occurred. Five branches, 1.5-2 cm in length, were broken off from each half colony. The five branches on the shaded side were each marked to correspond closely to five branches from the non-shaded side. Each branch was immediately put into a marked zip-lock bag and frozen in a freezer at -50°C.

The method for lipid extraction was adapted from Harland et al., 1992: method two. The method differs in that no decalcification was done before the extraction as that could lead to loosing lipids in the process (Grottoli-Everett, 1995).

Each branch was grinded with a 2:1 chloroform methanol solution. It was extracted for 1 hour, then filtered and rinsed with the same solution. Residue (organic and skeleton) would then be dried to a constant weight in a drying oven at 75°C. This would be weighed and then burned at 450°C for 6 hours in a muffle furnace. Sample was then weighed again to determine dry tissue biomass.

The lipid in the chloroform methanol solution would then be rinsed with 0.88% potassium chloride solution to remove excess salts from solution. It was then rinsed three times with a methanol water solution (1:1) to remove excess water and other impurities. The

remaining organic phase was then dried under a fume hood to constant weight in a pre-weighed aluminum pan.

Lipid content in the corals was reported as % lipid per gram dry tissue weight.

Results

The total lipid content of the branches in each coral colony is shown in Table 2 for *Porites compressa,* and in Table 3 for *Montipora verrucosa*. The tables include mean per colony with standard deviation and percent standard deviation. Figures 3 and 4 show the percent lipid in the branches of each colony of *P. compressa* and *M. verrucosa*, respectively. The mean percent lipid per colony (shaded and unshaded) are represented with standard deviation in Figure 5 for *P. compressa* and Figure 6 for *M. verrucosa.*

Using a General AOV test within each species showed that there was a significant difference in % lipid between the shaded and unshaded *Porites compressa* colonies (P=.0001).That analysis also showed that there was a significant difference between colonies and within colonies (P=0 and P=.0039 resp.), showing that the treatment might not be the significant factor. However, comparing the two *P. compressa* colonies in the tank showed that there is a significant difference between the shaded and unshaded (P=0) and not a significant difference between or within the colonies (P=.6388 and P=.4995 resp.).

For *Montipora verrucosa*, an experimental error occurred and resulted in the loss of one colony. Analyses of the two remaining colonies show no significant difference between the shaded and the unshaded parts (P=.3642), and within the colonies (P=.5318). It did however show a significant difference between the two colonies (P=.0492).

Discussion

This study suggests that the lipid content in the shaded coral is higher than in the unshaded coral. There is a definite trend among all colonies of both species, even though there is not a significant difference in all of them.

This challenges the results obtained by Stimson (1987) that showed a decrease in lipid concentrations in correlation with decreased light levels until the corals photoadapted after a few weeks and percent lipid increased. One limitation with this study compared to that of Stimson's (1987) is the number of colonies in this study was limited to three per treatment and species (N=3). This sample number is too small to strongly demonstrate the results. However, the trends need to be accounted for and considered as possible counterparts to that of Stimson's (1987). It is also noteworthy that Stimson's study suggested *Montipora verrucosa* to have lipid contents around 40% and *Porites compressa* having around 30% lipid of dry tissue weight. These values are much higher than the percent lipids obtained in this study.

There are many factors to take into account comparing the studies described above. The technique used by Stimson (1987) was evaluated by Harland et al. (1984). It is there recognized as having limitation as extracts could contain non-lipid material in addition to the lipid, and it was described to result in about 30% higher lipid content than compared methods. (The method used here was not evaluated by Harland et al.) In addition Stimson did not use KCl wash nor the methanol:water wash to protect from excess salts and/or water. These differences might account for the higher % lipid found in Stimson's work.

Looking at the increased amount of lipid in shaded corals in this study yields many additional questions and hypotheses. One issue to consider is that the corals used in the experiment were collected in shallow water (< 1.5 m) during the summer months and

consequently were subjected to high levels of light on a daily basis. It is therefore possible that the 50% shading relieved them from high radiation stress and they thereby would utilize less amount of lipid as an energy reserve (Harriott 1993), and could instead store lipids.

Harriott (1993) also showed that one species of coral *Acropora formosa* increased its lipid index after four weeks in darkness. Possible explanations by Harriott included lipids being mobilized from other regions of the coral toward the tips, or that corals might adapt to low light environment by devouring plankton or suspended particulates.

Another issue in this study to discuss is the spawning of these coral species. *Montipora verrucosa* spawns every month during the summer months on new moon (Cox, 1998) and could have spawned on June 4 and on July 4 (during the experiment). *Porites compressa* also spawns during the summer on full moon and the species spawned before and after the experiment;,on June 20 and on July 19 (Cox, 1998). It was not noted if these spawnings did occur in the actual colonies studied, but it was noted that spawning did occur in the area on these dates. Since lipid levels have been seen to decrease with planulation (Stimson, 1987), it is possible that the lower percent lipids in the unshaded corals could be correlated with the spawning events, and that the shaded coral did not spawn to conserve energy. It is important to note that the condition of this hypothesis is the coral actually experienced stress as it was shaded.

Looking at the *P. compressa* colonies we find a significant difference between shaded and unshaded in the colonies put into the tanks. The colony on the reef flat show the same trend, but there is not a significant difference there. It might be that both parts of the colony on the reef flat did undergo spawning as it remained in its natural habitat, and that the colonies in the tank altered their behavior and tried to acclimatize by letting only the

unshaded part spawn. Any of the above discussed reasons might also apply to why the colonies in the tank showed a significant difference whereas the reef flat colony did not.

Montipora verrucosa showed the same trends, but not a significant difference between shaded and unshaded. This species also showed a comparatively low lipid content overall compared to other studies (Stimson, 1987) that found it to be around 40%. It is possible that this lower number is due to the spawning season as the above mentioned study was conducted in the spring (before the spawning months of the species). Looking at the significant variability between colonies and within colonies, it is also possible that the tested colonies varied greatly in lipid content compared to other colonies in the area for a reason not understood. This emphasizes the importance of a greater sample number for future studies.

As shown in this study and others (Harriott, 1983; Stimson, 1987), the variability of lipid levels within coral colonies as well between colonies, is significant. This might correlate to the feeding preference of coral feeding Chaetodontids. It is essential to further investigate the significance of light levels in relation to the amount of lipids to determine how run off and other sources of coral reef pollution might affect lipid levels.

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Figure 1.

Design of cage placed on reef flat

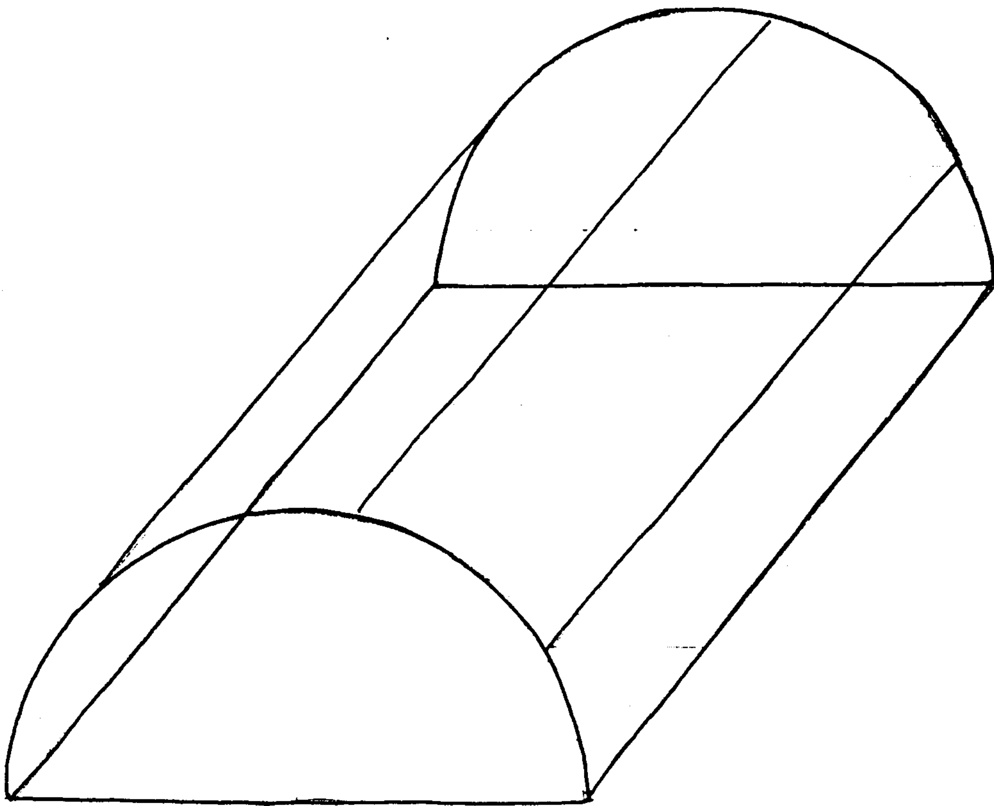


Table 1.

Mean air and water temperatures, and Quanta July 2 - July 9 1997

Date	Air temp. C	Water temp. C	Quanta; υmoles/cm2/sec.
July 2	25.2	26.5	385.8
July 3	25.3	26.6	471.0
July 4	25.3	26.8	402.1
July 5	25.4	27.0	521.6
July 6	25.3	26.7	419.6
July 7	25.6	27.1	504.3
July 8	25.8	27.0	395.5
July 9	25.2	26.7	225.4

Table 2.

The % lipid content of branches in each coral colony including mean, standard deviation and percent standard deviation

Porites compressa

Colony #1 Reef flat	Unshaded part	Shaded part
Branch #1	25.3	25.0
Branch #2	26.2	25.1
Branch #3	21.2	23.4
Branch #4	25.8	25.6
Branch #5	24.1	21.1
Mean colony #1	**24.5**	**24.0**
Standard deviation	**2.02**	**1.84**
% Standard deviation	**8.25**	**7.63**
Colony #2 Tank		
Branch #1	19.1	19.8
Branch #2	12.9	19.8
Branch #3	12.8	20.5
Branch #4	12.8	18.3
Branch #5	15.0	16.4
Mean colony #2	**14.3**	**20.0**
Standard deviation	**1.55**	**1.52**
% Standard deviation	**10.79**	**7.60**
Colony #3 Tank		
Branch #1	12.5	19.8
Branch #2	15.7	19.8
Branch #3	16.1	20.5
Branch #4	14.1	18.3
Branch #5	13.3	16.4
Mean colony #3	**14.5**	**19.0**
Standard deviation	**2.74**	**1.65**
% Standard deviation	**18.84**	**8.68**

Table 3.

The % lipid content of branches in each coral colony including mean, standard deviation and percent standard deviation

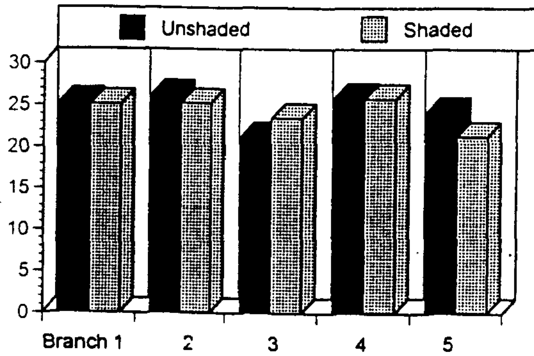
Montipora verrucosa

Colony #1 Reef flat	Unshaded part	Shaded part
Branch #1	14.8	8.87
Branch #2	7.5	20.0
Branch #3	8.7	9.3
Branch #4	9.3	6.5
Branch #5	10.4	8.4
Mean colony #1	10.1	10.6
Standard deviation	2.80	5.36
% Standard deviation	27.75	50.49
Colony #2 Tank		
Branch #1	15.4	16.8
Branch #2	6.6	11.4
Branch #3	16.7	16.5
Branch #4	10.4	16.6
Branch #5	14.2	15.5
Mean colony #2	12.7	15.4
Standard deviation	4.14	2.28
% Standard deviation	32.73	14.87

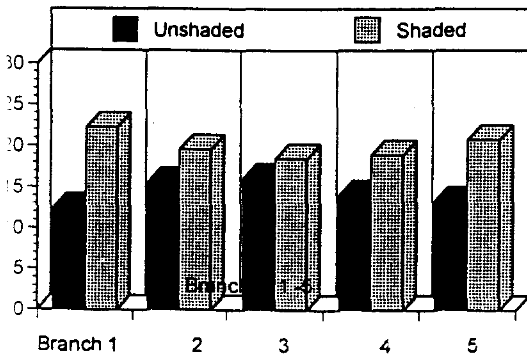
Figure 3.

Percent lipid in the branches of *Porites compressa*

% Lipid in the different branches of *Porites compressa* colony #1 (cage)



% Lipid in the different branches of *Porites compressa* colony # 2 (tank)



% Lipid in the different branches of *Porites compressa* colony # 3 (tank)

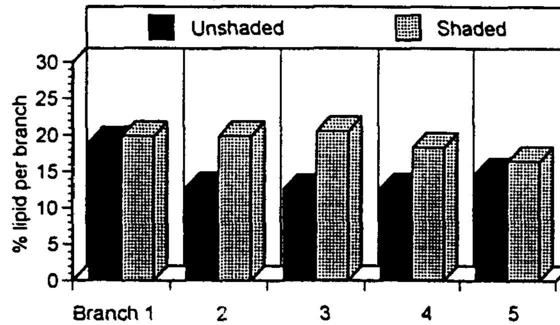
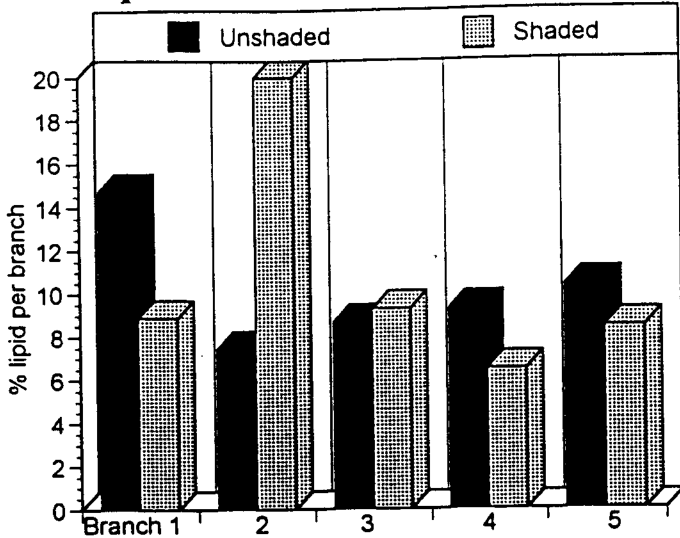


Figure 4.

Percent lipid in the branches of *Montipora verrucosa*

% Lipid in the different branches of *Montipora verrucosa* colony # 1 (cage)



% Lipid in the different branches of *Montipora verrucosa* colony # 2 (tank)

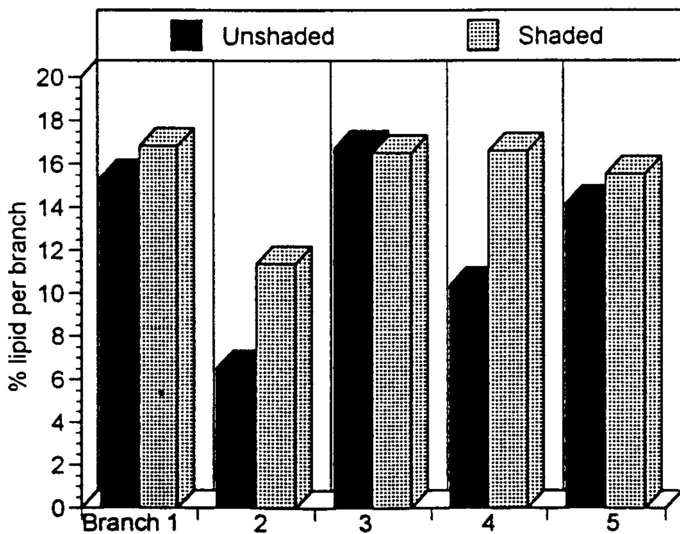


Figure 5.

Mean percent lipid in three *Porites compressa* colonies; half of each colony shaded and half unshaded

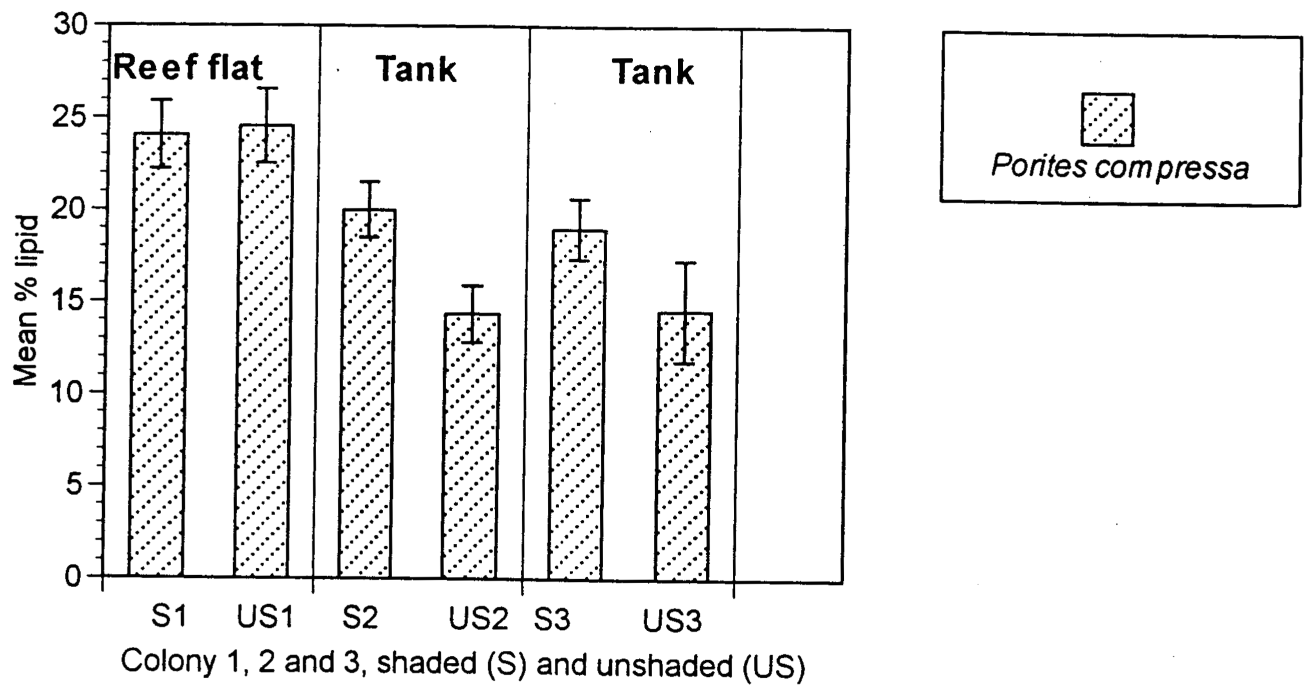


Figure 6.

Mean percent lipid in three *Montipora verrucosa* colonies; half of each colony shaded and half unshaded