Chapter 2 Spatial and temporal variation in tissue layer thickness

2.1 Introduction

Taylor *et al.* (1993) proposed simple models that described the formation of density bands in massive *Porites*. These models projected characteristic forms of skeletal density variation according to thicknesses of tissue representing different proportions of the annual skeletal linear growth increment. However, the thickness of the tissue layer of massive *Porites* corals is not a fixed quantity within or between colonies of any species of massive *Porites* used in density band studies. Nor is it fixed within a single colony over time. Preliminary studies Barnes & Lough (1992) and True (1995) indicated that variation in the thickness of the tissue layer in *Porites* occurs over several spatial scales, from within reef to within region. True (1995) also reported consistent tissue thickness variation between the species of coral colony and the depth of water it occurred in. Moreover, the study also reported what might be a seasonal pattern of variation in tissue thickness. Variation in the thickness of the tissue layer in space and time is an important concept, since it allows the possibility that the corals respond to differences in the nature of their environment. This possibility, in turn, allows the potential use of tissue thickness fluctuations as a means of diagnosing environmental degradation prior to coral community mortality.

Barnes & Lough (1992) reported that the tissue layer is thicker in larger colonies than in smaller colonies in a given locality. Darke & Barnes (1993) proposed that this was an indication that larger colonies have greater problems in accommodating new tissue by increase in skeletal size, which they attempted to solve by increasing the thickness of the tissue layer. Anthony *et al.* (2002) suggest that, as massive colonies increase in size, growth becomes increasingly dominated by the cost of skeletal growth, so that creation of tissue becomes comparatively cheaper.

Barnes & Lough (1992) also suggested that the thickness of the tissue layer represented the "nutritional status" of the colony, which could be used as a proxy for the "goodness" of the coral's habitat. They used the spatial patterns they found as examples of this. These workers proposed that identification of the proximal causes of such variability might allow isolation of biologically important factors that could be used as indicators of environmental degradation. The patterns reported by Barnes & Lough (1992) were inconsistent, however, and inexplicably reversed between the northern and southern regions. Subsequent analysis of an expanded data set by Lough *et al.* (1999) did not find any significant inshore/offshore or latitudinal patterns in tissue thickness variation for the GBR.

2.1.1 Intra-annual variability in TTL

The contradictory results of early spatial examinations of tissue thickness might therefore be explained in several ways. The colonies used in Barnes & Lough's (1992) and Lough et al's (1999) analyses were retrieved over

several years, at different times of the year. Moreover, the colonies were not of uniform size or species; indeed, the authors pooled colonies of many sizes and species to achieve sufficient to perform spatial statistical analysis. The preliminary data presented by Barnes & Lough's (1992) paper as well as True (1995, unpubl.) suggest that these factors may have contributed both to the apparent spatial patterns and to their subsequent disappearance.

Faster-growing colonies on the Great Barrier Reef tend to deposit 12-13 dissepiments within annual density bands (Barnes & Lough, 1992), strongly suggesting lunar periodicity. The uplift of tissue and formation of dissepiments has been assumed to occur rapidly – within the space of a few days, as it does in other massive corals. There is some evidence that *Porites* cease linear extension close to the full moon (Vago *et al.* 1997); a lunar cue provides a very convenient window into which to place the timing of tissue uplift. This suggestion would agree with lunar cues associated with reproduction in scleractinia (cf. Willis *et al.* 1985).

Barnes & Lough (1992) attempted to control collection times so that the regular (theoretically monthly) uplift of the lower margin of tissue did not occur between collection of the first and last colonies of a collection sequence, but did not conceive of the potentially confounding effect of intra-annual or seasonal variation. The unforseen factor of intra-annual tissue thickness variation has not been satisfactorily resolved to date.

Of the more than 20 species of massive *Porites,* there are only 5 species in the Indo-Pacific that grow to heights greater than 200mm; however these five species have been used indiscriminately for density banding studies and other proxy record research. These species (*P. lobata* Dana, 1846, *P. lutea* Edwards & Haime, 1860, *P. australiensis* Vaughan, 1918, *P. mayeri* Vaughan, 1918 and *P. solida* Forskål, 1875) are reported to be not significantly different with regards to size, linear extension rate, density, or tissue thickness (Lough & Barnes 1990).

Of the five species, *P. lobata, P. lutea* and *P. australiensis* are very similar in appearance and can be quite difficult to distinguish underwater (particularly the last two). True's (1995) report suggested, however, that several ecological differences exist between these three species. Tissue thickness was found to decline with depth across all species, on average, but the patterns of decline were not consistent between species: for instance, the thickness of tissue found in *P. australiensis* declined significantly between transects in water less than 3m and those in 6m; tissue thickness of *P. lutea* colonies did not significantly diminish between transects at 2.5m and 11m depth.

True (1995) also found that the three main species (*P. lobata, P. lutea, P. australiensis*) occurring at Davies Reef (central Great Barrier Reef, Australia) differed in their distribution around the reef. These findings accorded well with Pichon's predictions that ecological differences between the massive *Porites* species might be as important as

morphological differences (Veron & Pichon, 1982; Pichon, 1995 pers. com.). All of these findings suggest that much could be learned from a systematic examination of tissue thickness variation both within and between species.

2.1.2 Seasonal TTL variation and density bands

The existence of seasonal cycles in the thickness of the tissue layer may help resolve conflicting reports on the timing of density band formation (see e.g. Klein *et al.* 1992, Barnes and Lough, 1993; Taylor *et al.*, 1993; Lough and Barnes, 1997). All techniques for timing coral skeletal growth depend on knowing the position of the density band relative to the outside surface of the colony at the time that the band is formed. The timing of the most recent density band is usually calculated by estimating what proportion of the total annual linear growth increment has formed between the density maximum and the date of collection (Lough & Barnes, 1990). However, the timing of density bands is increasingly displaced from the timing of growth at the outer surface during the period when tissue thickness is increasing (Taylor *et al.*, 1993).

It then follows that intra-annual variations in tissue thickness will vary the intra-annual timing of density bands relative to times provided by the position of the outside edge of the colony. If tissue thickness is less in winter, the timing of the low-density trough will more closely approximate that of the outside surface than will the timing of the summer, high-density peak. Similarly, tissue from a coral growing in deep water is thinner than

that of a conspecific in shallow water (True, 1995); timing of density bands will therefore appear to be closer to the collection date in the deep colony than in the shallow coral. Therefore, if seasonal TTL variation occurs, it is critical to know the time of year that a colony used for skeletal chronology is sampled.

Even small differences in the thickness of the tissue layer between summer and winter will bias calculations of the timing of density maxima. A similar pattern of anachrony or skewness (i.e. displacement from median timing) was predicted by Lough and Barnes (1990) for intra-annual variations in extension rate. Thus, intra-annual variations in tissue thickness will further increase the temporal skewness of the density profile. The tendency of decreasing tissue thickness to bring the apparent date of the density band maximum forward in time will be exaggerated by a slowdown in linear extension. However, Lough *et al.* (1999) note that temporal skewness of density bands (asynchrony) averaged only 3-5% across 392 colonies of *Porites* from the length and breadth of the GBR. This may have been due to opposing asynchrony in affective environmental factors. Solar radiation shows a distinct opposing skewness for most of the GBR whereas SST is similarly skewed only north of 14°S (Lough *et al.*, 1999).

Lough and Barnes (2000) showed that coral calcification is very sensitive to temperature, and that seasonal change in temperature largely drives the calcification mechanism in shallow water corals to produce the annual

density banding pattern. This is mostly because shallow water corals are light-saturated for the entire year (Chalker et. al, 1983); temperature dependent rates of calcification and photosynthesis are therefore the determining factors in skeletal density. The existence of annual cycles in skeletal density even in corals whose habitats are not affected by large or regular semi-annual regimes of seawater temperature fluctuation likewise suggests that temperature may not be the only forcing factor. Gagan *et al.* (1996) suggested that other seasonally-linked biological functions, such as reproduction, may also contribute to the formation of density bands. In such a situation, seasonally variable energy reserves (proxied by the thickness of the tissue layer) may explain some part of the story.

In developing their skeletal growth models, Taylor *et al.* (1993, 1995) recognised that tissue thickness had the potential to vary in time as well as space, but chose to regard tissue thickness to be fixed within a colony to make the maths easier. They defined a term called "Effective Tissue Layer" to describe the proportion of annual skeletal growth lying within the tissue layer. If the depth to which the tissue occupies the skeleton is partly dependent on the time of year at which the measurement is made, however, such skeletal relationships as "effective tissue layer" are of limited value unless the exact relationship of tissue thickness variation to skeletal extension is known. Moreover, unless the ratio of tissue thickness to extension rate is fixed, resolving the contributions of the various components to skeletal density is extremely problematic. Since the function of Taylor *et al.*'s (1993) models was principally illustrative,

however, the simplification should not affect their usefulness. It does, however, indicate that knowledge of the functional parameters of skeletal density banding needs to be expanded. There is currently no published account of the scale or range of tissue thickness variation in space and time which examines these two parameters systematically.

2.1.3 Scope of this chapter

This chapter is an examination of some aspects of spatial and temporal variation in tissue layer thickness. Neither of these quantities has previously been the target of a systematic survey; the corals measured in the few published reports which mention tissue thickness were collected or sampled for entirely different reasons, and no attempt was made to standardise either time of collection or size and shape of the colonies.

I first determined the timing of the regular tissue uplift episodes which characterise the growth of *Porites* and identify the monthly timing of tissue uplift.

I then undertook a large-scale survey of tissue thickness in widely-scattered reefs on the Great Barrier Reef, Australia (GBR) was undertaken to try to obtain a snapshot of the scale of natural variation between different localities. Additional reefs were surveyed in the Coral Sea and Papua New Guinea at approximately the same time to provide a benchmark for comparison of any patterns of variation found. A further comparison survey was undertaken in the Gulf of Thailand on heavily

impacted reefs dominated by massive *Porites*. My overall objective was to gain insight into the scale of tissue thickness variation in different localities; this would allow me to place any results of the subsequent long term survey (below) into context.

I also commenced a long term program to measure the potential seasonal variation in tissue layer thickness. This study represents the first time a biological parameter such as tissue layer thickness has been monitored over an extended period.

2.2 Materials and Methods

2.2.1 Timing of tissue uplift

To identify the timing of monthly tissue uplift, small colonies of massive *Porites* spp. were stained with sodium alizarin sulphonate (alizarin red S, abbreviated simply henceforth to alizarin) - which substitutes for calcium in the process of skeletogenesis, leaving a visible pink stain in the skeleton - in an attempt to capture the day of formation of dissepiments.

Staining of colonies up to 1.5m in diameter was achieved by completely enclosing a hemispherical or globular colony of *Porites* in a transparent plastic bag, secured with elastic straps (Figure 2.1a). The bags were filled with water and placed in such a way that they did not touch the surface of the target colony. Concentrated alizarin-s was introduced into the bags at the rate of 0.02‰ (at which concentration, the water inside the bags was completely red). Bags were left in place overnight, since the bulk of new skeletal framework appears to be added at night at the tips of spines (whereas secondary thickening occurs mainly during daylight hours: Cohen & McConnaughey, 2003). Removal of bags and skeletal sampling occurred mid-morning the day after staining. Samples consisted of 2x 35mm cores, extracted according to the technique detailed below. Samples were air-dried and subsequently bleached in dilute sodium hypochlorite to remove tissue. Skeletal core samples were fractured using a sharp steel chisel and examined under an Olympus SZ-40 stereo dissector microscope.

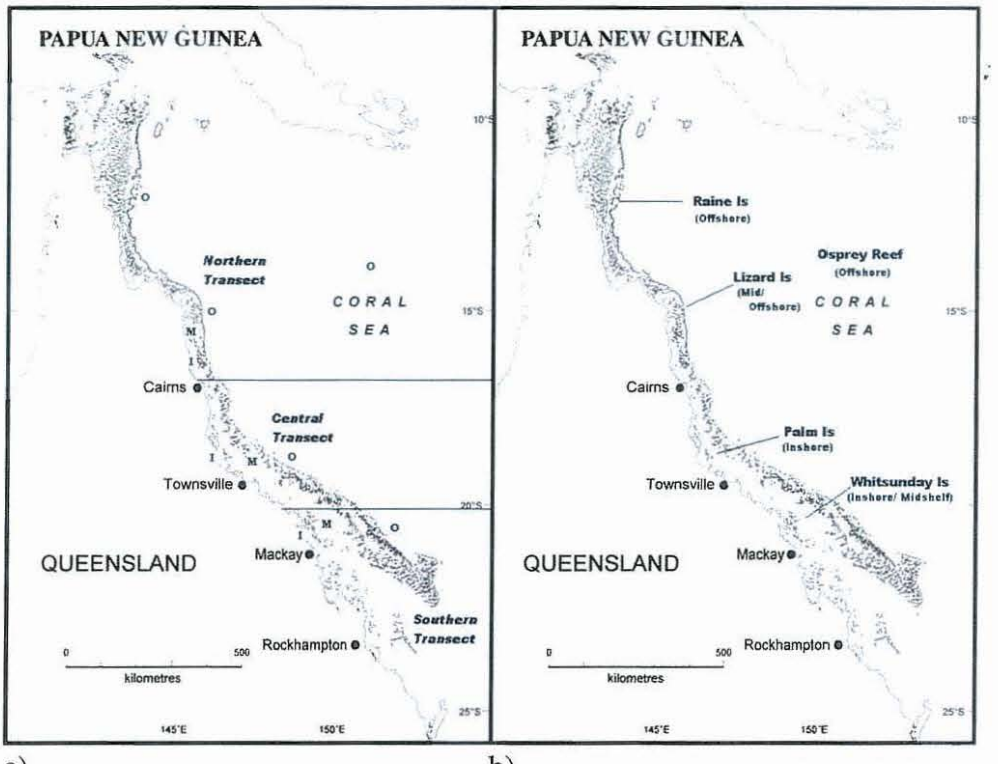
After approximately 20 colonies had been stained at random times during the lunar cycle, it was decided that dissepiment formation was likely cued by the full moon, rather than the new moon. Preliminary staining of colonies showed that staining during the middle of the lunar cycle tends to leave a stain throughout the tissue layer, suggesting that, not only does dissepiment formation not occur, but that secondary thickening of skeletal elements occurs throughout the thickness of the tissue layer. Further staining was therefore concentrated around the time of the full moon.

Vago *et al.* (1997) indicated that linear extension commenced approximately 5 days after the full moon, so this was taken as the limit of the uplift window. Therefore, groups of 3 small (~350-400mm diameter) colonies of *Porites* spp. were stained on each of 6 nights: 2 nights prior to the full moon in August, on the night of the full moon, and for 3 days subsequent to the moon.

2.2.2 Spatial study

In 1997, seventeen widely-scattered reefs at on the Great Barrier Reef were surveyed to determine the spatial variation in tissue thickness. Initially, three broad transects were envisaged for the GBR survey, running from the coast to the outer shelf reef in the northern, central and southern sections of the GBR (Fig.1a). Unfortunately, adverse weather caused problems coordinating shared ship time with other researchers resulting in the loss of four localities from the original twelve (Fig.1b). Barnes & Lough's (1992) survey suggested that cross-shelf trend existed in TTL.

These were reported reverse in the Northern and Southern sections of the GBR, and not exist in the central GBR. The survey could not therefore test the effects of distance from the coast, which produced such bizarre patterns in Barnes & Lough's (1992) study. What remained was a strict north-south transect, which isolates any effect of latitude on the thickness of the tissue layer of *Porites*, but cannot offer great insights into inshore/offshore gradients over the entire GBR.



a) b)

Figure 2.1a) Proposed structure of GBR tissue thickness survey (described in text). Cross-shelf transects were envisaged for three sections of the GBR, to simultaneously test for latitudinal and inshore/offshore trends in TTL. I=Inshore, M=Mid-shelf, O=Offshore/Oceanic; **b)** Actual sampling locations for the 1997 survey. Adverse conditions prevented access to the mid-shelf and offshore sections of the Central Transect, and the offshore component of the Southern Transect, as well as the inshore component of the Northern Transect.

Outgroups from Papua New Guinea (PNG) and the Coral Sea were collected at roughly the same times to determine the generality of the values recorded in this survey and to test for extensions to any latitudinal trends. The tissue thickness of colonies was examined on four reefs in the coastal area north-west of the Sepik River mouth in Papua New Guinea in May 1997, when long cores were extracted for palaeoclimatic study of the coral skeletons. The Coral Sea outgroup (from Osprey Reef) was collected to gain insight into the tissue thickness of corals in oligotrophic water distant from any terrigenous input. A further outgroup from the Gulf of Thailand was added in August 2003 to provide a snapshot from the northern hemisphere (i.e. completely unaffected by any potential regional factors which might influence corals in northern Australia or PNG), so that the relative scale of tissue thickness might be gauged in a wider context.

The colonies in all locations were selected for similarity in their size and shape. The tops of all colonies were located at 4.5 to 6.5 m depth relative to MLWS. Colonies were roughly hemispherical and approximately 1-1.2m across. Their upper surfaces were mostly free of fouling organisms, such as algae, endolithic barnacles and polychaetes. They were unlikely to be overtopped by adjacent organisms. Colonies of *P. lobata, P. lutea* and *P. australiensis* were sampled whenever they were encountered and satisfied the criteria above. These species have similar growth rates and skeletal densities (Lough & Barnes, 2000), and cannot be distinguished based on the thickness of the tissue layer (Barnes & Lough, 1992). The three species are also very difficult to separate taxonomically for non-

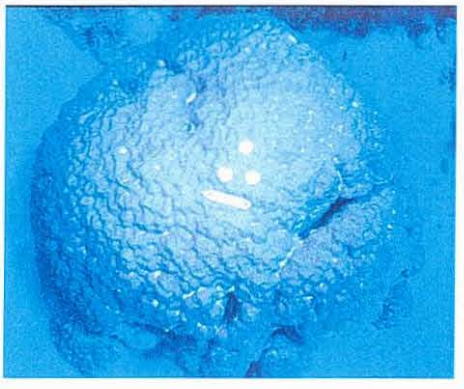
specialists; indeed, most studies using *Porites* coral cores for paleo-environmental studies identify them no further than genus. The problem of discriminating between the three main species is exacerbated underwater, particularly in turbid conditions. A fourth species, *P. solida,* which was occasionally used in density band studies and is grouped with the previous three by Barnes & Lough (1992) was not used, because its distribution is somewhat restricted (tending to be found mainly in very oligotrophic waters). Fortunately, this species is relatively easy to distinguish from the other massive *Porites* spp., even underwater.

Region	Sites	Lat.	Long. (E)	Inshore/ Mid/ Offshore	Month sampled
PNG (outgroup)	Mushu Is,	3°20'S	143°30'	I	May 1997
Sepik River / Wewak	Koil Is,	3°15'S	144°10'	I	
	BlupBlup Is	3°28'S	144°25'	I	
Central/Southern GBR	Deloraine Is,	20°15'S	149°10'	M	August 1997
(Whitsunday group)	Double Cone Is,	20°S	148°32'	I	
	Lupton Is,	20°20'S	149°5'	M	
	Double Is,	20°6'S	148°44'	I	
	Border Is,	20°10'S	149°1'	M	
	Hook Is (East)	20°9'S	148°58'	M	
	Hook Is (West)	20°10'S	148°54'	I	
Coral Sea (outgroup)	Osprey Reef	13°53'S	146°52'	O	September 1997
Northern GBR	Lizard Island,	14°40'S	145°27'	M	September 1997
	GBR 14-1039,	14°38'S	145°38'	O	
	Arlington Reef,	16°40'S	146°	O	
	No-name Reef,	15°20'S	145°44'	O	
	Harrier Reef	15°08'S	145°41'	O	
	GBR-15-1072	15°29'S	145°50'	O	
Central GBR	Orpheus Is,	18°37'S	146°23'	I	September 1997
(Palm Group)	Fantome Is,	18°39'S	146°26'	I	
	Pandora Reef	18°46'S	146°21'	I	
Far Northern GBR	Raine Island,	11°35'S	144°05'	O	November 1997
	Moulter Cay	11°25'S	144°04'	O	
	McLennan Cay	11°27'S	143°56'	O	
Thailand (outgroup)	Chumporn Province				
Gulf of Thailand	Ko Matrah	10°24'N	99°21'	I	August 2003
	Ko Ratchajiew	10°19'N	99°18'	I	
	Pratchuap Kiri Kan Province				
	Ko Singha	11°03'N	99°33'	I	August 2003
	Ko Sung	11°02'N	99°32'	I	

Table 2.1 Collection sites and dates of the spatial study of tissue thickness. The PNG and the Gulf of Thailand colonies would all fall under the "inshore" category, being collected from continental islands within 10nm of the mainland. The Thai provincial sample sites are separated from each other by an equivalent distance to the Palm Group and the Whitsunday Group in the GBR.

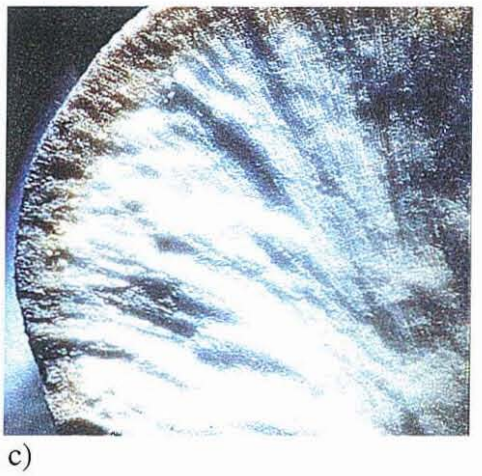
intervals (3 & 6 months) to determine whether such regular sampling had an adverse effect.

In the first 9 months of the study, colonies were sampled using a 52mm diameter high-speed steel core-drill. Subsequently, 35mm diameter cores were extracted once it was determined that smaller cores provided measurements having the same variance, while removing less coral material.



a)

b)



c)

Figure 2.2a) Typical 1m-diameter colony used in GBR survey after sampling. Core samples are 35mm in diameter, taken from the upper surface of the colony; **b)** Diver plugging holes left by sampling with pre-cast concrete plugs to promote healing and reduce potential colonization by boring organisms. This smaller colony was stained with Alizarin Red-S as part of the uplift timing study described in the text; **c)** Air-dried and fractured specimen of *P. lutea*, showing the tissue layer and dissepiments. Notice the uniformity of the lower margin of the tissue layer.

2.2.3 Temporal study

Twenty large hemispherical colonies of *Porites* spp in Pioneer and Harrier Bays (Orpheus Island, one of the Palm group of Islands, central GBR) were tagged and sampled for tissue thickness over a period of 21 months between October 1994 and July 1996 (Figure 2.3). Colonies were selected using the same criteria as for the spatial survey described previously.

Initial sampling of these colonies occurred on a monthly basis for six months from October 1994. The monthly sampling was initiated to determine what proportion of the *Porites* community actively uplift the lower margin of the tissue layer each month. As sampling progressed, such close-grained analysis was deemed unnecessary and so sampling intervals became wider to reduce the potential for stress to the corals. An extended period of bad weather prevented completion of the March 1996 sampling episode until July, when the condition of the shaded colonies (Chapter 3) predicated termination of the sampling. In each instance, sampling commenced on the fourth or fifth day after the full moon. One site was sampled per day, always in the same order, to standardise the time between samplings.

Colonies sampled over time were monitored closely for signs of morbidity that might be associated with repeated sampling. Also, after 6 months, measurements of tissue thickness from colonies sampled monthly were compared with those from control colonies sampled at much longer

2.2.4 Extraction and analysis of core samples

Cores were extracted using drill bits powered by an Ingersolrand™ or PowerTool™ pneumatic hand drill, supplied with compressed air from either a SCUBA cylinder or petrol-powered compressor mounted in a dinghy moored directly above the experimental site. Cores, once removed, were replaced with pre-cast concrete plugs (Figure 2.2) to enhance regrowth of coral tissue and inhibit settlement of endolithic parasites (cf. Hudson, 1981). Plugs were made locally from a rich mixture of Portland cement and (mainly coralline) beach sand which had been washed with fresh water. Three cores were removed from each colony during each sampling episode. Cores for visual inspection were air-dried in the sun before microscopic inspection and analysis.

Cores were fractured into two pieces longitudinally along a growth axis using a hammer and fine chisel in order to produce a clean fracture-surface suitable for measuring tissue thickness. Fracturing was found to be preferable to sawing or grinding for this purpose. The latter two techniques tended to shatter a large proportion of the delicate dissepiments, rendering the measurements less precise and skeletal details more difficult to elucidate. As well, the fracturing process tends to divide the corallite vertically, enabling proper measurement of the height of the theca. Sawing or grinding away the corallite is not guaranteed to enable this level of precision, and produce measurements vastly inferior to those obtained by fracturing the core.

Measurements of tissue layer thickness were made using an eyepiece micrometer fitted to an Olympus SZ-40 stereo dissector microscope. The tissue layer thickness was defined as the distance from the top of the calice wall to the level of the dissepiment forming the lower margin of the tissue layer (Fig. 2). To avoid the effects of random variation in calice height, three measurements were made close to the central axis of each core and averaged. Statistical analysis was performed using the SAS statistical package (proc GLM: SAS Institute, 1987). The model was a repeated measures design, comparing sites and time of year (colonies nested within sites, individual core samples as replicates).

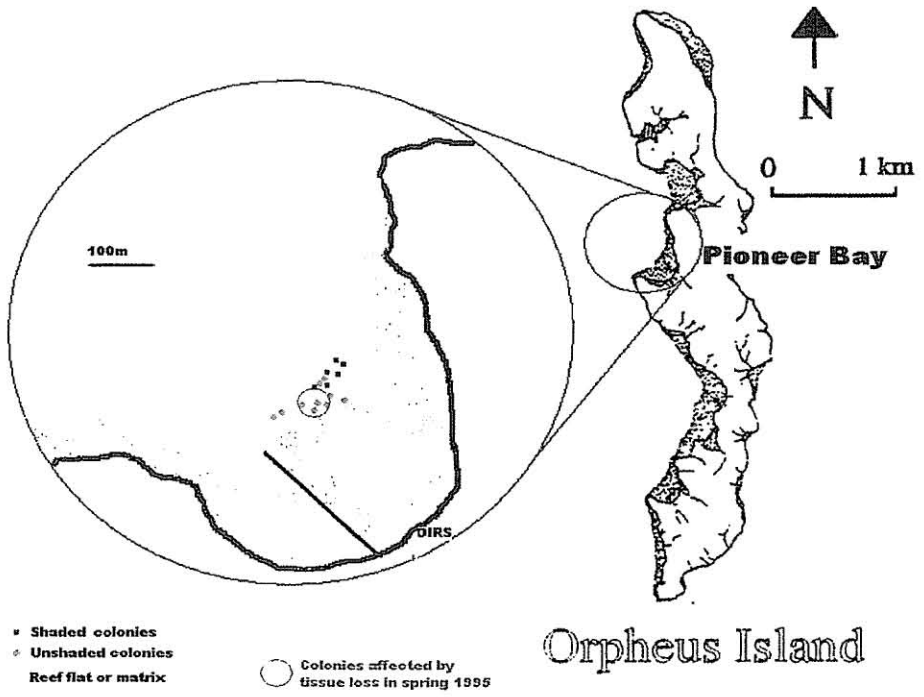


Figure 2.3 Location map of colonies used in the long-term study described in this chapter. The red circle shows the colonies affected by the unexplained tissue loss in spring 1995 in Pioneer Bay

2.3 Results

2.3.1 Timing of tissue uplift

The majority of colonies stained did not appear to incorporate the staining compound into dissepiments; the greater proportion being colonies stained before the full moon. This may have been for several reasons: the colonies did not calcify at all during this period; the colony did not form a dissepiment during this month; the dissepiment formed before or after the compound was introduced (and the colony was sampled). Of the colonies that were successfully stained, many incorporated the compound into the growing tips of skeletal spines. Those colonies that did incorporate the staining compound were predominantly stained on nights subsequent to the full moon. Dissepiment formation and tissue uplift were therefore taken to occur approximately 1-3 days after the full moon. More staining compound was incorporated into general skeleton on day 3 after the moon than day 2, suggesting that dissepiment formation may have been nearing completion and that normal growth processes were taking over.

In the light of these findings, subsequent sampling of tissue thickness was concentrated (where possible) in the period around the third quarter moon of a month (i.e. several days after the full moon, but not so far that an entire month's linear growth would skew the comparative value of the measurement).

2.3.2 Spatial study

No systematic variation in tissue layer thickness was found in regards to geographic location, either within the GBR or elsewhere. Variation between sites within localities was found to be at least as extensive as that between locations (see also Appendix 1).

While this survey could not analyse the effects of distance from shore within a latitudinal band, comparison of inshore/midshelf and midshelf/offshore locations (in the southern and northern regions, respectively) shows no indication that tissue thickness is affected by distance offshore.

	Sum of Squares	df	Mean Square	F	p
Inshore/offshore	1.250	1	1.250	1.147	.289
Reefs	62.155	57	1.090		
Total	63.405	58			

Table 2.2 ANOVA results testing for differences between midshelf and offshore sites in the Far Northern section of the GBR

	Sum of Squares	df	Mean Square	F	p
Inshore/Midshelf	3.128	1	3.128	2.280	.139
Reefs	53.512	39	1.372		
Total	56.641	40			

Table 2.3 ANOVA results testing for differences between inshore and midshelf sites in the Whitsundays section of the GBR

Exclusion of Deloraine Is from the ɔuthern-most region does, in fact allow a significant trend to emerge regarding inshore/mid-shelf location,

however this excision would be quite inappropriate, since this was, in fact, the most easterly site in the group.

No effect of latitude on tissue thickness was found. Samples were analysed according to the common group (i.e. mid-shelf locations). Surprisingly, there is a tendency for the southern-most sites to have thicker tissue layer measurements than the more northerly sites (Table 2.4). All of the sites north of 18°S have virtually the same average tissue thickness, with the exception of Moulter Cay, one of the the most northerly. The sites from the Whitsunday Group are much more variable (Figure 2.4) but average slightly more.

	Sum of Squares	df	Mean Square	F	p
Latitude	6.419	1	6.419	6.539	.015
Reefs	34.358	35	.982		
Total	40.777	36			

Table 2.4 Latitudinal effects within midshelf reefs (north v south)

Since there is no difference between the sites due to distance offshore within region or due to latitude, there is a good statistical argument to pool the regions to investigate inshore/offshore gradients. This argument, in all likelihood, would face strong resistance from biological analysts; the biological diversity of the GBR changes significantly along this north-south transect, probably reflecting important biological constraints or factors. Despite this, it is discovered that the previous result is not upset: there appears to be no difference in TTL in reefs at different latitudes on the GBR.

	Sum of Squares	df	Mean Square	F	p
Latitude	29.388	3	9.796	7.765	.000
Reefs	156.431	124	1.262		
Total	185.819	127			

Table 2.5a ANOVA testing latitudinal effects – significant result can be traced to palm group reefs. Exclusion of 2 anomalous (stressed?) sites in the central section removes any effect of latitude.

(I) LOCALITY	(J) LOCALITY	Mean Difference (I-J)	Std. Error	p
1 - North	2	.39669	.58032	.496
	3	1.01352(*)	.27193	.000
	4	-.35351	.22837	.124
2 – Coral Sea	1	-.39669	.58032	.496
	3	.61682	.60659	.311
	4	-.75020	.58835	.205
3 – Central/Palm	1	-1.01352(*)	.27193	.000
	2	-.61682	.60659	.311
	4	-1.36703(*)	.28867	.000
4 - Whitsundays	1	.35351	.22837	.124
	2	.75020	.58835	.205
	3	1.36703(*)	.28867	.000

Table 2.5b Tukey's LSD post-hoc comparison of the ANOVA from Table 2.5a. Within the Palm Group, Orpheus and Fantome Islands exhibit unusually low values for TTL from anywhere in the study, Pandora Reef (7km away, closer to shore) is not significantly different from other GBR reefs.

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	2	.75020	.58835	.205
	3	1.36703(*)	.28867	.000

Table 2.5b Tukey's LSD post-hoc comparison of the ANOVA from Table 2.5a. Within the Palm Group, Orpheus and Fantome Islands exhibit unusually low values for TTL from anywhere in the study, Pandora Reef (7km away, closer to shore) is not significantly different from other GBR reefs.

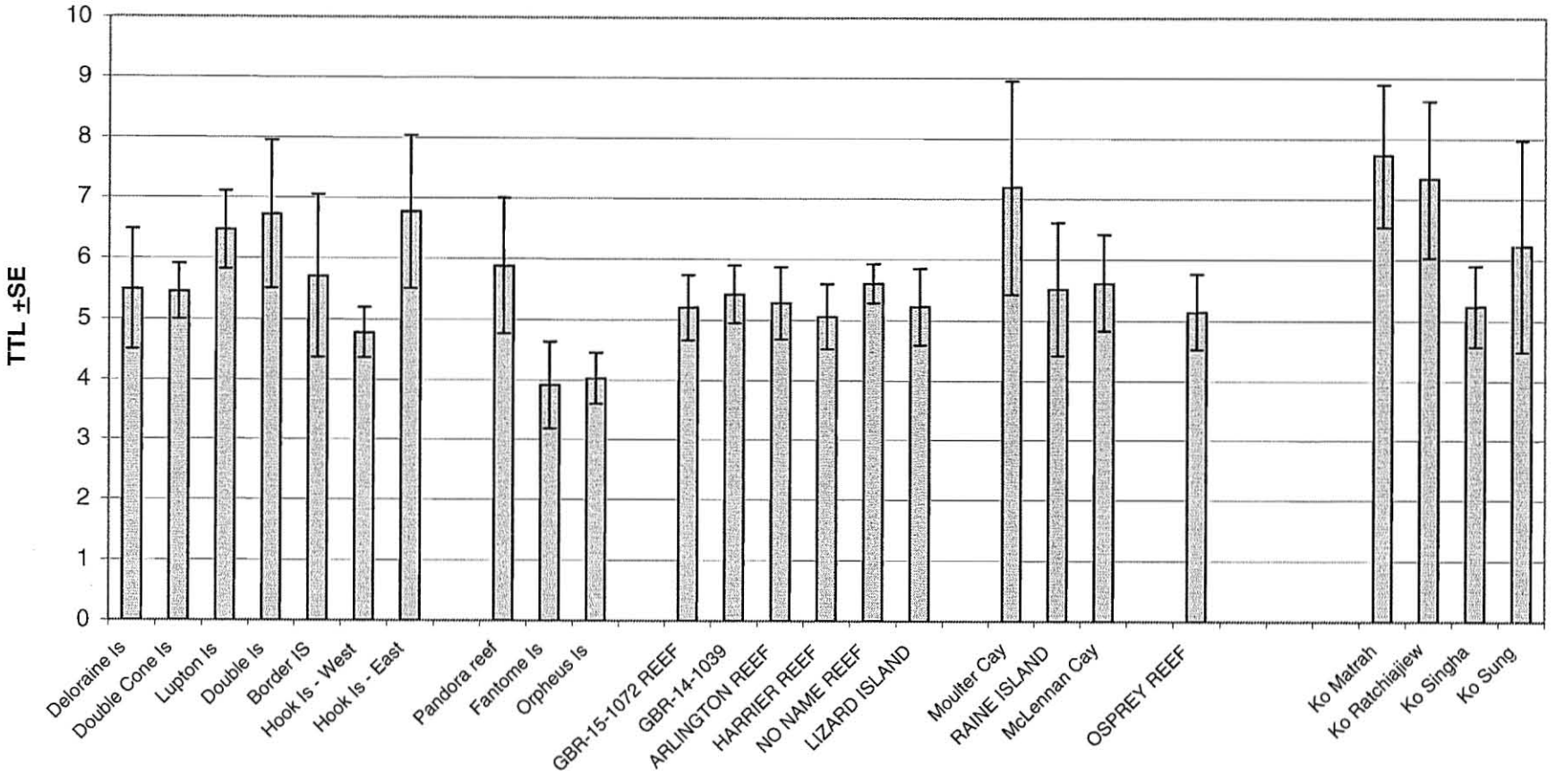

Figure 2.4 Average TTL values for sites along the east coast of Queensland and from the Gulf of Thailand (very roughly, L-R = south-north). TTL appears to be very conservative, reflecting mainly differences in the quality of habitats. Note the uniformity of the northern GBR reefs, which are mostly offshore, compared to the widely variable midshelf and inshore reefs.

2-24

2.3.3 Temporal study

Comparison of tissue thickness of control and treatment colonies revealed no statistically significant difference due to the initially frequent sampling (SAS, ANOVA, $F_{1, 25} = 4.02$, p=0.054).

The thickness of the tissue layer of the *Porites* colonies sampled at Orpheus Island varied from month to month with pronounced seasonality. In both years of this sampling program, the tissue layer was significantly greater in summer than in winter. The scale of seasonal fluctuations in tissue thickness was quite large, with mean summer maximum in tissue thickness reaching 5 mm compared to an average of approximately 3mm during winter. The minimum tissue thickness recorded throughout the study was 2.2mm.

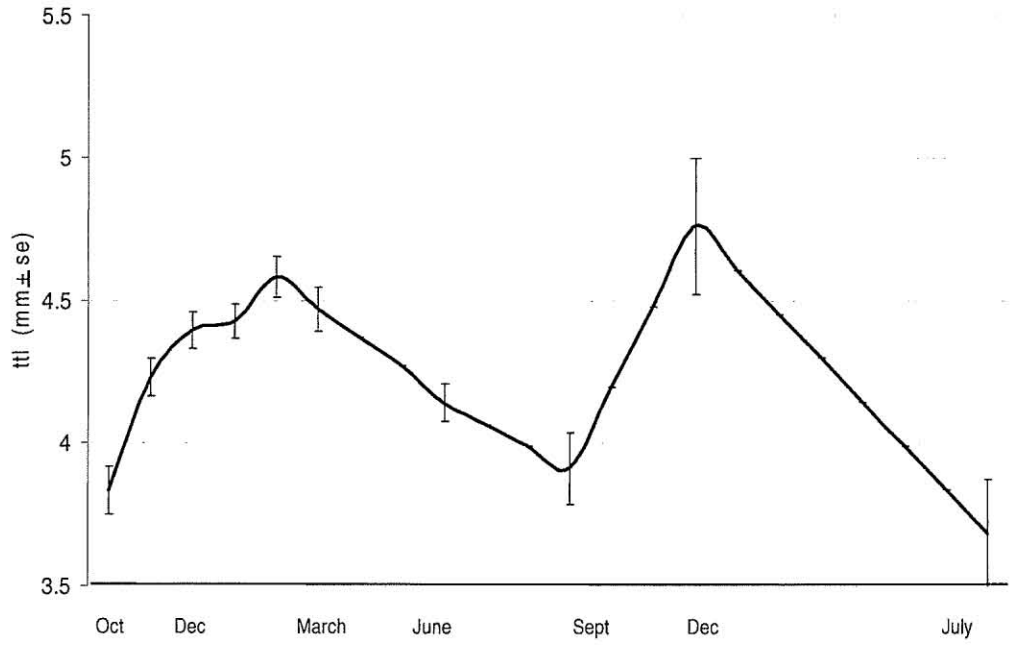

Figure 2.5 Average TTL of study colonies at Orpheus Island between 1994 and 1996

Several of the study colonies at the Pioneer Bay site suffered an unexplained loss of tissue thickness between September and December 1995 (Figure 2.6). The loss of tissue thickness relative to the unaffected colonies persisted until the final sampling episode in July 1996. Only two colonies (HB01and HB05 – both *P. australiensis*) exhibited a similar TTL loss amongst the Harrier Bay colonies. The affected colonies were all close to each other in the same depth of water, and were *P. australiensis* except for PB06 (*P. lobata*).

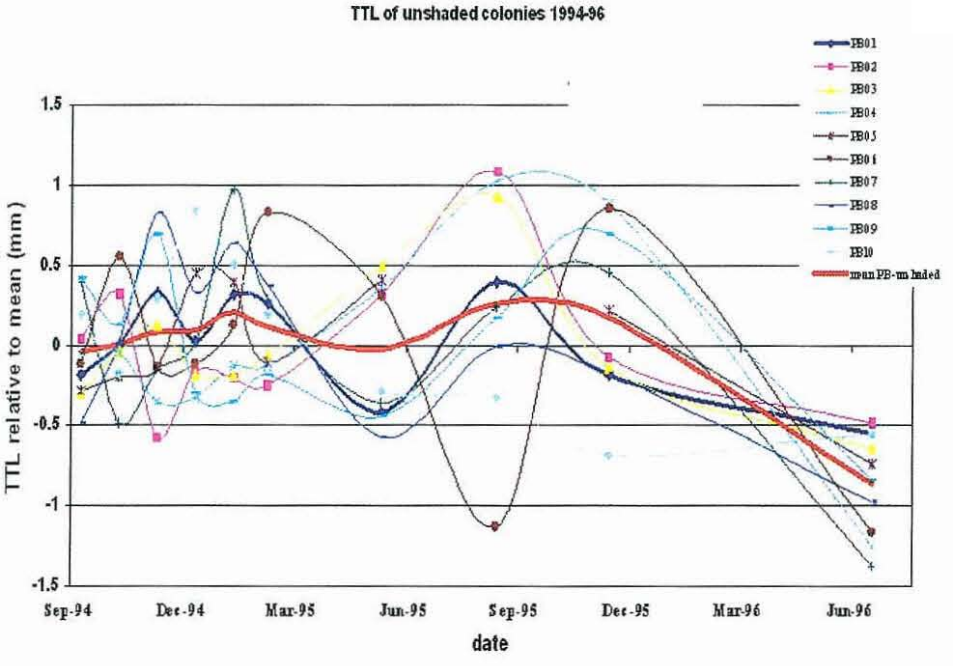

Figure 2.6 Detailed record of TTL variations during the study period in Pioneer Bay. Individual colonies were quite varied in their seasonal patterns; abrupt declines in the early summer may be linked to spawning behaviour. Notice that, while the average TTL trace follows a reasonably well-defined sine curve, very few of these colonies display uniform TTL variation. Of particular interest are the abrupt decline in TTL exhibited by PB01, PB02 PB03 and PB06, in the September-December 1995 period, and the apparent prolonged decline of PB10.

There was no evidence of bleaching in these colonies and there were no proximal environmental cues to explain this behaviour, which shows up clearly as a growth perturbation in the skeletal record of an affected colony (see Chapter 4). That the affected colonies were mostly *P. australiensis* is interesting, but not particularly informative, since little is known about potential pathogens or even species-level differences in ecology. An overzealous lab manager inadvertently destroyed tissue samples taken at the time for later analysis, so pathology of the affected colonies will remain a mystery.

2.4 Discussion

This survey found no concrete evidence of geographical constraints on the thickness of the tissue layer in massive *Porites* spp. Regions sampled on the GBR had the same mean TTL in each latitude. Inshore localities had the same mean TTL as those mid-way across the continental shelf and those well offshore, including the Coral Sea. As such, it appears that proximity to land does not in itself seem to affect TTL. There is a tendency for the northern sites to average thicker tissue (PNG, GoT), which may be due to the lack of seasonal temperature fluctuation (the average temperature range for the sampled regions on the GBR is ~6-8°C; northern PNG the Gulf of Thailand experience annual temperature ranges around 3°C).

The thickness of the tissue layer of massive *Porites* corals on the central GBR was found to vary seasonally, reaching a maximum in late summer and diminishing to a minimum in winter. Annual cycles in sea surface temperature vary with distinct seasonality at Orpheus Island, with summer maxima and winter minima. Temperature range for this locality is ~8°C, with winter minima approximately 22°C and maxima about 30°C. The annual cycle of insolation precedes that of temperature by approximately two months (Lough, 1994), and is the environmental variable that most closely approximates annual cycles in tissue thickness in this study.

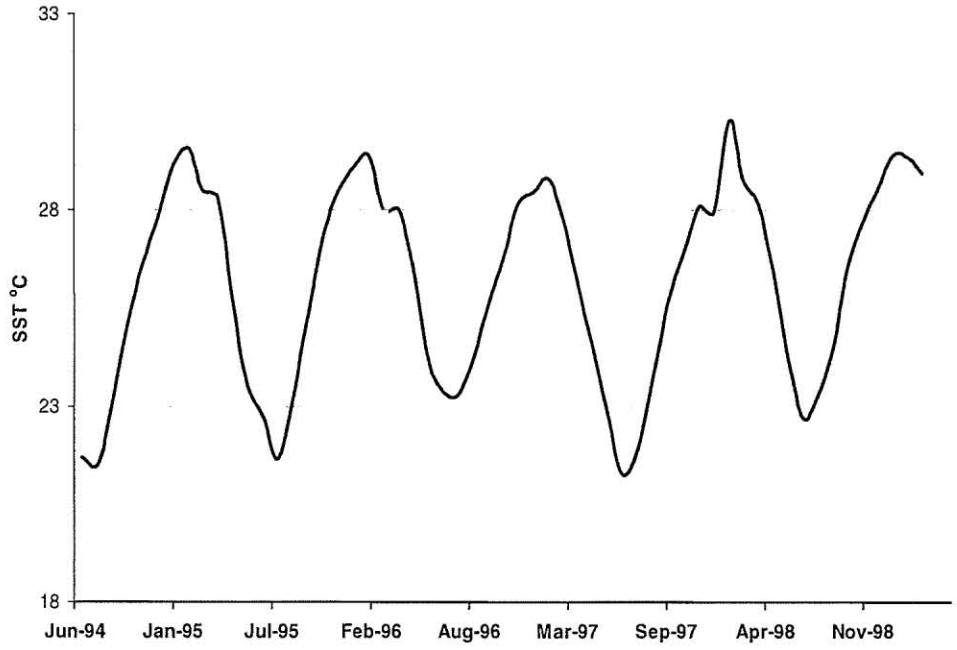

Figure 2.7 Seawater temperatures in Pioneer Bay during this study (source, GBRMPA).

Cycles in the thickness of the tissue layer may reflect a number of underlying causal relationships. They might reflect an annual cycle in availability of food or in factors that deplete reserves stored in the tissues. They may also reflect seasonal variations in extension rate, which are not matched by tissue uplift and formation of dissepiments. Skeletal densitometry studies (e.g. Lough and Barnes, 1997) indicate that the rate of linear extension of the skeleton is greatest in the summer months and least in the winter months. Oppositely, the density of the skeleton is greatest when laid down in winter and least when formed in the summer. Is there, then, a trade-off between density and extension, in that bulk deposition of skeleton is relatively constant throughout the year? Since

calcification rate is greatly influenced by temperature, one might expect that the greater rate of linear extension in summer to be offset by higher rate of calcification so that density of skeleton would not change significantly. Why, then, do density bands form? This counter-intuitive relationship is explained when it is realised that skeleton laid down in summer actually spends *less* time in the tissue layer than that laid down in winter, since tissue thickness declines in autumn. Secondary skeletal deposition is thus less apparent in summer skeleton than in winter skeleton.

Diminishing energy supplies at the end of summer, after solstice and sea surface temperature maximum, predicate the using-up of somatic energy reserves in the tissue and consequently declining rates of linear extension and calcification. The lower margin of the tissue layer thus begins to "catch up" to the leading edge of the colony, causing the lowest skeletal elements to exit the tissue layer at a lesser stage of thickening than those formed when conditions were better. The opposite process occurs in the early spring time, when the leading edge of the skeleton accelerates away from the lower margin of the tissue layer, allowing more time for secondary calcification of skeletal elements formed during that time. Since the tissue is subtracted from the lower margin of the tissue layer, missed uplift opportunities (in early spring) by colonies with fewer than 13 dissepiments per annum represent periods when the skeleton is "held" within the tissue layer. The formation of density bands is thus easily explained in terms of the varying energy supply available to the colony.

Density band formation in equatorial regions, with less pronounced seasonality, is less obviously explained than in higher latitudes, and may be tied more closely with reproductive effort than climate. Where temperature does not vary significantly, there are no reasons to suppose that growth rate or calcification rate changes are simply the result of chemistry. There are many gaps in our knowledge of growth and reproduction in equatorial corals. Closing those gaps will undoubtedly shed light on the annual energy budgets of corals and the timing and nature of density band formation in those regions.

2.4.1 Potential architectural mechanisms to allow rapid tissue uplift

Intuitively, the thickness of the tissue layer must increase with extension of skeletal elements at the outer surface of a colony. Tissue uplift involves the evacuation of the lowest portion of the tissue layer and the sealing off of the tissue from the emptied skeleton. The details of how this process occurs have not been satisfactorily explained thus far. In fact, the expression "tissue uplift" is perhaps a misnomer. Three major candidate mechanisms exist to explain the process of evacuation of tissue from the region below the new dissepiment during uplift episodes: (1) the tissue is withdrawn ("uplifted") and a new dissepiment formed; (2) there is no uplift and the new dissepiment grows through the tissue, cutting off a batch of tissue when it closes; (3) a combination of 1 & 2 in which tissue is withdrawn as the dissepiment closes.

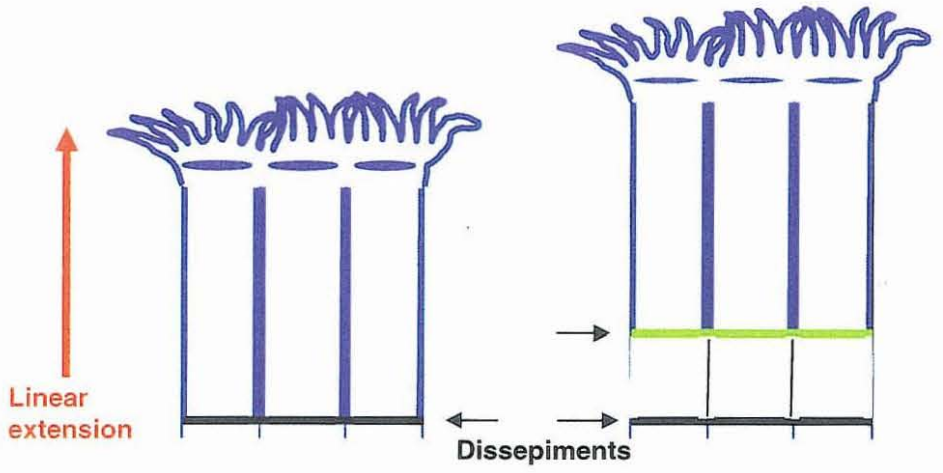

Figure 2.6 Idealised drawing of one month's growth in a massive *Porites* colony, indicating the formation of a new dissepiment to maintain tissue as a thin band. The drawing on the left represents time zero, that on the right, the situation after one month's linear extension; the new dissepiment seals off the polyp from the older skeleton, following evacuation of the lower part of the tissue layer.

The mechanism of the withdrawal of tissue from the evacuation zone is not understood, however. It may be that: (1) the tissue is physically withdrawn from the skeletal matrix (in the same way that a nautilus evacuates old chambers as new chambers are formed, leaving gas-filled compartments in the shell-spiral as it grows); (2) the tissue within the evacuation zone is digested and the dissociated components (being mainly proteins and lipids) are translocated into the remaining tissue as building blocks for new tissue to fill the newly-available space following linear extension.

The dissepiment is a single, essentially horizontal plate at the bottom of the tissue layer, hung between the septa and theca. In *imperforate* corals, a dissepiment can seal the bottom of an enclosed cavity between two septa

and the theca. Thus, it is possible for the tissue to uplift at different times within the several compartments of a calix or corallite.

The situation in *perforate* corals is necessarily different, since adjacent corallites communicate *through* the skeleton. The complex three-dimensional architecture of the skeleton comprising the framework of the lower part of the tissue layer of *Porites* precludes the simple "lifting" of a section of tissue seen in many imperforate corals at the time of dissepiment formation. Moreover, the complex interconnections between adjacent polyps of a perforate coral such as *Porites* means that uplift must occur simultaneously amongst many polyps to avoid leaving a gap between tissue and unoccupied skeleton. What must happen, then, is either a complex involution of the various tubules and pockets of tissue occupying the spaces between the skeletal elements or a decomposition of the tissue and relocation of the structural precursor components within the remainder of the tissue layer.

The tissue of the subgastric layer may reflect a certain degree of plasticity in the vertical architecture of the polyp, depending on the time of year and conditions. Certainly, the continual mobilisation of tissue at the lower margin in response to its episodic uplift allows for this possibility. For example, dissection of the subgastric region reveals that it occasionally contains distinct out-pockets of the gastrovascular cavity. During reproduction, these pockets can be observed to contain bulging gametogenic mesenteries deep within the subgastric tissue layer. At other

times of the year, however, it is difficult to distinguish the vertical gastric cavities separated by the septa below the level of the columella. Cross-linking between septa superimposes a three-dimensional skeletal lattice on the radial symmetry of the polyp, distorting the appearance of the coelenteric pockets. Whether this represents a change in the architecture (to allow extra room for enlarged gametocytes) or change of utility of the tissue during reproduction is not known. The season of reproduction on the Great Barrier Reef has, however, been linked to the period of formation of the less dense phase of skeletal density bands (Gagan *et al.* 1996). This may even be the principal cause of density band formation in equatorial regions where strong seasonal temperature (hence growth and calcification rates: Taylor *et al.* 1995) differences do not exist. Whether or not this is true, it is quite possible that the seasonal patterns in tissue thickness variation seen here are part of the corals' reproductive cycle. Alternatively, the reproductive aspect may be coincidental: the availability of abundant light and clear waters in the spring and early summer, coupled with rate increases in calcification and photosynthetic reactions due to increasing temperature may be sufficient explanation for TTL increases during the spring. Such an explanation does not, however, satisfactorily deal with the autumnal decline in TTL. Some aspects of the Chapter following may offer insight into this question.