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### Chapter 3 Influence of stress on the thickness and lipid content of the tissue layer

#### 3.1 Introduction

The results of Chapter 2 indicate that the thickness of the tissue layer (TTL) of massive *Porites* corals varies seasonally and between years and that differing conditions or impacts at one site may produce different patterns of variation. Coupled with data showing differences in TTL between corals located in various habitats within a reef (True, 1995), these results suggest that the thickness of the tissue layer may reflect differences<sup>-</sup> in the relative quality of a coral's habitat.

Since temperature and light vary seasonally on the central GBR (with incident solar radiation cycles preceding and driving sea surface temperature cycles: Lough & Barnes, 2000), either of these factors may drive the cycles in TTL described in Chapter 2. Indeed, Lough & Barnes (2000) suggest that temperature is the dominant factor forcing cycles of calcification and linear growth in *Porites* growing in shallow waters. The forcing behaviour of temperature is closely linked to the physical chemistry of calcification: higher temperatures drive the reactions at greater rates. The same is true of photosynthesis; at higher temperatures (albeit less than those which cause disruption of the xanthophyll cycling mechanisms), photosynthesis is more rapid, so the energy supply of the coral is enhanced by the increased efficiency of the zooxanthellae. Intuitively, then, TTL cycles should conform to the seasonal variations of SST. However, while

shallow water corals may be saturated year round (Chalker *et al.* 1983; Barnes & Chalker, 1990), the increase in summer insolation may thus drive the seasonal cycles observed in Chapter 2 in corals living deeper than constant-saturation depth.

True (1995) found that TTL of colonies of the same species located at different depth in the reef front habitat of Davies Reef declined with increasing depth of water. This finding led the author to suppose that light may be an important parameter controlling TTL, especially since other workers have described decreasing calcification and growth rates in corals from deeper waters compared to conspecifics in shallow habitats (e.g. Chalker *et al.* 1983; Marubini *et al.* 2001). This suggestion is very much in agreement with Barnes & Lough's (1992) proposal that TTL may, in fact, represent a kind of "nutritional status" measurement for these corals.

#### 3.1.1 Lipids in coral tissue

Coral tissue is rich in lipid, in both structural (e.g. cellular membranes) and storage roles. Oku et al (2002) reported that the lipids from *Montipora digitata* consisted of seven major subclasses: polar lipid (PL), sterol, free fatty acid (FFA), triacylglycerol (TG), two unknown lipids and wax ester. Examination of energy use patterns in shallow-water *Acropora* colonies by Crossland *et al.* (1980) indicated that zooxanthellae export lipids mainly as FFA and TG, while the corals tend to sequester sterols and wax esters in lipid bodies within the tissue. Since photosynthesis is a reducing reaction, the export of energy-rich substances by the zooxanthellae alleviates the oxidative stress within the algae, albeit passing it on the host. These workers argued that the lipid exports of the zooxanthellae in shallow water colonies far exceed the energy requirements of their hosts, forcing the corals to excrete lipids in fatty mucus discharges. *Porites* corals have been observed to excrete heavy mucus sheets at certain periods of the month (Coffroth 1991), perhaps coinciding with tissue uplift and concomitant reduced energy requirements of the colony (see Chapter 2).

Stimson (1987) reported that lipids of various classes constituted between 30-40% of the dry tissue weight in Hawaiian *Pocillopora* colonies from shallow water. This worker also reported that colonies kept in reduced light presented lower growth rates and lipid levels than unshaded colonies. Neibuhr (1999, 2001 abstr.) suggested that reductions in the proportions of various lipid classes (chiefly those identified with zooxanthellae) prior to bleaching events were an indication of energy crises in corals. Many researchers regard lipid sequestration as a major energy reserve for the corals (e.g. Davies, 1991; Oku *et al.* 2002). This suggests a possible linkage between lipids and tissue layer thickness; it begs the question of whether the thickness of the tissue layer maps directly to the health of the coral or via lipid intermediaries.

#### 3.1.2 Stress in corals

Corals respond to stress in many ways: changes in growth rates, loss of zooxanthellae, changes in metabolism and interruptions to reproductive activity (Edmunds & Davies, 1989, Michalek-Wagner & Willis, 2001,

Brown, 2003; Coles & Brown, 2003). Measurement of sublethal stress in corals has proven difficult because there are currently no simple techniques to quantify low- to medium-level stress in corals (Gates, 1990; Lesser *et al.*, 1990; Gates *et al.*, 1992; Al-Sofyani and Davies, 1993; Coles, 1993; Jokiel *et al.*, 1993; Brown, 1997, Jones, 1997). Little is known about baseline levels of stress in corals (Glynn *et al.* 1996) or the lasting effects of stress events (Michalek-Wagner & Willis, 2001). The problem with the most commonly used indicators of stress in corals and coral communities is that they do not allow for rapid assessment of the condition of the corals and often simply indicate that corals are dying rather than suffering incipient stress (Jokiel *et al.*, 1993).

One of the most commonly discussed "symptoms" of stress in corals is bleaching. Bleaching in scleractinian corals is defined in terms of zooxanthella loss (Jones 1997). Since the photosynthetic pigments of zooxanthellae provide most of the colour observed in a coral colony, the loss of these symbionts is an unmistakable sign that some great stress has occurred. However, colour (symbiont) loss is distinguishable only once it has occurred. In field situations, particularly, bleached are distinguished from unbleached corals by visual approximations of zooxanthella density, most often couched in terms of colour lost from the tissue layer (Jones 1997). In laboratory studies, zooxanthella loss can readily be quantified, but can only be calibrated against unbleached corals that are assumed not to have suffered the same fate. The condition of the animal part of the coral symbiosis prior to visible bleaching is more difficult to assay. Pre-bleaching or sub-bleaching responses to stress are seldom quantified or reported, some notable exceptions being records of diminished photosynthetic quantum yield of zooxanthellae reported by researchers using Pulse Amplitude Modulated (PAM) laser fluorometry which coincide with certain types of bleachingrelated stress (eg. Jones *et al.* 2000; Jones & Hoegh-Guldberg 2001).

Jones et al. (2000) demonstrate that quantum photosynthetic efficiency of zooxanthellae decreases markedly prior to experimental thermal-stress bleaching. While measurements of declining photosynthetic efficiency of the zooxanthellae indicate damage to the photo-system of algal symbionts of a coral that eventually did bleach, it provides no measure of how well the coral itself tolerates the damaged zooxanthellae. Nor does it provide any indication that some other factor may have been responsible for the bleaching response. It is now being understood that temperature-related bleaching is only one of many "bleaching" responses to a host of stress factors, each with its own suite of characteristics (Brown et al., 2002b; Jones et al. 2000). The ontogeny of the bleaching phenomenon in general, however, is poorly understood (Brown, 1997). Even in the case of high temperature/light-mediated bleaching, continued competency of zooxanthellae post-expulsion suggests that the host is exercising its options independently of the status of its symbionts (Ralph et al., 2001). The generic early precursors of bleaching stress in corals appear to be sufficiently cryptic to escape report.

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#### 3.1.3 Scope of this chapter

In this chapter I examine the behaviour of massive *Porites* corals under various stresses. Stress may be classified as either chronic or acute. In general terms, a chronic stress would be predicted to have greater impact per unit time than a transitory stress. Shading reduces the ability of the coral to access its main energy source. I first subjected a group of colonies to a long-term shading stress; to investigate the effect of chronically reduced light on tissue thickness over the same period (see Chapter 2). The aim of this experiment was to discover whether tissue thickness could be used as an analogue for the "nutritional status" of the colonies and wheth seasonal variation observed in Chapter 2 was preserved under chronic energy constraint. This experiment would, in some part, serve to separate the effects of temperature and light on seasonal cycles of tissue thickness and skeletal growth.

Additionally, I subjected small colonies to bleaching experiments in the laboratory, to investigate potential linkages between lipids and tissue layer thickness. The colonies previously part of the long term stress and monitoring experiment were used in a new analysis of natural bleaching and recovery following the 1998 mass bleaching event on the central GBR, again seeking linkages between lipid reserves and tissue layer thickness.

#### 3.2 Materials and Methods

#### 3.2.1 Shading experiment

Six large (~1m diameter) colonies of massive *Porites* spp. were selected from the population in Pioneer Bay (Orpheus Island, central GBR) in the manner described in Chapter 2. The colonies were a mixture of *P. lobata*, and *P. australiensis*. These colonies were all located within an area overlapping the area of the previously described study, in approximately 5-6m of water (MLWS).

For this study, 1.5m X 1.5m shade frames were constructed of 10mm welded steel rods to support a canopy of 70%-grade Nylex shade cloth (Figure 3.1). The frames were anchored by lugs secured to 2.5m steel posts driven into the substratum around each colony. These posts were sufficiently long that the shade cloth canopies were positioned approximately 0.3-0.5m above the upper surface of each colony. The shade frames were positioned so that the upper surface of the colony was entirely shaded from sunlight from directly above (Figure 3.2). Scattered light could reach colonies through the unshaded sides of the frame. Care was taken to ensure that the shade frames did not interfere with the colony in any other way. Anchor posts were positioned as far from the colonies as practicable and shade frames were orientated to minimise possible interference with the flow of water past the colonies.



Figure 3.1 Diagram of shading structure in situ

Photosynthetically active radiation (PAR) reaching experimental colonies was measured at intervals over the course of the study. A diver holding a LICOR<sup>TM</sup> underwater light meter descended and held the meter immediately above the shade cloth and then above the surface of the colony. Light above the shade-cloth averaged between  $63.6 \pm 22.4 \mu \text{Em}^{-2}\text{s}^{-1}$ on an intensely sunny day with patchy clouds in midsummer and  $11.4 \pm 0.7$  $\mu \text{Em}^{-2}\text{s}^{-1}$  on a dull day. The waters of Pioneer Bay are usually quite turbid (see Figure 3.2), particularly in the summer wet season. Absorbance of incident light by the 5m of water above the colonies thus accounted for a large proportion of radiation normally striking the surface (incident radiation at the water surface averages approximately 2200  $\mu \text{Em}^{-2}\text{s}^{-1}$  at noon in summer: Barnes, pers.com.). Such a low proportion of the incident light reaching colonies suggests that these colonies may be light-limited in normal conditions; shading would therefore be likely to have a profound effect on them.



**Figure 3.2** Shading structure in place. Direct sunlight is reduced to a small fraction of its original value (~5-30%), but reflected light from the environment penetrates under the shade.

Measured light below the shade structures ranged between  $4.4 \pm 0.2$  and  $4.2 \pm 0.2 \ \mu \text{Em}^{-2}\text{s}^{-1}$  immediately above the colonies. PAR at the colony was thus reduced from an average of about 3% of surface PAR to much less than 1%. Shading reduced incident light reaching experimental colonies by approximately 70-95% (Figure 3.3).



Figure 3.3 Average incident light above and below shading structures

The frames were installed in November 1994 and removed in July 1996, when the majority of shaded colonies appeared to be in obvious stress. Several colonies had, by July 1996, lost colour relative to the unshaded colonies and were exhibiting macroscopic signs of stress (see below). A decision was made to terminate the experiment before the colonies actually perished in order to see whether recovery from this type and magnitude of stress was possible.

Core samples were collected as described in Chapter 2. Samples were airdried and subsequently fractured to enable tissue thickness measurements as described in Chapter 2.

## 3.2.2 Lipid content of the tissue layer during the 1998 mass bleaching event

At the beginning of March 1998, it was noticed that corals of many families in Pioneer Bay, Orpheus Island, appeared to be in the early stages of bleaching. Large numbers of acroporids, pocilloporids, fungiids, faviids and soft corals were exhibiting colour loss consistent with bleaching. At the beginning of March, no massive *Porites* appeared to have bleached. The group of massive *Porites* colonies in Pioneer Bay, which had been subjected to stress from experimental shading, were resampled to determine their reaction to a different form of stress that was clearly affecting the wider coral community.

Core samples were collected as described in Chapter 2. One core was airdried for measurement of tissue thickness; the other was immediately placed into a -20°C freezer prior to lipid assay. Notes were taken describing the apparent condition of each colony. In early April 1998, one month after the onset of the bleaching event, the colonies were sampled again in the same way. By the time of the second sampling, most of the massive *Porites* colonies were showing clear macroscopic signs of bleaching; although only 5 of 16 colonies in Pioneer Bay had bleached completely (the remainder were bleached on the upper part only).

#### 3.2.3 Lipid analysis

Surface area of each sample was measured by the foil method. Tissue layer thickness was measured using vernier callipers under an Olympus SZ-40 stereo dissection microscope. Samples (including both tissue and skeletal material) were freeze-dried and macerated to a fine powder. Total lipid content of the tissue samples was estimated using the following gravimetric method (described by Anthony (1999)), modified from Folch *et al* (1957), Harland *et al* (1993) and Stimson (1987).

The soluble lipid/protein/chlorophyll fraction was extracted using a chloroform/methanol solvent. A total of three extractions were performed for each sample; the solutes from each extraction were combined. Solutes were washed three times in methanol before potassium chloride solution was added to remove non-lipid components.

Solvent fractions containing lipids extracts were then evaporated under air until only the extracts remained in the vials. The lipid extracts remaining in the vials were filtered through Micropore<sup>TM</sup> filter papers overlying precombusted glass filters. The filtrates were placed into aluminium trays (pre-combusted, weighed and uniquely identified) and dehydrated under a rising air column. The lipid extracts solidified on the aluminium trays were tared using a Metier brand three-place microbalance. Net dry weight of lipid extract for each sample was standardised using tissue volume (estimated by combining surface area and tissue thickness measurements) to arrive at a measure of lipid concentration in the tissue layer (mg/cc).

#### 3.2.4 Physical signs of bleaching in massive Porites

An observational experiment was undertaken to follow the physical signs of bleaching in massive *Porites* to gain insight into the progression of macroscopic signs of stress. Six small (~200mm diameter) colonies of *P. australiensis* were collected from 3m below MLWS in Pioneer Bay in August 1999. They were transported to an outdoor aquarium at Orpheus Island Research Station. The aquarium (4 metres diameter, 0.8 metre deep) was fed by coarsely filtered continuous running seawater at ambient temperature (~25°C). The aquarium was located in full sunlight (at that time, noon sunlight at the surface averaged >>1000  $\mu$ E/m<sup>2</sup>, representing a tripling of incident light for the colonies from 3m, as well as substantial change in incident spectrum).

Colonies were inspected daily for macroscopic signs of stress for four weeks, until a proportion of the colonies had perished. At the end of the experiment, the three colonies, which still retained some amount of live tissue, were returned to the collection site, where they survived until the following summer, when they apparently bleached and died.

## 3.2.5 Lipid content of the tissue layer during an artificial bleaching event

For this study, 12 colonies of *Porites* spp. were collected and transported to shallow aquaria at James Cook University in Townsville. Colonies were collected from a small site 3m below MLWS in Pioneer Bay, Orpheus Island in September 1999. The colonies were kept in a closed system supplying filtered flowing seawater. Each of four 50-litre aquaria was supplied with 20 l/hour of ambient temperature water (~26°C). A single Aqua  $Pro^{TM}$  100w in-tank aquarium heater purchased at an aquarium supply shop regulated water temperature in each aquarium. Repeated temperature measurements over the course of a day indicated that the heaters appeared to control temperature quite well: temperature variation in the treatment tanks was less than 1.5°C. Evaporation was limited by fitting each aquarium with a clear glass cover.

Colonies were habituated to a 12h night/day regime for two weeks prior to and for the duration of the experiment. Light was provided by a single 400W Eye<sup>TM</sup> Metal Halide 6500K aquarium light suspended 350mm above the top of each aquarium (manufacturer's literature indicated that each lamp produced a nominal ~20,000-lumen complete-spectrum output). Light from a single Phillips<sup>TM</sup> 25-watt "Natural Blue" fluorescent tube was applied for an additional half hour before and after the main lights came on. Total incident light was approximately ~180 µE/m<sup>2</sup> at the colony surface. Colonies were sampled at the beginning and end of the experimental period. Sample chips were removed from the colonies using a hammer and chisel and immediately frozen in a -20°C freezer. Two replicate chips were taken from each colony during each sampling episode. Initial tissue thickness measurements were used to divide the colonies amongst the aquaria (colonies appeared to fall into two basic groups separated by tissue thickness; approximately even numbers of colonies displaying "relatively thicker" and "relatively thinner" tissue were allocated to each tank). During the experiment, the temperature of each aquarium was identically raised by 1°C per week for four weeks. Almost all colonies had bleached by the end of four weeks, when the experiment finished. Analysis of lipids and tissue thickness proceeded as above.

#### 3.3 Results

#### 3.3.1 The effects of shading on tissue thickness

The thickness of the tissue layer decreased in shaded colonies within the first month of commencing the shading regime (Fig 3.4, Table 3.1). Thereafter, the general trend in the experimental group was for tissue thickness to decline until it reached a minimum in the first winter (June) of the study. Subsequently, average tissue thickness of the shaded group did not vary significantly for the remaining 13 months of the study, although some amount of variation was evident in individual colonies. In contrast, tissue thickness increased in control colonies between the first and the fourth month and subsequently followed the seasonal trend described in Chapter 2 (Table 3.1).

Source of variation	Type III Sum of Squares	df	F	р
Intercept	1745.47	1	1054.16	.0000
Shading	36.29	1	21.90	.0000
Error	21.53	13		

 Table 3.1: Analysis of variance (repeated measures) result for the effects of shading on tissue thickness over the two years of the survey (SPSS12.0).

This experiment was terminated in July 1996 because most of the study colonies (4 out of the 6) were exhibiting macroscopic signs of stress. Symptoms included paleness of tissue, thick coverings of sediment and an associated thickening of the mucus layer. One colony appeared to be undergoing moderate bleaching on its upper surface and tissue appeared to be dying off in patches. It was noticed also at this time that the scars of the previous sampling episodes were more visible on the shaded colonies than on the unshaded controls, suggesting that skeletogenesis and lesion healing rates were comparatively reduced. From observations made while sampling, it appeared that the processes of healing and tissue repair had been reduced in the shaded group than in the unshaded group of corals by the second year of the study. The apparent decrease in the rate of healing of shaded colonies in the second year may represent a long-term symptom of continued stress or severe energy limitation. A decision was therefore made to terminate the experiment as it was deemed unnecessary to kill 100-year-old colonies for the little additional information that could be gleaned before they died.



**Figure 3.4** Average TTL of shaded colonies at Pioneer Bay during the shading study, showing a clear and persistent trend in TTL reduction. Some colonies began to manifest macroscopic signs of stress in the winter of the second year (~May 1996).

#### 3.3.2 Natural Bleaching event – tissue thickness

During the 1998 mass bleaching event, the thickness of the tissue layers of the study colonies declined rapidly (Fig. 3.5). The colonies that had been stressed 2 years previously in the shading experiment appeared to suffer less tissue loss from the bleaching conditions than the unshaded colonies. By April, two-thirds of the study colonies were exhibiting visible signs of bleaching. In three colonies, the upper surface appeared moribund (examination one month later revealed that these colonies had, in fact, suffered extensive tissue mortality on their upper surfaces, although the sides of the colonies appeared healthy). All of the Porites colonies in this study survived the bleaching event, although approximately 60% of corals from other families in Pioneer Bay perished. Subsequent sampling, however, excluded the obviously damaged colonies (which had suffered tissue loss from their upper surfaces). This was because previous studies (e.g. Lough et al., 1999) indicated that sampling from regions other than the upper surface produced inconsistent results.

During the bleaching, the study colonies as a group lost a significant amount of tissue (Table 3.2). Within the previous experimental groups (shaded and control), the shaded group experienced significant tissue thickness reduction, however, the control group did not (although the reduction in TTL was marked: Figure 3.5). No difference in TTL loss was attributable to species of colony. The two groups, indistinguishable at the beginning of the bleaching event, were again separated by TTL after one month of bleaching conditions (Table 3.2, Figure 3.5).



Figure 3.5 Falling tissue thickness levels during the 1998 bleaching event. the previously-shaded group lost significantly more tissue than the unshaded group.

	Source	Sum of Squares	df	Mean Square	F	р
TTL	Between Groups	1.060	1	1.060	6.680	.027*
	Within Groups	1.587	10	.159		
	Total	2.647	11			

Table 3.2Analysis of variance results for changes in tissue thickness (TTL) duringthe 1998 bleaching event.

#### 3.3.3 Recovery from bleaching

Samples taken irregularly over the two years subsequent to the 1998 bleaching event tracked the process of recovery in the Pioneer Bay colonies. By 1999, the seasonal cycle appears to have reasserted itself (Figure 3.6). Unfortunately, samples taken in the spring and summer of 1998 (when tissue colour had returned to normal) were destroyed by an infrastructure failure at Orpheus Island Research Station (i.e. a freezer unit failed and station staff disposed of its entire contents in a landfill without consultation).



**Figure 3.6** TTL of colonies used in the shading experiment during the massbleaching event of 1998 and afterward. Shade structures were removed in July 1996 (at which time several colonies appeared to be on the verge of bleaching). Notice that the bleaching response in this group was evoked at a similar TTL. The variable effects of the stress event on different colonies is evident fro the large error bars on the postexperimental samples – indicating that the colonies are not reacting uniformly to the stress. Recovery of energy reserves from such an event is likely to take more than two years, since TTL of both groups after this period is still significantly less than the control group's normal winter minimum. The previously-shaded group appeared to "lag behind" the control group in terms of tissue recovery until the autumn of 2000, when the "shade" group presented tissue layers slightly thicker than the control group.

#### 3.3.4 Sunlight-induced bleaching (observations)

Colonies had bleached after 3 weeks exposed to extreme sunlight conditions. The first obvious sign of stress in these colonies was a thick mucus cocoon and a rapid diminishment of tissue mass. This was followed by loss of colour and the appearance of epilithic algae over the surface of the colonies. The algae first appeared in patches, before becoming more generally distributed, and was growing on patches of mucus which were not successfully shed from the surface of the colonies. After bleaching, tissue necrosis was rapid (3-4 days), possibly exacerbated by the algae overgrowth. In all cases, tissue on the upper surface was most affected by and algal overgrowth (Figure 3.7). Tissue on the lower sides of colonies did not exhibit signs of bleaching until tissue loss on the upper surface was extreme (in some cases, the tissue on the upper surface was completely replaced by algae before signs of tissue loss were obvious on the lower colony margins).





**Figure 3.7** Top left: Experimental colony at the end of the shading experiment. Some evidence of bleaching and tissue mortality is evident on the upper surface; small areas of pale tissue suggest impending bleaching. Top right: Natural bleaching in a colony of Porites (not part of the 1998 mass bleaching event). Note algal infection of the mucus sheath. Below left: Laboratory-bleached colonies of Porites. A colony in the final stages of a mortal bleaching. Algal invasion has occurred where the tissue has died off in patches. The bright white sections are bleached tissue which has not yet died. Below right: A colony which has only just bleached. The pink flecks visible in the image are symptoms of "Pink Spot Disease", which afflicts colonies at certain water temperatures and when they are under stress. Bottom: differential bleaching amongst Porites in Pioneer Bay during the 1998 event, highlighting between-colony differences in susceptibility to impacts.

#### 3.3.5 Lipid analyses

#### 3.3.5.1 1998 bleaching event

No significant patterns in lipid concentrations were evident during the bleaching event, according to previous shading history (Table 3.3). The previously shaded group had, however, significantly higher concentrations of lipids in their tissues than the control group both before and after the bleaching event. Neither group experienced significant change in lipid concentration during the bleaching event. Species of *Porites* does not seem to be an important factor determining lipid concentration, although the initial (pre-bleaching) concentration of lipids in colonies of *P.lutea* is much higher than the other two species (Figure 3.8)

Source	Type III Sum of Squares	df	Mean Square	F	р
Corrected Model	106.140(a)	3	35.380	2.263	.109
Intercept	1621.917	1	1621.917	103.737	.000
Time	10.676	1	10.676	.683	.417
History	91.518	1	91.518	5.853	.024
Time * History	4.981	1	4.981	.319	.578
Error	343.968	22	15.635		
Total	2022.598	26			
Corrected Total	450.107	25			

Table 3.3Analysis of variance results for the effect of bleaching on lipid storeswithin the tissues of the long-term colonies. The significant result for "history" representsthe large difference between the lipid stores of each group at both stages of the event(Figure 3.9). No significant depletion in lipid concentration appears to have occurred ineither group, although the shaded group lost an average ~3mg/cc of lipid during the event.



**Figure 3.8** Lipid concentration in coral tissue during and after the 1998 bleaching event showing differences between species of *Porites*. Time 1= March 1998 (prebleaching); Time 2= April 1998 (just bleached); Time 3= August 1999; Time 4= October 1999 (just prior to the annual reproductive event); Time 5= April 2000

While the history of the colonies appeared to have some bearing on the level of lipids (Figure 3.9), individual variation was larger than group variation. The colonies could be divided into groups by tissue and lipid: colonies with thicker tissue layers generally had lower concentrations of lipid within them. The converse was also true. By using an arbitrary midpoint to separate these two clusters, it was possible to see that colonies with initially high lipid concentrations (= less tissue) tended to lose a greater proportion of lipids compared to tissue. Those with initially thicker tissue layers (= lower lipid content) tended to lose tissue during the stress event.



Figure 3.9 Lipid concentrations in coral tissue during the 1998 bleaching event.

#### 3.3.5.2 Laboratory bleaching

Similarly to the field study, colonies used in the laboratory study could be divided into groups with either high or low initial lipid content in the tissue, although the separation of groups was more striking in this case. Colonies that had high initial lipid content suffered significant lipid loss before bleaching. In contrast, for colonies that had relatively thicker tissue and lower lipid content initially, tissue mass decreased markedly without significant lipid loss. In both cases, there was a threshold level of lipid content/tissue thickness where visible signs of bleaching begin to appear. At a tissue thickness level of approximately 2.2 - 2.6cm, colonies with 3 - 10mg/cm<sup>3</sup> of lipid in the tissue layer begin to bleach.

#### Group 1 (colonies which initially had thick tissue layers)

Colonies in this group, with TTL initially averaging 3.2mm, suffered significant tissue loss during the experiment (Figure 3.10). Total lipid content of the tissue layer (per cubic centimetre volume) did not change significantly during the experiment for this group. The amount of tissue lost was equivalent to the average distance between consecutive dissepiments, indicating that the tissue loss was comparable to normal monthly uplift.

Group 2 (colonies which had initially relatively thin tissue layers)

Colonies in this group did not lose significant amounts of tissue during the experiment (Figure 3.10). They did, however, lose significant amounts of lipid compared with the lipid concentration at the beginning of the experiment (which, incidentally, was significantly greater than that of the first group). This was in strong contrast with the first group.



Figure 3.10 Tissue/lipid relationships during laboratory bleaching experiments.

The two groups maintained a significant separation with regards to tissue thickness during the experiment (group 2 had significantly less tissue than group 1 at the beginning and end of the experiment). The groups ended the experiment with equivalent concentrations of lipid within the tissue layer, however. There was no difference in the incidence of bleaching between the groups.

#### 3.3 Discussion

The thickness of the tissue layer of massive Porites corals was found to vary seasonally, reaching a maximum in late summer and diminishing to a minimum in winter. Shading caused a distinct decrease in the thickness of the tissue layer, a decrease that continued for 10 months until a minimum tissue thickness between 2.0 and 2,5mm was reached. While individual colonies showed some amount of variation, the mean of 6 colonies remained remarkably stable at this level for the remaining 10 months of the shading period. Decreases in tissue thickness of shaded relative to unshaded colonies were detected within one month of the imposition of shading and 15 months before macroscopic signs of deteriorating health were observed, suggesting that the thickness of the tissue layer is a useful indicator of sub-lethal stress in corals.

In the colonies by the natural bleaching event, and those used in the experimental bleaching, when the bleaching threshold was passed, the upper surface of the colony bleached while the sides and lower margins remained apparently normal. As bleaching progressed further in the worst affected colonies, patches of the upper surface began to die off, apparently at random. Yet, the sides of the colonies appeared unaffected until necrosis of the upper surface was well advanced. In three of the field-bleached colonies, the upper surface died off completely and was overgrown by turf algae. The colonies survived, however, with the side areas growing normally over the subsequent years. The sides of a massive colony are shaded and protected somewhat from the conditions conducive to

bleaching. This palliative strategy may account for the remarkable persistence of massive *Porites* in reef communities subject to stress. In reefs badly affected by bleaching, *Porites* may in fact dominate the surviving coral community (Wilkinson, 1999).

The usefulness of tissue thickness as a measure of coral health lies in the fact that decreases may be detected weeks or months or even years before the appearance of macroscopic signs such as bleaching or tissue lesions. It is noted that in cases of extreme stress, such as thermal bleaching, the time scale for the appearance of macroscopic signs of stress may be compressed to days or weeks and thus sub-lethal indicators are of limited value as monitoring tools. In cases of low level or chronic stress, changes in the thickness of the tissue layer also appear to record changes in a coral's environment much more quickly than changes in skeletal growth parameters.

Anthony (1999) reported that reduced light levels decreased tissue mass, but did not affect calcification in *Porites cylindrica*. This result is similar to Barnes & Lough's (1999) finding from their investigation of the effects of sediments released from the Misima Island gold mine. While the thickness of the tissue layer decreased monotonically with increasing proximity to the mine dumping site, no stress-related changes were recorded in skeletal density, extension rates or calcification rates. Consequently, the thickness of the tissue layer represents a much more sensitive indicator of sub-lethal stress than either visual observations or measurements of skeletal growth characteristics.

High lipid levels in the tissue layer may reflect several things: 1) low expenditure of energy on somatic tissue growth for those colonies, or 2) the mobilisation of somatic reserves to make up energy shortfalls, or 3) "energy satiation", in which the coral is receiving a surplus of energy from all trophic sources. Low tissue lipid levels and relatively thicker tissue layer may conversely indicate that the colony strategy is to invest heavily in structural tissue at the expense of storage material, that the mobilisation threshold has not been reached, or that the colony is experiencing some sort of energy crisis.

Davies (1991) estimated that 10g nubbins of *P. lobata* stored enough lipids within their tissue to survive 71 days of sub-optimal (40%) energy harvesting. The large colonies in this experiment survived 600 days of ~90% reduction in PAR before exhibiting macroscopic signs of stress. This finding suggests that they could a) adapt somewhat to adverse conditions by becoming more efficient, b) had access to an alternative energy supply or c) extremely large stores of energy. The corals did, in fact, become more efficient; or, rather they reduced their investment in growth and calcification (see Chapter 4). This result concurs with several studies (e.g. Edmunds & Davies, 1989; Anthony *et al.* 2002). However, the severe reduction in tissue layer thickness preceding the overt signs of stress suggests that this measure did not solve their energy crisis entirely. The

bleaching experiments described here suggest that lipid reserves are finite, and may be insufficient to stave off the bleaching response. This leaves the possibility that either some form of trophic shift has occurred (which seems likely to be minor in light of Anthony's (1999) findings) or the corals have mobilised an alternative reserve of energy – the somatic tissue.

When bleaching conditions arise, large amounts of stored energy are mobilized to combat the oxidative stress resulting from the damage to the algal symbionts and to maintain normal metabolic functions. Whether this energy is derived from somatic tissue or from storage lipids depends on the colony's energetic strategy. Colonies in the high lipid group would tend to sacrifice lipid stores after the onset of the stressful conditions. Colonies in the low lipid group may metabolize somatic tissue to provide the energy to combat the bleaching stress. In both groups, it would seem, visible signs of bleaching occur only after a threshold level of tissue/lipid storage is reached.

This result corresponds to the minimum tissue thickness observed during prolonged shading stress by True *et al.*, (in review) and during sedimentation stress Barnes & Lough (1999) after which partial or total colony mortality was inevitable. It appears possible for colonies to thrive only if they have either much higher lipid levels or much greater tissue thickness than this. Observations from a previous study (True, unpubl.) suggest that the thickness of the tissue layer may be the more important parameter.

A remarkable relationship exists between the thickness of the tissue layer and the amount of lipid held within it. Grouped together, the Pioneer Bay colonies present a close linear relationship between tissue and lipid, deviating from this relationship only during the bleaching event (Figure 11). This result would appear to agree with Niebuhr's (2001) conclusion that lipid loss during bleaching corresponds primarily to loss of zoxanthellar membrane lipids, except that the deviation from the relationship corresponds to loss of tissue rather than lipids. Tissue lipid content after bleaching is therefore proportionately higher than prior to the stress event that caused expulsion of the symbionts.



**Figure 3.11** Relationship between tissue thickness and lipid content of the tissue over time. Diagonal line is fitted to the four non-bleaching sampling episodes. (Explanation in text)

Separating the groups into shade and control groups corresponding to the previous experiment, and an even more complicated story emerges (Figure 3.12). During the bleaching event, and for most of the two subsequent

years, the shade group has significantly less tissue than the control group. Both groups present exactly the same regression line, deviating from it during bleaching. By the second year, however, the two groups coincide remarkably. By examining the timeline of these samples, it appears that tissue and lipid content of the tissue increase in lockstep during the spring and decrease during the autumn (see Chapter 2). This is quite interesting: it suggests on one hand that much of the lipid content of tissue is either structural or that tissue contains a relatively fixed amount of lipid per unit.



**Figure 3.12** Lipid/tissue relationships in Pioneer Bay colonies during and after the 1998 mass bleaching episode. Colonies did not show macroscopic evidence of bleaching in March, but were bleached in April. The samples with the highest tissue lipid concentrations correspond to the spring of 1999. Circled sample is April 2000, suggesting that the period of anomalously high lipid/tissue ratios was ended.

On the other hand, the previously stressed group presented much higher amounts of lipid per unit of tissue during the bleaching and two years afterwards (Figure 11b). Bleaching occurred when the tissue lipid content reached a baseline; in the case of the shade group, tissue loss was insignificant, in the case of the control group, lipid loss was negligible (having already reached the baseline of lipid). The different points at which regression lines for these two groups intercept the tissue axis may explain the wide spread of tissue thickness minima reported here and elsewhere (e.g. Barnes & Lough, 1996); a colony with a previous history of stress may tolerate a thinner tissue layer than otherwise. The concentration of lipids is therefore indicative of the "available energy" of the coral tissue. The previously stressed colonies appear to prefer a larger supply at hand, perhaps reflecting a short-term adaptive response. In any case, there would uppear to be a relationship between lipids and bleaching in *Porites* that is more complicated than Niebuhr's theory would suggest.

It seems likely that massive *Porites* have an absolute minimum tissue thickness of 2.0 - 2.5 mm (cf., 2-3 mm given by Barnes & Lough, 1999; True, in prep.). Shading decreased the thickness of the tissue layer by approximately 2mm compared with unshaded controls, a reduction of almost 50% over 10 months, but tissue thickness did not fall below 2mm for the remaining 10 months or until tissue death occurred. Calices in massive *Porites* are about 0.5 mm deep (see Veron, 1986) and polypary structures such as mesenteries are obvious to a depth of about 1.5 mm. Below 1.5 mm the structure of individual polyps is difficult to discriminate and the tissue consists of a tangled mass of canaliculae within the porous skeleton.

It seems likely that the tissue layer below a distance of roughly 2.0-2.5 mm from the top of the calix might provide a store of resources that can be made available to the animal in times of need (i.e., the coral equivalent of the hump on a camel's back). Since the minimum sustainable thickness appears to be around 2.0-2.5 mm, reduction of the tissue layer below this level results in lesions and tissue death. The first signs of morbidity associated with shading were patches of tissue dying off, while islands of tissue between these patches appeared perfectly healthy. Such patches were probably maintained by resorption of tissue from other areas. I take this as further evidence of the high degree of interconnectedness of *Porites* colonies, in that during crises, nutrients appear to be translocated from moribund units to sustain less damaged portions of the colony. This pattern of patchy bleaching and tissue death was repeated during the mass bleaching event of 1998.

On average, massive *Porites* colonies lose around 15% of the thickness of the tissue layer approximately every month, when the lower margin of the tissue layer is raised and a new dissepiment formed (Lough *et al.*, 1999). This "uplift" appears to alternate with discrete periods of linear extension (Vago *et al.*, 1997). It is quite possible that the coral fuels calcification by subsuming resources from the lower margin of the tissue layer. The logical extension of this reasoning is that, during a stress event, once the easily metabolisable energy sources (e.g. lipids) have been exhausted, tissue is sacrificed to supply the additional energy demands arising from the stress event. In the worst case, where even this strategy is inadequate, tissue is

evacuated from the small areas worst affected and catabolized "for the common good" of the colony. While the stressful conditions persist, more and more of these areas are sacrificed until (eventually) the conditions ameliorate or the colony as a whole passes the lower threshold necessary for survival – thence perishes. Anecdotal evidence suggests that this strategy may also involve a sort of "hibernation" in which the tissue withdraws from the surface into the subgastric skeleton (perhaps better protected from malign influences), and re-emerges as conditions improve. Observers have documented the patchwork recovery of seemingly dead, algae-covered colonies after a bleaching event (E. Turak, pers. com.). The resilience of *Porites* appears to stem largely from the behaviour of the tissue layer under stress.

It has been demonstrated in the present study that bleaching is an extreme reaction to stress in massive *Porites*. The combination of high water temperatures and high levels of ambient light is demonstrated to be a lethal one for corals. It is evident that high temperatures destroy the electron transport system of the zooxanthellae (eg. Warner *et al.*, 1999, Jones *et al.*, 2000) resulting in a variety of oxidative stress reactions, all of which are energetically expensive (W. Dunlap, personal communication). It is far from the only combination of environmental stresses that can result in bleaching, however. The present study, amongst others (e.g., Jones *et al.*, 2000, Brown 2003) suggests that bleaching is most aptly described as the coral's inability to acquire sufficient energy to combat the stress factors operating upon it. These factors might be environmental (salinity,

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temperature, sediment) or endogenous (disease, damaged zooxanthellae, toxins); the determining factor in bleaching is that the combination of stresses depletes the energy reserves past a threshold level. Beyond this level, the energetic cost of maintaining zooxanthellae, damaged or not, is greater than the host organism will tolerate, resulting in the eviction or digestion of zooxanthellae.

The thickness of the tissue layer reflects the relative condition of the colony in terms of somatic energy reserves: relatively thick when the colony is healthy, relatively thin when the colony is suffering acute or chronic stress. Subsequent onset of bleaching symptoms would therefore appear to be subject to the ability if the coral to withstand the energy loss concomitant with the degree of damage. Rapid or unusual loss of tissue thickness is thus a sensitive early warning sign that the system is under stress. Monitoring of tissue layer variation in massive *Porites* corals may therefore provide valuable insight into the actual severity of environmental conditions thought likely to cause coral bleaching.

The existence of between-colony and between-species variation, however, suggests that *general* monitoring of TTL may not be the best methodology. Individual colonies appear to react uniquely to a given stress event, probably as a result of their individual history. Perhaps also because of genetic reasons (pertaining to either the coral itself or its algal symbionts), the response of a particular colony to a stress event will be difficult to predict in isolation. The relative merits of various monitoring

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methodologies is discussed in a subsequent chapter, inevitably concluding that the biological responses of corals are much too complex to be easily coerced into a reliable, quick monitoring protocol without referring to individual "exemplar" colonies, which represent a sort of norm. Certainly, the lipid content of the tissue layer, as well as the mass of tissue within the layer represent measures of coral "health" much more accurately and contemporaneously than gross measures of appearance or counts of zooxanthellae. The complexity of the organisms involved, and as yet unknown linkages between environmental parameters and species and genotype and host-symbiont relationships, however, betray the ease of measurement. Much fine scale physiological study must yet be undertaken to tease out the different strands of the story.