A STUDY OF THE INTERACTIVE BIOLOGY OF CORALS

Thesis submitted by

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ABSTRACT

The general ecology of the Magnetic Island, North Queensland, fringing reefs is described, together with the hydrological and biological effects of a tropical cyclone ('Althea', 1971) on this system. This particular cyclone allowed the mortality associated with the mechanical damage to be assessed separately from that of the reduced salinity caused by the flood rains, the effects of the reduced salinity being the most catastrophic. Interactions between coral colonies on the reef are commonplace and an analysis of these and artifically induced interactions by grafting corals have been performed.

This analysis reveals that only tissues of identical genotype will fuse and form fully integrated colonies. Tissues that are from the same species, but from different individuals, i.e. allogeneic, do not fuse, and remain mutually indifferent cytologically and morphologically. Foreign tissue interactions between different species usually show signs of rejection that vary from mild to severe, these culminating in extracoelenteric feeding aggressions, depending upon the species interacting. Histological evidence supporting morphological observations is presented, and the immunological implications are discussed.

Because of the unusual soft tissue and skeletal configuration the study of corals require a variety of histomorphological techniques to be performed. New methods of embedding and recording growth forms are described. If an embedding stage is introduced before decalcification, then distortion or separation of the tissue is minimised. By using the existing skeleton as an integrated

record of past calcification, it is possible to extrapolate and interpolate future and past isochronous surfaces and in this way analyse growth allometries.

The significance of the cyclonic redistribution and destruction of coral on the reef is placed in context with the knowledge gained on the ways in which corals interact. It appears that cyclones promote interactions on the reef, and may even aid in the vegetative reproduction by fragmentation of some species. The application of the grafting techniques to coral colonies will allow the contribution made by vegetative reproduction to be assessed in population studies of maintenance and recruitment on coral reefs.

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institute of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

J. D. Collins

30th November 1978

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A STUDY OF THE INTERACTIVE BIOLOGY OF CORALS

CHAPTER I GENERAL INTRODUCTION

(a) General Introduction

(a) General Introduction

Corals interact in three main ways by

(a) shading and overtopping (Connell, 1973),

(b) extracoelenteric digestion (Lang, 1971, 1973),

(c) cellular interaction.

It is more with this latter category that this study is concerned.

A fundamental tenet of an interactive system is the ability to recognise 'self' from 'non-self'. Burnet (1970) reviewed this ability in colonial marine animals and flowering plants. The work of Oka (1970) and Theodor (1970) showed that a primitive immune system exists in the Invertebrates and has led to more extensive searches for the precursors of the vertebrate immune system in lower animals, i.e. the phylogeny of immunity (Holdemann and Benedict, 1975). Within the coelenterates the tissue interaction of the hydrozoan Hydra have been reviewed by Campbell and Bibb (1970) and Shostak (1970, 1974). Du Pasquier (1974), working with the data of Hauenschild (1954, 1956) on Hydractinia, concluded that the degree of homozygosity is important in studying an interactive system. Natural interactions of the anthozoan Anthopleura also show a genetically based interactive response that allows the distinction between clones (Francis, 1973).

While animals may naturally grow to contact each other, by close initial settlement, it is quicker to force an interaction experimentally by transplanting and grafting tissues. Such forced interactions are sensitive indicators of immunoresponsiveness. Both natural and experimental grafts of gorgonid corals showed interactions that were controlled by genetic relationships. 'Self' recognition leading to

fusion, 'non-self' to a variety of rejection responses. The reviews on the phylogeny of Invertebrate immunity by Hildemann and Reddy (1973), Valembois (1973), Du Pasquier (1974) and Hildemann (1974) allow the cellular interactions in the coelenterates to be placed into perspective.

The grafting of corals was initially performed by the writer under the supervision of Hildemann, and is reported in Hildemann et al., 1974. This work revealed the basic interactions associated with zeno-, allo- and isografts in Scleractinia. Hildemann et al. (1975) have followed the initial coral work by determining the frequency of natural interactions in the field at Eniwetok, and Hildemann et al. (1976, 1977a,b) have shown that the essential precursors of the vertebrate immune system, specificity and memory, are present at the coelenterate level, at least for the coral Montipora verrucosa (Lamarck, 1816).

The anatomy, morphology and histology of the experimental grafts performed on Acropora sp. were analysed using light microscopical techniques. Earlier descriptions of the histomorphology of the Acroporidae are specifically mentioned by Fowler (1887) and Brook (1893). Many authors include sections on the Acroporidae, e.g. Hickson (1924), Wells (1956) and Ogilvie (1896) that provide useful comparative data, mainly on taxonomically important histomorphology. The summarised studies of the British Great Barrier Reef Expedition of 1928 to 1929 (Yonge, 1940) allow a physiological interpretation to be placed on coral morphology, culminating in the histological work of Goreau (1956). This latter work has allowed a visualization of the interaction between calcification and tissue growth and its expression in the morphology of the corals.

Yonge (1963) further clarifies and summarises these relationships.

The literature on corals has numerous descriptions of unusual interactions; von Koch (1892), Lacaze-Duthier (1899), Duerden (1902), Whitfield (1901) are but a few of the earlier reports containing statements on natural interactions. Later works by Yonge (1935), Stephenson (1931), Roos (1971), Lang (1971, 1973) and Connell (1973) have references in part, dealing with natural interaction. In these examples a general confusion in terminology and lack of realization of the significance of the soft tissue interactions has made historical interpretation difficult. New definitions and interpretations are proposed in this study.

Initially it was conceived that the sensitive test of biological interaction, mediated by grafting methods, might be applied as a tool in coral taxonomic problems, in a way similar to that suggested by Yonge (1963). This would have been particularly appropriate to the protean genus Acropora whose taxonomy is only now being clarified (Wallace, 1978).

This study was undertaken to investigate the physiological and taxonomic (hence genetic) implications of interactions. During this period, the unpleasant, but scientifically enlightening experience of a destructive tropical cyclone was experienced, presenting the opportunity to examine and assess the catastrophic effects on reef corals of this not uncommon natural phenomenon. Such damage has been described previously by others, e.g. Hedly (1925), Slack-Smith (1960), Banner (1968) and Goreau (1964). This particular cyclone ("Althea", December, 1971) allowed the effects of mechanical damage to be assessed separately from osmotic damage caused by reduced salinity.

The study is divided into separate but related sections:

(a) A description of the general high island fringing reef area, and the impact of cyclones on it.

(b) Experimental grafting of Acropora to elucidate interactive biological and physiological mechanisms.

(c) The survey of some natural interactions in both branching Acropora and the massive coral Goniastrea thus allowing the experimental work to be placed into environmental perspective.

(d) Histomorphological descriptions to aid with the interpretation of grafts containing details and accounts of the gross microscopical anatomy of Acropora species.

Each section has a separate introduction and discussion. The whole is brought together in a general statement of conclusions. A glossary of the more unusual scientific terminology is included.

CHAPTER II GENERAL ECOLOGY OF STUDY AREA

(a) Description of the Nelly Bay study reef.

(b) Cyclone damage to Nelly Bay reef.

(c) Coral regrowth following cyclone damage to Nelly Bay reef.

(a) Description of the Nelly Bay Study Area Reef

An area of reef in Nelly Bay, Magnetic Island, Queensland (Figures 1 and 2) was selected for study as this contained abundant healthy coral and laboratory facilities for the grafting studies were situated nearby (see Chapter III (a)).

The fringing reef at the southern end of Nelly Bay (Figure 3) is about 200 m wide with a platform extending from the reef flat at a depth averaging 1 - 2 m below mean low water springtide (MLWS). The outer edge of this platform slopes down to a sandy bottom at about 8 m depth. A diagrammatic cross section of this region of the reef is shown in Figure 4 which also indicates the structures that resemble incipient spur and groove formations that extend irregularly from the outer reef platform to the sandy bottom. It is unlikely that wave action alone is responsible for these structures, as waves of a sufficient magnitude to affect the reef to a depth of 4 - 7 m rarely occur. The uneven seaward growth of individual colonies, and the coalescence of numerous isolated coral heads that extend from the reef front across the sand are probably responsible for these particular spur and groove formations.

Hopley (1974d) has reviewed the sea level changes that have probably occurred on the Great Barrier Reef coast in recent geological times. He concludes that the relative position of the sea level has not changed by more than $\pm$ 0.5 m in the past 2,500 years. In the Townsville region rates of uplift prior to this time were estimated to be in the order of 2.5 m per millenium, thus 6,000 years ago the sea level may have been some 4.4 m below the present level. The reefs around Magnetic Island would therefore have basement foundations

little more than 6,000 years old. The reef's structure would also have to be mainly depositional as no aerial exposure due to falling sea level has occurred during their recent history.

It is assumed that these reefs are still in an active growth phase and that there is probably nothing exceptional about their present configuration and composition, as a result of the apparent stability of sea level during the past 2,500 years.

Early in 1971 the coral reef platform was dominated by at least two species of Acropora (A. formosa (Dana, 1846), A. pulchra (Brook, 1893) and the corals Turbinaria mesentarina (Lamarck, 1816) and Montipora ramosa (Bernard, 1896). Isolated colonies of Porites sp. were also common, while the favids Favia speciosa (Dana, 1846) and Goniastrea aspera (Verrill, 1865) were of scattered occurrence. The reef slope had a similar composition, but in addition more than 20 other genera were commonly present. Some of these formed large (often greater than 1 m in diameter), isolated colonies, e.g. Pavona decussata (Dana, 1846), Goniopora sp. and Lobophyllia sp. Many other genera were only represented by much smaller and infrequently occurring outcrops, both on the reef flat and in deeper water (approximately 69 species in total, Bull, 1977).

Although a modest number of genera were found in this inshore situation, which is 8 km from the coast and partially embayed, the coral fauna is impoverished when compared with the situation at the Palm Islands only slightly further offshore (19 km from coast) (Figure 1). Endean et al. (1956) considered that the distance from the mainland was not a factor of major importance in itself, but that the distribution of coral was probably a consequence of greater

wave action offshore. Furthermore, they considered that cyclonic disturbances and the associated heavy rainfall was probably the major factor in preventing the establishment of many species of coral in areas adjacent to the mainland. Greater water turbidity in shallow waters has often been cited as a factor preventing the growth of many coral species (Roy and Smith, 1971). However, even in shallow turbid water, the most extensive coral growth is found in the deeper parts of such regions. It is probable that both light attenuation and sedimentation causes the reduction in species diversity, but do not hinder the settlement and growth of the more resistant species. Roy and Smith (1971), noted that there was a higher percentage of ramose corals in turbid lagoon water, and that the presence of turbid water and a muddy bottom does not prohibit coral growth. A similar dominance by ramose corals is evident in Nelly Bay and other inshore reefs, e.g. Lawn Reef (Figure 1).

Salinity, temperature and turbidity measurements were taken throughout the study period in Nelly Bay, and were found to agree with the extensive measurements taken in Cleveland Bay by Grigg (1972), Kenny (1974) and Zann (1975). Water temperature in Nelly Bay ranged from a winter minimum of 21^{o}C to a summer maximum of 30^{o}C. For most of the year, salinity was measured at 33.5$^{o}/_{oo}$ to 36.0 $^{o}/_{oo}$, but during periods of high rainfall, especially in the summer months of January and February, considerable dilution of the coastal water can occur. Table 1 shows the effect of monsoonal rainfall runoff into the creeks entering Nelly Bay. Brandon (1973) has noted reduced salinities for these months in the oceanic water adjacent to the Barrier Reef.

TABLE 1

COMBINED JANUARY/FEBRUARY RAINFALL AND SALINITY MINIMA FOR 1972, 73, 74

Date	Combined Jan./Feb. Rainfall (cm)	Salinity Minima ($^{o}/_{oo}$) recorded in Nelly Bay
1972	77.1	17.0
1973	68.5	32.8
1974	131.8	19.0

Although the total rainfall and salinity are related, it is a high rate of rainfall that causes the most marked salinity changes. This is due to the close proximity of the catchment area and the swift runoff into Nelly Bay. A low rate of rainfall over an extended period of two months occurred in 1973, and the normal water circulation through Cleveland Bay prevented a large salinity reduction. Coral mortality resulting from salinity reduction further depends upon the depth of the diluted sea water, the rate of mixing, the degree of replacement, and the tidal regime.

The turbidity of the water in the whole of Cleveland Bay is directly related to wind strength, duration and, to a lesser extent, direction. Secchi disc readings taken in Nelly Bay ranged from 0.8 m to 6.5 m, with an average of 1.8 m. Wind strength in excess of 6 ms^{-1} considerably increased the turbidity of the water. The wind strength usually increased in the afternoon. As most of the Secchi disc measurements were taken in the afternoon, they represent the daily maxima for turbidity. Other factors known to influence the turbidity of the area include harbour and channel dredging operations, spring tide cycles, and blooms of the blue green alga Trichodesmium.

(b) Cyclone Damage to Nelly Bay Reef

On 24 December 1971, Cyclone (=hurricane) "Althea" struck Townsville, North Queensland, with wind speeds in excess of 190 kph, storm seas, and a storm surge. Severe and extensive damage to buildings and the foreshores of Townsville and Magnetic Island, including the study area, occurred (sea Bureau of Meteorology Report, 1972). Oliver (1973) has published a detailed account of the meteorology of cyclone "Althea", the main features of which are summarised below.

The cyclone, with a central pressure of 952 mb, approached the coast at a rate of 33 kph on a south-westerly track from the Coral Sea, crossing the coast at 0900 hours, 38 km north of Townsville (Figure 1). A mean windspeed of 130 kph, with gusts to 196 kph, were recorded at Townsville during the height of the storm. Prior to crossing the coast, the wind direction at Townsville was 180^{o}, backing sharply to 060^{o} as the eye crossed the coast and finally backed slowly over a period of three hours to 020^{o}. Hopley (1974a,b) has described the storm surge and wave action produced during the cyclone. The tide level rose 2.85 m above the predicted height at Townsville Harbour, and waves as high as 6 m were reported off Cape Cleveland lighthouse. The storm surge peak occurred 70 minutes after the predicted low tide, giving a total height of 4.17 m above datum, or 1.27 m above mean high water springs (MHWS) for Townsville (see Figure 5).

Flood rains were recorded during and after the cyclone. On the 6 - 7 January 1972, cyclone "Bronwyn" affected the Gulf of Carpentaria, and degenerated into a tropical rain depression which, moving south,

caused extremely heavy rainfall between 8 - 12 January (see Figure 6) on the Queensland coast. Unusually severe flooding resulted from the rainfall associated with "Althea" and "Bronwyn".

On 27 December 1971, the first inspection of the study area reef after cyclone "Althea" revealed extensive damage to the reef platform and widespread destruction of coral colonies. Many Turbinaria and Montipora colonies were overturned or completely fragmented. Few specimens of Acropora survived intact, most were reduced to fragments of 10 cm or less or left as broken stumps. However, most of the fragments and stumps were judged to be alive, still showing characteristic brown pigmentation three days after the cyclone. Considerable redistribution of unconsolidated coral rubble had occurred. A previously clean sandy depression about 20 m^2 in area near the outer edge of the reef was regularly used as an experimental site prior to the cyclone. Over half this area was now covered in coarse coral rubble. Similar extensive redistribution of coral debris over much of the reef resulted in the burial of living coral, particularly encrusting forms such as Montipora.

On the 14 January 1972 when the area was reinspected, the majority of Turbinaria and Montipora colonies and the larger fragments not covered by rubble were alive. In contrast, very few living branches or stumps of Acropora could be found and the numerous dead branches were covered in a film of algae. By the end of February 1972 less than 5% of Acropora on the reef to a depth of 2 m had survived, but in deeper water (below 2 m) on the seaward reef slope more than 15% was still alive even though severely fragmented. Tissue repair over the broken surfaces was evident in many of the specimens. The fragments of many of the deeper colonies were spread

up and across the reef in a west to south-west direction. A similar distribution of some experimental oyster shells that were spilled from holding baskets was noted.

Hopley (1974c) summarised the effects of a cyclone on a coral reef and concluded that there are four major factors which either singly or in combination could cause damage:

1. Wave activity may be temporarily extended well below the normal depth, resulting in direct structural damage either by fragmentation of coral colonies, e.g. Acropora, or the over-turning of the more massive species, such as Turbinaria.

2. Movement of coral sand and rubble over the reef may bury living colonies and, unless the material is redistributed within a few days, mortality of the polyps will occur.

3. Movement of fine sediment from the mouths of coastal streams may reach the fringing reefs and slowly bury the corals beneath silt and mud. This process may continue for several weeks after heavy rain.

4. Reduction of salinity by freshwater runoff may reach levels fatal to most species of coral.

Apart from the low salinities recorded in the summer wet season, the salinity of the water around Magnetic Island generally deviates little from $35^{o}/_{oo}$ (Grigg, 1972; Zann, 1975).

It has previously been noted that coral reefs show mass mortality following dilution by flood rains (Hedly, 1925; Rainford, 1925; Slack-Smith, 1960; Cooper, 1966; Banner, 1968; Goreau, 1964).

Salinities recorded over the reef in Nelly Bay and between Magnetic Island and the mainland, after the periods of heaviest rain, are summarised in Figures 7 to 12. The fresh water discharge from the small creeks No. I and II (Figure 3) was deflected to the south parallel to the shore, and salinities as low as $6^{o}/_{oo}$ were measured within 5 m of the shore at mid tide between creeks I and II. For intertidal organisms in this region to survive they would have to tolerate such low salinities during each tidal cycle while the creeks flowed. These creeks would usually flow once or twice during the wet season. During the winter months when they do not flow, longshore drift causes the formation of sand banks, closing the mouths of these creeks. The exact position of the mouth and the time when the sand bank is broached is difficult to predict. Similarly, the effects on the coral of a large volume of fresh water flowing for a relatively short, but very variable period, can never be accurately estimated even during a 'normal' summer. The flow of a creek may have a considerable effect on the coral in the immediate vicinity, the severity of which will vary with the tidal cycle and the wind strength and direction at the time of maximum flow. The creeks begin to flow rapidly into Nelly Bay very shortly after the onset of heavy rain as their catchment areas (7.6 km^2) lie in steep hills (500 m high) very close to the coast. The creeks were still flowing on the 17th January when the salinity measurements (see Table 2) of that date were made. The highest flow rate and consequently the greatest volume of fresh water would certainly have occurred in the preceeding week at the time of highest rainfall (see Figure 6). The extensive cyclone damage to the City and harbour facilities precluded scientific investigations during this period. Figure 12 is a salinity profile derived from data taken on 17th January.

It represents a period of time 1½ hours after a low water of 1.0 m above low water datum (LWD), on a tide that rose to a height of 2.4 m above LWD. Water approaching $32^{o}/_{oo}$ slowly displaced the less saline ($16 - 20^{o}/_{oo}$) and less dense water which had covered the shallower coral reef at low tide. At a depth of 2 m a prominent halocline was present, and this ensured that the coral below that depth would not be exposed to water of a salinity less than $32^{o}/_{oo}$. The continued stability of this halocline was aided by the tidal influx of high salinity water along the deep water channel to the immediate south-east shore of Magnetic Island (see Figure 2).

TABLE 2

SALINITY READINGS (PARTS PER THOUSAND) AT NELLY BAY REEF SAMPLING STATION

Date	Depth (m)				Depth (m) of Bottom
	Surface	0.5	1.0	2.0	
27 December	19.9	-	-	-	2.5
14 January	17.0	-	20.6	23.5	2.2
17 January	17.0	17.6	18.4	-	1.6

The immediate physical disruption of the reef was clearly a result of the wave action generated by the cyclone. Similar wave effects have been noted on other reefs in Queensland and elsewhere following a cyclone (Moorhouse, 1936; Stephenson et al., 1958; Banner, 1968; Stoddart, 1963). The heaviest rain and consequent land runoff did not coincide with the cyclonic winds, but reached a

maximum 10 - 14 days later (see Figure 6). The mortality in the upper region of the reef of Acropora fragments in this latter period was probably the result of the drastic salinity reductions and not the mechanical damage caused by wave action.

The tolerance of corals to lowered salinity is dependant upon the degree of dilution and duration of the exposure to it. Vaughan and Wells (1943) reported that some species of corals were able to withstand 24 hours' immersion in a salinity of $27.9^{o}/_{oo}$, but 4 out of 17 species they tested died after 24 hours in a salinity of $17.3^{o}/_{oo}$. Salinities as low as these were recorded from the surface of the reef in January 1972 (see Table 2). It is significant that species of Acropora showed a decrease in mortality with depth. Although the wave action was severe, it did decrease with depth, thus less physical damage was caused. The presence of a distinct halocline undoubtedly contributed to the better survival of this deeper living coral. These results also showed that the genera Montipora, Turbinaria, Porites, and the favid corals here were more tolerant to low salinity water than were species of Acropora.

It has been observed that the mass expulsion of zooxanthellae, which leaves the coral in a bleached condition, may follow exposure to physiological stress (Yonge, 1968). Goreau (1964) described the mass expulsion of zooxanthellae after exposure to sublethal dilutions of sea water following a hurricane in the Carribean. Bleached corals were only rarely observed in Nelly Bay in the post-cyclone period, but were common on the reef in Alma Bay, some 3 km to the north. Bleaching was not confined to any one species, but was most frequent in the genus Turbinaria. Observations on bleached corals in

running sea water aquaria showed that these corals regained their pigmentation by regrowth of zooxanthellae over a period of 2 - 3 months. Natural expulsion of zooxanthellae is common in corals during the summer months on inshore reefs near Townsville (D. Tarca, I. Croll, personal communications). Although the 1972 rainfall was exceptionally high, low salinities are obviously a recurrent but irregular feature of these reefs. This factor may be one of the major reasons for the lower species diversity so characteristic of inshore reefs. Considerable water turbidity is a characteristic feature of Cleveland Bay throughout the year and is particularly marked when the wind velocity exceeds 6 ms^{-1}. Such wind conditions are frequent during the year, and although the waters were extremely turbid for two weeks following cyclone "Althea", they were in the subsequent months not significantly more turbid than expected. Even though Hopley (1974c) observed a small deposition of fine mud on the reefs of the Palm Islands, this was not evident on the Nelly Bay reef and this may be discounted as a contributary factor to coral mortality. Once established, the coastal reef forming corals seem to be able to tolerate a normal silt load, their three dimensional structure being ideally suited for silt removal; indeed this may be the raison d'etre for a skeleton in corals (Yonge, 1968). On calm days in the months after the cyclone, the clearest water visibility in five years was experienced, with Secchi depths in excess of 7 m. It is possible that the cyclonic disturbances either flushed out the Bay or buried the fine silt normally resuspended by average wind action. Although Acropora species suffered more physical damage from wave action than other corals, the redistribution of coral rubble and the subsequent burial destroyed many of the encrusting species (e.g. Galaxea, Montipora, and smaller favids) in the shallower (less than 2 m)

reef zones.

Extensive banks of debris did not form, but burial of live coral under 10 - 30 cm of rubble was frequently observed. The overall effect on the reef front was one of planing off the higher outcrops, and the filling in of depressions. The coral rubble deposited in this zone was derived from locally generated reef front rubble with many live fragments and well worn rubble from the reef flat carried to the area by longshore drift and wave undertow. Considerable erosion of the upper beach provided additional rubble to the reef flat and also rubble to the storm beach that formed above the intertidal zone. The large stands of Sargassum algae of the lower reef flat were totally destroyed and deposited in the storm beach area.

Of the four factors detailed by Hopley (1974) as contributing to the destruction of live coral, as a direct result of cyclonic disturbance, it is considered that for Nelly Bay, in order of effect they were:

(1) Lowered salinity

The salinity dilution effects were limited to the shallow reef area, and built-up to maximum effect after the cyclone winds had abated. Acropora, the dominant coral of the area, was severely affected.

(2) Burial under rubble

This damage occurred along the entire reef front, but was more evident on the shallow reef front. Encrusting and small massive corals suffered extensively, while the already truncated

branching forms were often buried in their own fragments. The burial was not continuous, and many outcrops were only lightly covered.

(3) Physical damage and overturning

There appeared to be only minimal permanent damage attributable to these causes. Abraded surfaces either healed over or else damage was restricted to the crushed region. The settlement of algae on the exposed coral skeleton reduced the ability of the coral to repair damaged areas. Overturning of colonies was not fatal to the corals as many recovered by reversing growth polarities. Unless physical damage is accompanied by burial or salinity dilution it has little effect in killing live coral. This form of damage to the reef was the most spectacular to observe, but has proved to be the least effective in destroying the live reef.

(4) Silt and turbid water

In Nelly Bay, this factor presented little problem to most coral species. Light penetration was certainly limited in the first few weeks after the cyclone, but as the deeper corals survived this period no destruction can be attributed to these factors.

Salinity was the only physical factor that showed a rapid and substantial change in the period following the cyclone.

(c) Coral regrowth following cyclone damage to Nelly Bay reef

Five months after the cyclone there were distinct signs of regrowth from the surviving coral fragments. Many of the free Acropora branches had now firmly re-attached to the stable coral rubble substratum that had been partially consolidated by the growth of calcareous algae. New branches were present on the upper surfaces of Acropora fragments (Figure 13, lower). On the surviving over-turned and normally smooth undersurfaces of other corals, e.g. Turbinaria, new growths were apparent indicating that total reversal of growth polarity had occurred. Regrowth was regularly monitored and, in addition, specimens were collected from an area 2.5 m below low water datum where the coral had suffered the greatest fragmentation damage. The extent of Acropora regrowth from branch fragments (horizontal element) at 5, 15, and 40 months after the cyclone is shown in Figure 13. On the basis of these measurements, the growth rate of Acropora formosa is between 6 - 8 cm per year in Nelly Bay, compared with the 10 cm per year that Shinn (1966) reported for the Caribbean species Acropora cervicornis (Lamarck, 1816). In areas where coral fragments were most numerous these growths produced large colonies of Acropora as the centres of growth coalesced. The significance of this and the regrowth is further discussed in later sections. New colonies of Acropora resulting from the settlement of larvae had not been observed up to 15 months after the cyclone, when this section of the study was concluded. The wide-spread scattering of living fragments and their subsequent regrowth suggests that vegetative reproduction in the genus Acropora may be more important than originally thought, and as a consequence may be a major reason why the species diversity is so low on inshore reefs

where the conditions for the settlement of larvae are made unsuitable by silt and algal growth.

As the incidence of cyclonic influence at any point along the coast north of Rockhampton is greater than once in 20 years (Hopley, 1974d), coastal reefs must frequently be exposed to the conditions described for cyclone "Althea". The massive and encrusting species (that survive without being dislodged or buried) are more tolerant of lowered salinity (Vaughan and Wells, 1943) and are able to grow into the large coral heads frequently found along most coastal reefs, e.g. Porites, Platygyra, and Goniastrea. Since the cyclone, a greater number of small (10 - 25 cm in diameter) Goniastrea heads have been noted in the low intertidal zone (see Figures 4 and 40b) and as such they represent the most dominant intertidal coral in Nelly Bay. This particular species is very hardy and large colonies (up to 1.0 m across) have been found on the inner side of the Townsville Harbour breakwater (Figure 40c), a location with high turbidity, often reduced salinity and, on rare occasions, oil spillages. No other corals have been recorded from this location. Although Acropora species suffer high mortality in cyclonic conditions and are temporarily removed from the shallow growing front of the reef, they are able to regenerate sufficiently quickly to form large colonies before the likely onset of another cyclone in the same location. Indeed, the growth of this important reef forming coral may be stimulated as a consequence of the distribution of fragments in a cyclone, especially if the fragments are not subjected to a protracted immersion in low salinity water. The consequences of cyclone "Althea" to the coral were aggravated by the occurrence of excessively heavy rain produced by cyclone "Bronwyn".

The occurrence of two cyclones in the same area within a week of each other is unusual.

It is interesting to speculate on the lasting stability of this coastal reef system as the marked change in land use over the last 50 years, and the local activity of the harbour dredge have added considerably to the silt load of this coastal environment. The rapid increase in size of Townsville in the last ten years, and the consequent increase in industrial activity along the coast have increased the output of basic nutrients into the local bays. If this eutrophication causes an increase of algal growth on the local reefs, and if increased sedimentation does inhibit the settlement of planulae then the vegetative reproduction of corals by fragmentation will assume greater significance in the maintenance of a viable inshore reef.

FIGURE 1

Townsville and adjoining areas of the Great Barrier Reef.

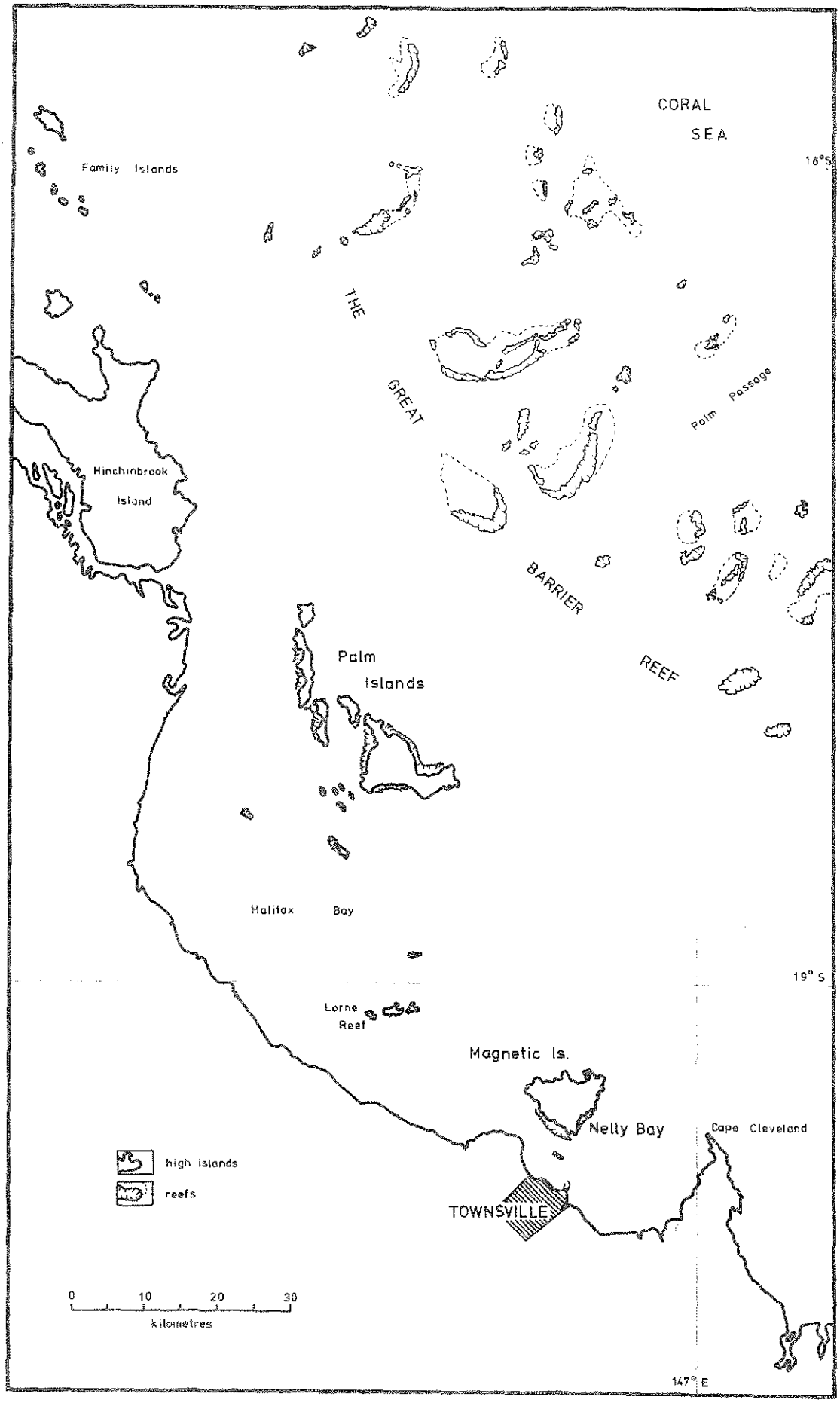

CORAL
SEA
18°S
Family Islands
THE
GREAT
BARRIER
REEF
Palm Passage
Hinchinbrook
Island
Palm
Islands
Halifax Bay
19° S
Lorne
Reef
Magnetic Is.
Nelly Bay
Cape Cleveland
high islands
reefs
TOWNSVILLE
0 10 20 30
kilometres
147° E

FIGURE 2

Magnetic Island and associated reefs.

Inset is the location of Figure 3.

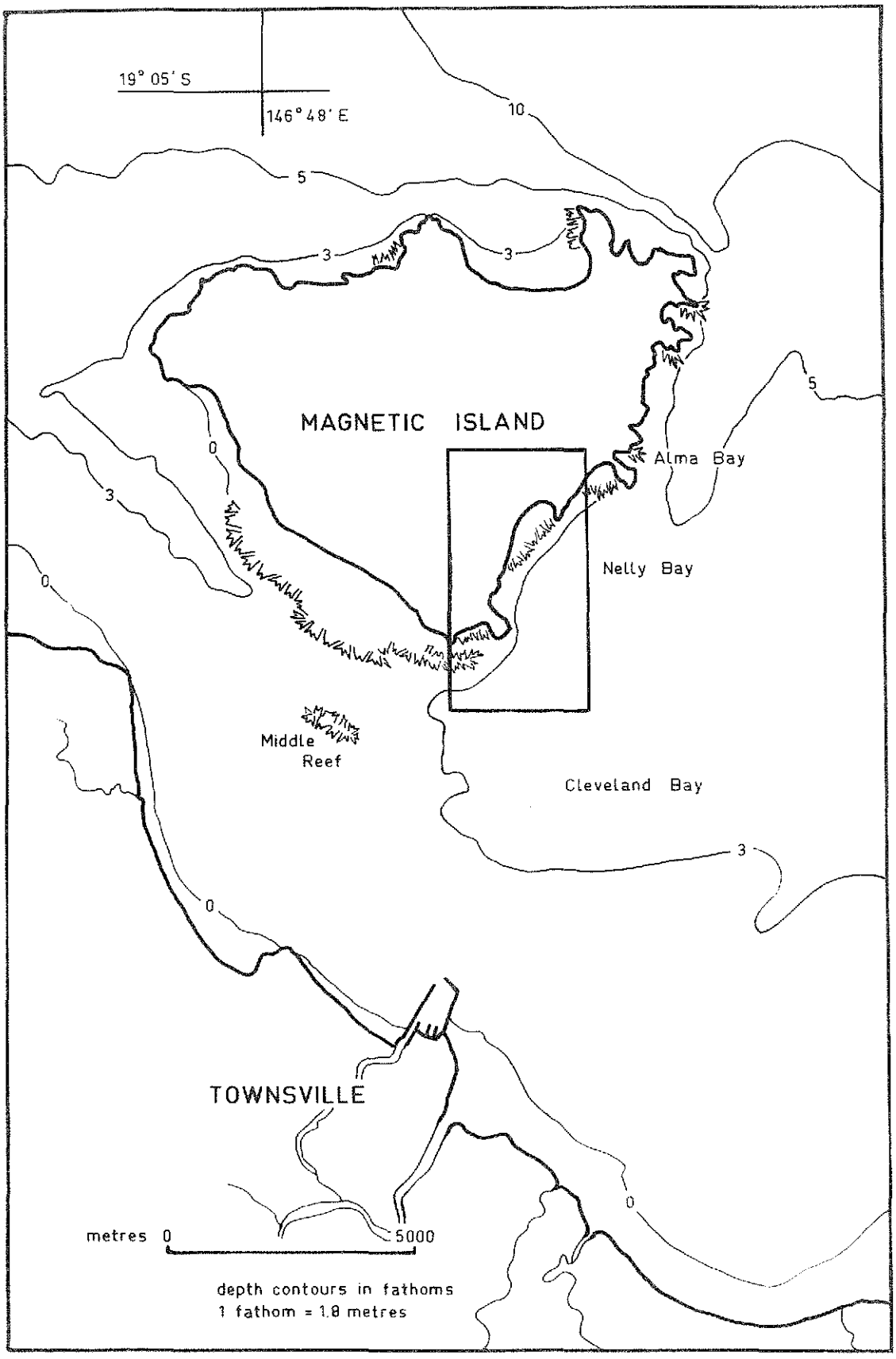

19° 05' S
146° 48' E
10
5
3
3
5
MAGNETIC ISLAND
0
3
Alma Bay
Nelly Bay
0
Middle
Reef
Cleveland Bay
3
0
TOWNSVILLE
0
metres 0
5000
depth contours in fathoms
1 fathom = 1.8 metres

FIGURE 3

The geomorphology of Nelly Bay, Magnetic Island.

The upper beach is composed of a medium to fine terriginous beach sand (bs). Below the spring-line at the change in slope of the beach is an area of fine sandy-mud (sm) that covers an older consolidated reef surface. The sandy-mud is more extensive at the northern end of the Bay, where there is also a band of coarse deltaic sand (d) associated with Gustav Creek, the larger of the three ephemeral creeks that drain into Nelly Bay in the monsoon season. The reef flat (rf) consists of a semi-consolidated reef rubble with shallow pools and small drainage channels. The outer edge drops steeply to form the reef slope (rs). At a depth of 6.5 m, the reef slope irregularly merges with the sea floor (sf), consisting of a fine sandy mud mixed with foraminifera, shell and coral fragments. The transect line a-b is profiled in Figure 4.

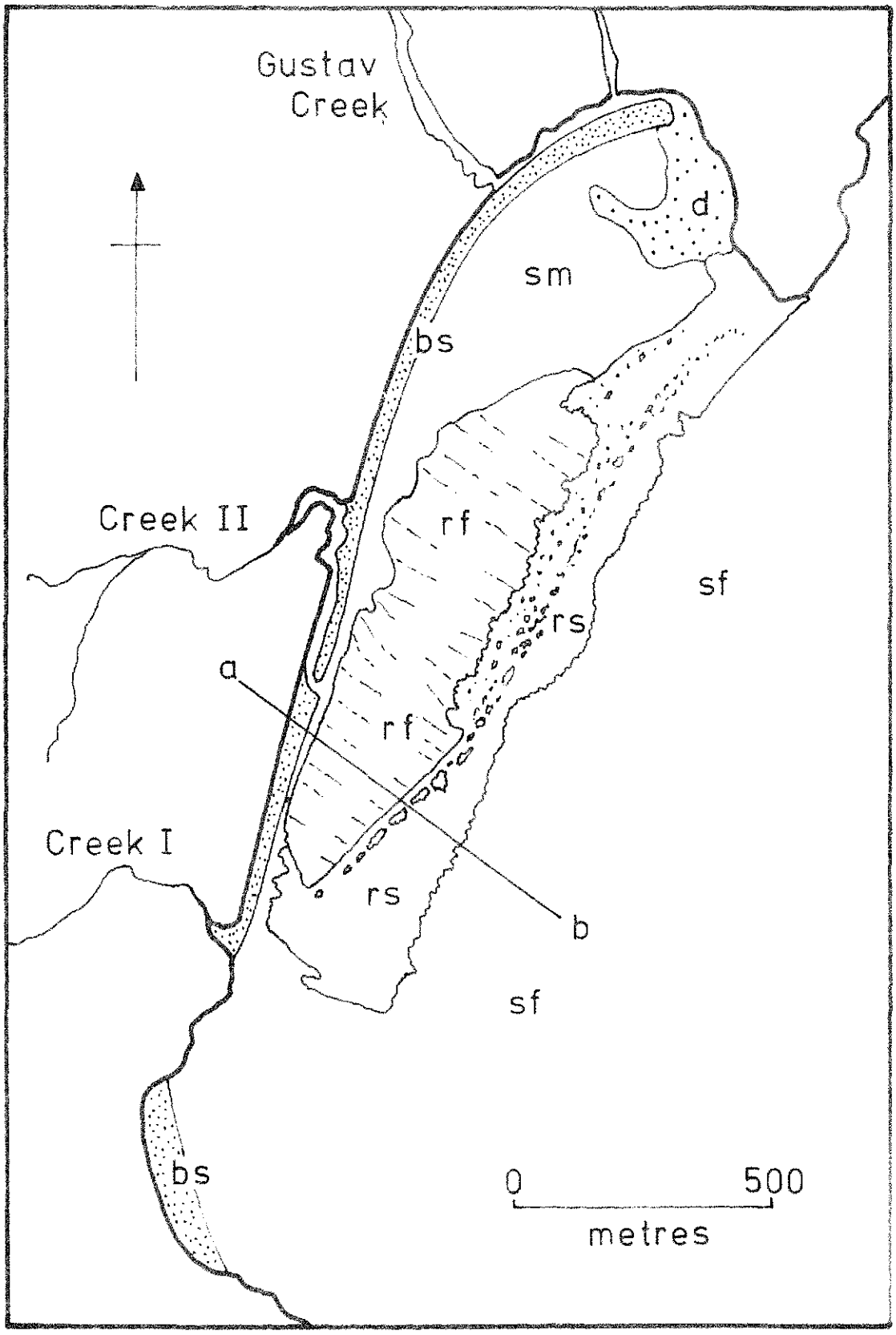

Gustav
Creek
d
sm
bs
Creek II
rf
sf
rs
a
rf
Creek I
rs
b
sf
bs
0
500
metres

FIGURE 4

Cross section of Nelly Bay, Magnetic Island.

The position of the transect is indicated in Figure 3. The distributions of the common Goniastrea aspera and Acropora formosa are indicated prior to cyclone "Althea" (December, 1971) and for the year after.

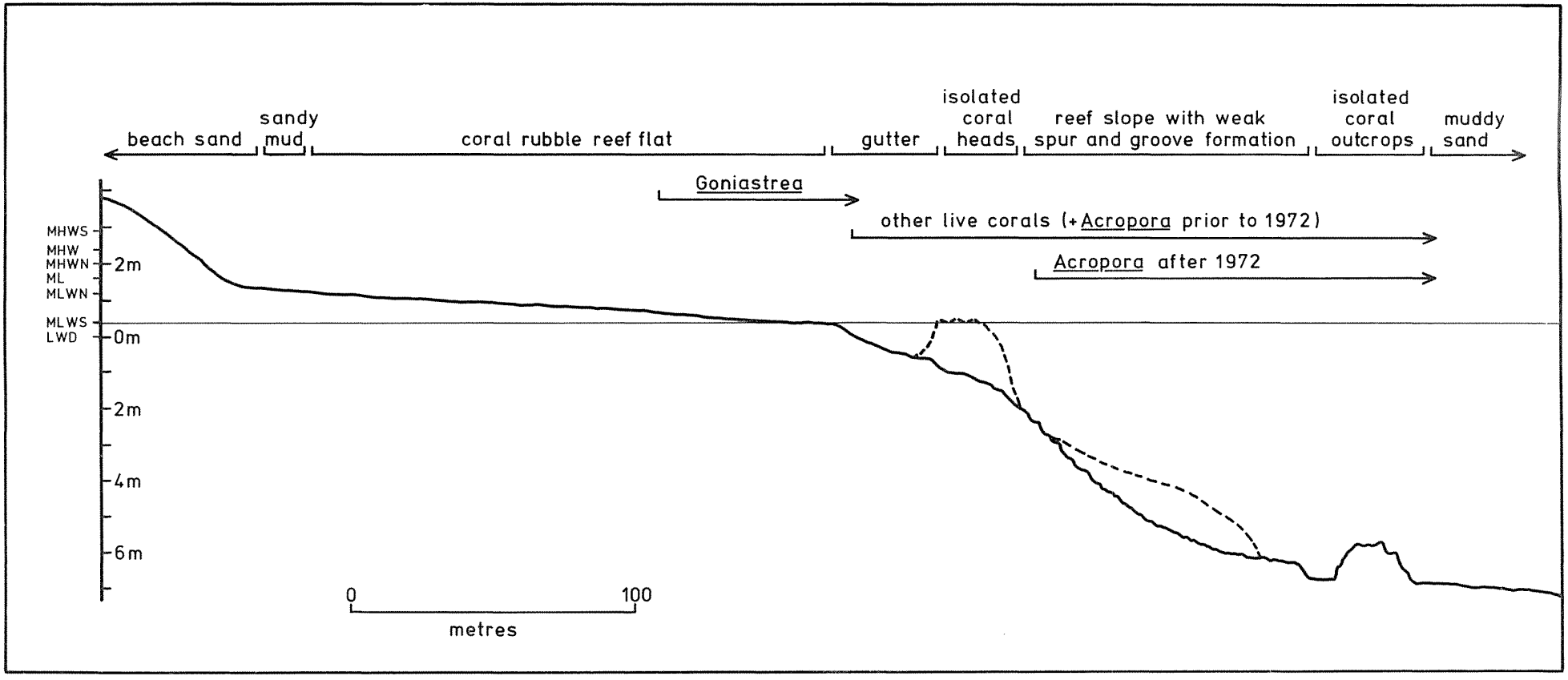

beach sand
sandy mud
coral rubble reef flat
gutter
isolated coral heads
reef slope with weak spur and groove formation
isolated coral outcrops
muddy sand
Goniastrea
other live corals (+Acropora prior to 1972)
Acropora after 1972
MHWS
MHW
MHWN
ML
MLWN
MLWS
LWD
2m
0m
2m
4m
6m
0
100
metres

FIGURE 5

Tidal prediction and tidal record from Townsville Harbour for the period of cyclone "Althea", 24th December, 1971.

The eye of the cyclone crossed the coast at 0900 hours and was accompanied by a storm surge 4.7 m above datum, 2.85 m above the predicted height for that time.

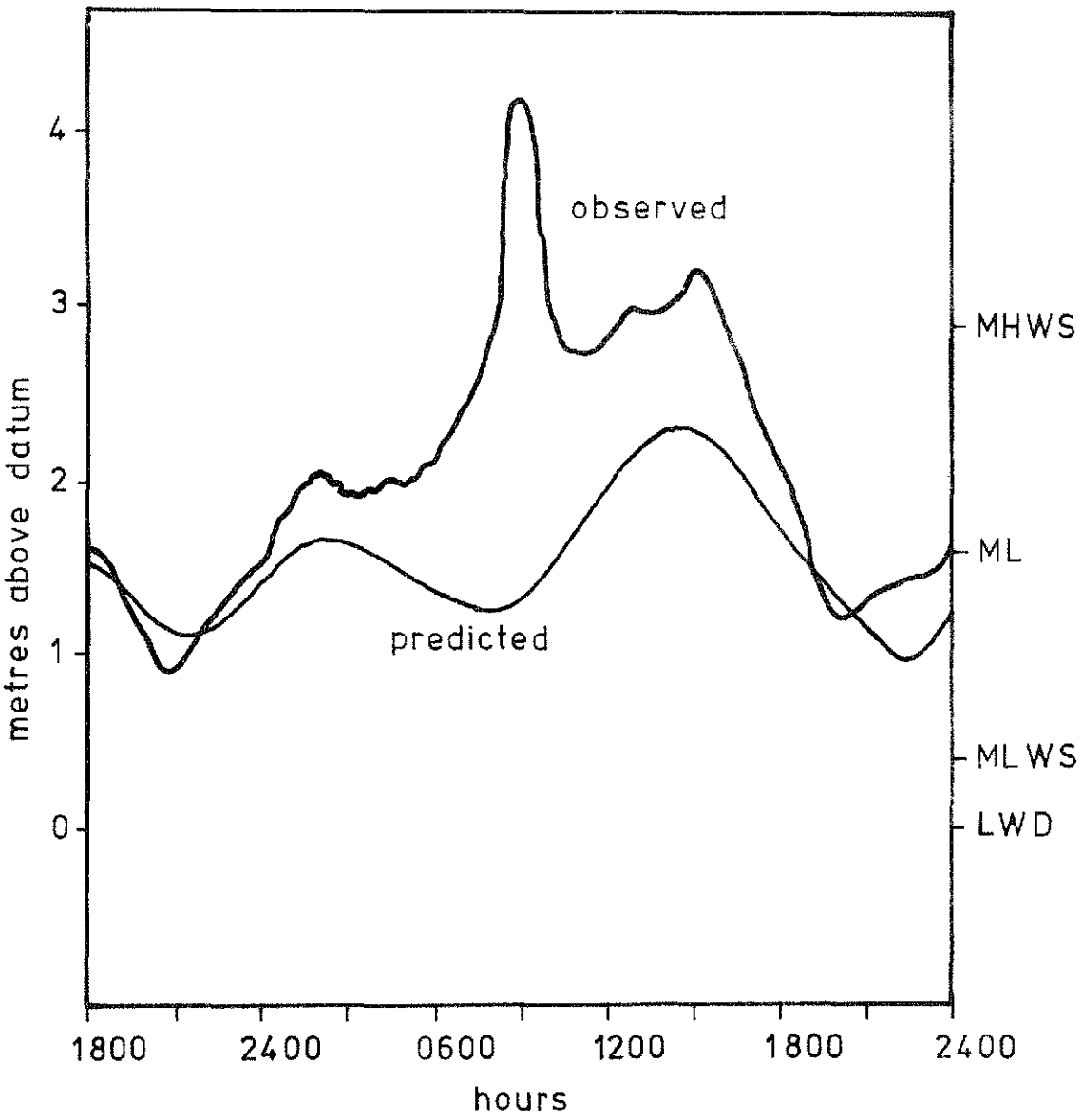

4
3
2
1
0
metres above datum
observed
predicted
MHWS
ML
MLWS
LWD
1800
2400
0600
1200
1800
2400
hours

FIGURE 6

Daily rainfall recorded in Townsville for cyclone "Althea" and the "Bronwyn" rain depression.

"Althea" deposited 31.8 cm and "Bronwyn" 60.5 cm to give a total of 92.3 cm of rain in 22 days. The yearly mean rainfall for Townsville is 112.8 cm.

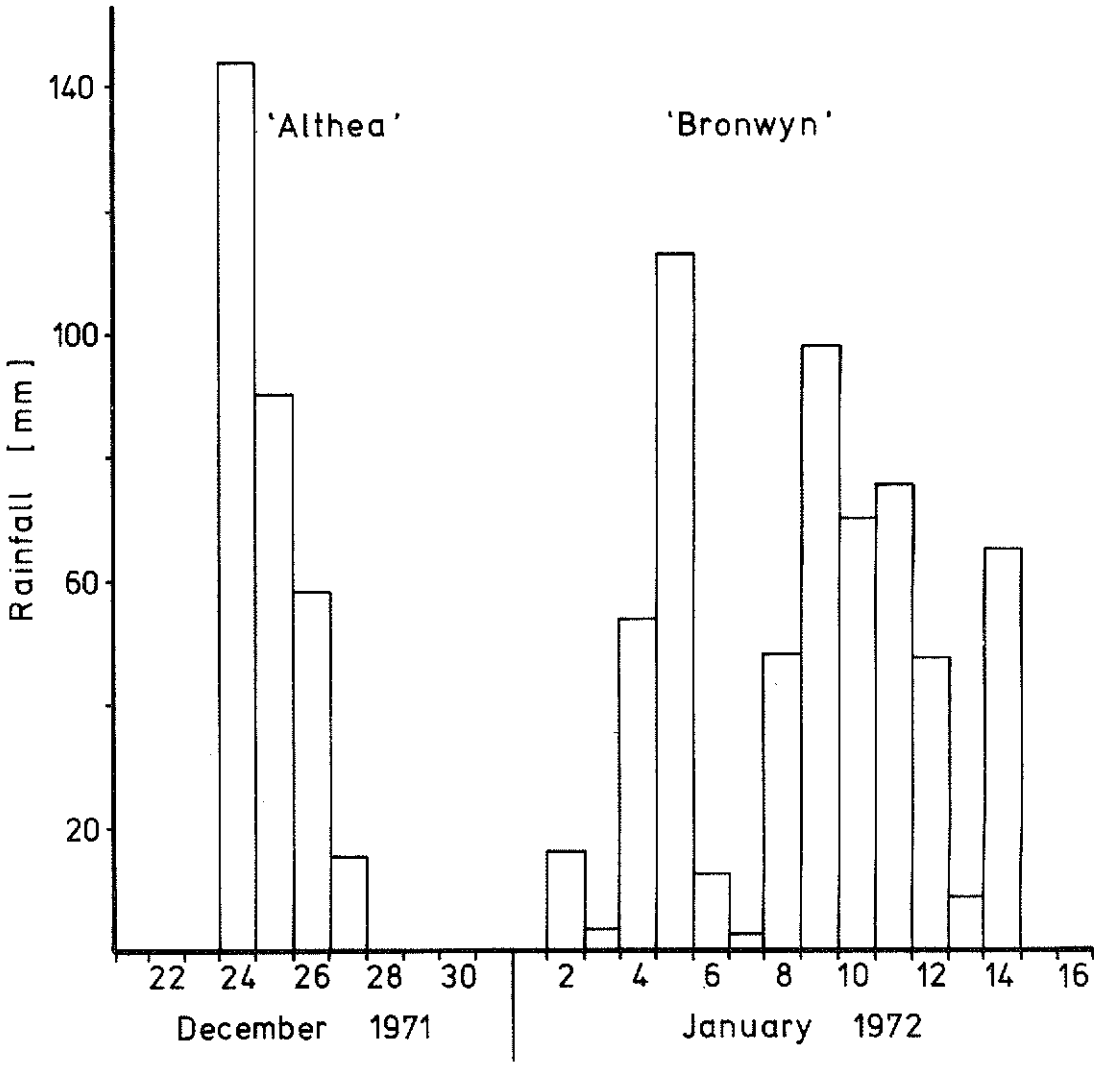

140
100
60
20
Rainfall [mm]
'Althea'
'Bronwyn'
22
24
26
28
30
2
4
6
8
10
12
14
16
December 1971
January 1972

FIGURE 7

Surface salinity values for the Western Channel region of Magnetic Island, 14th January, 1972.

A westerly current of 1.5 knots was found near Middle Reef. The salinity values increase regularly with distance offshore.

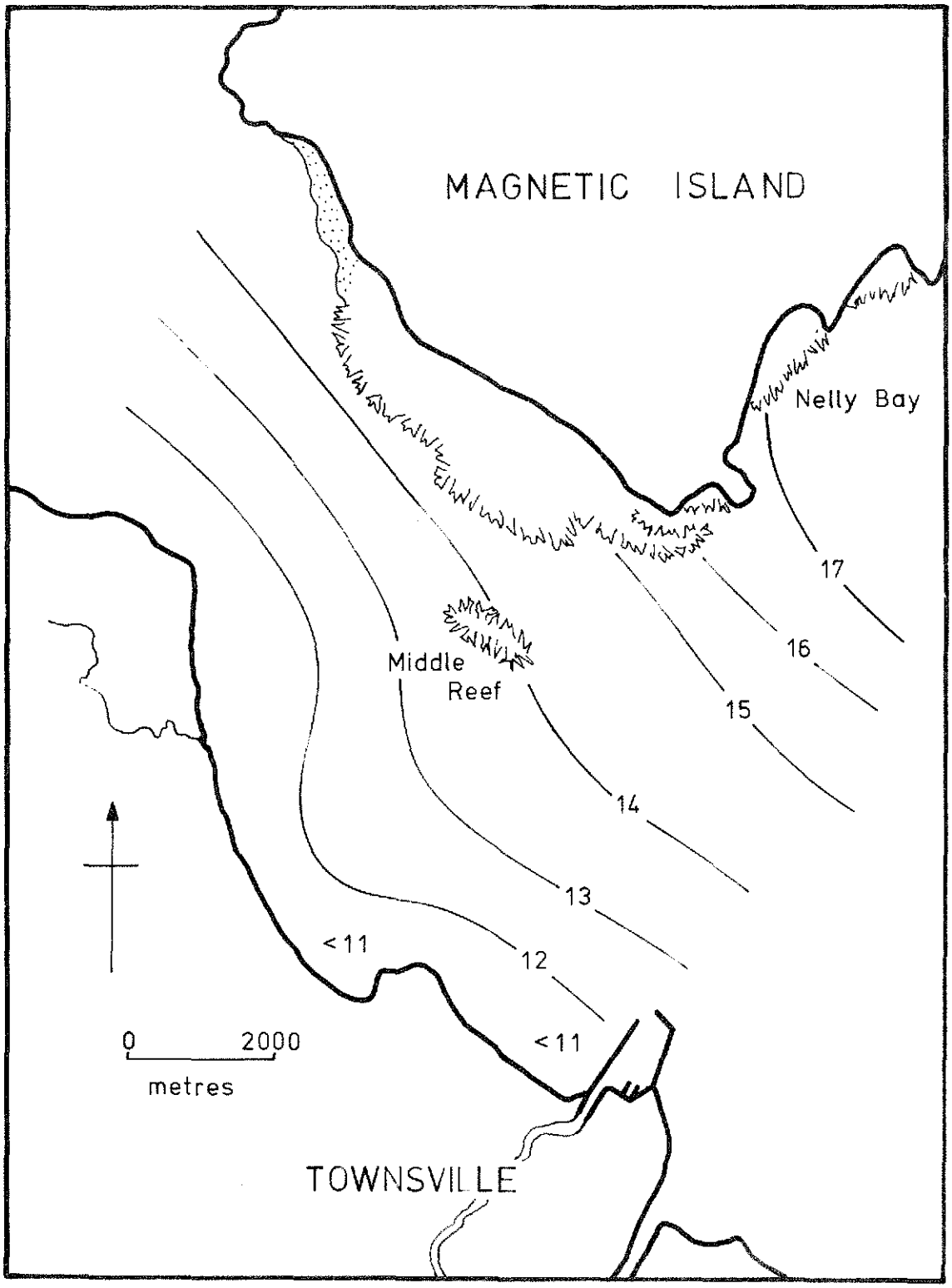

MAGNETIC ISLAND
Nelly Bay
Middle
Reef
17
16
15
14
13
12
< 11
< 11
0
2000
metres
TOWNSVILLE

FIGURE 8

Salinity values at a depth of 1.0 m for the Western Channel region of Magnetic Island, 14th January, 1972.

A similar gradual increase in salinity with distance offshore to that found for the surface values was seen.

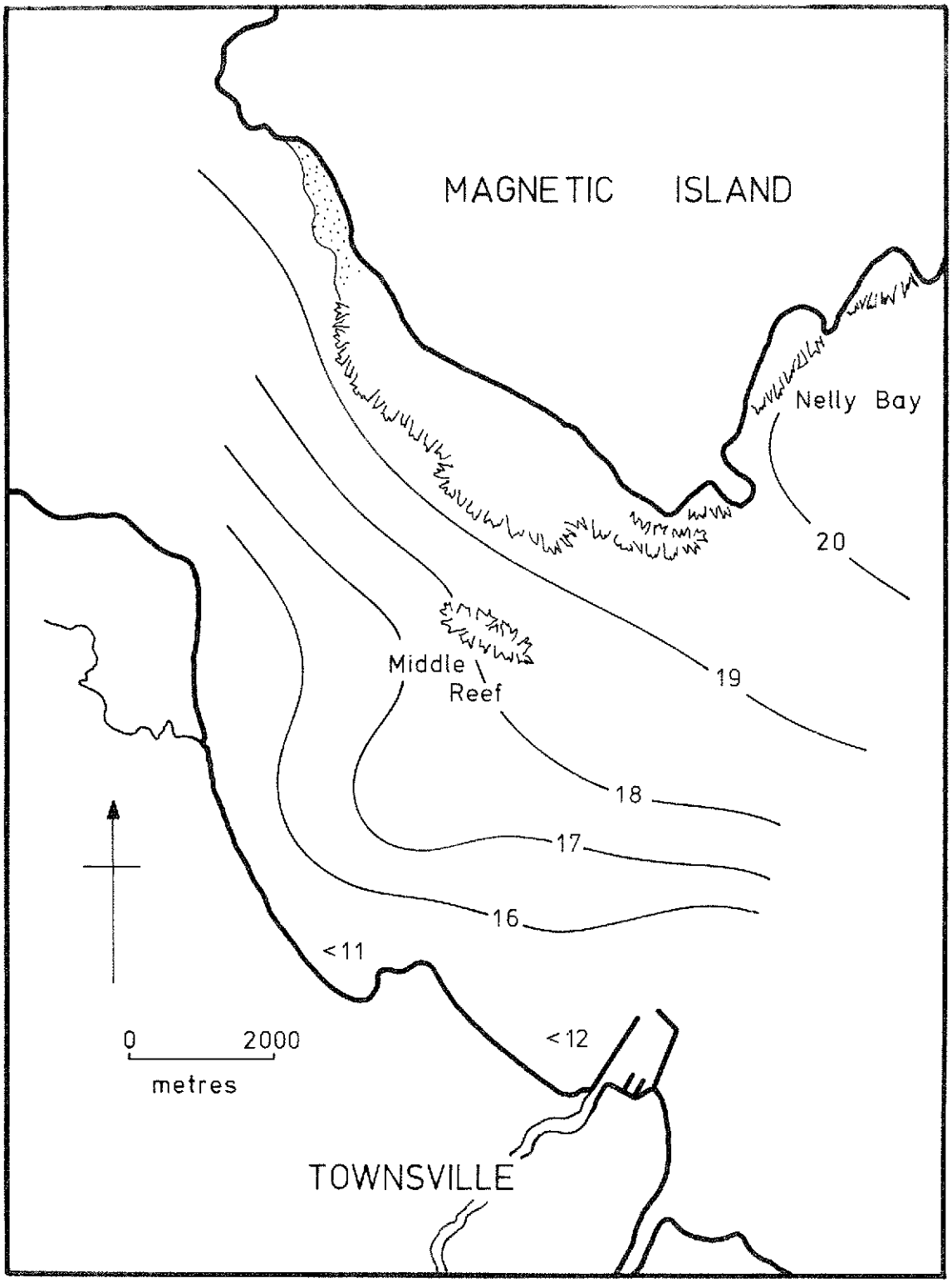

MAGNETIC ISLAND
Nelly Bay
20
19
Middle Reef
18
17
16
<11
<12
0 2000
metres
TOWNSVILLE

FIGURE 9

Salinity values at a depth of 5.0 m for the Western Channel region of Magnetic Island, 14th January, 1972.

A marked tongue of higher salinity water has penetrated beneath the lesser saline water, forming an estuarine like salt wedge. The higher salinity water penetration was facilitated by the deeper water channel along the eastern side of Magnetic Island.

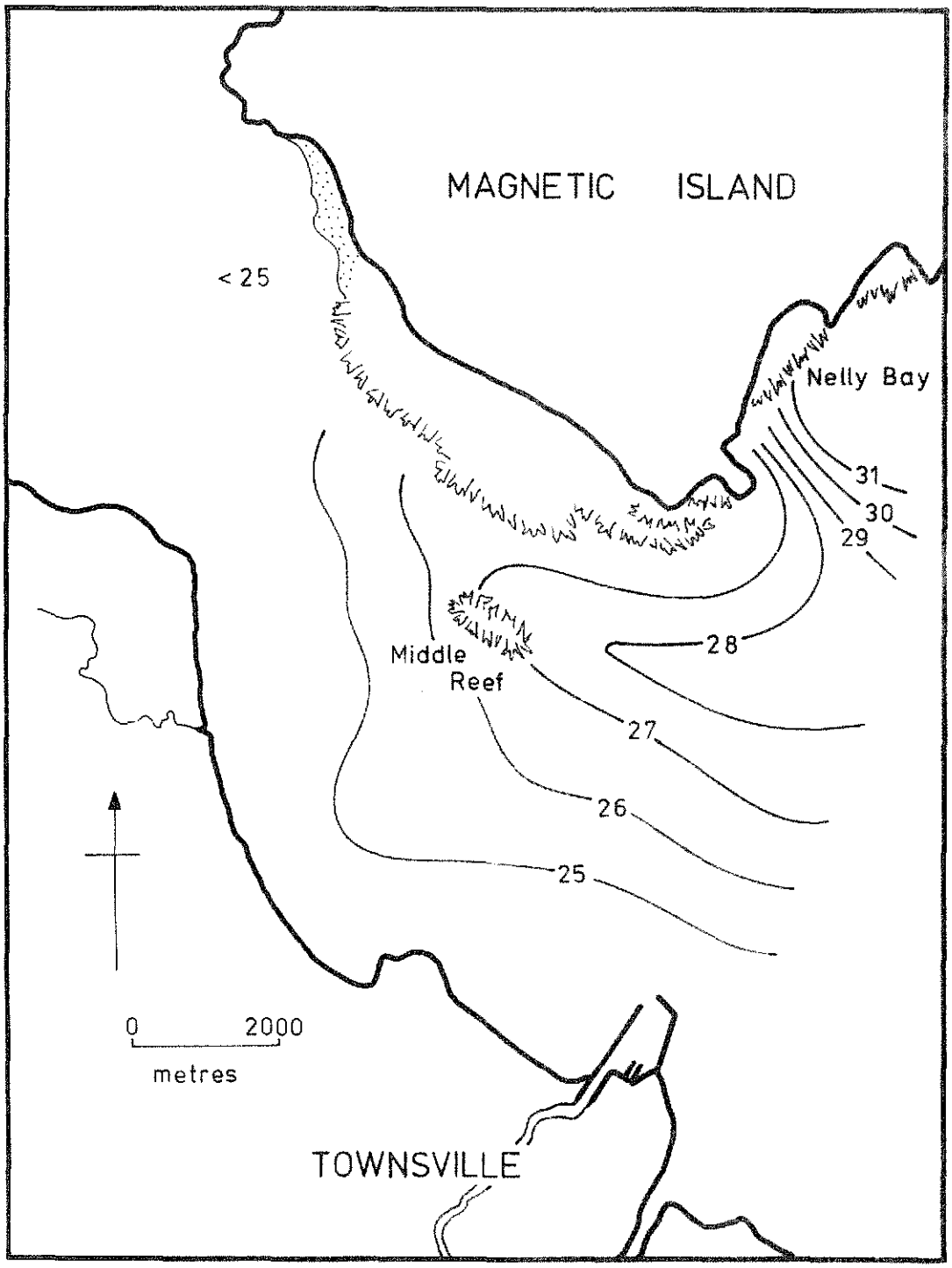

MAGNETIC ISLAND
< 25
Nelly Bay
31
30
29
28
27
26
25
Middle Reef
0 2000
metres
TOWNSVILLE

FIGURE 10

Surface salinity values for Nelly Bay, Magnetic Island, 17th January, 1972.

All the creeks were flowing at this time. The wind was light and from the north-east. The tide was rising, with a depth of approximately 1.0 m over the reef flat.
The transect x-y is profiled in Figure 12.

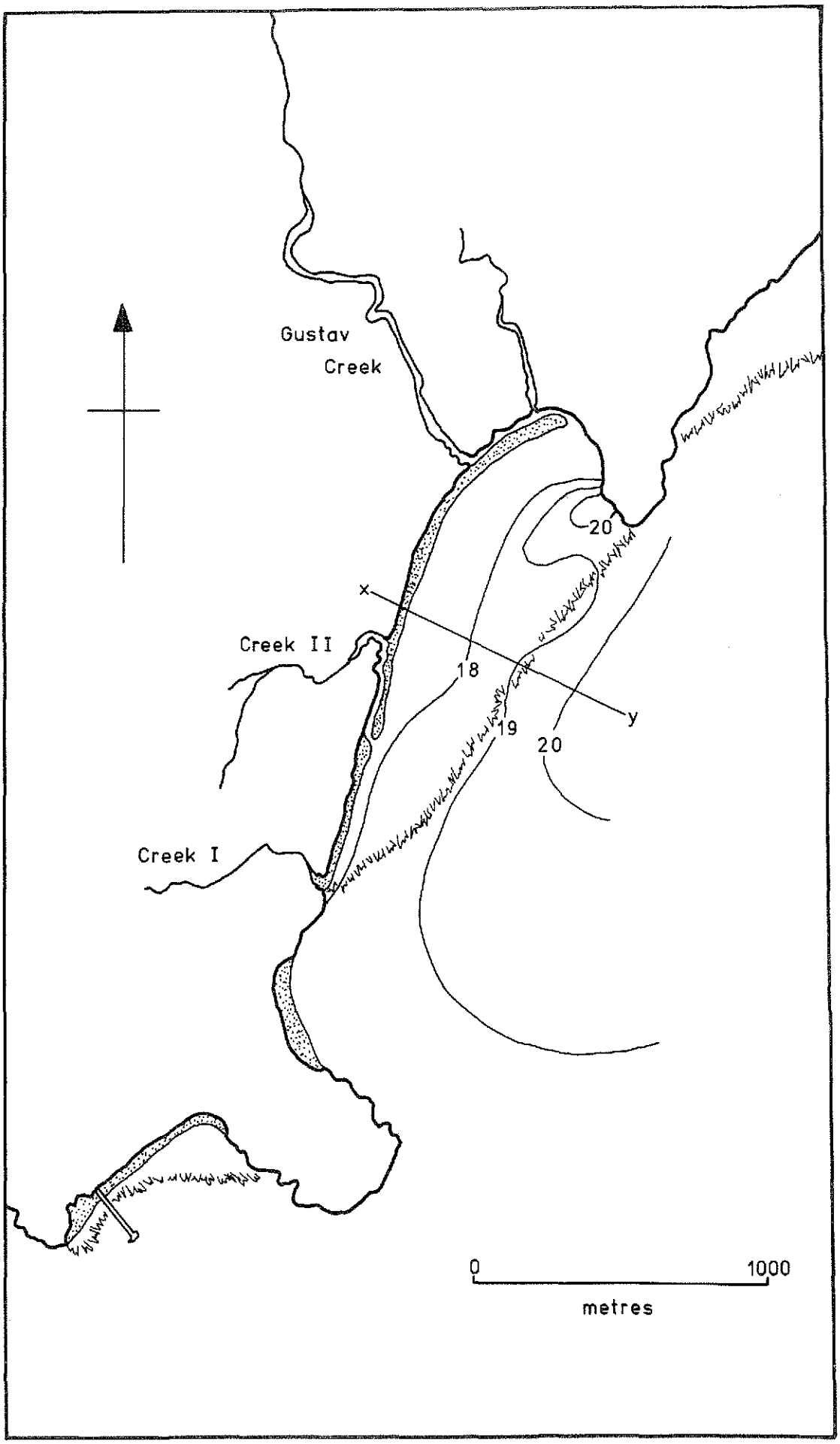

Gustav
Creek
Creek II
Creek I
x
y
18
19
20
20
0
1000
metres

FIGURE 11

Salinity values for 1.0 m depth for Nelly Bay, Magnetic Island, 17th January, 1972.

The higher salinity values to the northern end of the Bay are associated with the rising tide, that has allowed more saline water to penetrate onto the reef flat. The light north-easterly wind has moved the less saline water along the Bay in a southerly direction. The transect x-y is profiled in Figure 12.

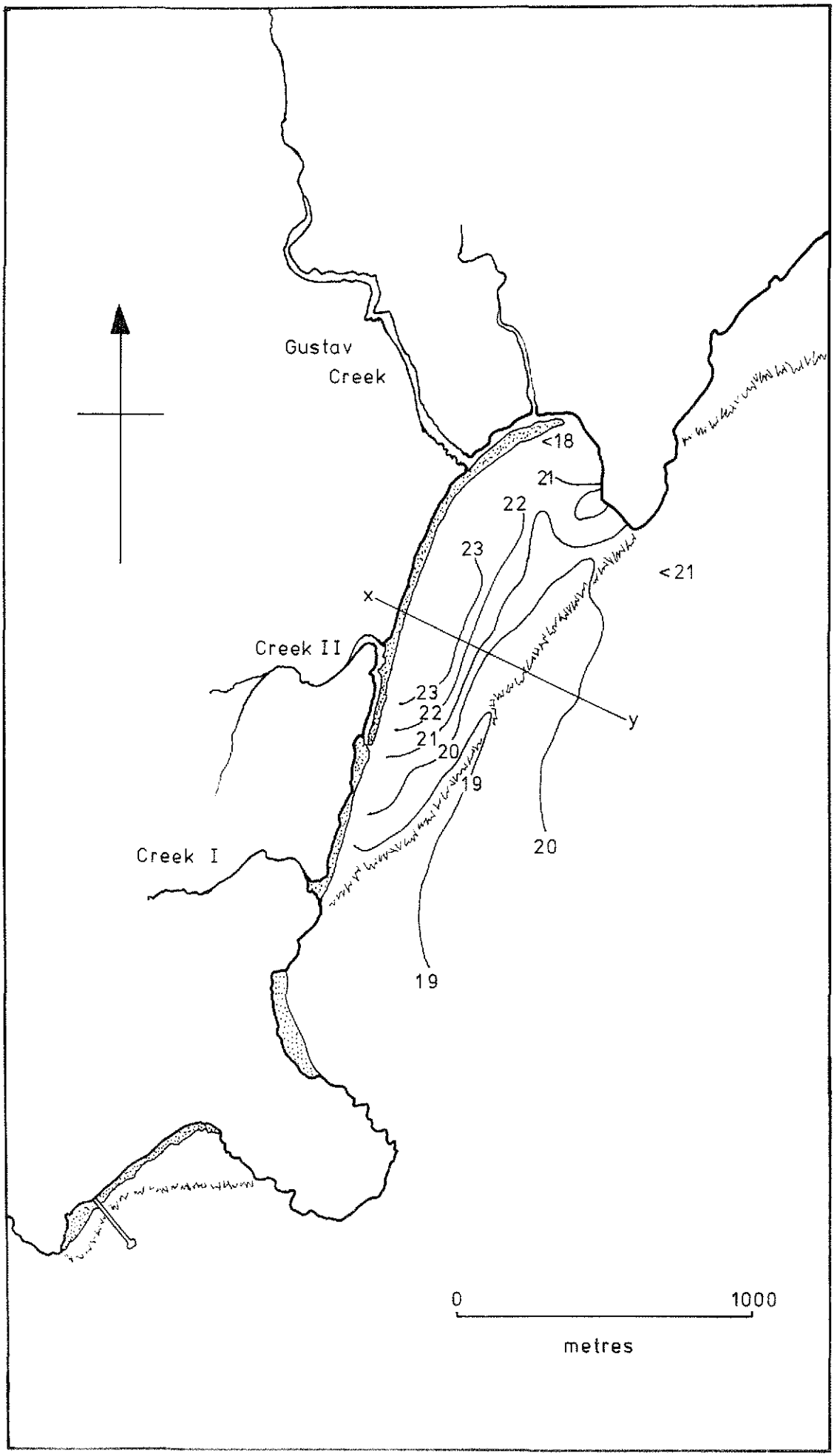

Gustav
Creek
<18
21
22
23
<21
x
y
Creek II
23
22
21
20
19
20
Creek I
19
0
1000
metres

FIGURE 12

Profile of Nelly Bay, Magnetic Island, for 17th January, 1972, along the transect x-y of Figures 10 and 11.

The rising tide, 1.5 hours after low water of 1.0 m, has allowed the deeper more saline water to rise over the reef flat. A distinct halocline was present at a depth of 3 m. The tidal movement allowed the less saline water to sit on the reef front to a depth of approximately 2 m below datum at low tide.

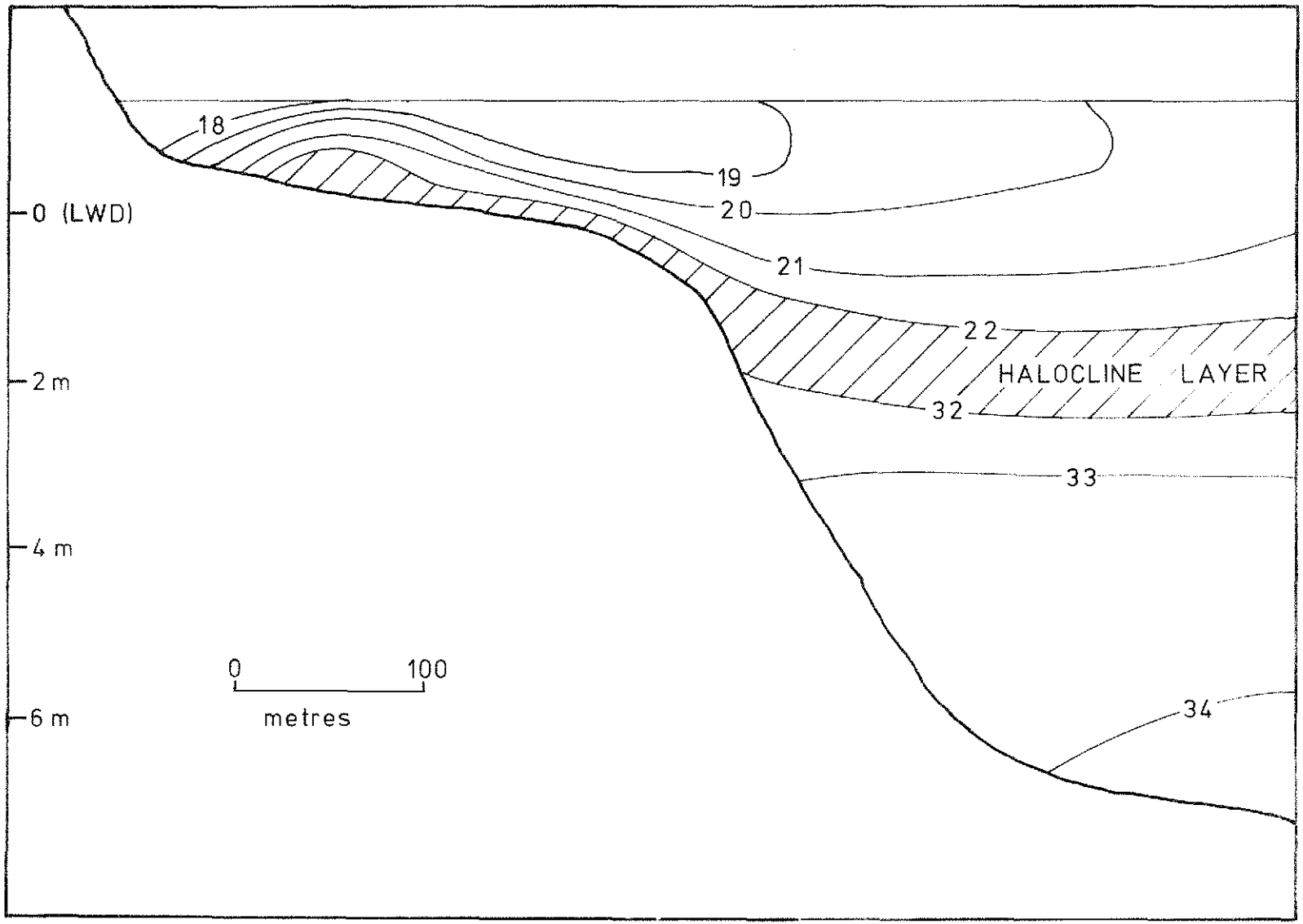
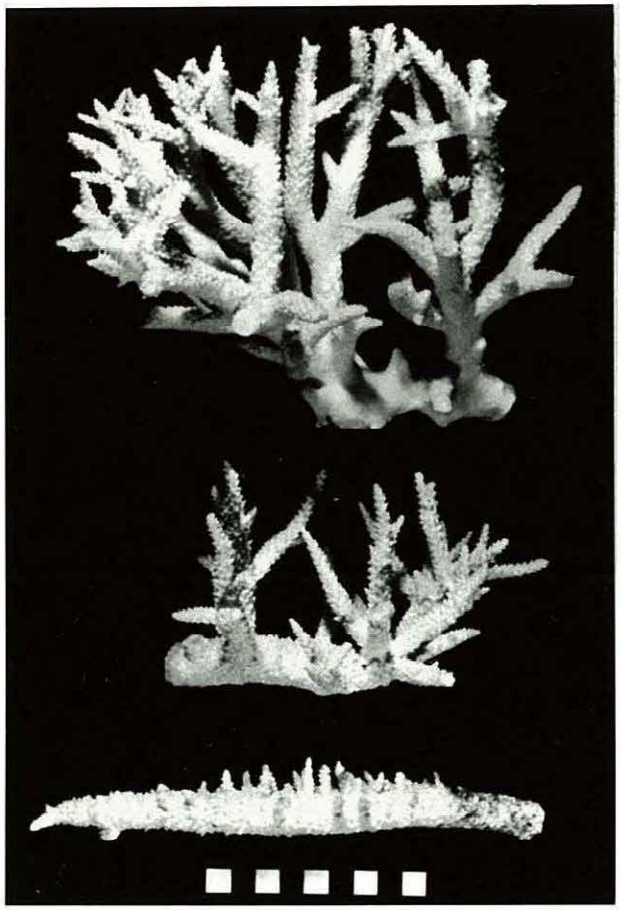

18
19
20
21
22
32
33
34
HALOCLINE LAYER
0 (LWD)
2 m
4 m
6 m
0
100
metres

FIGURE 13

Regrowth of typical *Acropora formosa* fragments from Nelly Bay, 5, 10 and 40 months after cyclone "Althea".

Note that there were many small incipient branches, but that only a few eventually became dominant.

Scale: 1 division = 10 mm.



CHAPTER III MATERIALS AND METHODS

(a) Maintenance of corals in aquaria

(b) Preparation of the skeleton

(c) Relaxation

(d) Fixation

(e) Grafting technique

(f) Double paraffin embedding with decalcification

(g) Resin embedding

(h) Staining procedures

(i) Xerography and X-radiography of coral slices

(a) Maintenance of corals in aquaria

Hermatypic reef corals require reliable and precise aquarium conditions for optimum survival over the long periods (1 week to 6 months) required for coral graft investigations.

Most grafting experiments were performed at the Marine Gardens, Magnetic Island, where near natural conditions were available in aquaria supplied with open seawater circulating and illuminated by natural sunlight. Minimum flow rates of two litres per hour through the experimental aquaria (95 x 51 x 31 cm) were maintained. The aquarium temperature and salinity closely paralleled those of the natural environment in Nelly Bay. Thus temperatures in the aquaria were found to be no more than $2^{o}C$ above or below Summer maxima or Winter minima recorded in the bay, and salinities showed no measurable variance.

Failure of the seawater circulation occasionally caused coral mortality, but continuous aeration of the water minimised losses on such occasions. In Summer, corals in both the aquaria and Nelly Bay were sometimes seen to bleach and expell their zooxanthellae, indicating environmental stress (Goreau, 1964; Yonge and Nicholls, 1931). If this occurred in the experimental corals, the experiments were terminated and the results discarded.

The aquaria were so placed to allow a maximum of two hours full sunlight, and strong reflected light for the rest of the day. Under these conditions excessive weed growths accumulated on the glass surfaces unless regularly removed. As most corals survived for long periods (greater than 150 days) and showed active calcification and rapid tissue repair, it was assumed that additional feeding was not

required, and that sufficient food, as microscopic plankton, was available in the circulating seawater.

When experimental corals were collected in the field, care was taken to avoid overheating, dessication and mechanical damage. Some early coral deaths were attributed to overheating and insufficient aeration while collecting. This stress was later minimised by the use of portable air pumps and polystyrene insulated containers.

(b) Preparation of the skeleton

Coral skeletal preparations were made by soaking the freshly collected specimens either in freshwater or bleach solutions at a concentration of 20 g calcium hypochlorite and 20 g commercial soda ash per litre. In freshwater, the soft tissues are usually decomposed sufficiently after three days of soaking to allow cleaning with a water jet. Bleaching was more rapid and less rigorous than a water jet which could easily fracture tenuous coral grafts. Skeletal material was labelled before cleaning with coded plastic tags, attached to the skeleton by heavy nylon monofilament.

(c) Relaxation

Wells (1932) suggests that corals may be fixed, in an extended state, by swift immersion in boiling sublimate and acetic acid. This method was impractical in the field and therefore was not used. Marr (1963) used menthol crystals to relax a range of animals, including corals, prior to fixation in 10% formalin-seawater. In his review article on 'Anaesthesia in Invertebrates', Kaplan (1969) tabulated a range of fixatives for Coelenterates, and for Anthozoa he indicated chloretone, magnesium chloride/sulphate + menthol, menthol+chloral hydrate and oil of cloves as applicable. Goreau (1956) used a 7.5% solution of magnesium sulphate to 'paralyse' some corals prior to fixation.

Other compounds found to induce nacotisation in other groups of animals were also tried on corals, and on Acropora in particular. Table 3 lists the narcotics investigated. Narcotisation was assumed when the expanded polyps did not respond to gentle tactile stimulation.

All the compounds tried produced narcotisation within 1 - 3 hours, however the responses of the corals were highly variable, and no one narcotic appeared superior in its action. Slight vibration could cause irreversible contraction at any time during induction. Contraction of tissues was frequently observed on initial contact with the narcotic, even when the narcotic was applied slowly over a long period. Wells (1932) noted that Anthozoan material responded in a highly variable way during narcosis.

TABLE 3

NARCOTICS AND METHOD OF USE

Narcotic	Method of Use
Chloretone	Crystals on water surface
Magnesium sulphate	Stock solution added to form 7.5% final concentration
Magnesium sulphate+ Menthol	As above, but with menthol crystals on surface
Chilling	Temperature reduced to 1-2^{o}C over 2 hours
Urethan	Stock solution added to form 10% final concentration
Quinaldine	Drops added to form a saturated solution
Propylene phenoxetol	As for Quinaldine
Menthol + chloral hydrate	As for Quinaldine

Although apparent narcosis of Acropora was achieved, judged by lack of withdrawal of the tentacles after gentle mechanical stimulation, contraction of tissues invariably followed fixation. The degree of contraction was similar to that found when fully expanded polyps were rapidly placed in 10% formalin-seawater. If corals were fixed in a contracted condition (either as a result of poor narcotisation or after strong mechanical stimulation) then the further contraction of tissues during the fixation process was so violent that skeletal spines protruded through the ectoderm. A similar condition can be induced as a result of constant mechanical stimulation of colonies in the field, especially obvious in the echinulate corals, such as Lobophyllia.

With Acropora it is apparent that fixation in an expanded state is difficult under field conditions and that swift immersion of fully expanded polyps into fixative will achieve the same result at the time consuming attempts at narcotisation. As long as the polyps were expanded prior to fixation then shrinkage artifacts, such as epidermal rupture, were avoided.

Wells (1932) has encountered similar difficulties with narcotised Anthozoan material, ".. many times when it is thought that the animal has been deprived of all sensitiveness, immersion in a reagent even of rapid action is sufficient to show a sudden and surprising contraction of tentacles and body". It is obvious that narcotisation in coelenterates needs further investigation.

(d) Fixation

A series of fixatives was tried on small samples of Acropora formosa (8 - 12 diameter, 4 - 5 cm in length), in order to determine a suitable fixative for the routine histological analysis of grafted coral. The following fixing solutions were used. Most were made up in seawater to minimise the osmotic effects that might otherwise have occurred. Figure 14 shows representative micrographs of coral fixed in these solutions.

Bouin (1897)[1], 75 parts sat. sol. picric acid, 25 parts 40% formaldehyde, 5 parts acetic acid. Saturated solution of picric acid in seawater.

Baker (1944)[1], (a) 90 parts distilled water, 10 parts 40% formaldehyde, 0.4% $CaCl_2$, and

(b) 90 parts seawater, 10 parts 40% formaldehyde, the 0.4% $CaCl_2$ omitted, due to the substitution of distilled water with seawater.

Zenker (1894)[1], 70 parts 7% $HgCl_2$, 30 parts 7.5% potassium dichromate with 3% Na_2SO_4, 5 parts acetic acid.

Heidenhains' Susa (1916e)[1], 15 parts seawater, 65 parts 7% $HgCl_2$, 20 parts 40% formaldehyde, 4 parts acetic acid, 2 parts trichloroacetic acid (0.5% NaCl of the original method omitted because of seawater substitution).

von Orth (1896)[1], 60 parts seawater, 30 parts 7.5% potassium dichromate with 3% Na_2SO_4, 10 parts 40% formaldehyde.

Flemming (1910)[1], 55 parts seawater, 10 parts 2% OsO_4, 37.5 parts 2% CrO_3, 5 parts acetic acid.

Lo Bianco (1890)[2], seawater 250 ml, 1.75 g $HgCl_2$, 25 g $CuSO_4$ hydrated.

Williams and Jackson (1956)[3], 90 ml seawater, 0.5 g cetyl pyridinium chloride, 10 ml 40% formaldehyde.

Lendrum et al. (1962)[4], 90 ml distilled water, 3 g $HgCl_2$, 10 ml 40% formaldehyde. (Seawater caused precipitation of mercury salts.)

References:

1. Grey (1973), table on pages 168 - 180.
2. Grey (1973), page 166.
3. Pearse (1968), Appendix 5, page 604.
4. Lendrum et al. (1962).

Of the fixatives tried, Bouin, Zenker, Heidenhains' Susa and Flemming could not be used as routine fixatives for graft analysis as they contained acid components that caused premature decalcification. The necessity for rapid fixation precluded the use of Lo Bianco, which also gave poor nuclear fixation. The fixative of Williams and Jackson caused considerable delamination of the epi- and gastroderm along the mesogloea. Although initially developed specifically for the fixation of muco-substances this fixative gave poor fixation in the mucus rich ectoderm. Of the other fixatives, Baker (with and without seawater), Lendrum and von Orth gave fixation qualities that appeared similar and satisfactory. The mercuric chloride component

of Lendrum did occasionally give a fine precipitate with seawater.

For convenience of preparation in the field and overall good fixation properties, the Baker's solution made up with seawater was used as the fixative for routine analysis of grafts.

(e) Grafting technique

Simple techniques can be used in branching Acropora species to provide viable grafts, without the use of narcotics or complex operative procedures. A 2 - 5 cm terminal piece of Acropora was broken off a donor colony branch with a pair of side cutting pliers to provide the grafting portion. This piece was tied parallel to a recipient branch with a single loop of 0.2 mm monofilament nylon. This brought donor and recipient into intimate contact with multiple points of potential interaction.

FIGURE 14

FIXATION SERIES

Magnification x 330. Stained in Haematoxylin-Eosin

a	Bouin (1897)
b	Baker (1944a)
c	Lendrum *et al*. (1962)
d	Baker (1944b)
e	Zenker (1894)
f	Heidenhains' Susa (1916e)
g	von Orth (1896)
h	Lo Bianco (1890)
i	Williams and Jackson (1956)
j	Flemming (1910)


a
f
b
g
c
h
d
i
e
j

Prospective grafting material was collected in the field by removing suitable branches, 10 - 20 cm in length, and placing them in plastic coated test tube racks. Great care was taken to ensure that the branches taken from separate colonies were placed in separate racks. Colonies from which branches were removed were identified at the time of collection by tying plastic tags to a basal branch with nylon filament line. In this way it was possible to repeat a series of grafts derived from the same colonies. The plastic racks and their branches were transferred to aquarium holding tanks and kept for 1 - 3 days before grafting. Recipient branches were left as collected, but donor branches were trimmed to 2 - 5 cm in length. This facilitated observation of the grafting processes and allowed a more uniform grafting procedure to be followed.

Grafts were usually inspected on the first, second, third and seventh days after grafting, and then twice weekly until the completion of the experiment. Experiments were conducted over periods of one week to six months. An Olympus stereo-zoom dissecting microscope was used to examine the grafts. The scoring of grafts was based on the following observational criteria:

(1) At the contact zone, the tissue fusion was apparently complete, perhaps with the formation of localised temporary 'scar' tissue.

(2) No fusion at the contact zone, but the two graft components were in close contact, sometimes with a paling (loss of zooxanthellae) along the junction line.

(3) The contact zone was necrotic or hypoplastic in the first few days and later showed regression of soft tissue from the skeleton, of either or both graft components.

With long term grafts, i.e. longer than six weeks, subsequent over-growth across the uncovered skeleton may occur.

(4) A sensitive test of functional graft survival could be made at an early stage by gently probing the polyp tissues with a fine needle, causing a wave of tentacular withdrawal (Horridge, 1957) to be transmitted over an area of 3 - 5 cm in diameter. A non functional graft, i.e. one in which tissues have not fused, will not conduct the wave of retraction, and no response is transmitted across the graft interface. In this way a functional graft may be identified as little as one day after grafting; an observation that could not normally be made until the seventh day using graft morphology alone.

The first three scoring categories separate the three types of graft that may be performed, i.e.

(1) Isograft: an intracolonial graft, derived from samples of the same original colony, hence of identical genetic constitution.

(2) Allograft: an intercolonial graft, derived from samples taken from different colonies of the same species, hence of different genetic constitution.

(3) Xenograft: an intercolonial graft, derived from samples taken from colonies of different species, hence of widely different genetic constitution.

The fourth scoring category will only give a positive response when applied to an isograft.

(f) Double paraffin embedding with decalcification for the routine examination of coral grafts

The Cnidaria have a low degree of cellular differentiation, with only simple tissue systems in which the function of the individual cell is often multiple (Chapman, 1974). Histological investigations must therefore be conducted at the cellular level. With corals, a further complication arises in that there is an extensive skeleton of almost pure calcium carbonate in the form of aragonite (Chave, 1954; Clarke & Wheeler, 1922). This, in association with the thin sheets of coral tissue, required special preparation before histological sections could be prepared.

Resin embedded ground sections (q.v.) proved invaluable for the interpretation of soft and hard tissue relationships but were both too long and imprecise (in the sense that the graft site was hard to locate) for the routine analysis of graft sites. A method had to be devised whereby thin serial sections (4 - 7 μm) of the graft tissue zone could be taken without the disruption of the cell structure or distortion of the graft tissue during decalcification and subsequent processing. Serial sections were necessary as the coral grafts were often restricted to a small area of contact.

When fixed material was decalcified by immersion in acidic solutions, such as Gooding & Stewarts' formic acid - formalin solution (Culling, 1963), gas bubble formation disrupted the still tenuous grafts. To overcome this decalcification with chelating agents (e.g. EDTA, Lillie, 1965) was tried, but proved to be too lengthy a process to be of value, taking 3 - 7 weeks in some cases. Both acid and chelation decalcification left the tissue sheets

unsupported and the grafts easily separated. The surface decalcification method of Culling & Barkoczy (1972) for mammalian bone was not successful on coral as the proportion of skeleton to tissue was too high, and insufficient support with too few tissue connections always caused the section to collapse on cutting. To overcome these difficulties the following method was developed, which proved successful for the routine investigation of coral grafts.

The entire graft specimen (i.e. a 10 cm branch with a 4 cm graft tied alongside it) was fixed in 10% formalin seawater, dehydrated infiltrated and embedded in a large block of paraffin wax. Slices 3 - 5 mm thick were then sawn from the block, and the coral skeleton dissolved in Gooding and Stewarts' fluid (Culling, 1963). The infiltrated soft tissue remained protected by the wax from the action of the decalcifying fluid, gas bubble evolution and distortion, as the skeleton was removed.

When decalcification was complete, the slice containing the wax impregnated tissues (as free standing tissue sheets) was washed in distilled water, dehydrated in an alcohol series, and allowed to dry in a dessicator at room temperature. The tissue slice was then cast into a block of the desired size for cutting by slowly re-melting at 60^{o}C with additional wax. This did not disrupt the delicate graft and permitted the cavity left by the skeleton to be completely infiltrated.

Procedure:

(i) Fixation. For routine analysis of coral grafts 10% neutral formalin - seawater was used (Baker, 1944).

After fixation, specific graft areas to be sectioned were marked with cotton loops.

(ii) Dehydration I. Whole fixed specimens were treated as follows:

two changes 70% ethanol, 3 hours each,

one change 80% ethanol, 3 hours,

two changes 96% ethanol, 3 hours and overnight,

two changes of absolute ethanol, 2 hours each, and

two changes of xylol, 2 hours each.

(iii) Embedding I. After clearing in xylol the whole specimens were infiltrated with two changes of paraffin wax (MP 58°C) at 60°C, for four hours each. Using L-pieces, each specimen was then cast into a block of clean wax, with at least 5 mm of wax surrounding the specimen.

(iv) Decalcification. The block was held in a small sawing box and a fine bone saw was used to remove a slice of tissue 3 - 5 mm thick containing the graft. The wax held the graft zone firmly, but only slow strokes of the saw were used to avoid frictional melting of the wax with consequent displacement of tissues. The slices were decalcified in Gooding & Stewart's fluid (Culling, 1963), i.e.

Formic acid (SG 1.21)	100 ml
Formalin (40% formaldehyde)	50 ml
Distilled water	850 ml

Each specimen slice was treated with three changes of this solution over a 24 - 36 hour period. Complete decalcification was determined by a modification of Arnims test (Brain, 1966):

Reagents: Concentrated ammonia (SG 880),

Saturated ammonium oxalate solution.

2 ml of decalcifying fluid was placed in a test tube, to which a small piece of red litmus paper was added. Concentrated ammonia was added from a dropper until the paper just turned blue. Five drops of saturated ammonium oxalate solution were then added and the tube left for ten minutes. If calcium was present in the fluid then a white precipitate of calcium oxalate developed. The test was found to be sensitive to 50 ppm of calcium in solution.

The test was applied 24 hours after initiating decalcification, just prior to the third change of fluid. If calcium was detected, the procedure was continued for a further 12 hours or until decalcification was complete. Placing the material under partial vacuum two or three times towards the end of decalcification for periods not exceeding 30 minutes aided the final stages of calcium carbonate removal, although Brain (1966) considered this unnecessary. However, in this technique the fine tubular cavities left by the removal of the skeleton may require the extraction of carbon dioxide (generated during the reaction) to allow for complete penetration of the decalcifying fluid. The decalcified slices were washed well in distilled water prior to dehydration.

(v) Dehydration II. The washed, decalcified slices were dehydrated as follows:

one change of 70% ethanol, for four hours,

one change of 96% ethanol, for three hours,

two changes of absolute ethanol, for two hours each.

Absolute ethanol was allowed to evaporate from the specimen by placing the slice in a dessicator overnight.

(vi) Embedding II. The slice was orientated and placed in a mould of the required size. Hot wax was then poured over the specimen, and the mould placed in an oven at 60°C. When the wax was homogeneously melted the specimen was checked for air bubbles and orientation. Air bubbles were removed by gently probing the tissue with a fine needle. At this stage the tissue sheets and the graft are unsupported and require careful manipulation to avoid damage. The block was then allowed to cool, the structure of the wax being improved by rapid chilling in cold water.

(g) Resin Embedding for Ground Coral Sections

To maintain the true relationship between the hard and soft tissues of coral, a technique of resin embedding similar to that used by Barnes (1972) was investigated.

Specimens fixed in 10% neutral formalin-seawater were dehydrated through a series of alcohols, followed by dry acetone. The material was infiltrated with a series of Araldite-acetone mixtures of increasing resin content. Curing of the resin during the long infiltration stage was prevented by refrigeration to 4°C. Throughout the embedding procedure the samples were gently agitated on a rotor at 5 revolutions per minute. Specimens were finally embedded in a small volume of pure resin and cured at 60°C for three to four days.

Procedure:

Specimens fixed in 10% formalin-seawater

2 changes 70% ethanol for 3 hours each,

1 change 80% " 3 hours,

2 changes 96% " 3 hours and overnight,

2 changes absolute ethanol for 2 hours each,

2 changes Acetone for 2 hours each.

Freshly prepared Araldite resin (CIBA-GEIGY), in the following proportions:

Araldite No. 502	27 ml
Hardner, DDSA	23 ml
Accelerator, DMP-30	1 ml

25% Araldite resin - 75% acetone for two days, at 4°C,

50% Araldite resin - 50% acetone for two days, at 4°C,

75% " 25% " " "

pure resin, 2 changes, 2 days each, 4°C.

Embedded in pure resin and cured at 60°C for three to four days.

The block was trimmed using a diamond impregnated lapidary saw. The exposed face was ground on a series of wetted carborundum papers (150, 300 and 600 grade) fixed to a sheet of plate glass to obtain a flat ground surface. A glass slide with one ground face was then fixed to the face of the block with Araldite resin, and a thin slice 1 - 2 mm thick was sawn from the block. The cut surface of the slice mounted on a slide was then ground on a series of carborundum papers as before, resulting in a section 5 - 10 μm thick. In the final stages of grinding, the specimen was frequently examined to avoid grinding the section away completely.

Sections were stained in 1% Toluidine blue in 1% Borax for one minute. A coverslip was mounted on the prepared section using Araldite resin as xylene based mountants were occasionally found to cause degradation of the resin.

Initially the sections were ground as petrological sections are on a lapidary wheel using carborundum powders. This technique caused abrasive grains to become embedded in the resin. This is of no consequence in petrological sections, where there is no infiltration of the specimen. In coral sections, however, infiltration of the soft tissue is essential and the numerous black grains of carborundum obscured much histological detail.

Hand grinding took much longer to complete but little or no carborundum particles remained to obscure the histological detail.

(h) Staining Procedures

Routine:

For the routine study of histological sections, Ehrlich's Alum Haematoxylin - Eosin (Culling, 1974, p. 212) and Picro-Gomori (Gurr, 1962, p. 375) were used. The picro-Gomori trichrome gave very pleasing results, and was preferred to the haematoxylin. No unusual modifications to the published methods of either technique was required. All sections were fixed in 10% formalin-seawater, and embedded in paraffin wax as they also were for the histochemical mucosubstance investigation.

Histochemistry of Mucosubstances in Coral Tissues - Procedures:

Periodic Acid-Schiff technique (after McManus)

The method reported in Pearse (1968, Appendix 10) was followed. No counterstain was used. Hexose containing mucosubstances (1 - 2 glycol group) stain various shades of purplish-red. Glycogen stains deeply.

Azure A

The method followed was that of Spicer et al. (1967). The pH range was made as follows:

pH 1.0 — 1:5000 Azure A in N/10 HCl,

pH 2.5 — 48 ml 1:5000 Azure A in distilled water,
2 ml M/10 citric acid,

pH 4 — 48 ml 1:5000 Azure A in distilled water, 1.25 ml M/10 citric acid and 0.75 ml M/5 Na_2HPO_4.

Generally, acid mucins stain blue. Sulphated mucosubstances stain metachromatically at pH 2.0 or below. Some sulphated muco-saccharides stain with a masked azureophillia at pH 3.5 - 4.5. Many sialomucins stain metachromatically at pH 3.0 and above, while hyaluronic acid is metachromatic at pH 4.0 and above.

Alcian Blue (after Steedman, 1950)

The method reported in Pearse (1968, Appendix 10) was followed. No counterstain was applied. The pH range was made as follows:

pH 1.0 1% Alcian Blue 8GX in 0.1 N HCl,

pH 2.5 1% Alcian Blue 8GX in 3% Acetic acid.

At pH 1.0 sulphated mucosubstances stain selectively, with the most strongly acidic chromotropes staining blue the least strongly. The pH 2.5 Alcian blue stains weakly acidic sulphated mucosubstances, hyaluronic acids and sialomucins dark blue. Strongly acidic mucins show little or no staining at pH 2.5.

Alcian Blue - PAS (after Mowry, 1963)

A modification of the method in Pearse (op. cit.) was used.

1. Stain in Alcian Blue (either at pH 1.0 or 2.5), for 30 minutes.
2. Wash for 5 minutes after pH 2.5 or blot dry after pH 1.0.
3. Oxidise in 1% aq. Periodic acid.
4. Wash for 5 minutes.
5. Stain in Schiff reagent (de Thomasi reagent, in Culling (1974), p. 216) for 10 minutes.

6. Rinse in water for 3 minutes.

7. Dehydrate, clear and mount.

This procedure stains periodate-unreactive, Alcianophyllic muco-substances blue; periodate-reactive and Alcianophyllic components are bluish-purple and periodate-reactive, non-Alcianophyllic components are red. Acid mucosubstances staining blue at pH 2.5 will be weakly sulphated, non-sulphated sialomucins or non-sulphated hyaluronic acids. At pH 1.0 only the sulphated acid mucins will stain.

(i) Xerography and X-radiography of Coral Slices

To investigate the history of massive corals, e.g. Goniastrea aspera, the corallum is cleaned in bleach solution (see section (b) of this chapter) and sliced using a diamond impregnated rock saw. The past growth patterns can be seen in the cut cross-section. It is possible to obtain a rapid and good paper copy of such sections by placing them directly onto a Xerographic copying machine. Such copies often show a superior definition of skeletal structure when compared with photographic reproductions. This appears to be due to a property of the thin plane of light that is used to sweep the original in xerography. This limits the backscattering and diffusion of light by the skeleton and so produces a sharper image. This technique of illumination could well be emulated in standard photographic reproduction to enhance skeletal details. Figures 43 and 48 are examples of direct xerographic reproductions of sliced corals.

If slices 1 - 2 cm thick are removed from a coral head, then they may be X-rayed onto mammography film to show growth banding. Figures 44 and 46b were obtained from such coral slices using the technique described by Isdale (1977). A comparison between the photographic and X-radiographic techniques can be seen in Figures 46b and 46c respectively. The X-rayed section was taken from the surface of the cut face of Figure 46c, prior to photography and is therefore not strictly homologous with the illustrations in that figure. X-radiographic and Xerographic techniques may be compared in Figures 44 and 43 respectively.

CHAPTER IV HISTOLOGY AND MORPHOLOGY OF THE ACROPORIDAE

(a) Introduction: A review of the morphology, anatomy and histology of the Acroporidae

(b) Results: Further studies on the histomorphology of *Acropora*

(c) Discussion: A discussion on coral histomorphology

(a) Introduction: A Review of the Morphology, Anatomy and Histology of the Acroporidae

Early descriptions of the Scleractinia centered on the taxonomically important morphology of the skeleton, and have been extensively summarised by Vaughan and Wells (1943). Of the few accounts of the anatomy and histology in the early literature, the works of Brook (1893), Ogilvie (1896), Fowler (1887), Duerden (1902), Matthai (1923) and Hickson (1924) were specifically consulted in the following account. Goreau (1956) provided a detailed account of the biology and histochemistry of corals which together with the following general accounts of Coelenterates by Hyman (1943), Moore (1956), Lenhoff and Loomis (1961), Bouillon (1968) and Muscatine and Lenhoff (1974), greatly facilitated the present investigations.

The Acroporidae form a large, structurally and morphologically diverse family of corals. Grafting studies were generally confined to species within the arborescent Eumadreporarian group and the following description (see Figures 15 to 20) refers specifically to these forms.

The following definitions have been applied in this work:

Coenosarc- extrathecal tissue layers connecting polyps

Coenosteum- extrathecal skeleton interconnecting corallites

Coenenchyme- collective term for both coenosarc and coenosteum.

As the mesentaries of the interseptal loculi are not found in the intercostal loculi, a true 'edge zone' as defined by Ogilvie

(1896) does not exist. Duerden (1902) remarked that among all the forms of coral with a perithecal continuation of the gastric cavity that he has studied, the Madrepora (Acropora) is the only genus in which the mesentaries are not prolonged perithecally. Ogilvie (1896) suggests and Duerden (1902) concurs that the term coenosarc should apply to the tissue that forms an extrathecal part into which the mesentaries do not extend. The corresponding calcareous structure deposited from calicoblastic coenosarc being referred to as coenenchyme.

Hickson (1924) refers to the use of the term coenenchym (= coenenchyme) for corals as etymologically and historically incorrect as the word was introduced by Milne Edwards and Haime (1857) for the fleshy substance between the polyps in Alcyonaria. He recommends the use of the term coenosteum for the skeletal elements deposited beneath coenosarc. Moore et al. (1956) define edge-zone in the same sense as Ogilvie, but reserve the term coenenchyme for a general term applied to both coenosteum and coenosarc.

The corallites are synapticulothecate with a very perforated wall, especially in the rapidly growing apical regions. Most corallites have ridged walls but as there is no strict homology between intra- and extramural structures (septa and costae respectively) the ridges must be regarded as pseudo-costae. In older and more basally situated corallites, or in slower growing areas, where secondary thickening with stereome is evident, the wall becomes spinose and little differentiated from the coenosteum, which is also heavier and thicker in this region (Figure 15b). The more apical coenosteum is fragile and reticulate, delicately spinose and sometimes striate on the surface. The perforate skeletal wall

(Figure 15a) allows the interior and exterior coenosarc and the intramural gastrovascular cavity of the polyp to be interconnected via a network of canaliculae. The perforate character of the skeleton belies its true origin in that it is always secreted by the calicoblastic epidermis and is therefore wholly exoskeletal, only becoming deep seated and 'internal' by subsequent changes in its relationship to the soft tissues.

Ricart y Menendez and Friedman (1977) considered the terminal corallite walls of Acropora cervicornis to be septothecate, and the extramural structures to be costae rather than pseudocostae. Until further work can be performed on the nature of the corallite in A. formosa the original view of Ogilvie (1896) and Vaughan and Wells (1943) will still be used, namely that the walls are synapticulothecate and the external ridges are pseudocostae.

Budding is extratentacular and usually occurs outside the corallite margin in the coenosteum. Buds are initially formed by trabeculae arching over to form a hood. Costae are subsequently formed on the surface. Following the development of twelve protocnemes, the septa are added internally.

Of these, two are always in the space between the directive mesenteries and are thus termed the directive septa. The other primary septa are inserted in alternate intermesenteric spaces, thus the first cycle of six septa are entocoelous. A further cycle of six septa may be added in the six remaining intermesenteric spaces, forming an exocoelous cycle. The costae and septa are usually imperforate, and structurally composed of simple trabeculae inclined in a series from the synapticulothecate wall and terminating in fine granules.

The outer wall of the corallite may show greater development than the inner surface (see Figure 15a), especially if the radial corallites are ascending and adpressed so as to become dimidiate.

There is a basic plan of two septal cycles in the Acroporidae, but a third cycle, giving 24 septa in total, has been reported by Brook (1893). The axial corallites show greater development, both in size and the number of septa. Most species have six large primary and six smaller secondary septa. Radial corallite septa are often variable in size and number, even within the same species. Usually only six primary septa are present. These are often incomplete, reduced to spines or absent altogether. The directive septa of the radial corallites are frequently the largest, and may be unequally developed, in which case the outer (abaxial) directive invariably shows the greater development. There is no true columella development in Acroporidae, but the primary septa of the axial corallites, and the directive septa of the radial corallites may in some species become confluent towards the base and form a pseudo-columella.

In the following account, mainly on the structure of the soft tissues, an attempt has been made to describe acroporan histo-morphology only to a level required to interpret tissue interactions. The only specific study on Acropora soft tissues has been that of Fowler in 1887. He worked with poorly preserved specimens and some of his interpretations, e.g. the dimorphic polyps of Madrepora Durvillei, might have been fixation artifacts. A more extensive histological investigation of the Acroporidae is long overdue.

(b) Results: Further Studies on the Histo-morphology of Acropora

The tissue layer covering the visible external surface of the coral is composed of three histologically distinct layers, the ectoderm, gastroderm and mesoglea. In many corals and in the Acropora, in particular, the column wall of the polyp and the coenosarc of the intercorallite region are morphologically identical, and have a positional significance only. The tissue layers rest on the calcium carbonate skeleton deposited beneath the calicoblastic body wall. The free body wall is supported by pseudocostae or echinulations (Figures 16, 17, 18). The superficial coelenteron is formed into simple canals between the pseudocostae, and into an anastomising network of tubules around the echinulations. The superficial coelenteron is also in communication with the deeper coelenteron via numerous tissue lined skeletal perforations (Figure 16a). In older specimens, the continued deposition of skeleton occludes the inner cavities, reducing the living coral to a series of fine tubules, each with a reduced coelenteron (Figure 16b). The thickness of the free body wall tissues is somewhat variable, but for the commonly grafted Acropora formosa, the following dimensions were obtained:

Ectoderm	20 μm
Mesogloea	less than 0.5 μm, and
Gastroderm	9 μm

The apparent thickness is also dependant on the angle of sectioning and the state of relaxation of the specimen.

Where calicoblastic body wall and free body wall come into proximity over the extremities of the echinulations and pseudocostae

the coelenteron and gastrodermal layers are excluded, while the mesogloea of each fuse (Figures 16c, 17, 18). Often, and usually as a result of violent stimulation, the natural contraction of living tissues over a skeletal spine causes disruption of the free body wall ectoderm, a thinning or breaking of the mesogloea, and a compression of the gastroderm (Figures 17 and 18). In extreme cases, the calicoblastic ectoderm is also pulled from the spine. Fixation and decalcification processes can also disrupt tissue over a spine.

Over the pseudocostae and the echinulations of the intercorallite areas, the gastroderm is thinner, the reduced number of zooxanthellae impart a lighter colouration to the bodywall of the living coral. Thus, where the skeleton forms ridges, a linear colour pattern is produced and where echinulations are random, a stippled pattern results (Figure 28c).

The polyp in Acropora when extended has a typical Anthozoan structure. The mouth is elongated in the saggital plane, with the invaginated stomodeum and oral disc supported with up to twelve mesenteries. The directive mesenteries, located in the saggital plane, and one mesentery (the 'transverse mesentery') on either side of them are more important and penetrate to a lower level than the others (Figure 20). According to Fowler (1887), the transverse mesenteries are the longest and are the only ones to bear gonads. However, in many sections more than two mesenteries have been observed with gonads, and on one occasion six mesenteries were involved in gonad production. The mesentery retractor muscles are located on the outer surfaces of the directives and inner surfaces of the other pairs, in the normal Zoantharian fashion. The free end of each mesentery bears a mesenterial filament, which in the more

basal region is free to form an acontial filament. Extrusion of the acontia in Acropora is not common. Permanent cinclides that are occasionally found in the body wall of certain Actiniaria were not found in Acropora. However, acontial filaments are extruded through the body wall via temporary perforations. Such filament extrusion has been observed on occasions when there is excess food on the body surface. In Figure 21 acontial filament extrusion extended up to 5 cm, possibly aided by small air bubbles trapped from the well aerated water and an excess of mucus. Numerous filaments were also extruded through the stomodeum. This particular response was caused by over-feeding on Artemia nauplii. Filaments have also been observed to extend short distances (0.5 - 1.0 cm) in response to local mechanical disturbance of the body wall. In a location close to the damaged area several small blisters were observed on the wall of the calyces. Within minutes these ruptured and single filaments were extruded, in an endless loop. The extension was accomplished by both extrusion and lengthening of the filament. If the coral is left undisturbed for 24 hours then the filaments are withdrawn and the temporary opening unobtrusively sealed. Lewis (1971) found mesenterial filament extrusion to occur in response to poor environmental conditions.

The number and form of the tentacles in the Acroporidae has been the subject of discussion in the past (Brook, 1893). In the Acropora species commonly used for grafting, the following condition was noted: Axial polyp, six tentacles of equal length; radial polyp, twelve tentacles, one of the twelve located over the abaxial directive sclerosepta, being considerably larger than the rest.

Damage to the overlaying coral tissues may expose skeleton. In Acropora the repair overgrowth of a damaged area has the distinctive morphology that makes it a homologue of the true free edge of the basal region. Namely that the growth over the substrate (or occasionally free space) is preceeded by a basal plate with an epitheca, behind and over which normal reticulate coenosteum is deposited (see Figures 22 and 23). Calices are not formed near the edge, their appearance probably being determined by the depth of the deposited coenenchyme and distance from the edge, both a function of the 'maturity' of the repaired area. The repaired area will eventually closely resemble the undamaged area that it overlies, thereby maintaining the overall colonial integrity. The regrowth over the exposed skeleton will require the formation of a local meristem so that the expanding calicoblast and free body wall can be generated.

Where the original skeleton is damaged or obscured by advantitious settlement of other organisms, then an entirely independant skeletal structure may be formed. A similar surface following growth was found by Duerden (1904) in the undamaged basal plate growth of newly settled Siderastrea radians Pallas. Little or no descriptive information has been published on the basal region once the coral has settled and passed through a juvenile stage. In branching forms the 'holdfast' area continues to grow in a controlled way throughout the life of the colony resulting in an extensive pad of calcium carbonate. The holdfast is composed of an coenenchyme with the occasional small polyp. A similar feature is found on the undersurfaces of horizontally growing branches and is especially evident on table corals, e.g. A. corymbosa (Lamarck, 1816), where the holdfast area grades into, and cannot be distinguished from, the

undersurface. At the periphery of the basal region of an Acropora colony the epitheca grows ahead of the skeleton in a similar way to the "unattached basal plate" described by Barnes (1972) for Montastrea annularis (Ellis and Solander, 1786).

A basal region morphology, acting as an holdfast area, may be formed at any part of the colony, in response to contact with a substratum. The early stages in the formation of a holdfast are, of course, the same as the initial growth of the skeleton after settlement of a planula larva (see Duerden, 1904; Boschma, 1929 and Stephenson, 1931). Initially a basal plate is deposited on the substratum, followed by a peripheral development of epitheca. A rapid proliferation of coenenchyme develops and a distinct pad is formed (Figure 23b). Characteristically, polyps are either absent or poorly developed in holdfast regions.

In the case of a newly settled coral, when the pad achieves a specific size, the proliferation of polyps from the growth centre, with a growth polarity into free space, becomes evident (Figure 23c). The type and frequency of branching eventually shows the specific morphology of that coral. The holdfast area does not expand indefinitely over the substratum, and has a distinct allometry with the size of the colony it supports and the environment in which the coral grew. The relationship between holdfast size, colony size and environment would be worthy of further investigation.

(c) Discussion: A Discussion on Coral Histo-morphology

From this study it is concluded that if damage or a substrate is encountered by the coral surface, that a local meristem and an epithecal structure will form, and either tissue repair or a holdfast will develop. These two processes appear to be identical; it is only the final outcome that is shaped by the integrative control of the coral colony that is different, i.e. a repaired surface resembling the original one, or a holdfast.

In the relationship between the skeleton and soft tissues, Barnes (1972) does not consider the lappet cavity tissues of the epitheca as being meristematic, as he observed no dividing cells in this region in the many sections that he inspected (Barnes, pers. comm.). He invokes a complex migration of the free body wall epiderm over the epitheca to form a calicoblastic layer. During this migration, all the specialized epidermal cells would be excluded, in a way he does not explain. From his illustrations it appears that about 4 cells per section are required to be produced to cover the daily extension of the epitheca. The dense body of cells that appear at the apex of the lappet cavity contain about 50 cells per section. It is therefore not surprising that dividing cells are not seen, as only 8% per day need to divide, and may do so synchronously. The lappet apex cells have properties that are to be expected of meristems, i.e. they are a densely packed undifferentiated body of cells, with differentiated cells on either side. A similar cytomorphology is exhibited in the gastroderm. It is therefore considered highly probable that this region is a meristematic zone. It is interesting to note that in the many sections of coral tissue that Barnes has studied, he has only observed one dividing cell

(not in the lappet region) (Barnes, pers. comm.) and none have been positively identified in many hundreds of sections in the present study. No doubt the small size of coral cells contributes to these negative findings.

Vandermeulen (1975) offers corroborative information from his description of the changes in epiderm morphology when Pocillopora damicornis (Linnaeus, 1758) planulae settle. He states that the undifferentiated columnar epithelium has an exposed area of 2 - 3 μm^2 which compares with about 25 μm^2 of the typical calicoblast cells exposed area. Thus, only 8% - 12% of the columnar cells would require to be transformed to form an equal area of calicoblast. These figures are in general agreement with those derived from Barnes' (1972) work.

The disruption of tissues over spines and echinulations has led some authors, notably Duerden (1902) to state that . . . "the ectoderm overlying the canals passes inwardly as the calicoblastic layer.". It seems likely that rough pre-operative treatment and slow fixation caused the tissue artifacts that have been interpreted as invagination of the epiderm. Araldite embedded ground sections of carefully prepared coral tissues with the skeleton in situ have shown that numerous internal spines and irregular shapes are deposited well away from the free body wall. All require an increase in the surface area of the calicoblastic layer to accommodate their growth. This calicoblast can only be derived from local intercalary growth or perhaps temporary meristematic areas. It is difficult to assess the in vivo juxtaposition of calicoblast and skeleton but it would appear that rapidly extending regions (identified by their thinner processes) have a closer calicoblast-skeleton relationship than elsewhere (Figures 16c and 18). It is reasonable to assume that the

genetic control of the shape of the coral skeleton would be exerted by these growth centres, switched on and off by the general colony co-ordination processes. Once a series of leading spines and shapes have been so constructed, general deposition of skeleton below the whole calicoblastic layer would increase the thickness of the templates. This will increase the structural strength properties concomittantly with the requirement to support a greater extension of branch as the coral grows (Figure 16b). It is the genetic control of the local growth and meristematic centres that will hold the key to many calcification and taxonomic problems and deserves considerable attention in the future.

FIGURE 15a

Skeletal morphology of Acropora formosa.

Magnification x 14.

c = calice

cc = costate coenosteum

p = skeletal perforation

ac = axial corallite

r = radial corallite

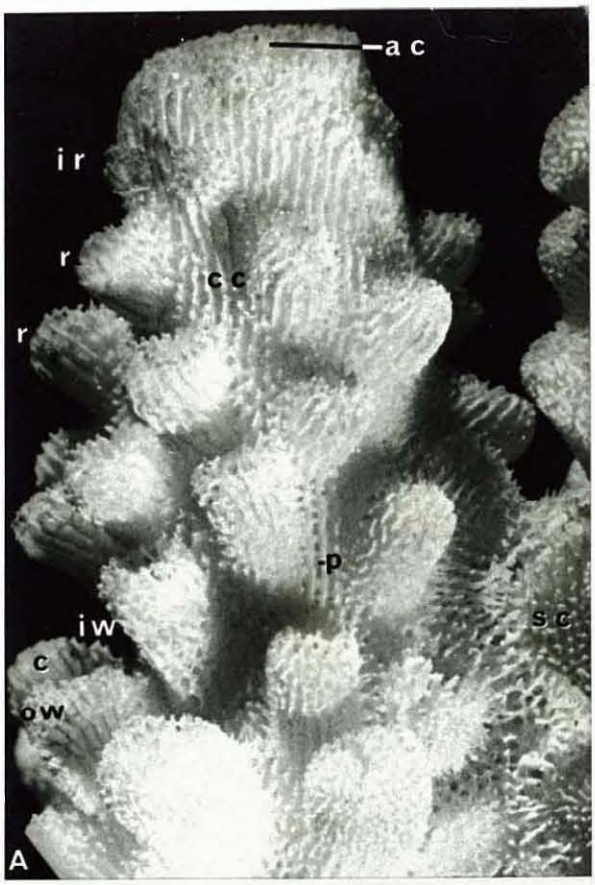
ow = outer or abaxial wall of corallite

iw = inner or axial wall of corallite

ir = incipient radial corallite.

FIGURE 15b

Acropora formosa skeleton preparation. Magnification x 18. The distinction between costate coenosteum (cc) and spinulose coenosteum (sc) is shown. These specimens are from the same colony, the costate piece coming from a rapidly growing upper surface, while the spinulose piece coming from a slower growing, shaded, branch undersurface.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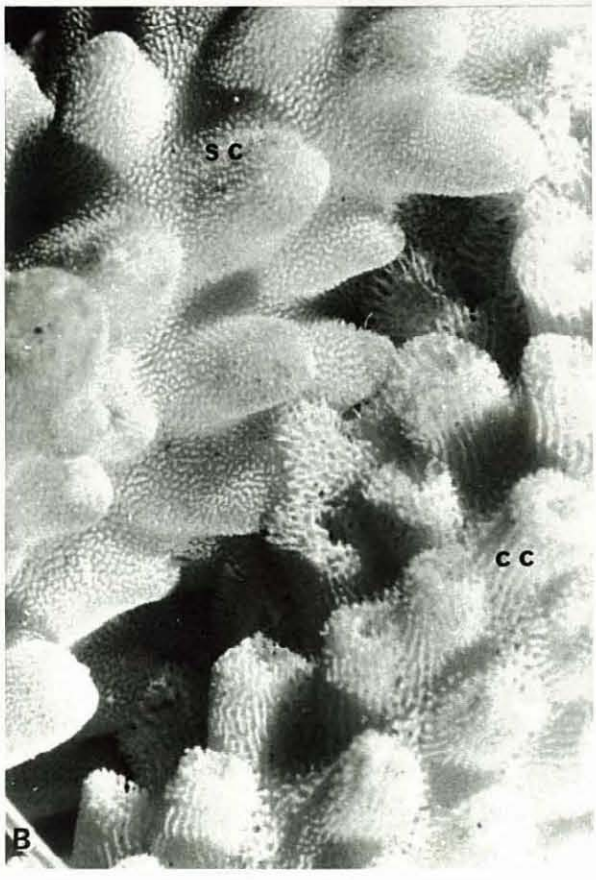
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c c
B

FIGURE 16

Resin embedded ground section of Acropora formosa.
Stained in Haematoxylin-Eosin.

(a) Magnification x 100.
The stomodaeum of a polyp and its corallite are shown.
The section was taken approximately 15 mm from the tip of a branch.

st = stomodeaum
p = skeletal perforation
t = contracted tentacle
ds = axial directive septum

Mesentaries are numbered according to pattern of Figure 20.

(b) The section was taken approximately 80 mm from the tip of a branch. Magnification x 40.
Note the heavy secondary thickening with stereome(s) and the centres of calcification (cc). It is interesting to note that the axial corallite (apc) is still invested with living tissue at this distance from the tip of the branch.

(c) Section shown is immediately to the left of (a) above, and shows the epidermal structure of the free body wall. Magnification x 143.

e = epiderm
m = mesogloea
g = gastroderm
sk = skeletal spine, probably part of a costal ridge in this terminal region of the branch.
cc = centre of calcification.

Figure 18 is a higher power montage of this region.

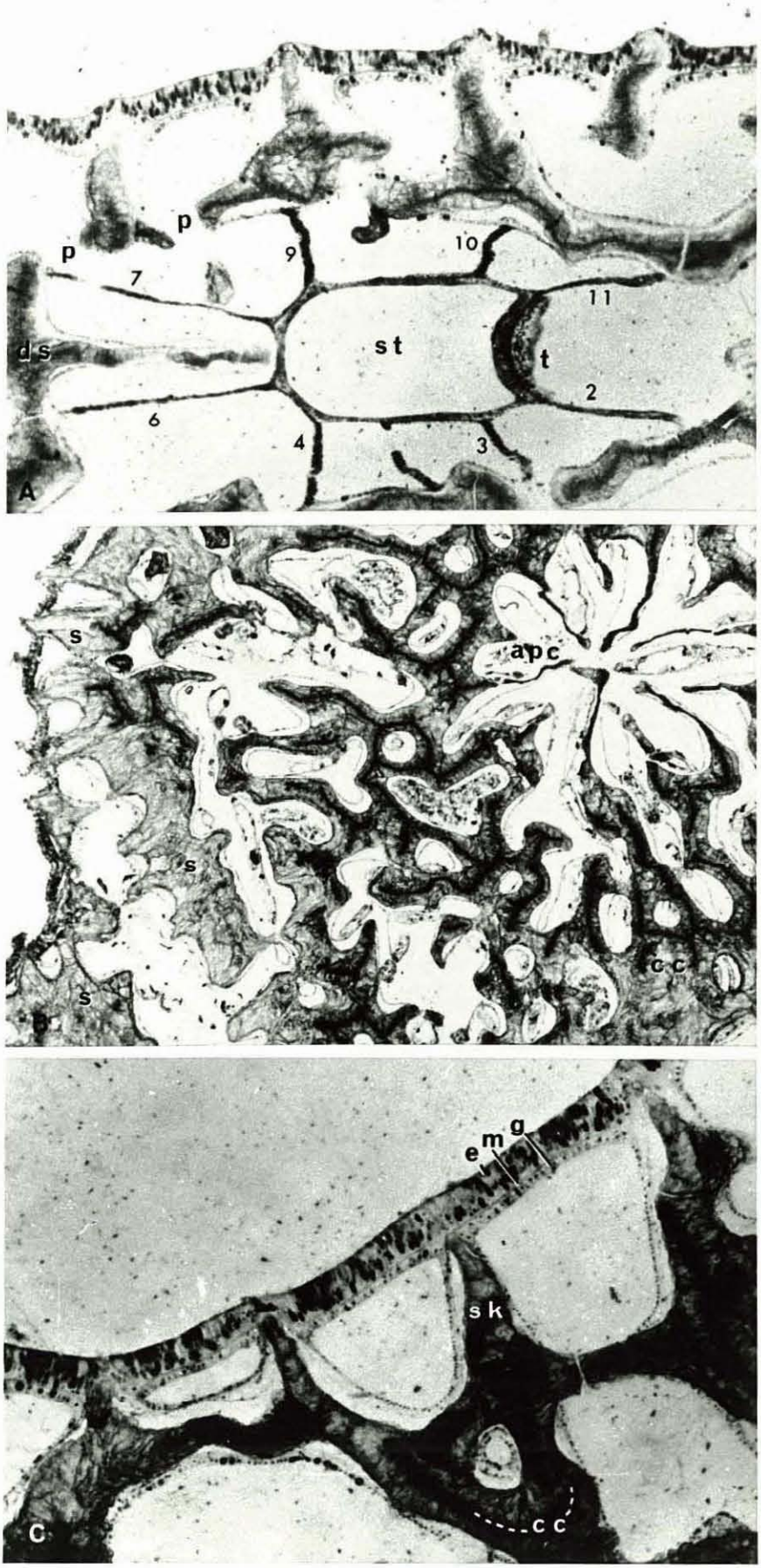

p
p
p
9
10
7
11
d s
s t
t
2
6
4
3
A
s
apc
s
s
c c
g
e
m
s k
c c
C

FIGURE 17

Diagram to illustrate the terminology and morphology of *Acropora formosa* coenenchyme.

ss = skeletal spine

z = zooxanthella

sp = spirocyst

mu = mucus gland cell

sk = skeleton

sc = superficial coelenteric canal or canalicula.

ce = calicoblastic epiderm
m = mesogloea
cg = calicoblastic gastroderm
} of calicoblastic layer (cb)

fg = free body wall gastroderm
m = mesogloea
fe = free body wall epiderm
} of free body wall (fbw)

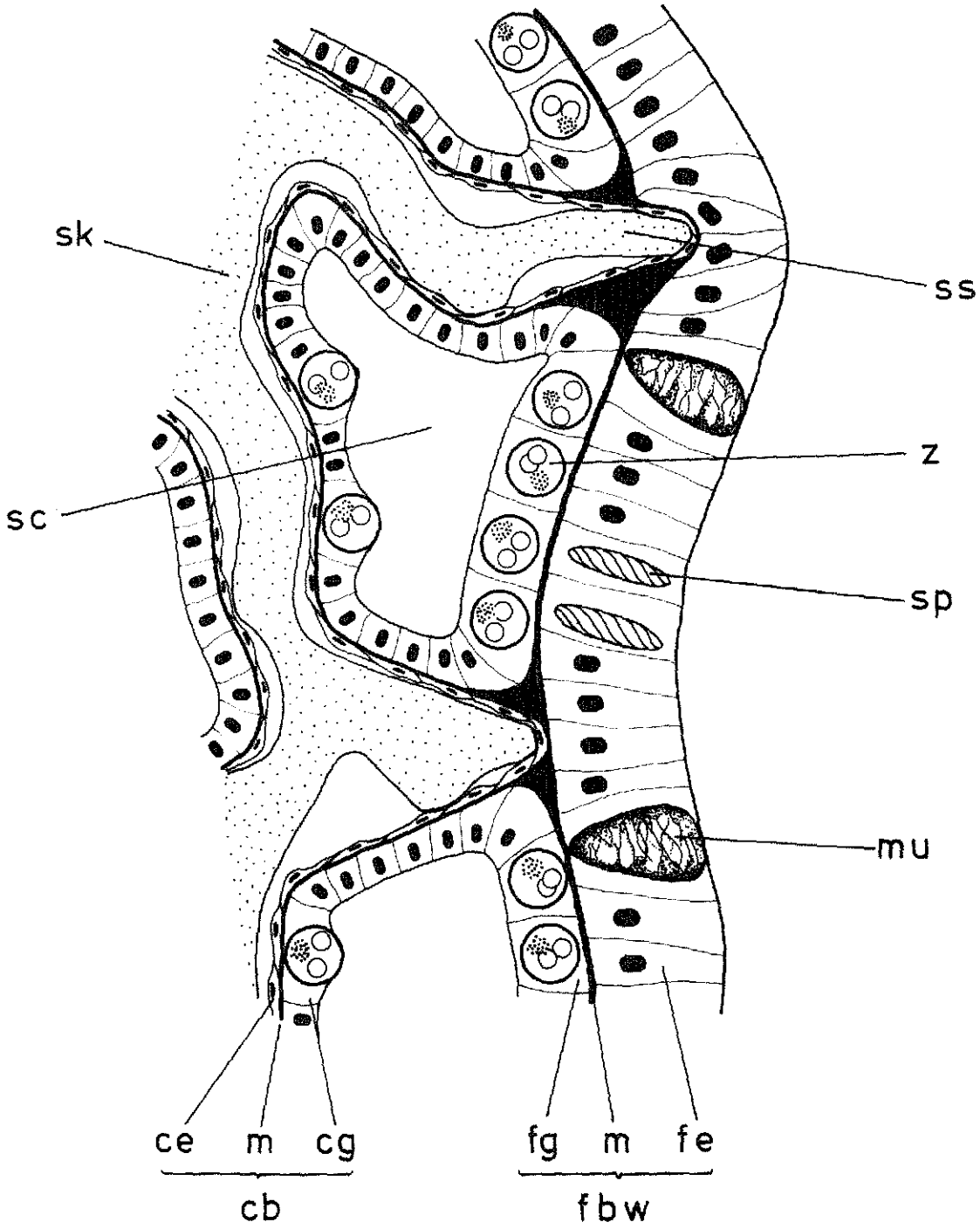

sk
ss
z
sc
sp
mu
ce
m
cg
fg
m
fe
cb
fbw

FIGURE 18

A photomicroscopic montage to illustrate the morphology of the coenenchyme of Acropora formosa.
Magnification x 233; stained in Haematoxylin-Eosin.

s = skeletal spine

sc = superficial canal of coelenteron

e = epiderm
m = mesogloea
g = gastroderm
} of coenosarc or free body wall

zcg = zooxanthella in calicoblastic gastroderm

ce = calicoblastic epiderm

Note the close application of the calicoblastic epiderm to the actively growing skeletal spines. The extreme stretching over the tips of the spines may be a fixation artifact.

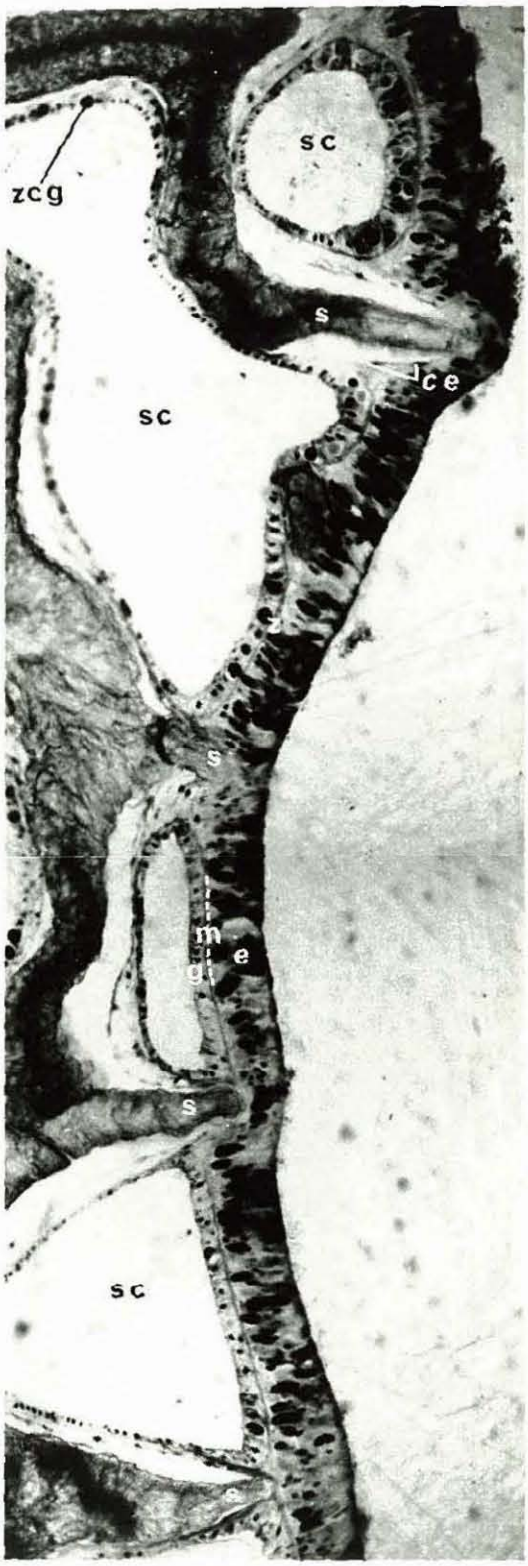

zcg
sc
s
lce
sc
s
s
mg
e
s
sc

FIGURE 19

Histology of the polyp of Acropora formosa.

(a) Section through the contracted oral disc and tentacular crown. Magnification x 92. Picro-Gomori stain.

xd = position occupied by axial directive septum
ad = position occupied by abaxial directive septum
t = contracted tentacle
1 to 12 = mesenteries, numbered as in Figure 20.

(b) Section through stomodaeum. Magnification x 92. Stained in Haematoxylin-Eosin.

k = cavities from which skeleton has been removed during decalcification.

Note the numerous interconnections of the coelenteron through and around the skeletal elements. This structure allows the rapid and free passage of any food material that passes down the stomodaeum (st) throughout the colony.

(c) Section through the lower stomodaeum region. Magnification x 240. Stained in Haematoxylin-Eosin.

This slightly oblique section passes through the lower region of the stomodaeum where the stomodael epiderm (se) continues along the outer margin of the mesenterial filament (me). The stomodael gastroderm forms the mesenterial gastroderm, which together with the epiderm form the cnidoglandular portion of the mesenterial filament. These two layers are separated by a 'Y' shaped mesogloeal layer. The former positions of the axial and abaxial directive septa are indicated (xd and ad respectively).

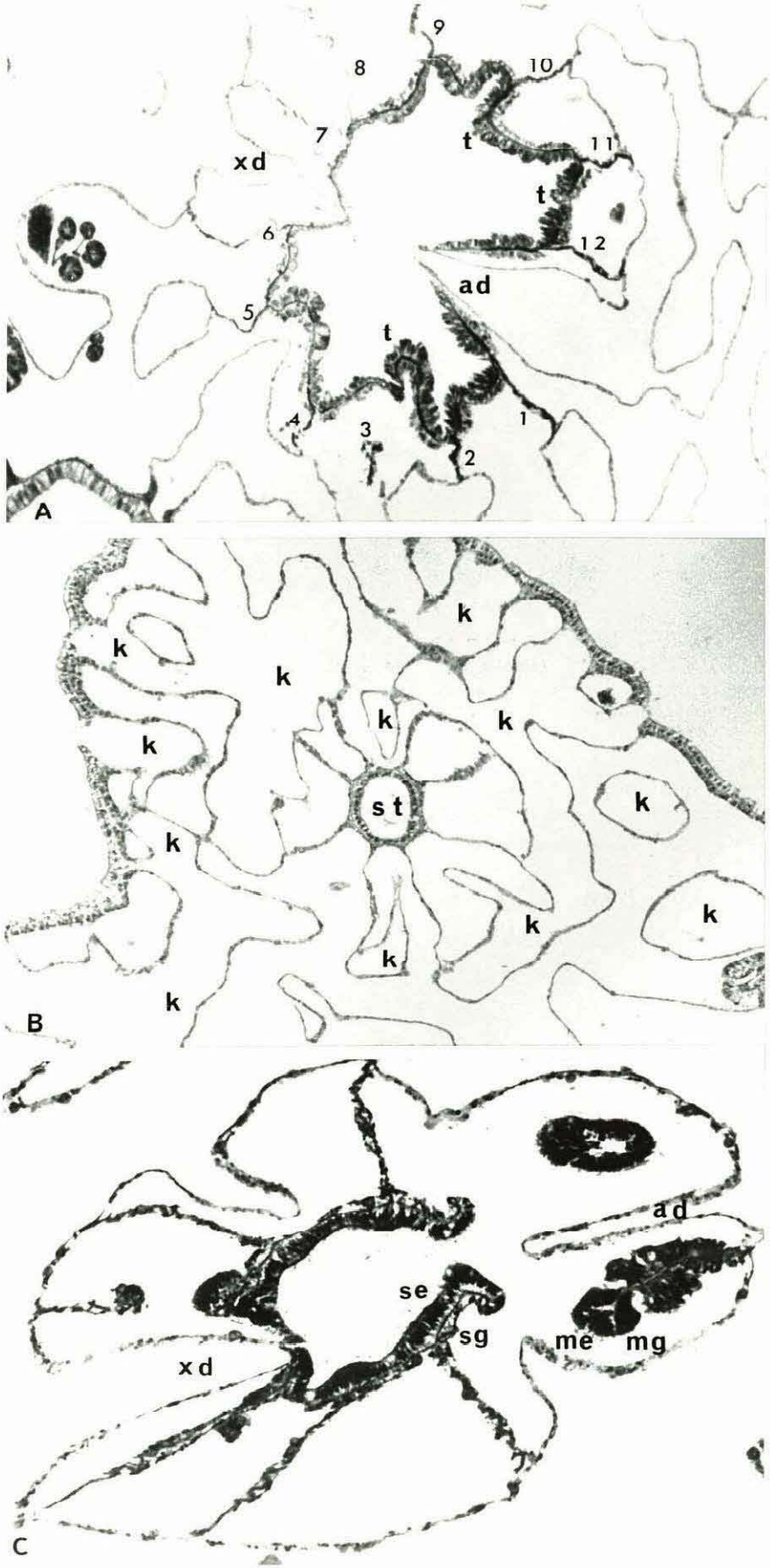

9
8
10
7
t
11
xd
t
6
12
ad
5
t
4
3
1
2
A
k
k
k
k
k
k
k
st
k
k
k
k
k
B
ad
se
sg
me
mg
xd
C

FIGURE 20

Idealised sections through

(A) an axial polyp and corallite at the level of the stomodaeum, and

(B) a radial corallite and polyp below the stomodaeum.

I = first cycle of septa

II = second cycle of septa

ds = directive septa, orientated as axial and abaxial in the case of the radial corallite.

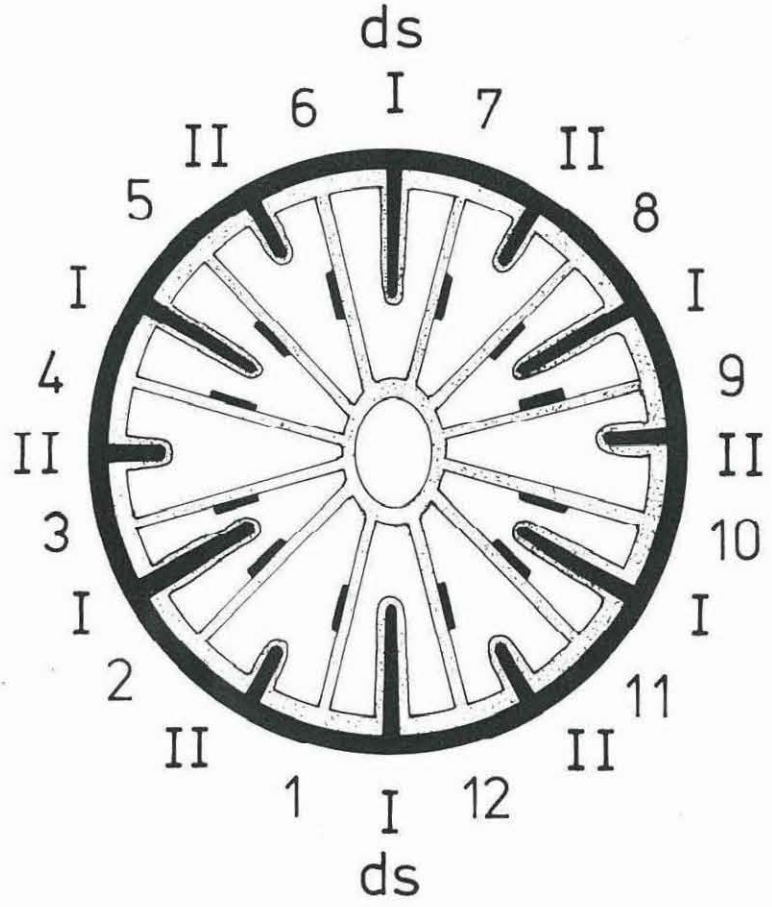
1, 12, 6 and 7 are the directive mesenteries

1 to 12 represent the 6 pairs of protocnemes

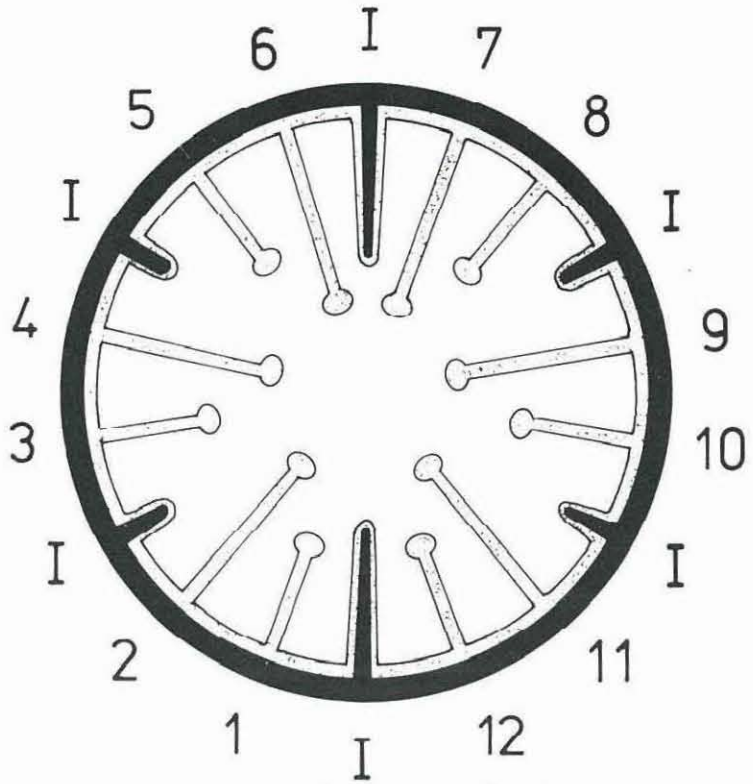
2, 4, 6, 7, 9 and 11 at least are normally present in the radial polyps.

If a third cycle of mesenteries are to be added, these metacnemes are inserted as unilateral pairs, always in the spaces between the protocnemes. In this way the directives remain as directives throughout the life of the polyp. The mesenterial retractor muscle blocks are indicated in the axial polyp, and are in the same relative position in the radial polyps.

A.



B. axial [inner] ds



abaxial [outer] ds

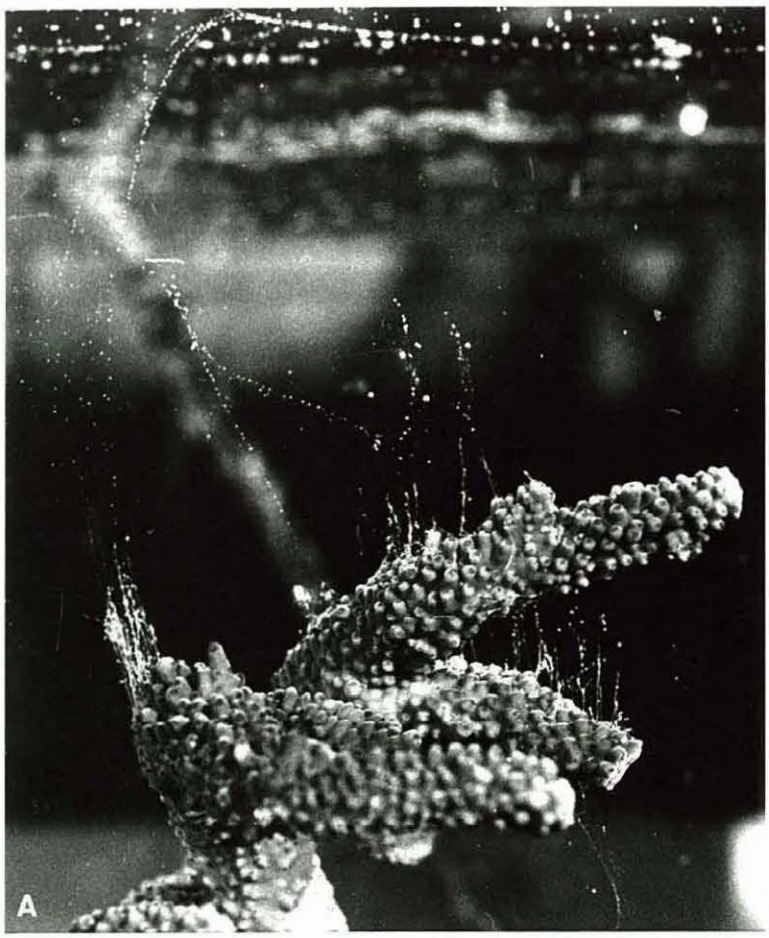
FIGURE 21

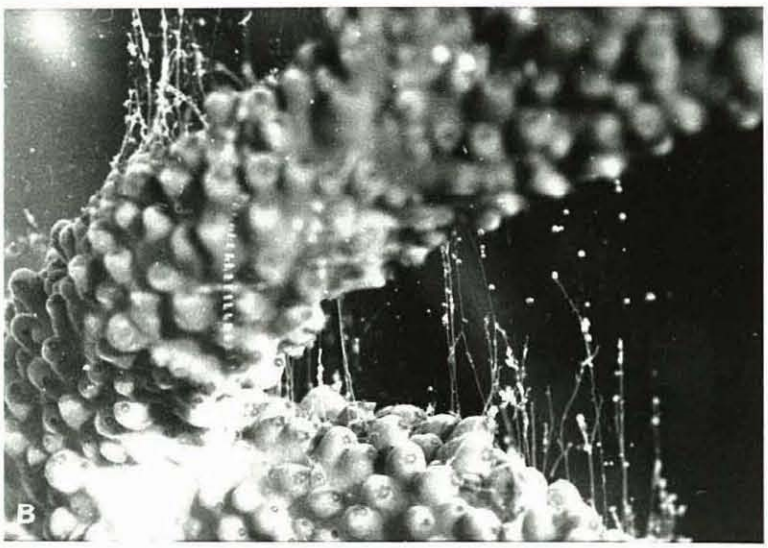
Acontial filament extrusion in Acropora formosa.

(a) Filament and mucus extrusion. Magnification x ¾.
The acontial filaments of the mesentaries are supported by strands of mucus buoyed up by small air bubbles. This response was due to over feeding with Artemia naulpii, introduced into the tank 30 minutes prior to this photograph.

(b) Close-up of the filaments, Magnification x 2.3
Many of the filaments consist of paired strands, issuing both from the stomodaeum and body wall of the polyp.

(c) The initial stages of the over feeding cycle. Magnification x 1.3, five minutes after addition of larvae. The polyps remain in a partly extended position. Numerous Artemia nauplii can be seen enmeshed in mucus along the upper portion of the branch.


A


B

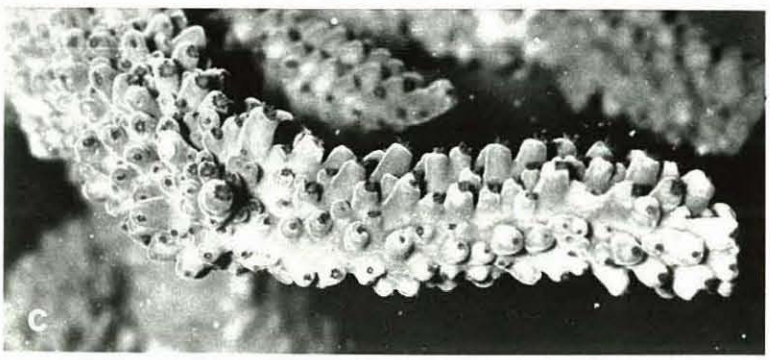

C

FIGURE 22

Regrowth of skeleton over damaged areas.

(a) A fold of tissue layed down an epithecal rim, followed by the deposition of undifferentiated coenosteum. New polyps form only at a distance from the repaired edge. Magnification x 25.

(b) The regrowth of skeleton in this area has formed a holdfast pad. Epitheca can be seen lining the fold of skeleton on the left. Magnification x 6.

(c) The epitheca that is formed during the repair sequence shows marked growth banding. Magnification x 65.

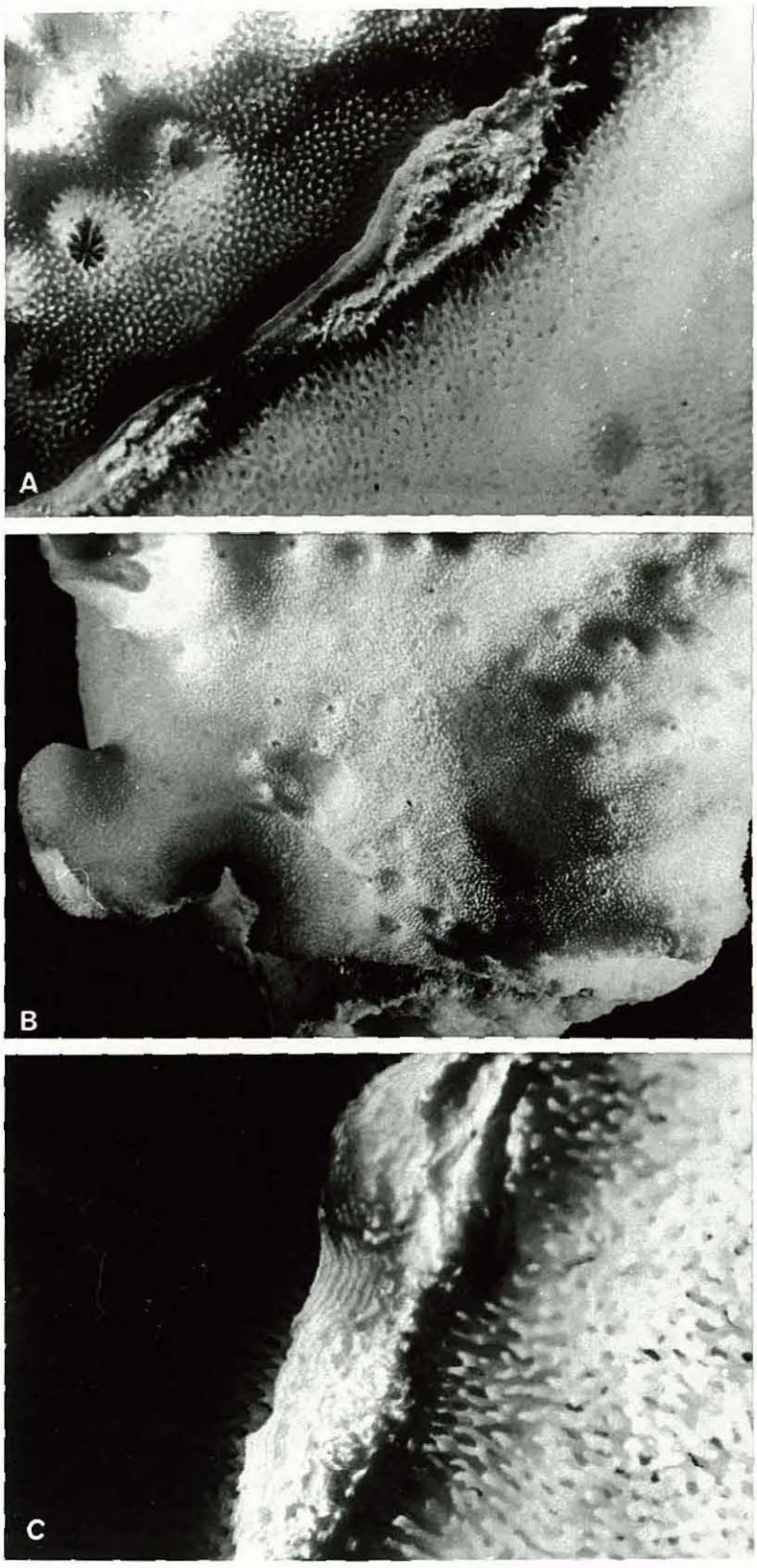

A
B
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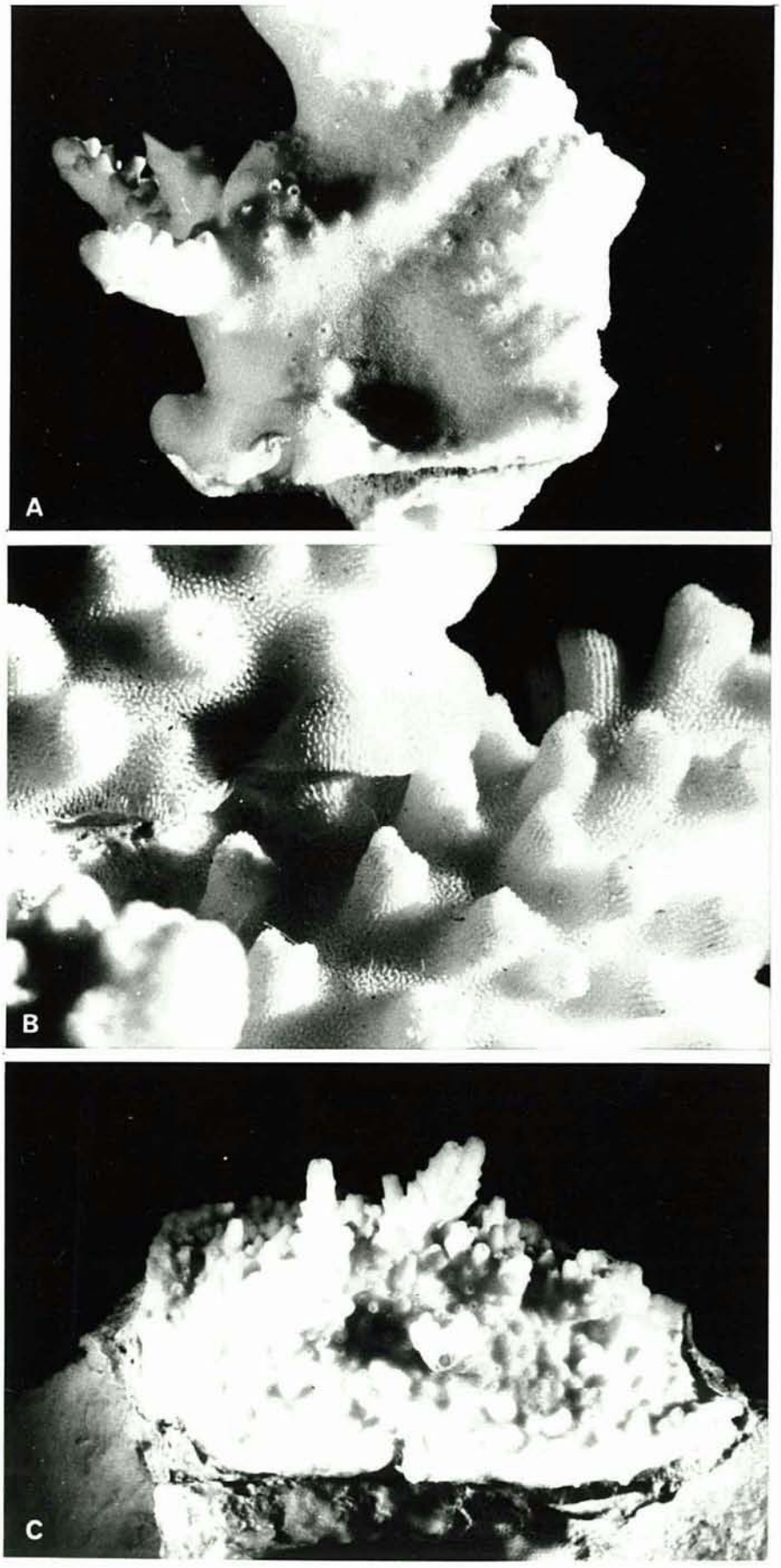
FIGURE 23

Holdfast basal morphology of Acropora formosa.

(a) A basal holdfast formed as the result of damage to the lower region of a branch.
Note the spinulose coenosteum and the reduced number of calices. Magnification x 3. A higher power view of this specimen is also illustrated in Figure 22b.

(b) A holdfast pad formed in response to contact with another coral (allogeneic interaction q.v.).
This is a mid branch holdfast. Magnification x 10.

(c) Juvenile colony, probably of Acropora formosa.
Note that a large holdfast pad forms before branches expand upward. Magnification x 2.


A
B
C

CHAPTER V TISSUE INTERACTION IN CORALS

(a) Introduction: Tissue interaction in coelenterates

(b) Results: Experimental grafts

(c) Results: Histomorphology of the graft region

(d) Results: Histochemical investigation of the mucosubstance composition of interacting corals

(e) Discussion: Discussion of coral grafts

(a) Introduction: Tissue Interaction in Coelenterates

When two colonies of coral come into contact, either by natural growth or mechanical disturbance, a tissue recognition reaction is observed (Hildemann et al., 1974). The study of such tissue reactions has recently been given added impetus from immunological studies, as the precursors of vertebrate immunological systems are thought to lie in the invertebrates. The phylogeny of immunoresponsiveness and the phylogenetic aspects of the genetic control of histocompatability reactions have been the subjects of reviews by Hildemann and Reddy (1973), Hildemann (1974), Hildemann et al (1977) and Du Pasquier (1974). The evolution of immunity in terms of the recognition of 'self' and 'non-self' in colonial marine forms and flowering plants was reviewed by Burnet (1971) and reinforces the basic concept of cellular recognition, in some form, to all multicellular organisms. Cooper (1974) acknowledges the two approaches that have developed in this field; "there are those who are interested in the ambiguous biological roots of recognition, sensitivity, specificity and anamnesis, and those with predetermined views searching for the characteristics of the immune systems to be found in primitive organisms". This dichotomy led Shostak (1974) to pursue the compromise question, "what does a primitive organism do in response to a challenge a vertebrate would respond to with its immune system?". It is more with this latter approach that the tissue interactions in corals have been investigated.

Tissue interaction in the Cnidaria have been extensively studied in the Hydrozoa. Hydra tissue graft reactions have been reviewed by Campbell and Bibb (1970) and Shostak (1970, 1974). Orthotopic isografts will quickly fuse, the grafted region becoming indistinguish-

able and persisting indefinitely. However, if morphogenetic fields and polarities are deranged (i.e. the graft is heterotopic with respect to biochemical gradients, rather than tissue type, which is homologous in this case) then unstable graft combinations between isogenic tissues can be found. In Hydra the allograft reactions are not clear cut. Lenhoff (1965) performed allografts between an abberrant genetic variant and normal parental stock and obtained a "heterocyte" with one cell genotype dominant. The chimeras healed normally and remained grafted indefinitely. Brien et al. (1956) showed that the allografts between a "stolonizing" mutant and normal animals (but presumably not parental stock) were incompatible, leading to poor healing and eventual graft separation, the apical graft becoming dominant. Campbell and Bibb (1970) reported that Shostak (no ref.) working with geographically isolated strains of Hydra found that all combinations of allografts were incompatible, and stable grafts could not be formed.

An explanation of the tissue reactions in Hydra allografts may have been found by Du Pasquier (1974). By reworking the data of Hauenschild (1954, 1956) on the grafting of the marine Hydrozoan, Hydractinia echinata (Fleming), it is apparent that if the allografted tissues have at least one haplotype in common in their major histocompatibility genetic region, then successful graft fusion will occur. The success of an allograft depends upon the degree of homozygosis (or inbreeding) of the grafted tissues which, in the case of Lenhoffs' (1965) Hydra grafts, was higher (mutant to parental stock) than in Briens' (1956) grafts (mutant to natural stock).

In their review of transplantation in coelenterates, Campbell

and Bibb (1970) conclude that xenografts in Hydra and marine hydrozoa are unstable and that their ability to heal is dependant upon the genetic composition of the donor and recipient. The formation of 'chimeras' between two distinct species of Hydra (as opposed to the intraspecific chimeras mentioned above) lead to the eventual elimination of one genetic cell type (Campbell and Bibb, 1970). Similarly the variety of cytological reactions that are exhibited in Hydra xenografts will depend upon the genome of the grafted species.

Ivker (1967, 1972) working with Hydractinia echinata noted that hyperplastic stolonization and overgrowth occurred only between strains of that species. Contact with other hydrozoans, human hair or agar did not evoke such responses. An intermediate incompatibility expressed as absence of overgrowth and lack of fusion occurred between parent and offspring and half siblings; a further example of a response dependant upon the degree of homozygosis of the interacting individuals. Colonies of Hydractinia derived asexually from the same source (i.e. isogeneic) retained their ability for compatible interaction even after long periods of separation, irrespective of their exposure to interactions with other stolons, different environmental or physiological conditions. Strong conspecific incompatibility was not found to affect the sexual reproduction between individuals.

The discharge of acrorhagial atrichous nematocysts of the anthozoan Anthopleura, observed by Francis (1973), occur preferentially in response to contact with conspecific, but genetically different anemones, and only rarely with other Anthozoans. Such behaviour facilitates the formation of clones, the advantages of which have been discussed by Francis (1973).

Theodor (1970) has shown that the Gorgonid corals behave in a similar way to the hydrozoan coelenterates in that there is complete fusion of isografts that persist indefinitely. The allograft reactions show incompatibility, but the histopathic effects appear at a slower rate than in xenogeneic graft combinations. In both allograft and xenograft combinations the eventual collapse and destruction of the tissues concerned occurred, a far more distinct reaction than that found in the hydrozoan coelenterates, but similar to the destructive interactions between allogeneic Anthopleura interactions.

The reviews on the phylogeny of immunity in the invertebrates by Hildemann and Reddy (1973), Valembois (1973), Du Pasquier (1974) and Hildemann (1974), allow the tissue and cellular interactions of the coelenterates to be placed into perspective. Lafferty and Crichton (1971) state that invertebrate responses to foreign material bear little resemblance to classical immunological reactions exhibited by higher vertebrates. In these higher forms the ability to produce circulating antibody, together with accelerated and functionally more effective responses to second challenges with antigen, are considered fundamental. However, if immunity is defined in terms of functional significance to the biological system, then a wider concept of immunological reactions can be accepted, that is, applicable to both invertebrates and vertebrates. The basis of this hypothesis lies in the ability of an organism to recognise "self" and "non-self" components, but does not tie this recognition to the characteristics of the higher vertebrate system, i.e. production of antibody.

The types of reaction that have been found in the lower invertebrates show responses that increase in complexity with

phylogenetic order. Allogenic transplants in Protozoa are found to remain viable, while xenogeneic transplants result in eventual death (Hildemann, 1972). Humphreys (1970) has shown that sponge cell aggregation depends upon the binding affinities of cell surface glycoproteins, and is not dependant upon an immunological surveillance system. Hildemann (1974) points out that no cellular damage occurs when incompatible sponges are grafted and that this appears worthy of more investigation as the next phylogenetic group, the coelenterates, show distinct immunorecognition, with cellular necrosis occurring in xenogenic interactions.

Hildemann and Reddy (1973) and Hildemann (1974) have introduced descriptive terms for invertebrate tissue interactions. The type of reaction shown by sponges when they are either grafted or allowed to reaggregate after dispersal is referred to as "cell or species specific aggregation". The capacity for 'non-self' recognition of allogeneic tissues, followed by incompatible reaction has been referred to as "quasi-immunorecognition" or "immunorecognition/immunocompatibility", and is characteristic of the coelenterates, tunicates and vertebrates. "Primordial cell mediated immunity" (PCMI) reactions are found in the Annelids, echinoderms and primitive fishes and are characterised by allogeneic incompatibility with at least a short term memory component. The more advanced immunological reactions shown in the advanced fishes and higher vertebrates, showing "integrated cell and humoral antibody immunity" are not found in the invertebrates.

Quasi-immunorecognition has already been shown to occur in scleractinian corals as reported by the author in Hildemann et al. (1974), and further confirmed by Hildemann et al. (1975).

The evidence for the involvement of a primitive immune system comes from the specificity shown by orthotopic grafts and the delayed tissue destruction in allo- and xenografts. Hildemann (1975) states that "enzyme-substrate or metabolic incongruities do not provide sufficient explanation for long delayed antagonistic reactions". The intercolonial interactions shown in sea anemones (Francis, 1973) and the interspecific 'aggression' shown in corals (Land, 1971, 1973) although showing a degree of specificity, are rapidly initiated following contact between antagonistic pairs and therefore show a marked contrast with the slowly initiated immunorecognition/ immunocompatibility reactions.

The preliminary studies on Scleractinian grafting were reported by Hildemann et al. (1974), covering grafting in corals (author), Echinoderms (Hildemann) and Molluscs (Dix). Hildemann et al. (1975) have furthered some aspects of coral grafting by ascertaining the regularity and specificity of natural immunorecognition reactions in a large group of species from numerous genera in a crowded reef environment at Eniwetok. An important conclusion of this work was that a 'sequentially structured hierarchy at the generic level is definitely an oversimplification', which is in contrast to the ideas of Lang (1973). A series of papers by Hildemann et al. (1976, 1977a,b) have now shown that, at least for Montipora verrucosa and probably for other hermatypic colonial Scleractinia, on contact, both immunological specificity and short term memory are unequivocably present. These surprising observations show that the advanced immunological system of the Vertebrates, at least with respect to histocompatibility and transplantation immunity reactions, has its roots extending to the lower Metazoa.

(b) Results: Experimental Grafts

Isograft

Over 120 isografts were performed over a three year period using specimens of Acropora formosa. In all, with the exception of a few technical losses, the tissue of the graft zone healed completely and showed little trace of the junction after 4 - 8 days, although its location was always easy to locate by the distinctive skeletal morphology. Occasionally small white areas (i.e. devoid or much reduced in numbers of zooxanthellae) 0.5 - 1.00 mm in width, and 2 - 4 mm in length, would appear at the graft junction. This pale tissue would invariably disappear, usually within one month and was therefore referred to as 'temporary scar tissue'.

Several combinations of graft orientation were investigated, as illustrated in Figure 24. The combinations were termed:

(a) Terminal/terminal, lateral graft

(b) Terminal/terminal reversed, lateral graft.

(c) Basal/terminal, lateral graft.

The type (a) terminal/terminal lateral graft was preferred for its ease of construction, but in all other three conditions compatible fusion of soft tissue was observed. Surprisingly, type (c) graft showed no lack of fusion or skeletal deposition in the grafted zone. Therefore no marked morphogenetic fields or polarities, or senility barriers to isograft orientation were apparent.

Three methods of inducing a graft were tried:

(i) by grinding the branches together and tying tightly with monofilament, thus forming a large graft bed with numerous contact areas,

(ii) by carefully resting the branches together and lightly tying with monofilament so that water currents would not disturb the graft,

(iii) by wedging the branches in racks so that the tissues were close (1 - 4 mm) but not in contact.

In each case, compatible fusion was observed. However, in the first case, small pieces of tissue were often trapped and damaged, leading to temporary necrotic areas. In extreme cases, infection (culminating in the appearance of large numbers of ciliate protozoa) caused large dead areas. The exposed skeleton in these areas was quickly covered with diatoms and small filamentous algae over which the coral tissues were observed to regrow only slowly.

Grafting by method (ii), in which tissue destruction was minimal, proved desirable with the fusion of contiguous soft tissue occurring in less than two days. The diurnal expansion of coral tissues allowed contact of adjacent epidermal tissue across gaps (measured when in the contracted state) of up to 2 mm. Such contact resulted in tissue fusion that soon occluded the smaller spaces between the irregular surfaces of the donor and recipient tissues. Tissues of adjacent specimens with a separation of more than 2 mm took much longer to fuse, and not until the tissue and consequential skeletal growth had brought them closer together. In regions where donor and recipient calyces were in opposition and separated, the diurnal expansion of the polyps allowed the oral disc, tentacles or upper part of the polyp column to come into mutual contact. In no case was there ever any fusion between these areas. The actual tissue of the polyps proper maintain their integrity while perfect compatible fusion of adjacent non-polyp epiderm of the coenenchyme occurs freely.

The same features were noted in the third grafting method. However the time taken for fusion to occur was usually longer and more variable because the specimens were not physically touching at the start of the experiment. Again it was apparent that those parts of the graft less than 2 mm apart in the contracted state would fuse quickly because they are able to touch when expanded. This latter method more nearly represents the natural situation where colonies might grow into one another. For practical reasons, it was not possible to set up such long-term experiments.

Experimentally grafted specimens that were allowed to grow for long periods (in excess of three months) developed considerable skeletal deposits along the graft zone. In many cases the infilling of the graft area allowed the graft position to be identified only as a shallow depression (Figure 25a). The infilled region between existing calyces appears at first as undifferentiated coenenchyme (Figure 25b), but with continued growth further calyces develop. Eventually the infilled area develops a field of polyps resembling those either of the donor or recipient. Often a line of more numerous smaller polyps was noted lying along the line of the original graft contact zone. Similar lines of numerous small polyps have been observed in the axils of mature branches and in natural isografts of this genus. These structures form as a consequence of a general rule that seems not to have been stated previously, viz. 'that once formed, a polyp tends to be persistent'. In the case of the laterally grafted corals, polyps grow out normally to the surface as the coenosteum infills in the contact zone thus forming a band of crowded, small polyps (Figures 26 and 27a). In a similar way, the thickening of a lateral branch will cause a crowding of the polyps

in the branch axil (Figures 26c and 27c).

Allograft

Branches derived from widely separate colonies (greater than 100 m) of the same species were used to form allografts. In all cases, grafts were made by terminal/terminal lateral combinations lightly tied together with nylon monofilament.

Initially tissue reactions appear morphologically similar to those of an isograft. In contrast to the isograft, at no stage were any waves of tentacular contraction transmitted across the graft junction. By the sixth day, the tissue of the graft contact zone often showed a paling, restricted to within 1 mm of the contact line. This paling is due to the reduction in the number of zooxanthellae in the endodermal tissue beneath the contacting epiderm. In areas where the pressure of contact caused cellular disruption, the irregular contact zone was observed to round up and heal from either side, the two components remaining functionally separate but often in physical contact. Areas of the graft tissue that were excessively damaged, such as that over the lips of the corallites, may become necrotic, revealing a small area of exposed skeleton. The necrotic zone is usually restricted to 1 - 2 mm, but occasionally, with the aid of infection, may spread up to 5 mm from the graft. After 20 days the graft is distinctly composed of two components, the donor and recipient. Infilling of small cavities does not proceed initially as fast as in the isografts, this being especially evident where tissues remain in physical contact. However after long periods (in excess of 3 months) the grafted area does become infilled, usually

by bilateral growth. Throughout this time, epidermal tissues may have been in intimate contact, but at no time was any fusion of allogeneic tissue ever observed.

If one component of the grafted system suffered an initial infection, the equal bilateral growth gave the undamaged side an advantage and allowed it to overgrow the other. Excessive and continued overgrowth, with the simultaneous regression of the overgrown tissue was not observed in any undamaged allografts. Overgrowths were always associated with the initial graft conditions. Continued growth tended to negate any initial disadvantages. It must be emphasised that these were experimentally contrived grafts using similarly sized pieces of coral orientated so that neither was at an advantage with respect to light or water currents and that the parent colonies, although separated by more than 100 m, were from the same environmental reef zone.

In long term allografts the mutual infilling proceeds further than that observed in isografts, and produces large pads of tissue covered skeleton resembling the holdfast region, but with more polyps over its surface. As the two holdfasts are growing on each other, an area of growth greater than the original diameter of the reacting branches can be attained. The periphery of the discoid contact zone is often crenulated giving the impression that local growth advantages alternate from side to side of the graft but are on average perfectly balanced so that the graft contact zone lies in much the same position as it was initially.

Xenograft

Xenografts were usually made between specimens of the genus Acropora but with such distinctive species that their inter-specific relationships could not be confused with intra-specific forms. Similar grafting technique was used as for iso- and allografts, but the size and shape of the branches were not always possible to match.

In all cases, strong cellular reactions occurred within 2 - 3 days of grafting. By the sixth day there was usually extensive necrotic tissue in the graft zone, but limited to 1 - 3 mm of the contacting area. Infection often increased the size of this dead area to 5 - 6 mm.

The above reactions were observed in each of the following xenografts between A. formosa and either A. cytherea (Dana, 1846), A. cerealis (Dana, 1846), A. tenuis (Dana, 1846), A. elseyi (Brook, 1893), A. pulchra, A. robusta (Dana, 1846), A. millipora (Ehrenberg, 1834), or Montipora ramosa. This range of grafts was continued for 4 weeks. At the end of this period little or no evidence of over-growth was evident. In nearly all specimens the initial necrotic areas had healed, leaving a variable width of skeleton exposed. In areas of the grafts where tissue was separated by a gap of 2 mm or more, no reactions were observed, leaving the tissue intact.

A series of grafts between A. formosa and A. tenuis, and A. formosa and Montipora ramosa were left as a long-term sequence, and observed for a period in excess of three months. A. formosa consistently overgrew A. tenuis in these longer experiments, irrespective as to whether it was the donor or recipient (Figure 28a). In all other grafts the recipient was the longer piece of a pair of

similar sized branches but in the dissimilar sized A. tenuis the lack in diameter and length were allowed for by taking a group of branches. In this way, a similar amount of living tissue was taking part in the reactions.

Occasionally small and localised areas of the A. tenuis side of the graft were observed to resist overgrowth, and even grew onto the A. formosa for a short distance. These small reversals of growth dominance appear to be associated with unusual configurations at the graft surface that places the A. formosa in a disadvantageous position, for example in a cup shaped depression surrounded by A. tenuis tissue. At all stages of xenograft growth a gap between the opposing epiderms of between 1 - 3 mm was maintained thus exposing a small area of skeleton. If the exposed area remains stationary, i.e. neither side is advancing or if the area increases in size, when an aggressor retreats advantageous settlement of algae, bivalves and/or polychaetes have been noted (see Figure 29). When this settlement occurs, further overgrowth is restricted, and a more stable boundary is formed.

In the long term reactions between A. formosa and M. ramosa the latter appears to be the aggressor. The area and speed with which it overgrows is less than that shown when A. formosa overgrows A. tenuis. This appears to be a consequence of the type of growth exhibited by the Montipora and the lesser quantity of growth in a unit time during the study period. In all cases, the Montipora formed a pad of tissue and skeleton on the surface of the A. formosa, and as it increased in size there was a concomitant withdrawal of Acropora tissue, maintaining the characteristic 1 - 3 mm xenograft separation. Occasional exceptions to Montipora dominance have on

occasions been noted, e.g. specimen in Figure 28b. This is the result of an infection at the graft junction that was initially exploited by the A. formosa.

In both the A. tenuis and M. ramosa long term xenografts with A. formosa, small interbranch gaps, 2 - 4 mm in size, were observed to infill by mutual growth. When this gap had closed to less than 2 mm the more dominant tissue was observed to damage the other, exhibiting a similar reaction to the initial graft.

In the grafts involving Montipora infection appeared to cause more extensive damage to the Montipora than the Acropora. This often placed the Montipora at an initial disadvantage.

In all the xenograft reactions it appeared that once a pad of tissue and skeleton had been formed that was of a similar size to a holdfast region, normal growth processes limited any further increase in size.

(c) Results: Histomorphology of the Graft Region

All sections used fixed in 10% formalin - sea water, using the double embedding with decalcification technique (see Chapter III (f)). Haematoxylin - Eosin was used as a routine stain.

Isograft:

The histology of the isograft region confirms that soft tissue contact is maintained and that complete fusion of tissues occurs within 4 - 8 days of grafting (Figure 30a). However, the junction presents a somewhat confused histology, particularly in the region of temporary scar tissue, with a reduced number of zooxanthellae for the initial 14 - 20 days. During the fusion process, epidermal cells such as nematocysts which become trapped in an internal position (Figure 30b) eventually are either eliminated or migrate back to the epidermis. In experimentally grafted corals at a temperature of 20 - 25^{o}C such cellular migrations take up to 30 days to complete. Intercommunication between the donor and recipient coelenteric cavities appear during the first few days of grafting (Figure 30c).

If an area of the graft junction becomes necrotic then typical repair processes develop in which the damaged and exposed skeleton are overlayed by a new epithecal layer, followed by normal tissue layers. This accounts for small areas of epitheca seen in the contact zone of some isografts (Figure 28).

Allograft:

Allografts never show fusion of tissues even in the early stages of grafting. Gross observations appear to show temporary fusion, but

the histological sections of such areas always show a total lack of fusion. Damaged tissues always round off and contract back to their own side of the graft. Necrotic areas are more prevalent. Although in close proximity, the opposing tissues never seem to interact, but show a mutual indifference (Figure 32). As a result of the initial tissue regression, a small and variable sized gap is formed, with the opposing branches mutually supported on the spines of the exposed skeleton. Tissues trapped or isolated in the contact area regress and die. A marked thickening of the coenosarc develops as the donor and recipient form the growth morphology of an holdfast pad (Figure 32). Coelenteric canals are sealed off at depth within the coral structure and are never in communication.

Xenograft:

The histology of the xenograft is initially similar to that of the allograft, but mutual tissue regression is more pronounced (Figure 33). In older grafts (i.e. greater than 30 days) the encroaching tissue edge has a typical edge-zone morphology, but the separation between the encroaching and regressing tissues remains at 1 - 3 mm.

The histological analysis of coral grafts failed to reveal any areas of necrosis that could be wholly attributed to opposing cellular interaction. Numerous necrotic areas were found in grafts up to 12 days old, but in all these cases this necrosis could not be divorced from the initial trauma and disruption associated with the graft formation. Where tissues are in the act of regression at an interface, the cellular material appears to atrophy and/or

migrate away (e.g. Figure 32c). This reaction is undoubtedly due to the presence of the opposing tissue. The further analysis of this type of cellular interaction is not possible using light microscopical techniques. Electron microscope techniques have not been available to investigate this further.

(d) Results: Histochemical Investigation of the Muco-substance Composition of Intracting Corals

Goreau in his Doctoral thesis on the Biology and Histochemistry of Corals (Yale University, 1956) stated that (p. 123) . . . "mucoid secretions of all kinds serve to protect and lubricate epithelial surfaces. A large number of mucopolysaccharides also possess anti-coagulant and immunological properties". He later (p. 142) speculated on the possibility of a boring algae causing changes in the calico-blastic layers, resulting in the formation of mucous glands. On numerous occasions in the field it was noted, as others have, that one of the first responses a coral makes on being disturbed is to secrete copious quantities of mucus. For those reasons, it was felt that an investigation of the muco-substance histochemistry of interacting corals may have been profitable.

An initial survey was conducted using the Periodic Acid-Schiff technique (PAS); Azure A at pH 1, 2.5 and 4; Alcian Blue at pH 2.5 and 1, and the general muco-substance technique of Alcian Blue + PAS

(see Methods Section). Such techniques allow an initial characterisation of muco-substances (using the definitions of Pearse, 1968) into the following groups:

Neutral mucins	
Strongly sulphated	Acid mucins
Weakly sulphated	
Non-sulphated sialomucin	
Non-sulphated hyaluronic acid	

It was proposed that if the initial study showed any intensified activity, or increase in number of mucous gland cells in the ectoderm of interacting coral tissues, then a further characterisation of the mucins would be warranted.

Table 4 indicates the staining properties for mucosubstances shown by two commonly grafted corals Acropora formosa and A. cytheria. This table is indicative of the general distribution of mucosubstances not only in Acropora but in many other corals as well (see Goreau, 1956). The following descriptions amplify the differences noted between this study and Goreau's work. As the main interacting tissues are ectodermal, more attention has been given to this area than others (the mesenterial filaments were not observed to 'interact' in these Acropora grafts).

Ectoderm:

Staining reactions similar to those found by Goreau (1956) indicate that this tissue layer in Acropora is not different to those corals he investigated for muco-substances.

TABLE 4 MUCOSUBSTANCE STAINING IN ACROPORA FORMOSA AND A. CYTHEREA

Cell or Tissue	P.A.S.	Azure A pH 1.0	Azure A pH 2.5	Azure A pH 4.0	Alcian Blue pH 1 + PAS		Alcian Blue pH 2.5 + PAS		Alcian Blue pH 2.5	Tentative Analysis of Mucin
(a) EPIDERMIS										
Mucus Locule[1]	-	-	-	Weak Ortho.	Weak +	-	+	-	+	Weakly acid
Mucus net[1]	Weak	-	Weak Ortho	Meta	Purple		Purple		-	Neutral and acid mixture
Mucus cell[2]	-	Meta	Meta + Ortho	Meta + Ortho	Weak +	-	+	-	-	Weakly acid sulphated
Spirocyst	-	-	-	-	-	-	-	-	-	"
Nematocyst	Weak	-	-	-	-	-	-	-	-	Neutral acid sulphated
Supporting cells	Weak	-	-	-	-	-	-	-	-	"
Mesoglea	+	-	-	-	-	+	-	+	-	"
(b) CALICOBLAST										
Epiderm	-	-	-	-	-	-	-	-	-	"
Gastroderm	-	-	-	-	-	-	-	-	-	"
'Active sites'	Weak	Ortho	Ortho + Meta	Weak Ortho	Weak +	-	+	-	+	"
(c) FILAMENTS										
Supporting cells	Weak	-	-	-	-	Weak	-	Weak	-	"
Granular gland cells	+	Meta	Meta	-	Purple		Purple		-	Neutral, probably strongly sulphated acid mixture
Mucus locule	-	-	-	Weak Ortho	+	-	+	-	+	Weakly or strongly sulphated acid
Mucus net	-	-	Weak Ortho	-	+	-	+	-	+	"
(d) STOMODEAUM										
Granular gland cells	+	Meta	Meta	-	Purple		Purple		-	Neutral and probably strongly sulphated acid
Mucus Locule	-	-	-	-	+	-	+	-	+	Weakly or strongly sulphated acid
Mucus net	-	-	-	-	+	-	+	-	+	"
Supporting cells	-	-	-	-	-	-	-	-	-	"
(e) ZOOXANTHELLAE	+[3]	-	-	Ortho[4]	+[5]	+[3]	+[5]	+[3]	-	Neutral and strongly sulphated and weakly sulphated mixture

1 = Acropora formosa only
2 = Acropora cytherea only
3 = PAS + ve Pyrenoids
4 = Nuclear material
5 = Outer membrane of zooxanthellae

The mucous cells showed a peripheral PAS positive layer that sometimes extended through the cell as a fine network. The enclosed locules stained strongly in Alcian blue at pH 2.5, often showing a strong metachromasia. This indicates that these cells have a neutral mucin sheath, with occasional cross connections, enclosing locules of acid mucins that are either weakly or non-sulphated. Other cells in the ectoderm showed little or no mucin activity.

Mesogloea:

Showed positive PAS, in common with other corals (Goreau, 1956).

Calicoblast:

The calicoblast layer in Acropora usually showed small (less than 1.5 x 3 u) "active sites" dispersed throughout the coral sections. These sites did not appear to be correlated with calcification areas, although in a decalcified section this is by no means certain! These active sites gave positive indications to all the mucin stains. They appear to be diffuse in nature and located in the calicoblastic gastroderm (Figure 34). They also take up nuclear stains such as neutral red.

Gastroderm, Mesenterial Filaments and Stomodeum

Showed the mucin staining reactions reported by Goreau (1956).

This study was particularly looking for:

(a) signs of unusual activity associated with the cells in or around the areas of tissue interaction, and

(b) increased numbers of 'mucin' bearing cells in the interacting area.

In no section was any such increased cell number or activity observed. These somewhat surprising negative findings were checked in all iso-, allo- and xenografts, at times ranging from 2 days to 19 days after grafting. Sections of grafts less than 2 days were not taken as the after effects of mechanical stimulation during the grafting manipulations may still have been present.

One interesting sideline was noted. In the many sections examined, a considerable variation in the relative number of mucus and granular gland cells was strongly evident. These variations were not in any way associated with graft sites or tissue interactions, but appeared more likely to reflect the overall activity of the branch from which they were initially removed. The number of ectodermal mucin cells showed little variation around the periphery of any one individual branch. The effect was most evident in the paired sections of xenografts (Figure 35) and allografts, but was occasionally seen in functional isografts. These pairs of sections were of course treated in an identical way, as indeed was the whole graft since it was first constructed. In some sections it is possible that the relative abundance of mucous cells may be caused by the recent discharge of mucus from the cells, making them less obvious in the section. This may indicate either feeding or cleaning activities occurring at different times on either side of the graft. This type of difference was restricted to xeno- and allografts where there is no functional contact across the graft. In one isograft formed by removing the distal 8 cm of a branch and forming the graft alongside the proximal stump, a large difference

in mucous cells of the respective ectoderms was noted. This suggests a change in the relative abundance of cell types along the branch. In this case, the mucous cells were more evident in the proximal portion, and hence 'older' part of the branch.

Although interesting, this distribution of cells was not followed further as it deviated from the central theme of the interactive investigation.

(e) Discussion: Discussion on Coral Grafts:

Table 5 summarises the range, cellular response and genetic relationship of coral interactions. Isogeneic contacts show fusion of tissues in most experimentally induced interactions. Hildemann et al. (1977b) reported that Fungia scutaria (Lamarck, 1801) tissues rarely, if ever, fused once they had been released from the anthocaulus. Fusion of isogeneic tissues may occur while the anthocyathus is still attached.

Figure 51 shows a fused Fungia fungites (Linneaus, 1758) skeletal preparation that has arisen from two centres of growth at the terminal portion of the anthocaulus. These had been formed during the regeneration of the surface after releasing an earlier anthocyathus. Mature Fungia are considered to be solitary polyps.

In numerous isografts formed with Acropora formosa, the polyp tissues, i.e. oral disc, tentacles and column, although often in intimate contact, refused to unite. The isogeneic fusion was limited to the coenosarc only.

It would appear, that as polyp tissues per se do not enter into isogeneic fusions, they behave as individuals and retain their integrity and independance. The inter polyp coenosarc behaves as a true colonial tissue and fuses with homologous isogeneic surfaces, irrespective of their position or age. Polyps can form in coenosarc tissue as a result of extratentacular budding, but once formed they retain an independance, with respect to further morphological modification, and a strong persistence to exist, even the pressure of crowding caused by topographical changes in morphology (see Chapter V (b)).

TABLE 5 SUMMARY OF CORAL INTERACTIONS

Category of Interaction	Descriptive Term	Cellular Response	Genetic Relationship	Examples
Self recognition	Fusion	Fusion	Isogeneic	Fused colonies. A. formosa from same colony. Fig. 25.
Passive recognition	Contact avoidance and mutual indifference	Cell contact, but non-fusion	Allogeneic	A. formosa from different colonies. G. aspera aggregated colonies. Fig. 40.
Quasi-immune system involving memory; evident mostly in early stages of contact	Allogeneic contact incompatibility	Short term cellular destruction and infective necrosis	Allogeneic	Early stages of Montipora verrucosa allograft. (Hildemann et al., 1977).
	Chronic xenogeneic incompatibility	As above, but with more cytotoxicity	Xenogeneic	A. formosa - M. ramosa xenograft. Fig. 28a. A. formosa - Galaxea sp. xenograft. Fig. 37.
Digestive hierarchy	Interspecific aggression	Extracoelenteric digestion of cells by mesenterial filaments	Xenogeneic	Scolymia cubensis - S. lacera, see Lang, 1971, 1973.
Non-contact	Overtopping	Growth rate and form dominance via shading	Xenogeneic, but may be allo-isogeneic	Diploria sp. - A. cervicornis. Shinn (1972). Acropora - Montipora Connell (1973).

These properties of the polyps may allow a partial solution to the problem of which path the evolution of the polystomous meandroid corals may have taken. These corals could have originated from either cerioid or solitary flabellate growth forms (Wells, 1956). If they evolved from the former, then they would represent 'the highest level of integration known in the zooantharian corals' according to Coates and Oliver (1973). If all coral polyps are as independant as those of Acropora then it would seem more likely that the polystomous condition arose from a single polyp, by the insertion of more mouths, rather than the fusion and loss of polyp walls if derived from a cerioid form. However, Lang (1971) observed that the solitary monostomodeal coral Scolymia cubensis (Milne Edwards and Haime, 1849) occasionally formed both skeletal and tissue fusions with larvae that settle nearby.

In a discussion involving individuality, it would seem that the polyp is the individual, especially in the solitary corals, e.g. Fungia. In most organisms, individuals of a species are usually genotypically different in a panmictic population.

This is not the case with the asexual offspring of a fungiid anthocaulus, where the anthocyathii are isogeneic. One polyp on an acroporan colony will be isogeneic when compared with another as they also have been formed vegetatively.

From the genetic viewpoint, the individual has a specific unique genome, i.e. an individual is 'the total product of a fertilized ovum' (Huxley, quoted in Hickson, 1924). With the possible exception of the fungiids, it is now possible to identify and map the genome of colonies on the reef flat using grafting techniques.

If Huxley's definition of individuality is accepted, then separate colonies of Acropora, formed as a consequence of regrowth following cyclonic fragmentation of a single colony, must be considered one individual. This definition of individuality is not a practical one, any more than it is to regard a single polyp of an Acropora colony an 'individual'. This latter case is made even stranger when the highly perforate nature of the skeleton and the way in which all the polyps are functionally connected via coelenteric canals is considered.

Following the lead of Hickson (1924) the individual in a sessile colonial animal must be regarded as the spatial colony itself, and all the components of the colony must be structurally interrelated by living tissue.

Although acroporan polyps exhibit individuality, they are not the individuals; that distinction is reserved for the colony alone.

The definitions do, however, become philosophically intractable when widely spread isogeneic material from apparently separate colonies, grow together to form one united colony.

Hildemann et al. (1977a,b) found that allografts of Montipora verrucosa showed an accelerated reaction to a second graft with the same colony, but showed a variety of time responses to a third party allograft. Such experiments indicate that the inducible 'memory' component of the immune system characteristic of higher animals is present at the Coelenterate level of organisation. For M. verrucosa they reported that fusion of the coenenchyme occurred in 3 - 5 days (observed by stereoscopic examination at the interface of direct tissue contact) and that the allograft separation occurred by

cytotoxic reaction in 16 - 20 days.

Extensive observation of Acropora formosa allografts using steriomicroscopical and histological techniques failed to show the initial fusion even where tissues were in direct contact. At no stage was there any nervous transmission between the allogeneic tissues, as judged by the non-transmission of waves of tentacular contraction across the junction. The paling of tissues and rounding up of damaged tissues that were observed by about the sixth day in A. formosa were considered to be the initial signs of the formation of an epithecal morphology, a consistent feature noted at all mature allogeneic interfaces.

It seems likely that there are two stages to a recognition reaction. The first is a very quick assessment of the contact, and an immediate classification into 'self' or 'non-self'. If 'self' is recognised, then fusion is immediately initiated. However, if the 'self' is polyp tissue, either surface properties or growth gradients inhibit fusion. It is an obvious disadvantage to a coral if polyp tissues have a tendency to fuse with any isogeneic surfaces they touch. As polyp tissues are effectively the only moving part in a colony, and therefore the most likely to encounter this problem, they need this contact inhibition protection. For the coenosarc tissues, it is advantageous to unite with any isogeneic surface that they encounter, either by growth or accident.

If 'non-self' is recognised, then a slow quasi-immune recognition process is initiated. Fusion is immediately blocked, and a contact indifference situation is maintained until further classification of the foreign surface is possible. Nematocyst action is

discussed later. In the case of an allograft, the outcome of this immunological refractory period is a reorganisation of the tissues giving rise to a holdfast morphology. The key structures that are formed are a new meristematic zone and an epitheca. Small areas of necrotic tissue may be formed as a consequence of both mechanical damage and cellular rearrangement. Occasionally, such areas lead to infection and further tissue damage. The speed of the rearrangement will depend on the previous encounters with the foreign tissue, i.e. immunological memory.

The functional significance of immunological memory to allograft coral interactions is hard to explain. The amount of growth a coral can achieve in the time saved by accelerated responses (approximately 12 days, Raison et al., 1976) would appear minimal when compared with the eventual result of an allogeneic interaction. The significance of this response most probably lies with the tissue and their responses to other challenges rather than in the short term coral growth interactions that have revealed these properties.

An interesting question that arises from this work is the extent to which memory is communicated to other areas of the colony. Experimental work was confined mainly to one branch. It is not known if this memory is related to the longevity of an 'exposed' cell or if there is an ability to pass this 'learned' information onto other cells. No information is available on the lifespan of individual coral cells or of the immune responses. If information is not transmitted around the colony, then memory will only be significant in one local area of repetitative contact.

While agreeing with Raison et al. (1976) that 'Histological

investigation at successive stages of allogeneic reactivity in Montipora is needed', from the histological investigations of Acropora this would need to be at the electron microscope level of cellular fine structure. Recognition seems to be a general function of coenosarcal epidermis, and is one of cell to cell identification, well below the resolution of light microscopy.

There are many organisms that can remain in close contact with corals and are not affected by nematocysts. Some of these prey on the coral tissues or may be juveniles of adult animals which are capable of burrowing into the corallum sub-structure (Robertson, 1970; Knudsen, 1967; Bouligand, 1966). A review of these nematocyst immune animals reveals that there are representatives from nearly every main taxonomic group. The question remains 'why are they immune and why do corals kill and capture only specific food?'. It seems probable that in most cases when an organism has persistent contact with a nematocyst bearing surface that rather than becomming immune to the stings, a method of recognition is adopted that does not permit nematocyst discharge in the first place. Picken and Skaer (1966) state that nematocysts are independent effectors in which 'the threshold and retention may be modified by other factors'.

The method of camouflaging oneself as 'self' so that nematocysts are not discharged has been investigated in the anemone-clown fish association by Mariscal (1971, 1970) and Schlichter (1972). The subterfuge is probably accomplished by the fish acquiring a surface coat of mucus from the anemone. The mucus or substances that it contains, effectively inhibit the nematocyst discharge. It appears from their analysis of removal and replacement of fish from one

anemone to another that a fish acclimated to Anthopleura elegantissima (Brandt) has immunity to that species as well as to A. xanthogrammica (Brandt); however the reciprocal acclimation does not confer any interspecific immunity.

The discrimination of nematocysts would thus appear to be similar to those shown in coral tissue interactions, and may overlap in some areas.

Corals will apparently only use nematocysts on one another in the extracoelenteric digestion reactions shown in some xenogeneic interactions. Most of the natural and experimental grafts in this study were confined within the order Scleractinia. The one unusual interaction involving a non-scleractinina that was studied was of a hydroid found ramifying through the tissues of Acropora formosa. No exceptional responses were detected in the coral as a result of this foreign tissue invasion.

As the hydranth structure had numerous feeding tentacles with nematocysts, giving the host coral a piliferous appearance, it is probable that the relationship of coral to hydroid was one of commensalism rather than parasitism. If further specimens are located, this relationship will be clarified.

FIGURE 24

Methods in which grafts were constructed in branching corals.

(a) Terminal-Terminal Lateral Graft

(b) Terminal-Reversed Terminal Lateral Graft

(c) Basal-Terminal Lateral Graft

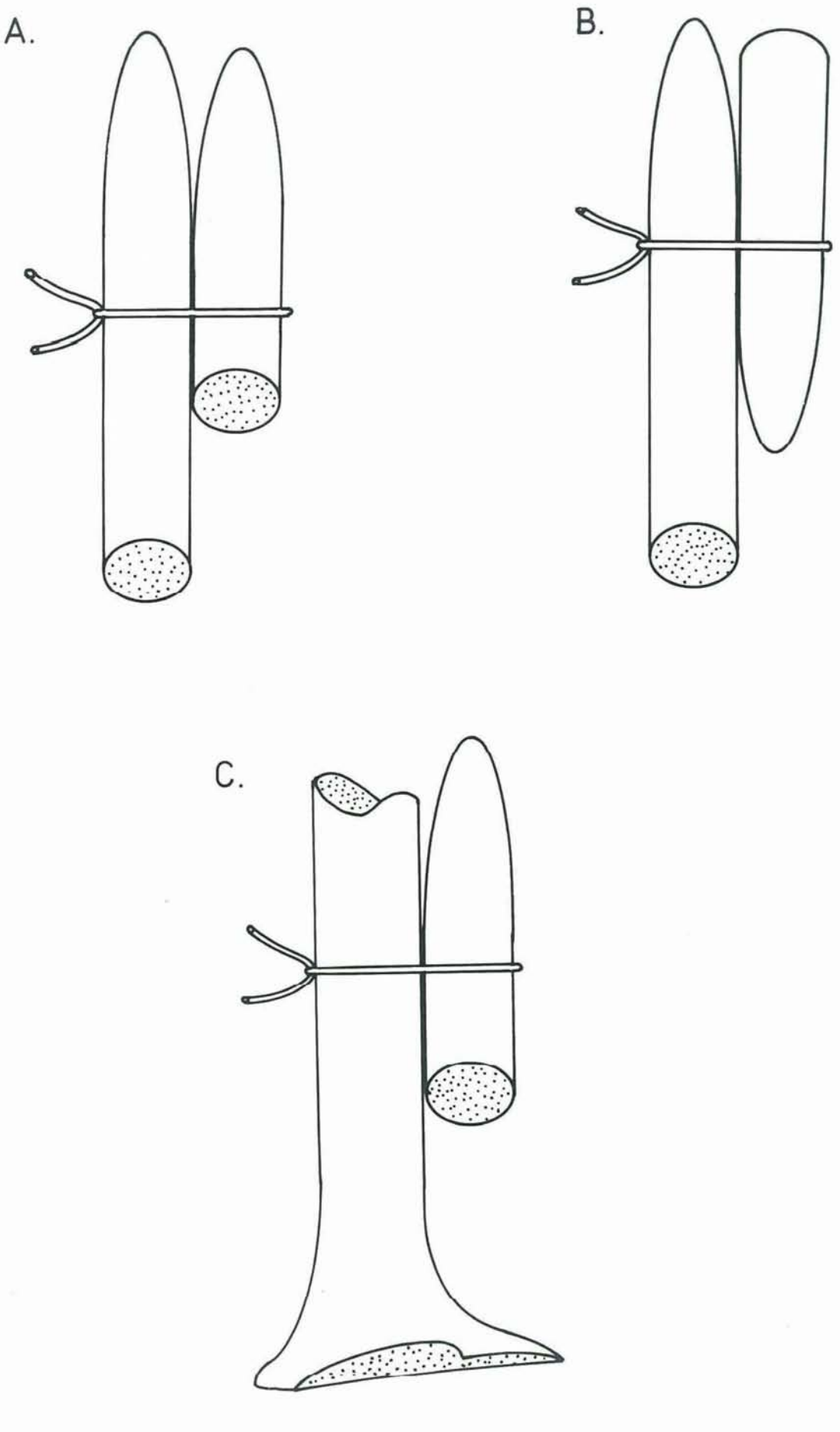

A.
B.
C.

FIGURE 25

Isograft skeletal morphology.

(a) Isograft of 2½ months.

Note that the suture of monofilament has been covered with skeleton, and the extent of the infilling between donor and recipient. Magnification x 3.5.

(b) The complete infilling of an isograft junction with undifferentiated coenosteum. Magnification x 9.


A

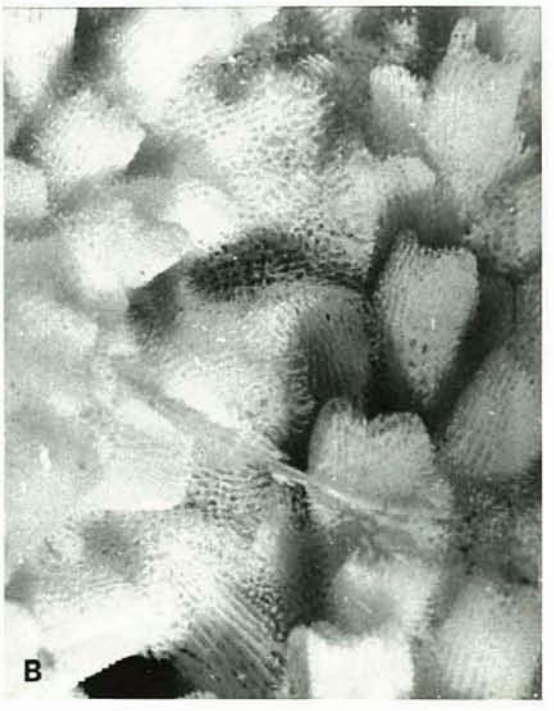

B

FIGURE 26

The formation of numerous small calices at the junction of an isograft.

(a) and (b) show a line of small polyps in the junction of an isograft. Magnification x 4.

(c) Small, numerous calices in the axil of a branch. Magnification x 2.

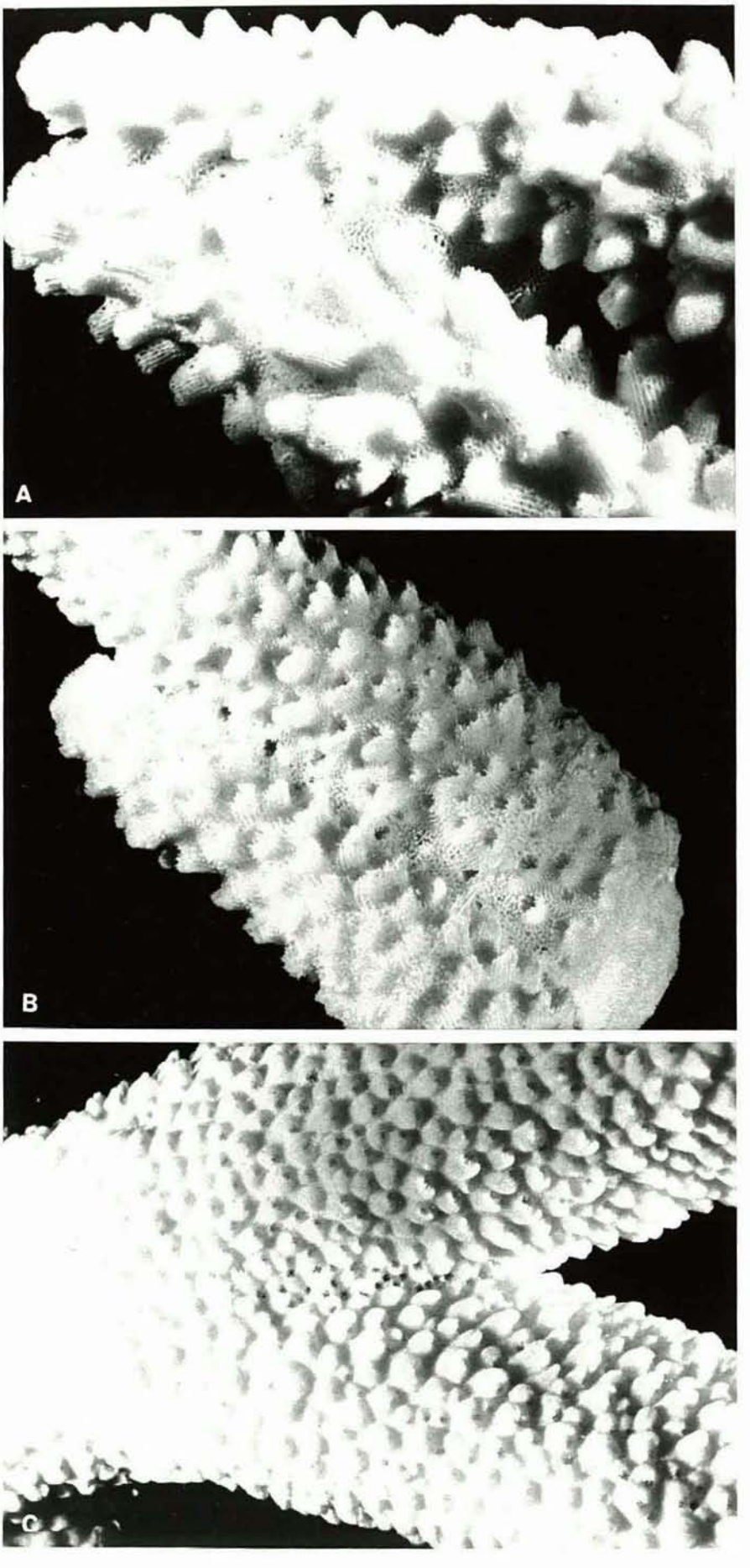

A
B
C

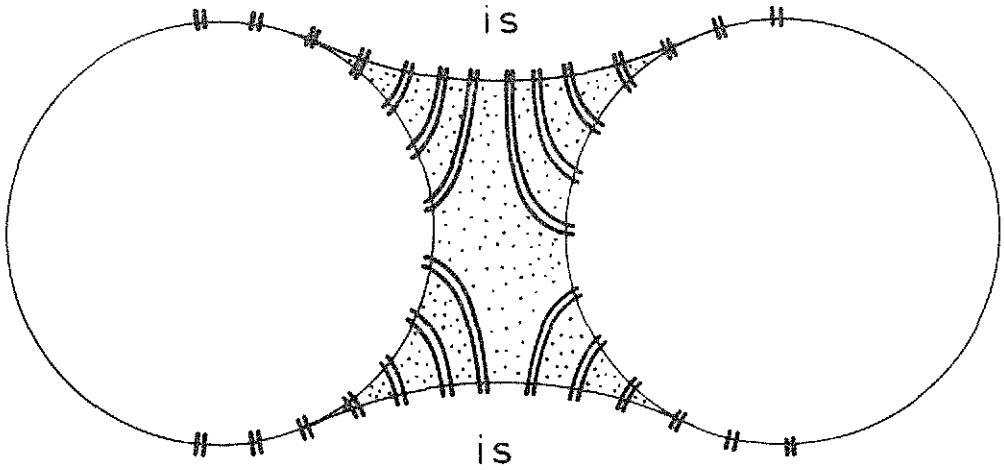
FIGURE 27

Diagram to illustrate the formation of numerous polyps in graft junctions and in branch axils.

(a) As the infilling proceeds, polyps alter their orientation and remain normal to the newly formed surface. The new surface has less area than that around the original branch and the polyps become more crowded, as there is a strong tendency for them to persist once they have been formed. The concentration effect becomes more pronounced if the branches are closer together.

(b) A similar process to that described above occurs in the axils of branches as a result of the growth from the surface a-a to b-b.

A.



B.

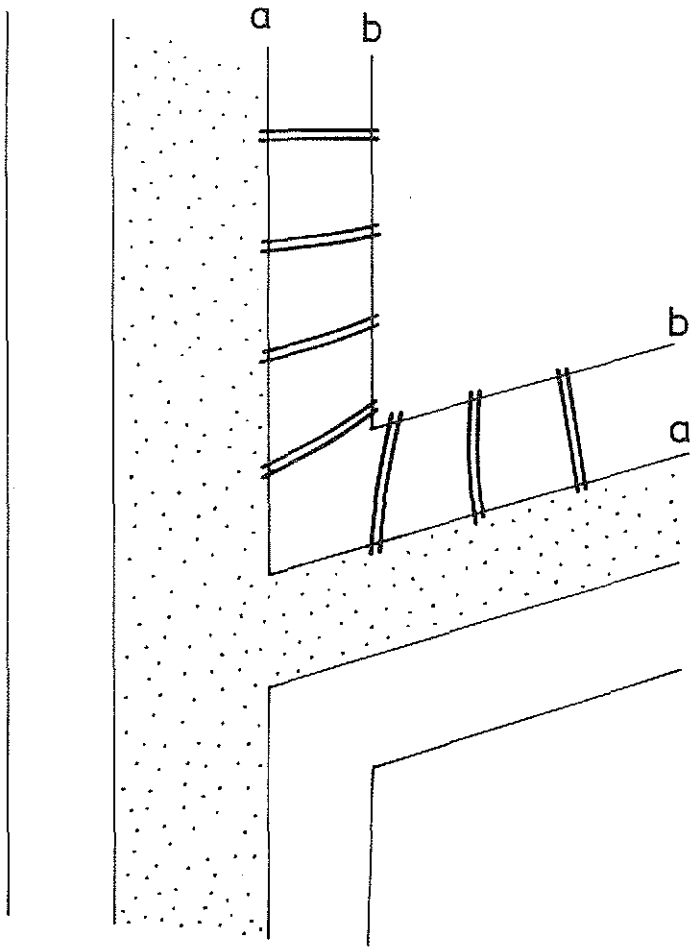


FIGURE 28a

Xenograft between Acropora formosa and Montipora ramosa.

This is a good example of the long term result of a xenograft. The reacting tissues are separated by a gap of 3 - 4 mm and at least one coral has formed a holdfast pad morphology. This specimen is unusual as Montipora ramosa more usually contributes equally or more material to the mutual holdfast region. In this specimen the M. ramosa suffered a heavy initial infection. Age of graft 3 months. Magnification x 2.4.

FIGURE 28b

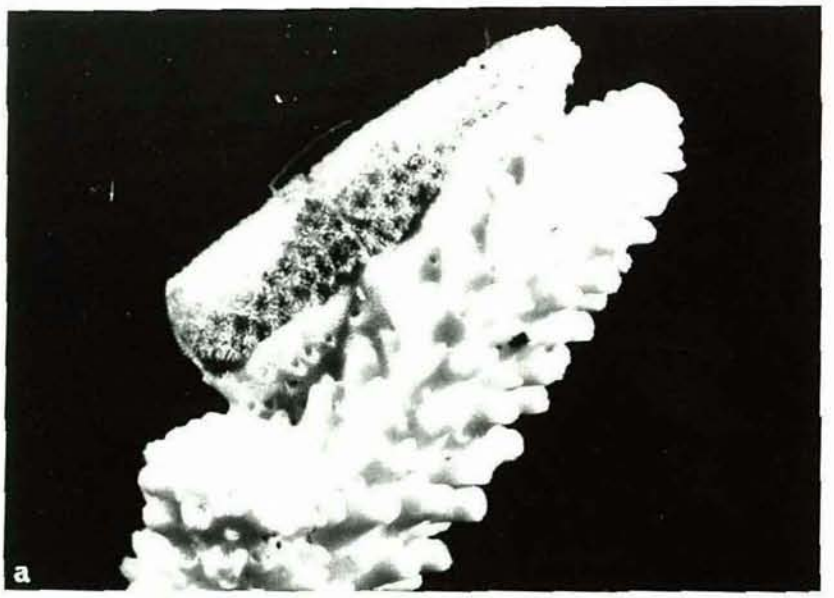
Xenograft between Acropora formosa and Acropora tenuis.

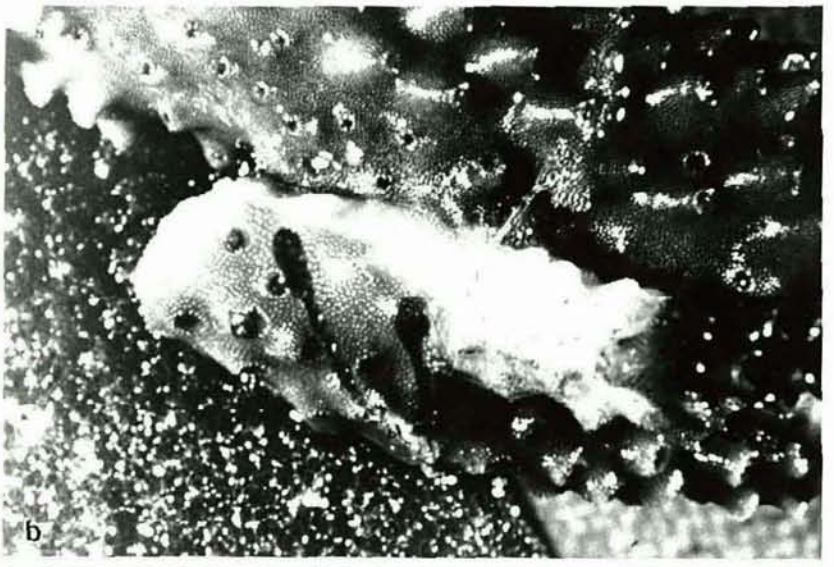
This living specimen shows incompatible reactions following grafting at about five weeks. Coenenchyme has partly covered the nylon suture. The upper A. formosa has started to overgrow the exposed coenosteum of the A. tenuis, whose regressing tissues have a marked epithecal rim. As the A. formosa tissues advance, a concomitant withdrawal of the opposing tissue is to be expected. Magnification x 5.

FIGURE 28c

Isograft of Acropora formosa.

This specimen shows no signs of any soft tissue derangement in the graft zone. The zooxanthellae are present in normal numbers and any temporary scar tissue has been overgrown. Graft is five weeks old. Magnification x 2.5.


a


b

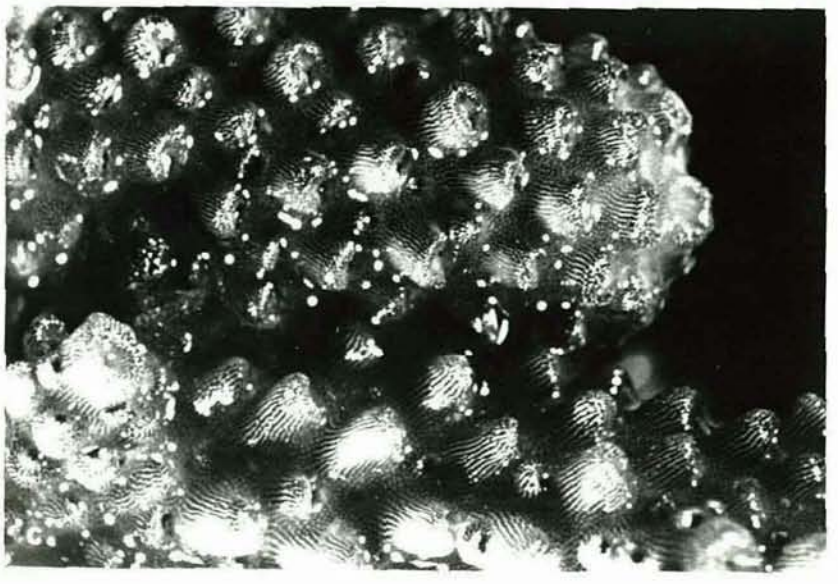
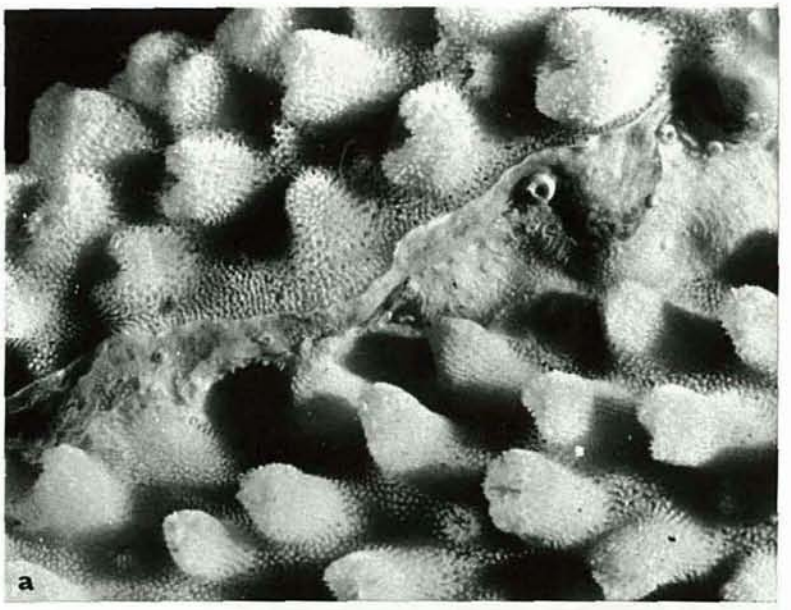


FIGURE 29

Advantitious settlement in xenograft junctions.

(a) The separation of tissues by 2 - 3 mm has allowed the advantitious settlement of spirorbid polychaetes on the exposed skeleton. This type of settlement further supports the view that these tissues free areas are kept free by coral interaction that does not involve mesenterial filament activity. Magnification x 5.

(b) and (c) These views of the same specimen are of the inferior junction of Acropora formosa and Acropora hyacinthus of Figure 38. In this example, the settlement has been of oysters. It is interesting to note that once settled these bivalves have been able to encroach onto the coral. In (b) the growth banding in the epitheca of the underlying coral can be seen through the transparent oyster shell. Magnification (b) x 8.7
(c) x 5.9.


a


b

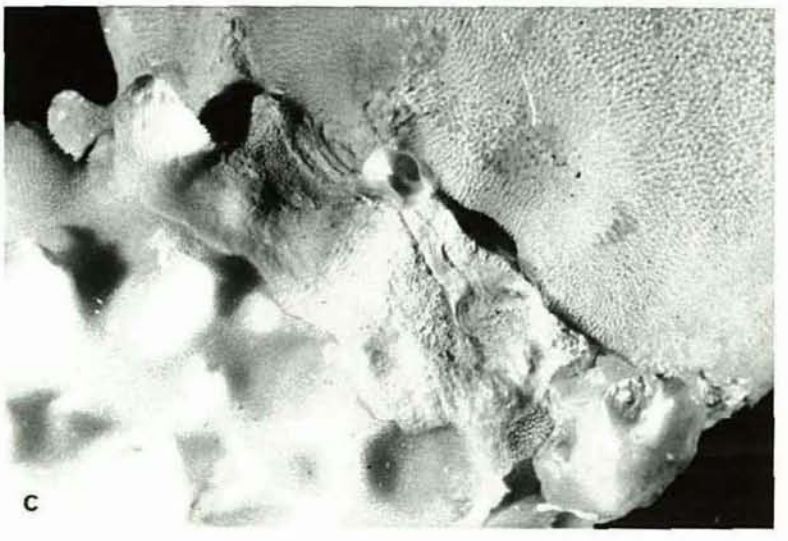

c

FIGURE 30

Histology of 4 day old Acropora formosa isografts at 22°C. Stained in Haematoxlin-Eosin.

(a) Low power view of an isograft junction, indicated by the line a-b. Magnification x 40.

(b) Higher power of isograft junction showing the trapped epidermal tissues containing nematocysts (n). Note also the disorganised tissue in the epidermal junction (ts). This view is of the 'x' side of the graft illustrated below. Magnification x 300.

(c) An isograft indicating the intercoelenteric communication c-c across the junction x-y. The cavities that contained skeletal elements are indicated by stippling on one side of the graft and cross hatching on the other. Magnification x 175.

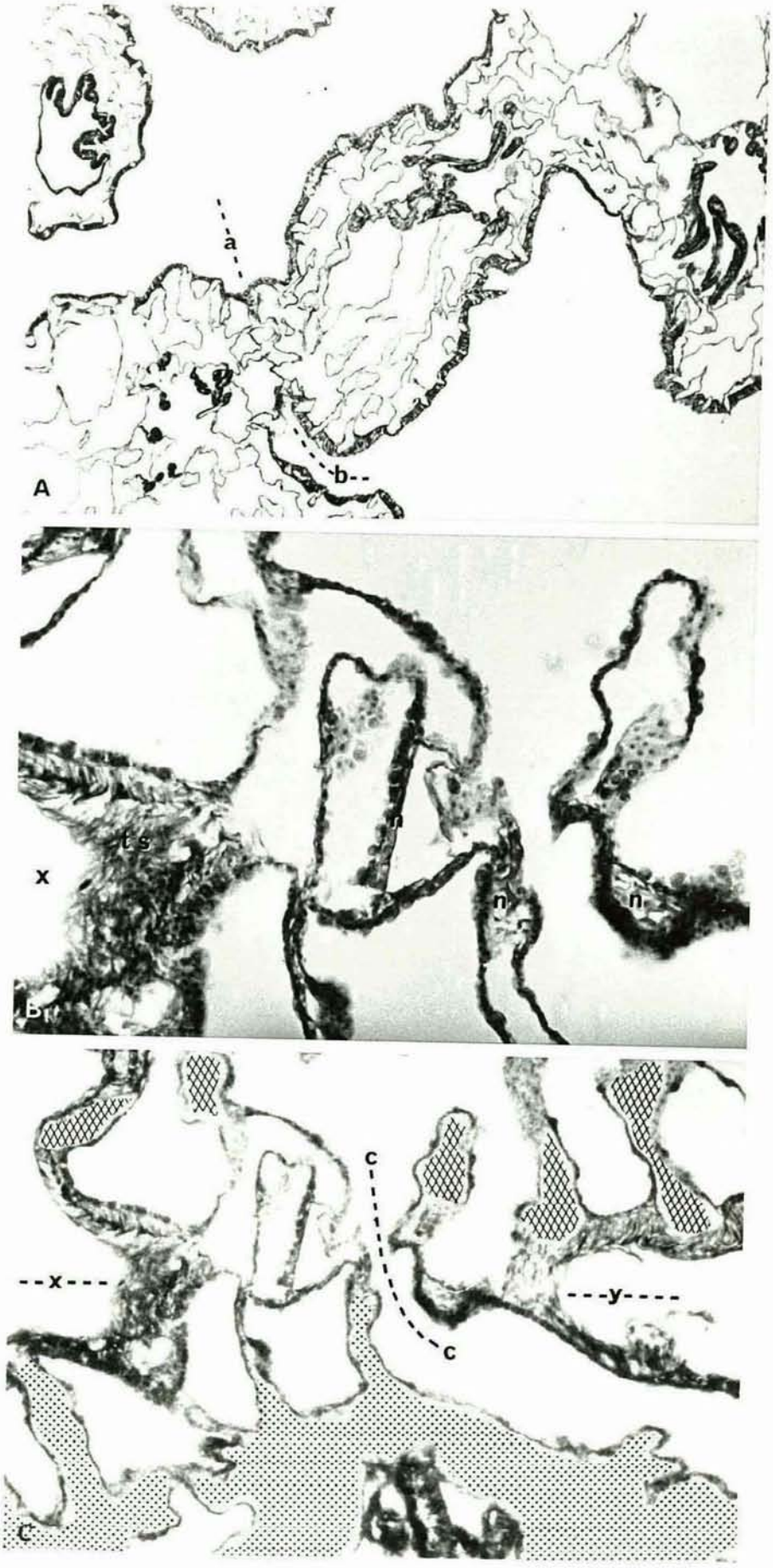

a
b
A
x
ts
n
n
n
B
c
c
x
y
C

FIGURE 31

Formation of temporary scar tissue in isografts.

(a) High power view of the 'b' side of the isograft junction of Figure 30a. The area of tissue over the skeletal spine (sk) has become necrotic (nt). Before tissue regrowth can cover this spine, an epithecal structure will have to be formed. Repair will be effected by the mutual growth of such structures from either side of the damaged area. These temporary structures will remain in the coral skeleton as coral tissues cannot resorb deposited skeletal calcium carbonate. Stain Haematoxylin-Eosin. Magnification x 320.

(b) Skeletal preparation of an isograft junction showing the formation of temporary epitheca (te). Magnification x 40.

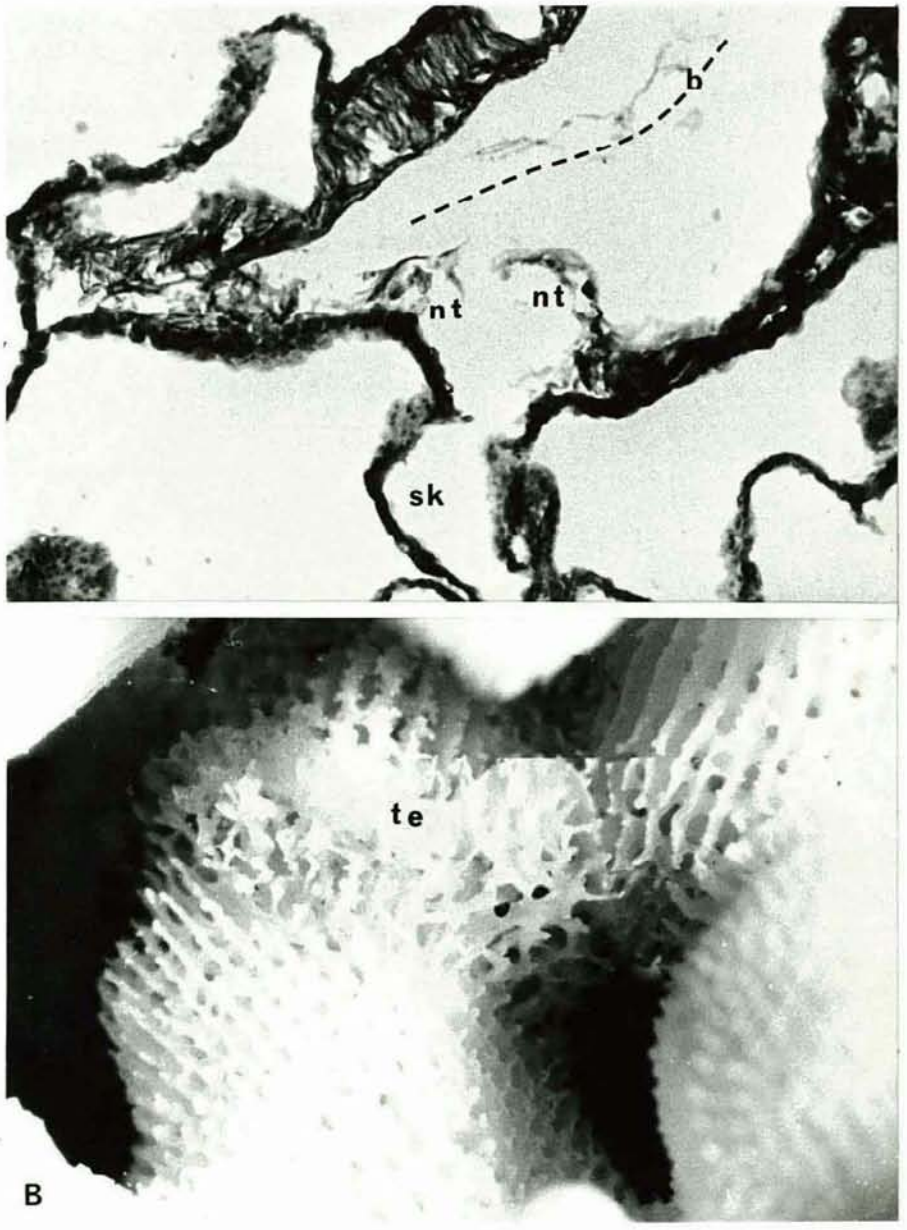

b
nt
nt
sk
te
B

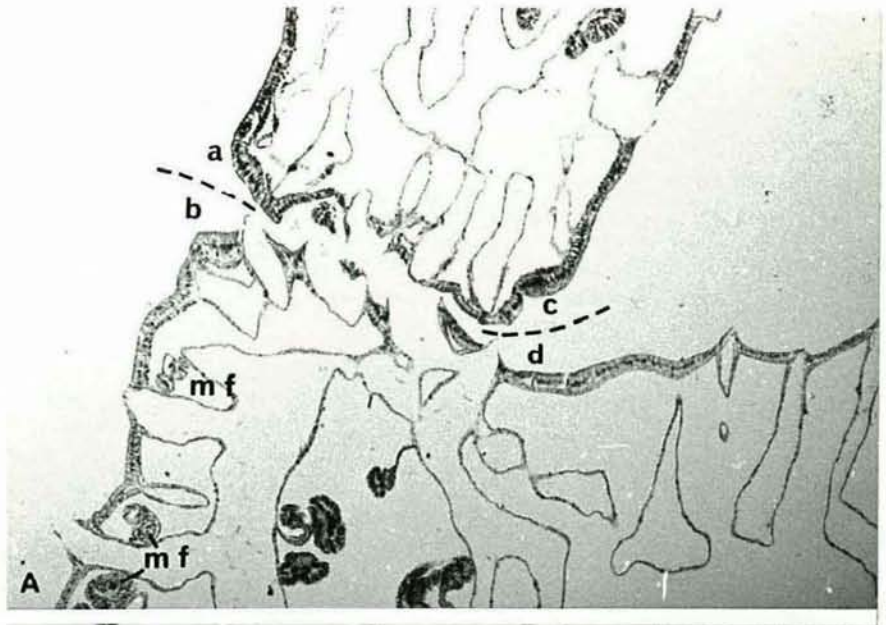
FIGURE 32

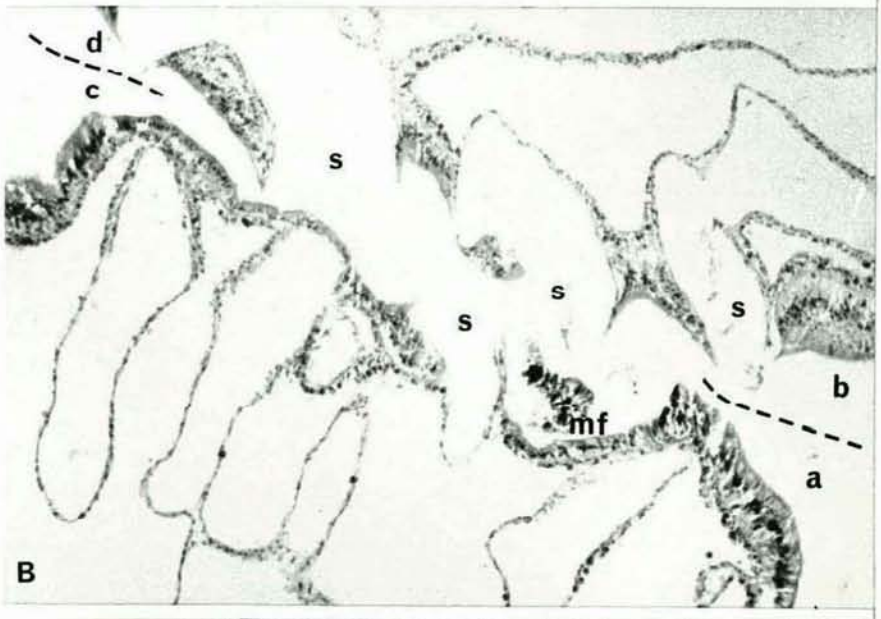
Histology of Acropora formosa allografts. Stained in Haematoxylin-Eosin.

(a) Low power view of a 4 day old allograft at 22° C. The opposing coral tissues a-c/b-d remain distinctly separate, with the tissue free spines maintaining the separation. Although mesenterial filaments (mf) are to be found in the vicinity of the junction, they do not take part in the cytological interactions. The tissues at the periphery of the graft are beginning to round up and form an incipient holdfast area. This is particularly evident at a, b, and c. Magnification x 51.

(b) Higher power view of (a) above. Note the skeletal spines (s). A single mesenterial filament is present in the graft zone (mf), but does not appear to be causing any digestive damage. Magnification x 140.

(c) Low power view of a 19 day old allograft kept at 25°C. This section cut at 4 µm to aid in cytological observations, and has poor contrast characteristics for photoreproduction. The graft junction between w-y and x-z is indicated. Skeletal spines (s) maintain the tissue separation. Holdfast pads are forming at the periphery of the graft. Tissue regression (r) is occurring in the graft junction.


a
b
c
d
m f
m f
A


d
c
s
s
s
s
b
mf
a
B

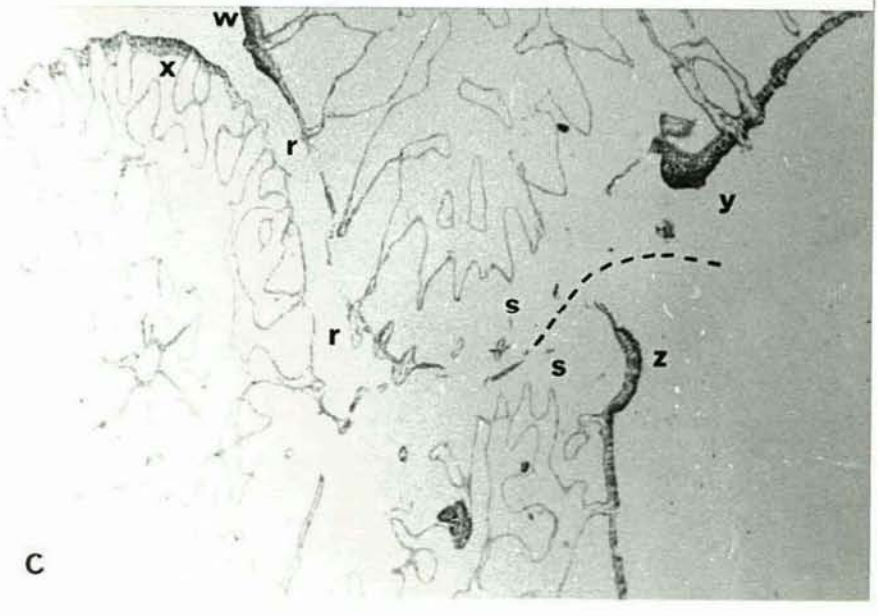

w
x
r
y
s
r
s
z
C

FIGURE 33

Histology of *Acropora formosa* and *Acropora pulchra*

5 day old xenografts, kept at 22^{o} C.

(a) and (b) Note the mucus and cellular debris in the graft junction, and the slightly further separation of the tissues. In graft (a), incipient holdfast pads are forming at a and b. The mesenterial filaments (mf), although in close proximity to the graft zone, are not involved in the cytological reactions. Stained in Haematoxylin-Eosin.

Magnification (a) x 33,

(b) x 37.

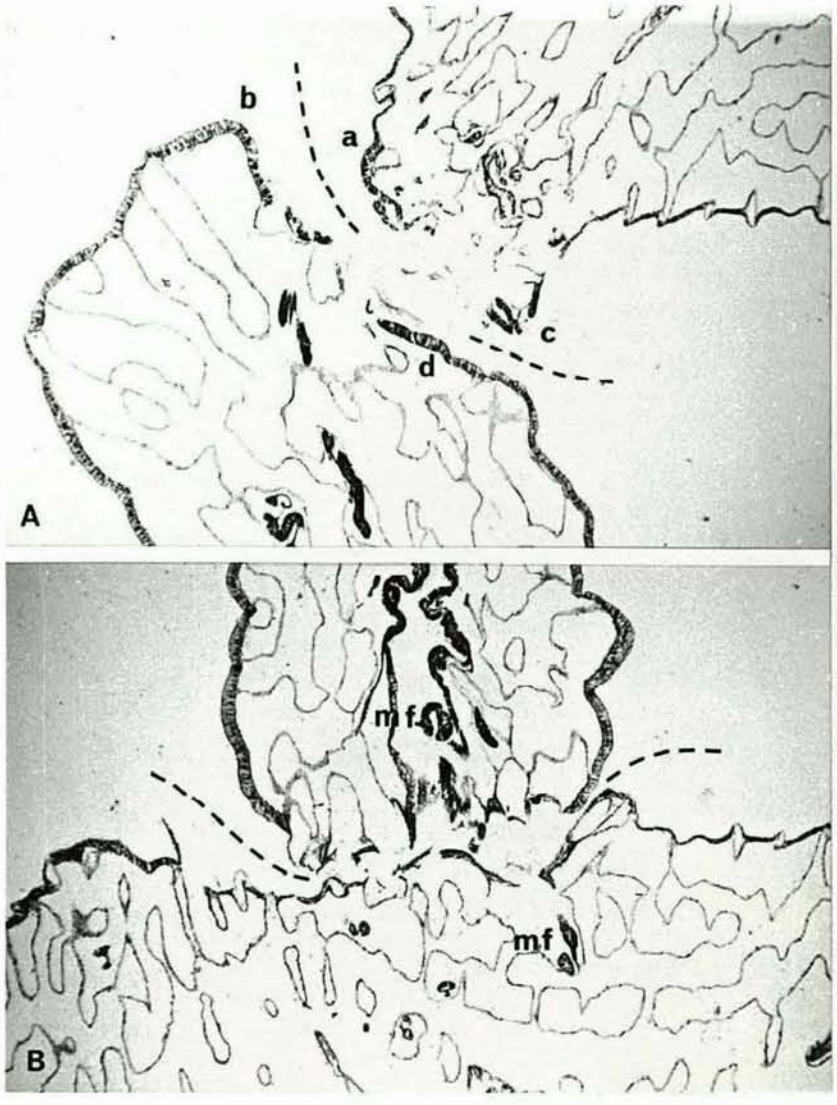

b
a
c
d
A
mf
mf
B

FIGURE 34

'Active sites' in Acropora formosa.

(a) and (b) Sections stained in Alcian blue at pH 2.5 and P.A.S.

sc = skeletal cavity

cc = coelenteric cavity

a = active sites

z = zooxanthella

g = gastroderm of calicoblastic layer

c = calicoblastic epiderm

m = mesogloea

Magnification (a) x 360

(b) x 1014.

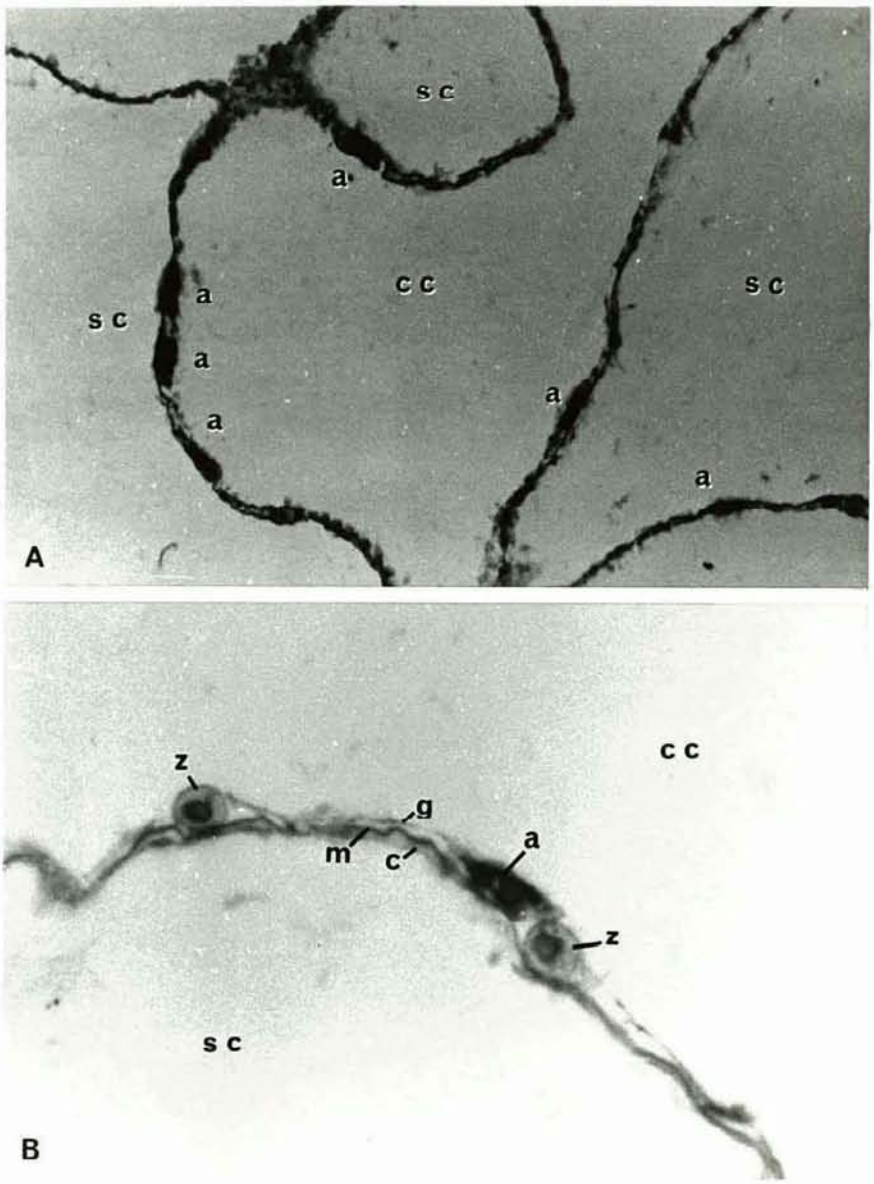
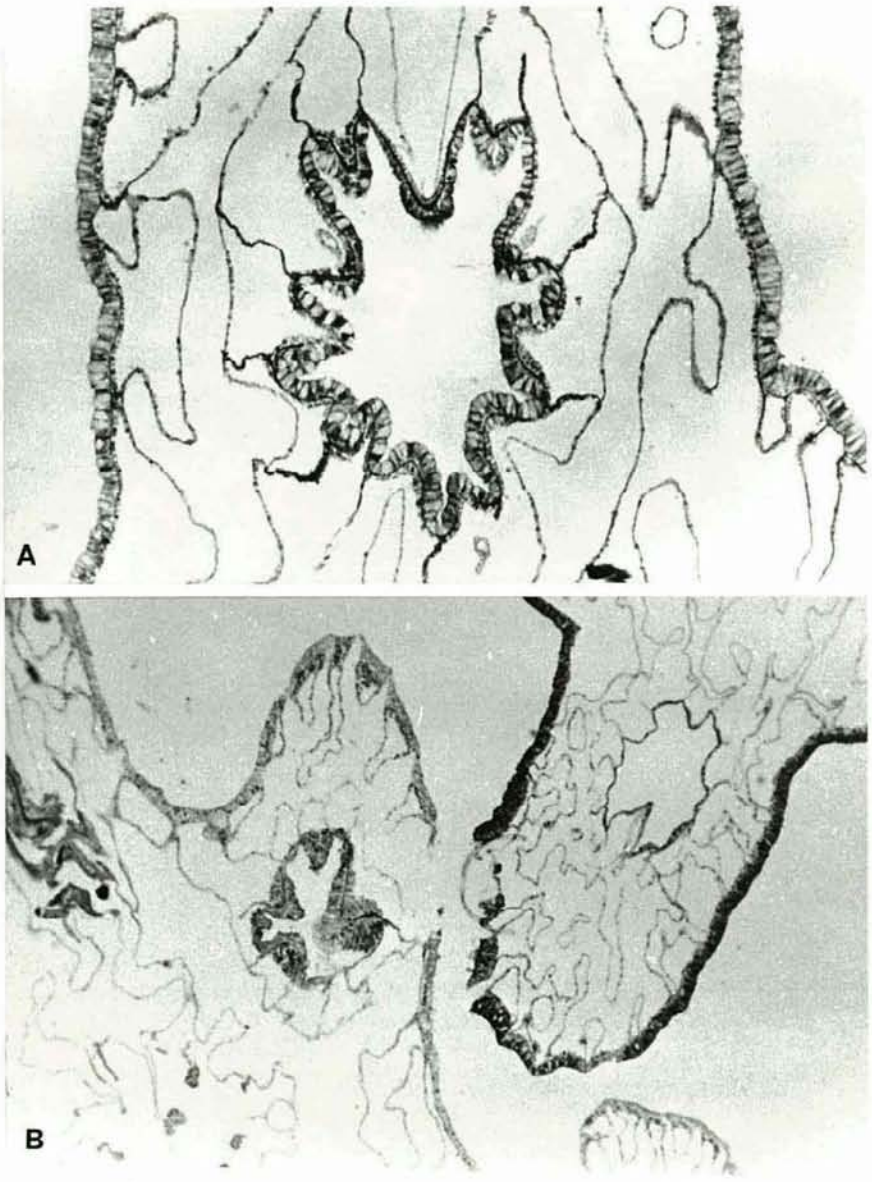

s c
a
c c
s c
a
s c
a
a
a
a
A
c c
z
g
m
c
a
z
s c
B

FIGURE 35

Variations in mucus cells and staining reactions.

(a) Stomodael section showing the enlarged number and nature of mucus cells that have occasionally been observed in some sections of Acropora formosa. Picro-Gomori stain. Magnification x 90.

(b) Section of a xenograft between Acropora formosa (right) and Acropora pulchra (left), stained in P.A.S. Note the intensity of staining and large number of mucus cells in the A. formosa. P.A.S. stains the locular network of the mucus cells, and not the inclusions. Magnification x 36.


A
B

CHAPTER VI NATURAL INTERACTIONS IN CORALS

(a) Introduction: Natural Interactions in Corals

(b) Results: Observations on some Natural Interactions

(c) Discussion: A Discussion on Natural Interactions

(a) Introduction: Natural Interactions in Corals

Natural interactions between corals in a crowded reef environment are commonplace. Reports of interactions have been made by Lang (1971, 1973), Hildemann et al. (1974), Hildemann et al. (1975), Roos (1971), Yonge (1963) and Whitfield (1901). The anastomosis of intracolonial branches is a normal consequence of growth for many branching forms occurring as a specific characteristic, conferring considerable structural advantage to the coral. However, the separation of the growing tips and the expansion of the branches of the colony into illuminated free space appears also to be a morphological dominant feature of the corals.

The growth of a colony may bring it into the sphere of influence of another colony, i.e. heterogenetic interaction. Interactions can proceed in a variety of ways, extracoelenteric digestion (Lang, 1971, 1973); contact avoidance, allogeneic contact incompatibility, chronic xenogeneic incompatibility and acute intraspecific/inter-specific aggression (Hildemann et al., 1975); and the 'non contact' overtopping (Connell, 1973; Maguire and Porter, 1977). The orderly and controlled genetic and environmental competition for space shown by individual branches of the same colony contrasts directly with the confrontation that is characteristic of inter colony competition.

There can now be little doubt that the violent intergeneric extracoelenteric digestion and the insidious 'overtopping' inter-action control many aspects of competition for space within a reef coral population once settlement has occurred. Intergeneric interaction has now been well documented and it is therefore with the intraspecific interactions that this section is more concerned.

Two quotes from Connell's (1973) review on the population ecology of reef building corals are particularly pertinent in that . . . "it is probably safer to conclude that most coral colonies on a reef have grown from a single larva, although some instances of fusion undoubtedly occur". Later he states as an alternative hypothesis to explain irregular recruitment of Porites in a study patch . . . 'that planulae are not carried far in the plankton, or that they choose to settle near others of the same species'.

It is the positioning of the planulae on the reef as well as the growth of the colony that predetermines the subsequent interactive sequence. Von Koch (1892) noticed colonies derived from more than one planulae and called such colonies 'aggregates'. He defined such aggregated colonies as those which have been formed through the secondary fusion of individuals that were originally distinct, thus distinguishing them from most other Anthozoan colonies which are produced by the budding and fission of a single individual. The coral with which he worked, Balanophyllia, is normally solitary. Some of his specimens showed that fusion may occur if planulae settle in close proximity. As the basal regions of the fused corallites were separate, the fusion must have occurred after settlement. One specimen was derived from four individual planulae and as the corallites were of a similar size they were presumed to have settled practically at the same time.

In another solitary coral, Caryophyllia, Lacaze-Duthier (1899) found aggregated colonies, but generally the corallites were of unequal size, suggesting a sequential settlement of planulae. Duerden (1902) describes a similar unequal size aggregation for Manicina. His analysis of aggregations in the Silurian rugose

coral Streptalasma led him to the conclusion that 'the corallites of the different colonies were dissimilar in size, but the members of any one colony are practically equal'. As in the case of Von Koch's Balanophyllia, this suggests that the aggregations are derived from synchronously settled planulae and do not bear the relationship of parent/offspring that is possibly the case in Caryophyllia.

Duerden (1902) induced the formation of aggregated colonies by rearing the planulae of Siderastrea radians (Pallas, 1766) under crowded conditions. The synchronous settlement of planulae in close proximity formed large aggregate colonies as they flattened out and formed polyps. Aggregates of up to 32 individuals were observed. He does not record if the aggregates were formed by planulae from only one parent colony. After observing numerous aggregates in Siderastrea, he concluded that the growth of individuals of an aggregate colony is slower than single individuals, but that given favourable conditions, the primary crowding would be outgrown and a normal colony would subsequently be formed.

Yonge (1935) has shown that, in Manicinia at least, the overall shape of the colony is not affected even if it has arisen from three separate planulae. It is possible to detect the original number of planulae contributing to Manicinia by counting the number of separate valley systems, as each will relate to a single, often branched, self contained valley system. Yonge considers that it is important for aggregates to maintain the overall shape of the colony as, in this coral in particular, such a regular shape will aid survival if buried in sand.

Boschma (1929) described aggregated colonies derived from the close settling of planulae in Maendra (= Manicinia). He states that 'with the exception of one instance, the aggregated colonies always remained separated from one another by a distinct theca' and that 'this would probably have remained in the further development of the colonies'. This results in the formation of, for example, two colonies that 'form a separate entity, though the skeleton is fused with that of the other colony'. He then cites the example of Manicinia areolata (Linnaeus, 1758) figured by Wilson (1888) Plate I, as an existence of this type of aggregation, the theca existing in the skeleton as a distinctly visible ridge. Yonge's (1935) specimens would appear to be very similar to that of Wilson's (1888).

This raises the important question: does the soft tissue completely fuse in aggregated colonies, or is it just the fusion of the skeleton in close juxtaposition? Undoubtedly in the previously mentioned examples skeletal fusion had occurred, but there is a distinct lack of information on the condition of the soft tissues in the junction region.

Duerden (1902) states that with Siderastrea, 'I could not assure myself that interconnection was established. The intervening external epitheca would for some time interfere with such a possibility, but no doubt this structure, along with the basal plate, would be left behind in the upward growth of all except the marginal polyps'. He did not rear his corals long enough to show this.

Edmondson (1929) comments on the 'fusion' of planulae of Dendrophyllia while still swimming. His illustration indicates that the planulae and the colonies that they give rise to are not of the

fused type but aggregated, i.e. the soft tissues remain distinct. In contrast to Duerden, Edmondson states that if aggregation occurs 'it is obvious that a coral colony would develop more rapidly than if it originated from a single planula'. A conclusion greatly opposed to Duerden (1902). Again no experimental evidence is invoked to support this proposition. His statements concerning the 'fusion' of colonies of the same species, Porites evermanni (Vaughan, 1907), where 'the calicules of the two specimens merge and fused perfectly at the point of contact', again refer to the skeletal structure and do not refer to the state of fusion of the soft tissues. Although the specimen is perfectly united, his illustration (Plate IV, 1929) shows a distinct line of dissimilarity between the two colonies. It is interesting to note that he describes a more distinct suture consisting of a curved and compressed ridge between colonies of different species, viz. P. evermanni and P. compressa (Dana, 1846).

It would appear probable that soft tissue fusion did not occur in Edmondson's specimens.

Stephenson (1931, page 124 et seq.) refers to aggregated settlements of planulae in Pocillopora, where on flattening and coming into contact 'the fusion becomes so complete, both as regards flesh and skeleton, that the independant organisms become one'. This is the first conclusive statement on the condition of the soft tissue in aggregated colonies. Later (p. 130), Stephenson refers to the skeleton of fused colonies stating 'the skeleton (but not the soft parts) of individual polyps may lose their identity altogether ...' and referring to Porites (p. 133) he mentions the rims (epitheca ?) that 'may or may not occur between the fused corallites' and that

this variability 'should not be the case if the presence of the rims is a specific feature'. His plates of Pocillopora show similar somewhat variable rims.

(b) Results: Observations on Some Natural Interactions

The branching and space filling mode of growth shown by arborescent species of Acropora promote frequent extra-colonial contacts as the branches grow and intersect those of other colonies (Figure 36). Numerous inter- and intrageneric contacts will thus be made, e.g. Figure 37, an intergeneric natural xenograft of Acropora formosa and Galaxea astreata (Lamarck, 1816). A typical xenograft morphology is exhibited, with a wide tissue free zone separating the recent epithecal deposits by 2 - 5 mm. This graft is certainly older than six months and possibly much older. The initial advantage favoured A. formosa which had formed a holdfast pad of normal proportions. An incipient holdfast morphology is evident in the flared epithecal edge of the G. astreata and a small area of recently dead A. formosa holdfast can also be seen (Figure 37b).

Natural interactions that may have been proceeding for two or more years have been observed, and as a general conclusion, no excessive overgrowth that could not be interpreted as a normal property of that particular coral, has been observed.

Figure 38 is an elegant illustration of this point.

The horizontal growth of the Acropora hyacinthus (Dana, 1846) has intersected the vertical growth of A. formosa. This specimen unfortunately broke above and below the interface during collection. Although the lateral extension since the intersection was made is approximately 11 cm, the extension of the A. hyacinthus in the vertical plane along the A. formosa stem has not exceeded 2 cm in either direction. The latter coral had a diameter of 12 mm when it was enclosed and further growth was prevented (see Figure 39). It is interesting to see that since that time the superior part of the A. formosa colony has increased in diameter to 16 mm. The inferior piece, some 6 cm in length between the junction and its dead base has, however, not significantly increased in diameter. The increase in diameter of the superior part from 12 to 16 mm is equivalent to an extension upward of 5.7 cm (using the method described in Appendix 1). The superior junction of this natural graft is shown in Figure 39b. It has the usual xenograft morphology, viz. a separation of tissues bounded by a distinct epitheca and a holdfast structure of undifferentiated spinulous coenosteum. It is interesting to note that the inferior junction (Figures 39c and 29c) was not so 'tight' and consequently room was left for the advantitious settlement of oysters. The actual age of this graft is not known, but as the collection locality (Feather Reef, 17^{o} 30'S: 146^{o} 25'E, near Innisfail, Queensland) is on the Barrier Reef proper, rapid growth of these species could be expected. The graft certainly has an age in excess of six months.

As the growth style of massive corals is more compact, interactions are usually peripheral. Goniastrea aspera provided a useful supply of such interactions, which contrast well with the branching

corals. This coral is particularly abundant on the reef flats of Magnetic Island (Figure 40c) and as such represents the dominant coral in many areas. Its distribution within the low intertidal zone (see Figure 4) is often patchy, ranging from 4 to 38 colonies per 25 m^2 on the Nelly Bay reef flat adjacent to the transect line A-B (see Figure 3) prior to Cyclone Althea (24.12.71).

Within the north eastern breakwater wall of the Townsville Harbour, numerous colonies of G. aspera are located on the rocks below a level corresponding to 0.3 m above local datum (0.1 m below MLWS, see Figure 4). The distribution of colonies within the Harbour is slightly lower than that seen on the reef flat, possibly due to the diminished effect of wave action behind the breakwater.

One consistent feature of the Goniastrea colonies in both locations is that a large proportion are composed of two or more intraspecific interacting groups, e.g. Figure 40a illustrating a 4 colony, and Figure 40b a 6 colony interaction. Only rarely on the reef flat is Goniastrea found interacting with other genera (Figures 41 and 45) and on the breakwater it is limited to intraspecific interaction as no other corals are present.

Many such Goniastrea composite colonies have been studied in the field and laboratory aquaria. The composite corallum of the three interacting colonies in Figure 42a was sectioned along its major axis, and a thin slice (17 mm thick) removed and X-rayed onto mammography film using the method of Isdale (1977). An analysis of this specimen (see Figures 42a, 43 and 44) reveals that there were three initial settlements, two in very close proximity, and the other some 8 cm away. The settlements were on the dead skeleton of

a Porites species. This initial settlement substratum has been noted on numerous occasions as the foundation for Goniastrea colonies. The closely settled pair grew in harmony to form a hemispherical head until growth allowed contact with the third colony. In both contact zones, an allograft morphology exists. The tissues did not fuse or have any functional histological contact. The transmission of waves of tentacular contraction in response to stimulation (Horridge, 1957) stopped at the allograft junction; e.g. the left hand side of the composite colony in Figure 42b, has been stimulated, while the right hand side of the junction remained with tentacles extended, thus revealing an otherwise almost invisible allograft (c.f. Figure 42c). The tissues thus show characteristic allograft indifference. A close examination of the skeletal junction reveals an epithecal ridge, its structure contributed to equally by both graft components. It is interesting to note that when an epitheca is formed in this way, it does not reveal growth banding (Barnes, 1972). This apparent single epitheca contrasts with the morphology found in the branching corals where a mutually formed structure appears to be very rare.

The X-ray reveals growth banding which indicate isochronous planes. An analysis of the growth of skeleton between the extant surface and such a plane shows that growth allometries attempt to preserve or establish a convex shape, characteristic of the species. The non-equivalence of growth allometries in the xenograft situation will not allow the apparent co-ordination of shape shown in composite intraspecific colonies. This co-ordination of shape occurs without histological fusion of soft tissues. The cilliary action responsible for the removal of silt and detritus from the colony surface is not co-ordinated, with the junction being treated as a free edge and

accumulating rolls of mucus deposited from the surface of either side.

Although not common, Goniastrea retiformis (Lamarck, 1816) can be found on the Nelly Bay reef flat. This coral also exhibits composite colonies (Figure 46). The X-ray reveals growth banding and a basal zone of regrowth over damaged coenenchyme which is a common feature of the reef flat corals. It is interesting to note that since this basal portion was killed, the main colony has extended 7 cm vertically and 4 cm radially. The regrowth zone has maintained a similar allometry by extending 1.6 cm radially and 3 cm proximally (i.e. in a vertical plane, but in an opposite direction to the main colony growth). The allometric ratios of radial to vertical growth are 1:1.75 and 1:1.88 for the main colony and the regrowth respectively. The settlement nucleus for this composite colony was again a Porites skeleton.

The periodic disturbance of colonies growing in shallow water and on the reef flat situation is common. The often short duration but very intense rainfall (over 300 mm/hour have been recorded by the Townsville Meteorological Office) associated with the summer monsoon often cause marked inshore salinity changes (see Chapter II). The wave action associated with cyclones along the Queensland coast (not necessarily those close enough to cause wind damage) will produce rougher than usual waves in areas such as Nelly Bay, and cause damage to reef flat corals. Figure 41c shows a head of Goniastrea aspera that has been dislodged and has regrown over damaged areas on at least eight occasions. The movement of sediment over the reef flat and the reduced salinities produce conditions that kill coral. However, such conditions eventually result in new surfaces for the settlement of

coral planulae. A coral showing such a history has been analysed in Figures 47a and 48.

The principles exhibited by this specimen are typical although the concentric shape of this specimen is in some respects atypical. Examination of the gross morphology showed a small isolated, allogeneic colony positioned on the crown of the main Goniastrea aspera colony. The corallum was sectioned along the major axis to reveal the internal morphology and hence history of the specimen.

The entire corallum had grown to a diameter of 12 cm and a height of 3 cm. It was proportionally flatter than most other G. aspera colonies on the Nelly Bay reef flat, when it was damaged probably by fresh water, along the line 1-1 on Figure 48. This killed the coral around the periphery and over the crown, leaving an annulus (AA, BB) of living tissue varying between 1.5 to 3 cm. This tissue subsequently grew to form the present upper surface. A further killing of tissue is evident along the line 2-2 on Figure 48, but does not have an homologous zone on the inner side of the annulus. At a time after the first killing of tissue (line 1-1) the allogeneic colony settled on the exposed corallum of the crown. As the growth of this colony has matched that of the main colony, and it does not have a wide basal zone, this settlement probably occurred when the annulus was closing to approximately 2 - 3 cm in diameter. The initial growth rate of the Goniastrea is not known so these figures must be regarded as approximations only. The subsequent infilling of the annulus by the inserted coral and the original colony have produced the form of Figure 47a. The graft junction and the behaviour of the soft tissues were entirely characteristic of an allogeneic relationship.

The specimen shown in Figure 49 was collected in a shallow pool near the edge of the Nelly Bay reef flat. In the field it was first thought to be a xenograft between a Porites sp. and an Acropora sp.. The absence of polyps over the surface of the dome-shaped growth, and the contiguous tissue contact with the recipient acroporan colony lead to the assumption that it was an isogeneic situation, possibly induced by a gall forming organism. Stereo-microscopical examination of the coenosarc revealed that there was a lower than normal concentration of zooxanthellae, but otherwise the tissues showed a normal morphology for undifferentiated spinulose coenosarc. The skeleton was prepared and sectioned across the growth. No macro-organism was found to have caused the isogeneic structure. An examination of the parent colony led to the conclusion that considerable damage had caused the crushing and removal of the branch tips, thus rendering positive identification of this specimen impossible. At the time of collection, the damaged tips had regrown some 2 mm. The sectioned coral showed that the concentric growth of the isogeneic material was centered on a level with the damaged tips, and it is probable therefore that this growth is a neoplasm originating from one tip that was induced by damage to the parent colony (Cheney, 1975). The neoplastic growth has a diameter of approximately 5 cm. Since damage, the tips have added approximately 0.018 cm^3 of growth while the neoplasm has added 49 cm^3. A volume ratio of 1 : 2700 results. The neoplasm has a structure that resembles a normal acroporan hold-fast region. In growing over other branches it has overridden and obliterated polyp tissue and normal growth morphology. The neoplastic growth ignores all the rules governing normal colony growth, and the usual iso-, allo- or xenogeneic interactions.

Two coral specimens of Acropora formosa (one from Feather Reef near Innisfail, the other from Nelly Bay, Magnetic Island) were observed in the field to be covered in a fine hair-like material. On closer inspection, this was found to be an extensive covering of athecate hydrozoan polyps. Histological sections of this material revealed extensive hydrorhizal ramifications between the calicoblastic ectoderm and the deposited skeleton of the coral (Figure 50). Although this basilary stolon superficially appears internal, it remains as an ectozoon both morphologically and possibly functionally. In many hydroids, the stolonary tissue is the only lasting tissue during adverse ecological conditions, and retains the ability to recover and form new colonies. The cover of live coral tissue affords this particular stolon considerable extra protection. The hydranths erupt through, and stand exsert, by 5 mm from the coral tissue layers. They usually appear adjacent to skeletal spines or protrusions where the investing layers are thinnest. There appear to be no exceptional responses by the coral to this penetration.

(c) Discussion: A Discussion on Natural Interactions

With branching forms of coral, the original settlement of the coral is obscured by the growth of the colony and often further complicated by extensive vegetative growth centres formed as a consequence of fragmentation. Natural interactions between different colonies are difficult to delineate as each contact point has to be carefully inspected to determine the type of interaction. In contrast to these branching forms, the massive coral Goniastrea aspera allows an easier analysis of the ontogeny of natural interactions. Although the intercolony distances of this coral may be greater than 2 m, a very high proportion of the population are composed of such closely settled individuals that extensive interactions are inevitable. Only rarely are the aggregates composed of more than one species and then it is usually an intergeneric aggregate rather than an interspecific one that is formed.

An expansion of von Koch's (1892) definition of aggregated colonies is needed to encompass recent findings:

Aggregated colonies are formed by the growing together of originally distinct individuals to form a composite growth. The secondary unit results from the settlement of a larva close to the original. There is no interconnection of tissue.

Fused colonies are aggregated colonies where the soft tissues are interconnected so that the coelenteric canals are in communication, with the components of the secondary unit showing complete co-ordination.

These definitions clearly delimit the loose association of

aggregation which may be no more than a growth phenomenon from the specific and distinct phenomenon of fused colonies, which obey the laws of tissue interaction based upon genetical relationships.

Stephenson's (1931) observations on the variable nature of Porites epitheca may be explained if both aggregated and fused colonies occurred together. The aggregated colony should have a fused skeleton, but no tissue fusion. This promotes the formation of a distinct epitheca. Elsewhere fused colonies would not exhibit epitheca within the colony system.

Aggregated colonies may be formed by the close settlement of planulae of the same species, but with little genotypic similarity. Fused colonies are formed planulae from the same species, considerable genotypic similarity, e.g. one haplotype in common.

An alternative suggestion is that coral planula larvae do not have a fully determined recognition system at the stage when they settle near other corals. The possibility of an invertebrate 'fraternal twin' situation, as described by Burnett (1976), has immunological implications that with present knowledge of corals does not allow further consideration.

Only aggregated colonies of Goniastrea have been observed at Nelly Bay and in the Harbour. If fused colonies were present, then they would have been readily identified in the sawn sections by the growth pattern of the calyces. These would radiate out from the initial settlement centres until they coalesced. No patterns of this nature were seen. All bi-, tri-, etc. composite colonies had distinct corallum and tissue discontinuities at their common junctions.

Porites skeletons have been observed to be the initial settlement sites for Goniastrea in Nelly Bay. Living Porites solida (Forskal, 1776), P. australiensis (Vaughan, 1918) and P. lobata Dana, 1846, were found in the Goniastrea zone, usually located toward the rims of small shallow pools and in areas where water is present even at low tide levels. Slight movements of sediment or excess rainfall may kill and thus expose clean Porites skeleton for Goniastrea settlement. Their narrow bands of substratum effectively concentrate the settlements and allow numerous intercolonial contacts as they grow.

The formation of Goniastrea colonies in the Harbour and Nelly Bay indicates that they are reproducing sexually, and that a steady successful recruitment of this genus onto these inshore reefs is continuing. It is interesting to note that Roy and Smith (1971) were able to show that ramose corals were more dominant in silty water in Fanning Lagoon (3^{o} 55'N, 259^{o} 23'W), while in the most turbid waters of the Barrier Reef a massive coral, such as Goniastrea, is the sole species.

At Goniastrea allogeneic junctions (Figures 40b, c) the opposing corals react to each other as if they were inanimate substrata. The irregular shape that the contact area often exhibits (Figure 47b) indicates that the growth potential of the interacting pair is balanced, and that the contact line is thrown either way as new polyps arise by intratentacular budding. Goniastrea aspera colonies in Nelly Bay develop to a diameter of 10 cm as hemispheres. Beyond that diameter (or length of maximum axis) the diameter to height allometry changes from 2:1 to 3:1. In the harbour, the colonies are usually flatter for a given diameter than those from Nelly Bay. Barnes (1973) and Jaubert (1977) relate a flattening of the growth morphology as an

adaptation to a lowered light intensity. As the mean Secchi disc value for the harbour is 1.6 (range 1.0 - 3.2) and Nelly Bay is 1.8 (range 0.8 - 6.3) this probably indicates that the observed flattening is in response to a lowered light intensity.

When two colonies of less than 10 cm diameter form an aggregate greater than 10 cm, then the further growth of the composite colony assumes the more flattened shape of a large colony. In the aggregate colony illustrated in Figures 43 and 44, the junction of the two closely settled colonies allowed an initial hemispherical growth, while the growth at the second junction is in the process of filling in to preserve a colony shape ratio of 3:1, related now to the 20 cm axial length.

These natural aggregation growth forms may be summarised by a growth rule 'aggregate colonies grow so as to preserve the natural colony shape'. Evidence from other coral studies suggest that this rule may be widely applicable (Yonge, 1935).

In xenogeneic interactions where different growth forms and allometries exist (Figures 41a, b), the eventual outcome of an interaction is that the one that grows quickest is the biggest! It is only if one of the corals exhibits the rare occurrence of extra-coelenteric digestion that the outcome is anything else (Lang, 1971, 1973). The spectacular nature of these interactions probably far outweight their ecological significance. The more usual interaction shown for instance by Goniastrea do not invoke any further mechanisms than those the coral have to use in normal growth.

Occasionally in arborescent species that do not usually show natural interbranch anastomosis, e.g. Acropora formosa, the growth

of one branch will intersect that of another, and perfect isogenic fusion of tissues will result. The whole area of contact then becomes indistinguishably unified with time. Agencies other than natural growth will allow intracolonial contact of branches. The breaking of branches singly due to the activity of larger reef animals, such as fish, or massive destruction by wave activity during storms, will allow intracolonial fusion interactions to occur. In the case of cyclone "Althea" described earlier, the distribution of the coral from one colony was probably very extensive, with isogenic material spread more than 200 m. The continued survival and growth of these fragments would eventually bring isogenic material into contact, resulting in the compatible fusion of apparently separate colonies. Such fusion will be identical with that obtained if a single branch was broken and laid across the rest of the colony. Storms and the activity of coral-rock boring organisms will often weaken the 'holdfast' area of a coral colony, and allow fracturing and subsequent transport to occur. In these branching forms, such disruption and disorientation caused the coral to re-orientate growth towards the light at a new angle. This has been observed to cause frequent contacts of growing tips with other branches.

It is interesting to note that Potts (1976) has shown that the outcome of allograft reactions vary depending upon the environment where the coral was growing. It would appear from his results that phenotypic expression remained unaltered during the year his corals were translocated to another environmentally different site. Such apparent conservatism of phenotype may place corals that are regrowing in a different environmental zone, for example as a result of cyclonic disturbances, at a disadvantage.

Yonge (1963) suggested that the fusion or non-fusion of planulae might allow 'a means of determining the effects of genetic conditions and the environment' in coral research. The analysis of grafting reactions and field observations has now allowed his proposed tool of fusion/non-fusion to be implemented. Earlier observations in the field have not been applied with sufficient attention to soft tissue anatomy to determine whether composite colonies were of the fused or aggregated type (see page 138 for relevant references). Some recent observations (Roos, 1971) are similarly in error for the same reasons.

His interpretation of xenogeneic interactions between Acropora cervicornis and A. palmata (Lamarck, 1816) as being fused and therefore 'hardly grown out of the subspecies level', cannot be accurate, as no case of xenogeneic fusion has been, or is likely to be seen in corals. If such tissue interaction techniques are to be used, they require the most scrupulous observation, combined with a precise and well defined description for them to be of any value. The methods employed in this present study should allow an analysis of the most recondite of interactions to be performed with precision. In this way the genetic mapping of reef colonies can, for example, allow the contribution of sexual vs sexual reproduction to be assessed in recruitment of corals to the reef. An immediate local application of this is required. The present alterations of land usage along the Barrier Reef coast may be causing excess silt and restricting settlement of planulae. The present inshore reefs may be maintaining themselves, mainly by vegetative reproduction processes. If, as the result of a single catastrophy an inshore reef is killed, then it will remain dead until planulae are able to re-settle. If siltation

remains or is further increased, then that inshore reef will cease to exist.

FIGURE 36

Natural allografts in Acropora formosa.

These natural allografts have been formed as a result of branches falling onto another colony. The holdfast pads are characteristic of allografts, with the tissues showing mutual indifference.

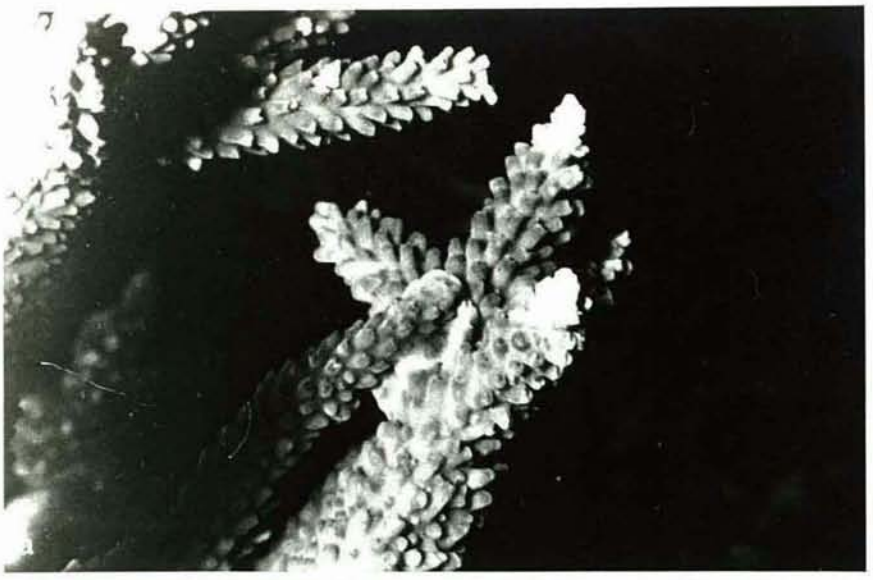
Note that at the free growing edge (a decreased) number of zooxanthellae. A similar paucity is to be seen in the actively growing axial polyp region.

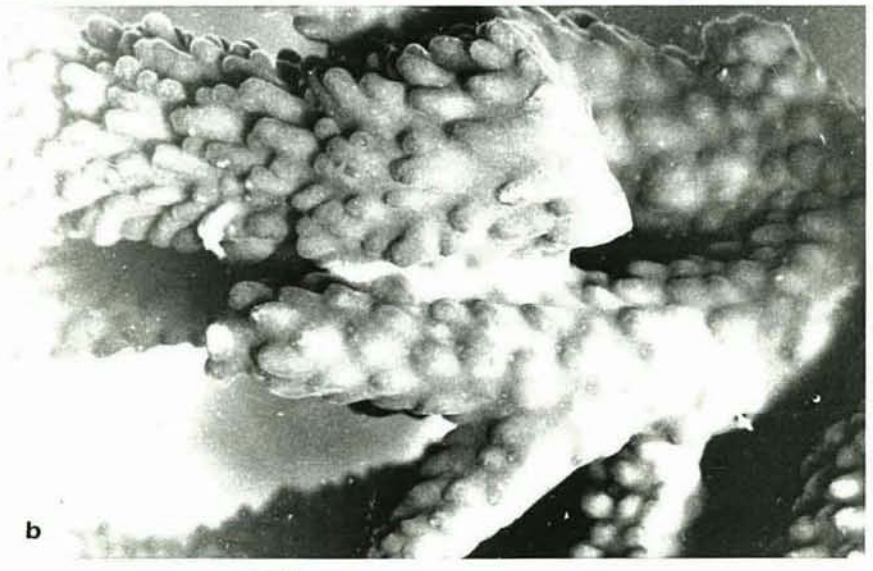
Figure (c) is a higher power view of (a).

Magnification (a) x 1.8

(b) x 2.4, and

(c) x 2.7.





b

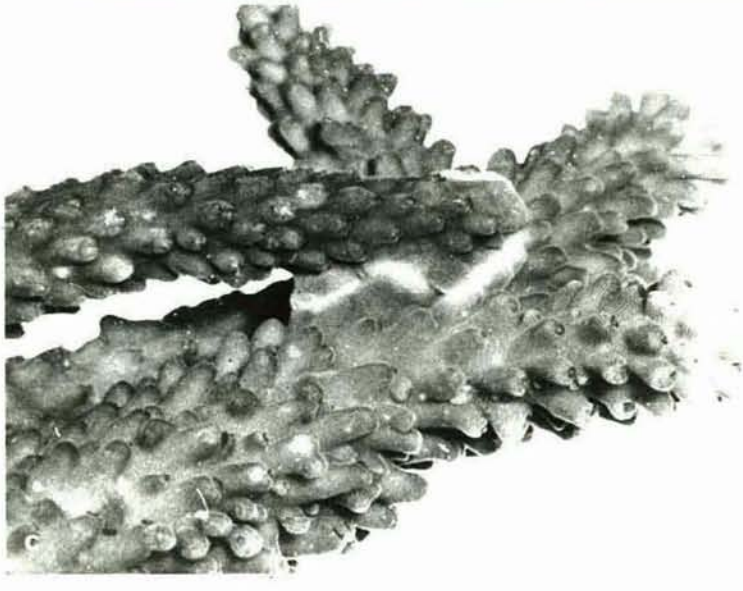


FIGURE 37

Acropora formosa - Galaxea astreata natural xenograft.

(b) Is a higher power view of the junction region of (a). Note that the initial area infilled by A. formosa is showing dead areas. The relative rates of growth of the opposing corals cause a variable contribution of material to the mutual holdfast areas. In this case the initial advantage went to the acroporan, but it would appear that the incipient overtopping growth of the Galaxea is causing local regression of the acroporan tissues. Magnification (a) x 0.8

(b) x 2.4.

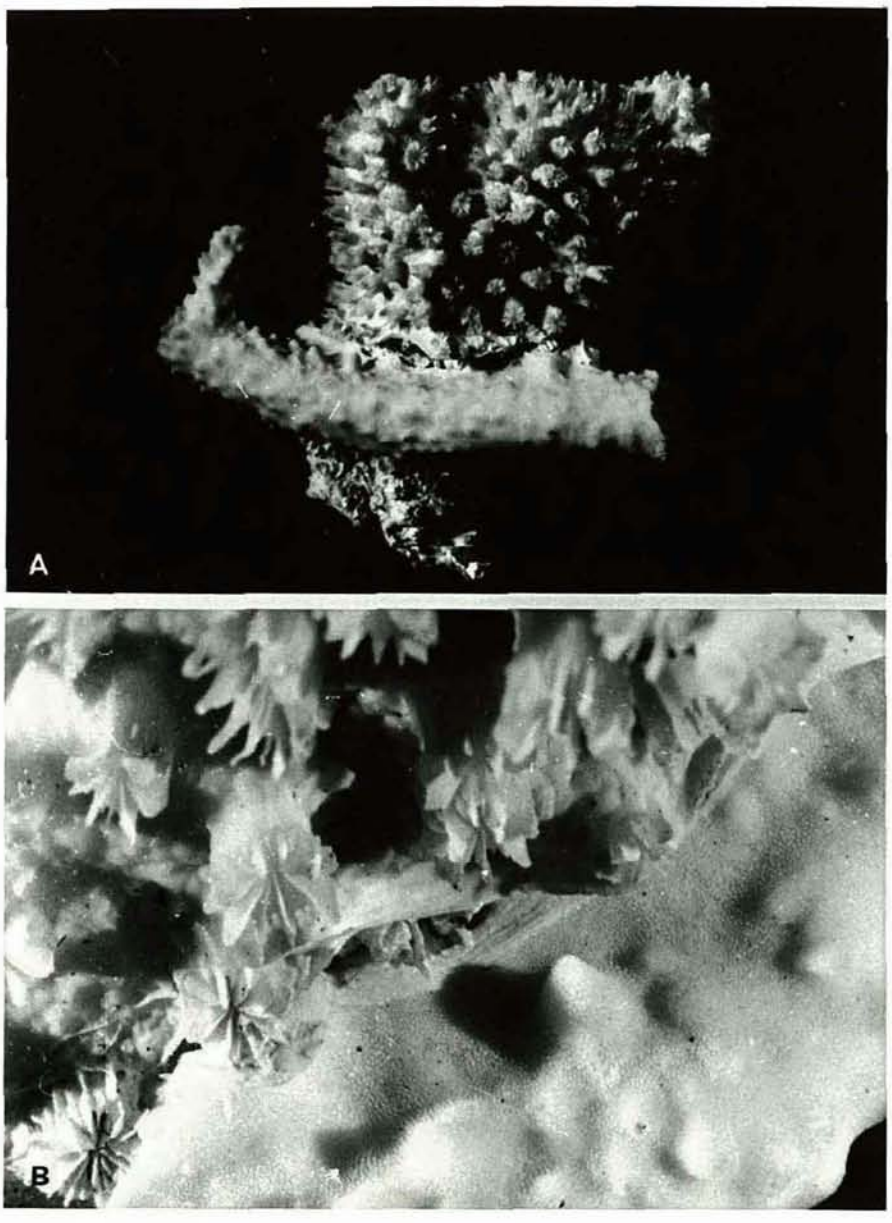

A
B

FIGURE 38

Natural xenograft of Acropora formosa and Acropora hyacinthus.

This natural xenograft was readily noticeable as the A. formosa passed vertically through the horizontally spreading plate of A. hyacinthus. The upper and lower junctions of this graft are illustrated in Figures 29 and 39.

Scale 1 unit = 10 mm.

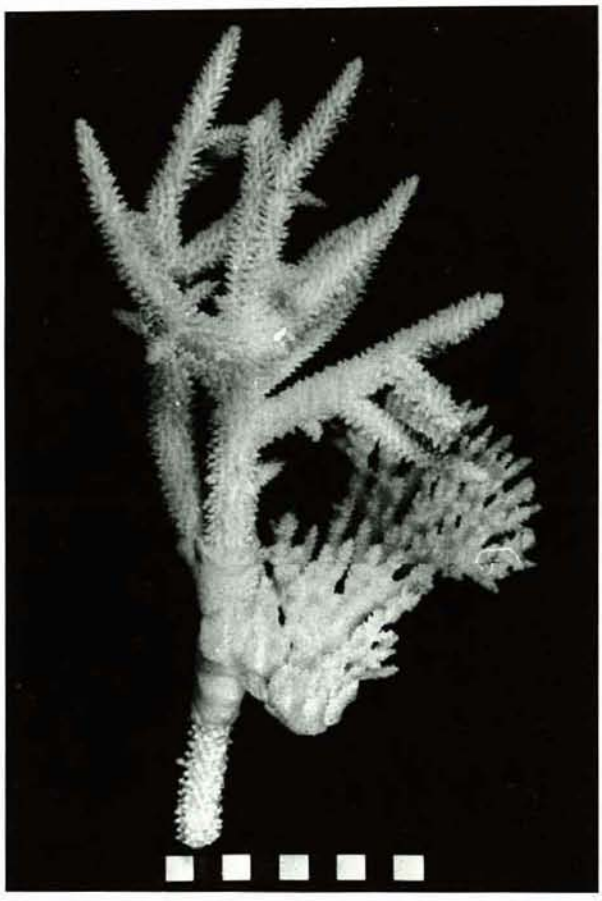


FIGURE 39

Natural xenograft between Acropora formosa and Acropora hyacinthus. Skeletal detail.

(a) A cross section in the plane of the A. hyacinthus, showing the encircled A. formosa. Magnification x 2.4.

(b) The superior junction. Note the relatively close nature of this junction, as opposed to the inferior one illustrated below and in Figure 29. There is a strong mutual growth to form the holdfast pad in the superior junction. Magnification x 4.

(c) The inferior junction.
Magnification x 3.7.

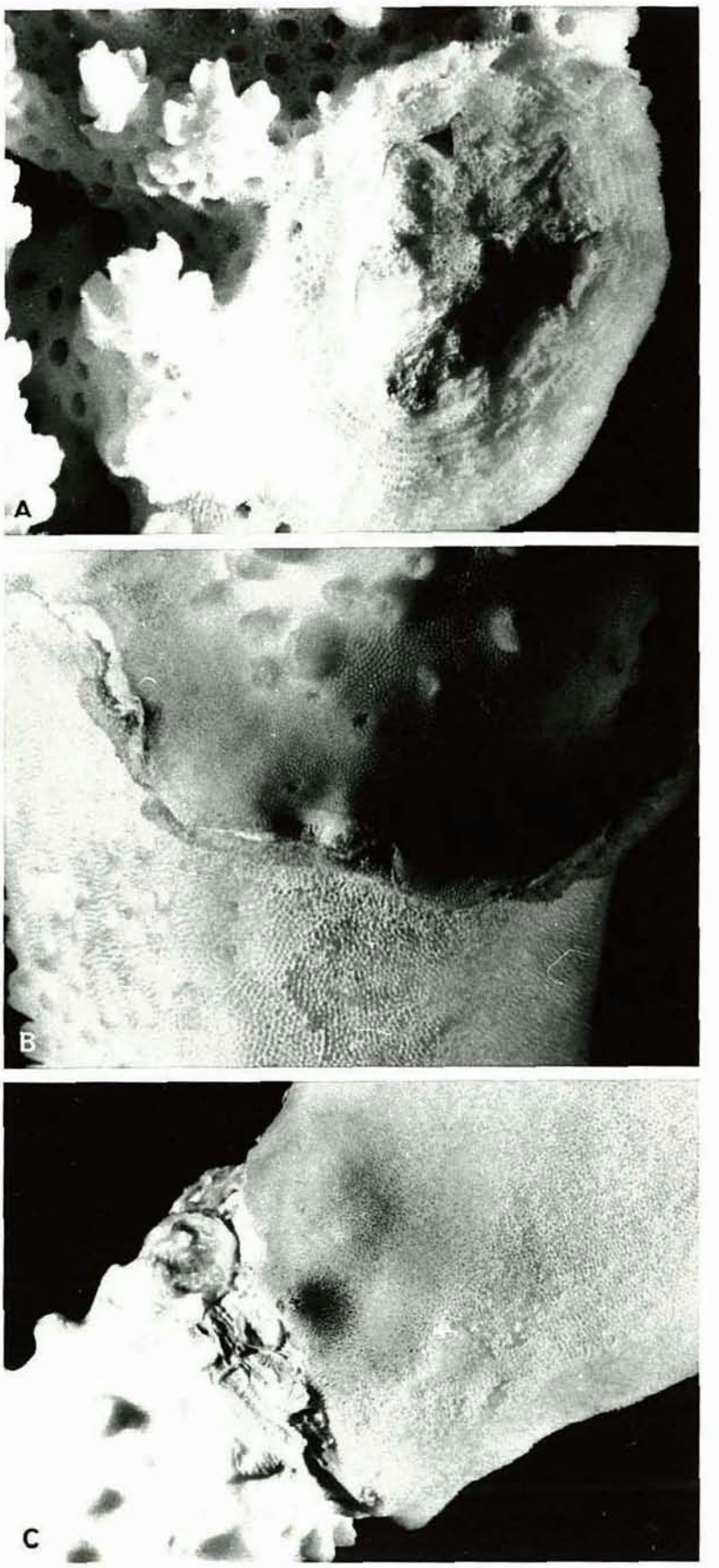

A
B
C

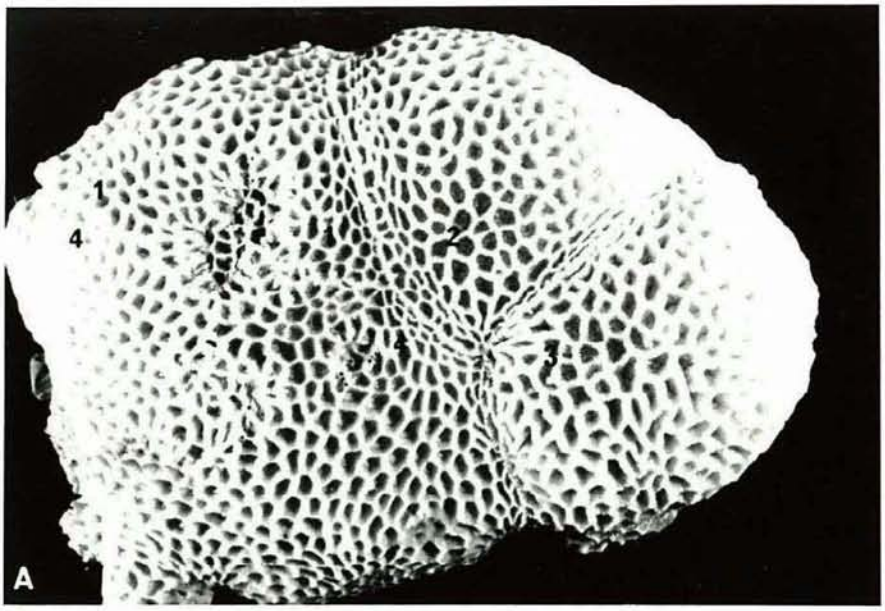
FIGURE 40

Goniastrea aspera and natural interactions.

(a) A composite colony with 4-way allogeneic interactions. 1 reacting with 2 and 4; 3 with 2 and 4; 3 and 1 do not interact. Magnification x 0.5.

(b) The Goniastrea aspera zone on the Magnetic Island reef flat.

(c) Goniastrea aspera colonial complex of at least six allogeneic colonies, growing on the inner wall of Townsville Harbour breakwater.
Note the silt-laden mucus (sm) and the turbid water.


1
4
2
3
A


B

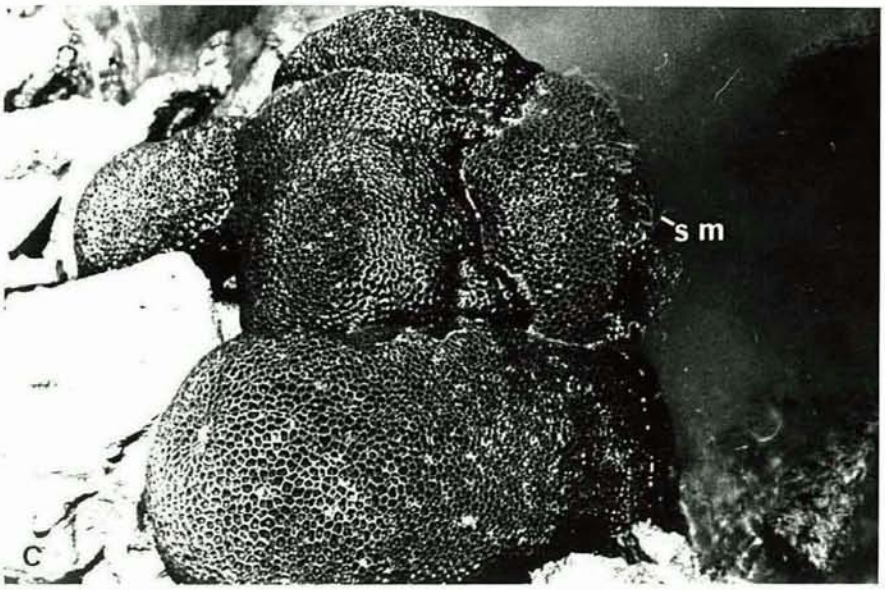

s m
C

FIGURE 41

Goniastrea aspera, natural interactions and growth form.

(a) and (b) Natural xenografts of G. aspera and Favia palida.
Magnification (a) x 1.5
(b) x 1.0

(c) Goniastrea colony on the Nelly Bay reef flat, showing numerous successions of growth and destruction. Magnification x 0.1.

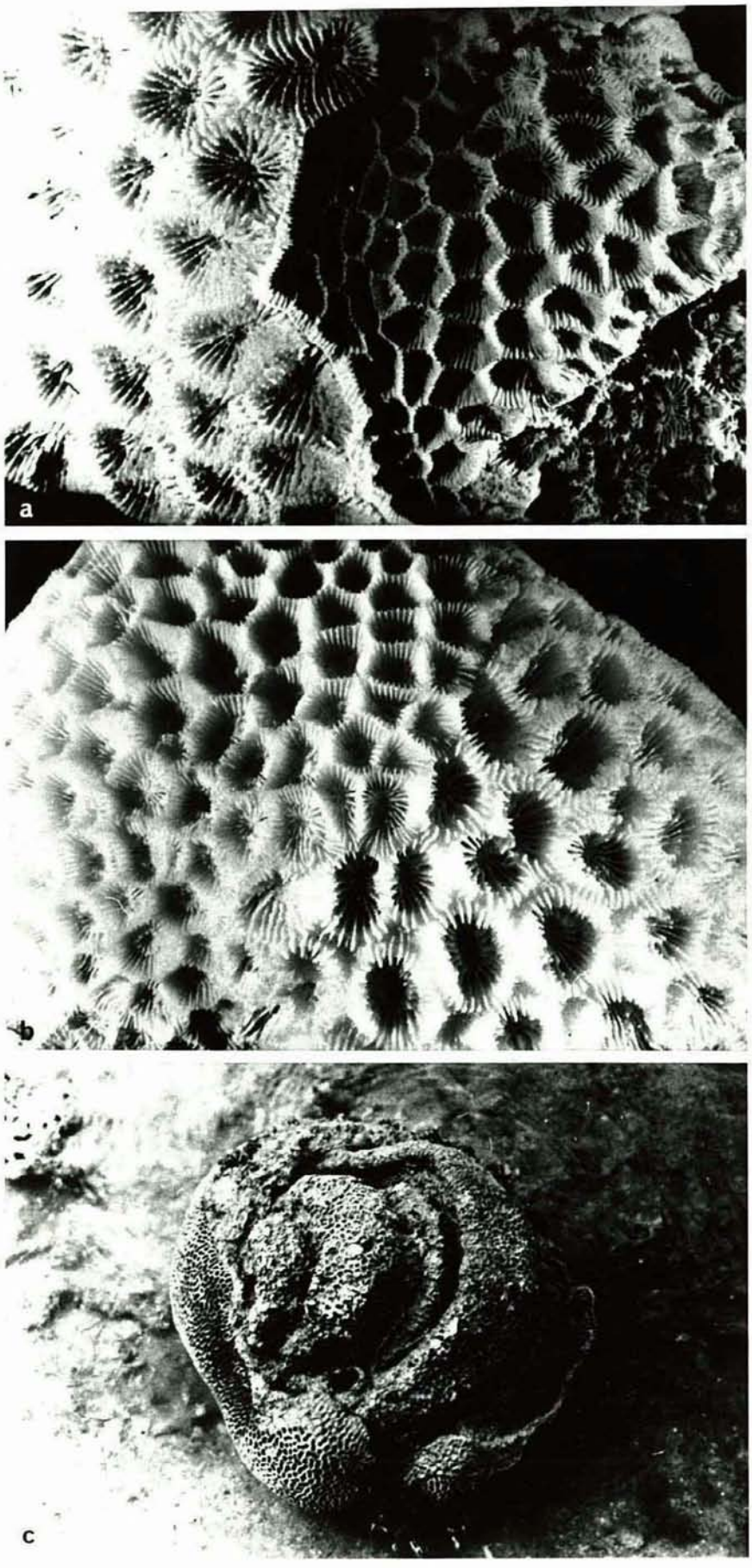

a
b
c

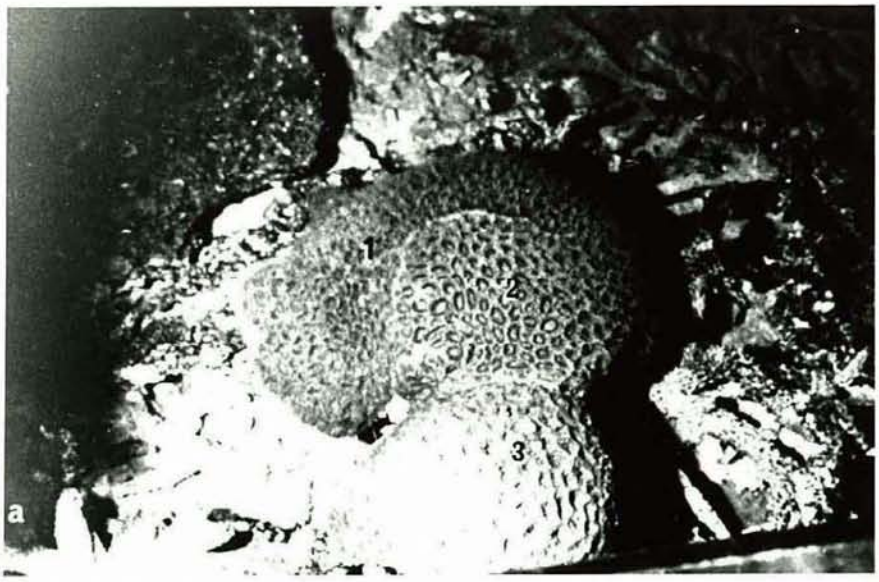
FIGURE 42

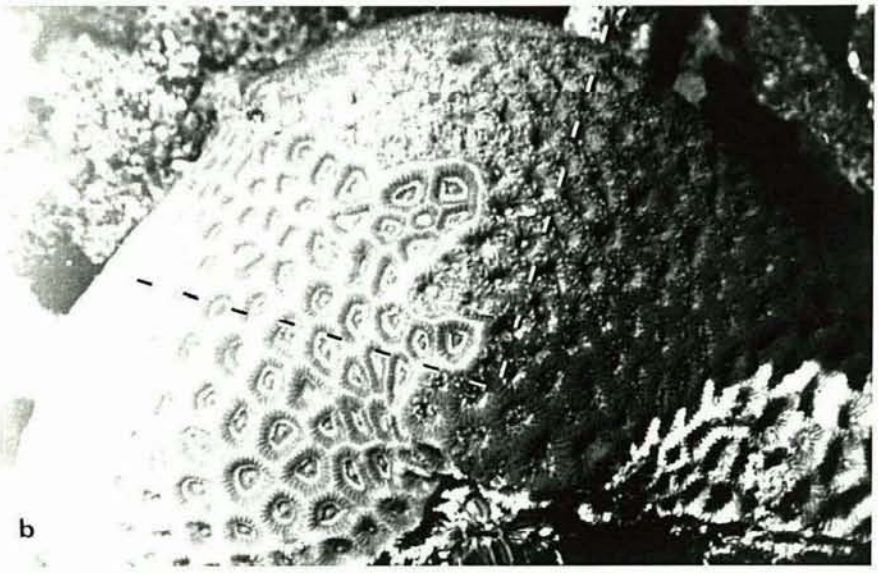
Goniastrea aspera natural interactions.

(a) A tri-allogeneic interaction. This colony is further analysed in Figures 43 and 44.

(b) A sinuous graft junction of a bi- allogeneic composite colony. The left hand side has been mechanically stimulated. The wave of nervous contraction does not pass the allogeneic junction. Magnification x 0.5.

(c) A view of (b) above of the area indicated, before the left hand side of the graft was stimulated.
Note the close juxtaposition of allogeneic tissues and their mutual indifference. The very indistinct nature of the junction when the tissues are expanded have led other workers to confuse the true nature of the soft tissue relationships in allogeneic and xenogeneic reactions. Magnification x 0.9.


1
3
a


b

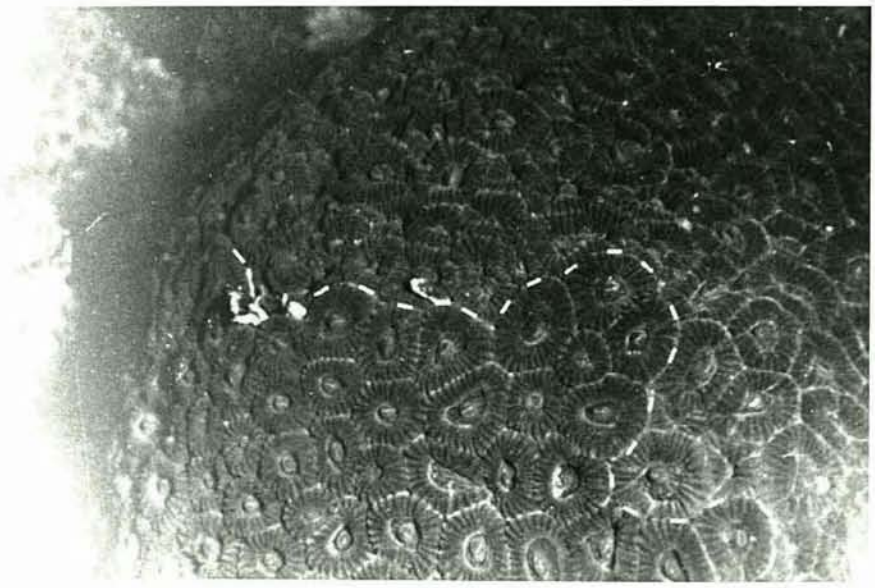


FIGURE 43

A direct Xerographic reproduction of a slice of skeleton of a tri-allogeneic graft of Goniastrea aspera.
Magnification x 1.0.

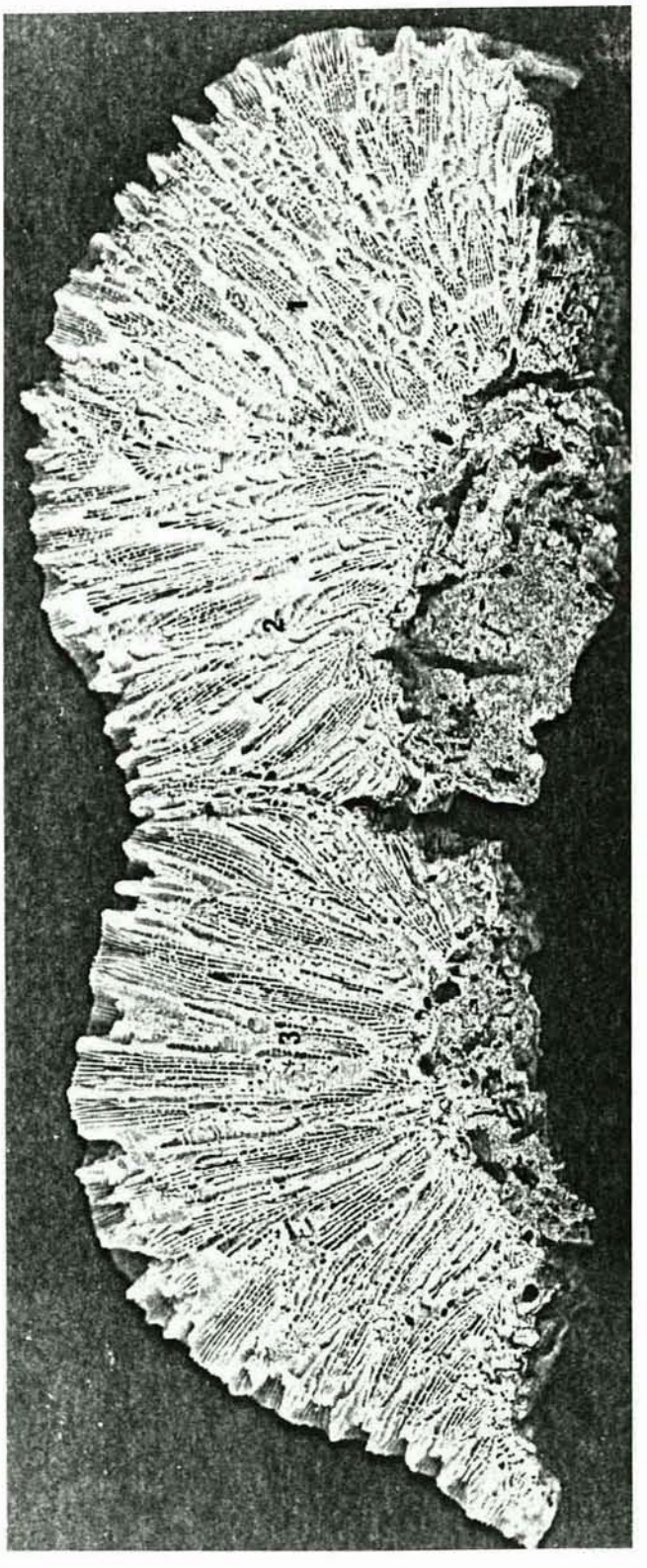

1
2
3

FIGURE 44

Contact print of an X-radiographic plate of the sliced section of Goniastrea aspera of Figures 42a and 43. Magnification x 1.0.

3
2
1

FIGURE 45

An analysis of Goniastrea aspera skeletal interactions.

(a) An isograft junction, formed where two regenerating surfaces met. Note that there is no epithecal structure at the interface. Magnification x 20.

(b) High power view of the allogeneic interface of (e) opposite. This is also the junction between 1 and 2 of the tri-allograft illustrated in Figures 42a, 43 and 44. Note that the epitheca separating the corallites is composed of two components. Magnification x 30.

(c) Xenograft junction of G. aspera and Favia palida illustrated in Figure 41a. Note the similar epithecal structure to that in (b) above. Magnification x 30.

(d) Xenograft junction of G. aspera and F. palida of Figure 41b. Magnification x 8.

(e) See (b) above. Magnification x 8.

(f) See (c) above. Magnification x 8.

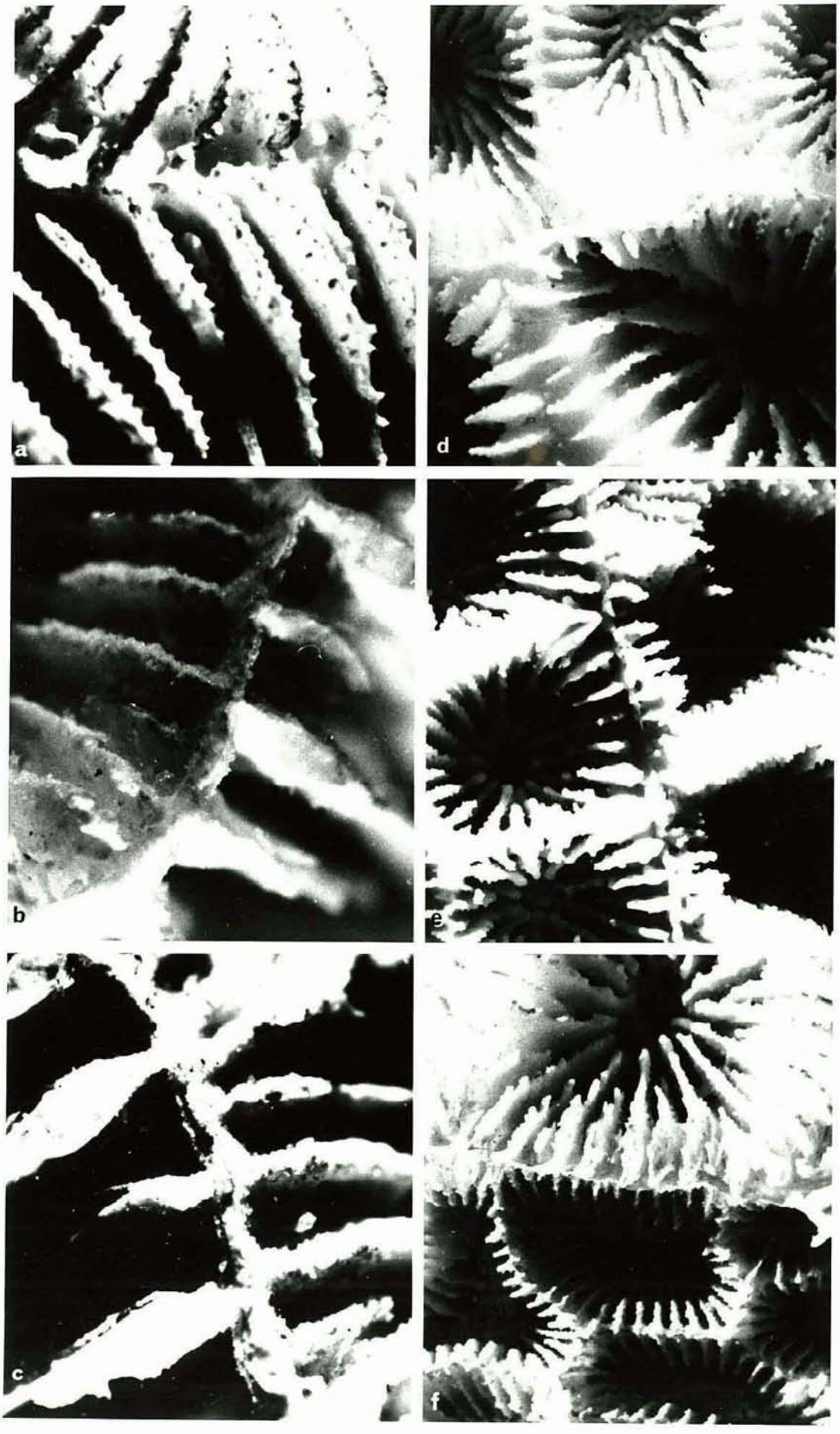

a
d
b
e
c
f

FIGURE 46

Goniastrea retiformis natural allograft.

(a) A surface view of the allograft.

Magnification x 0.6.

(b) An X-radiographic image of a slice of the allograft.

Magnification x 0.8

(c) A surface view of the cut surface of the allograft.

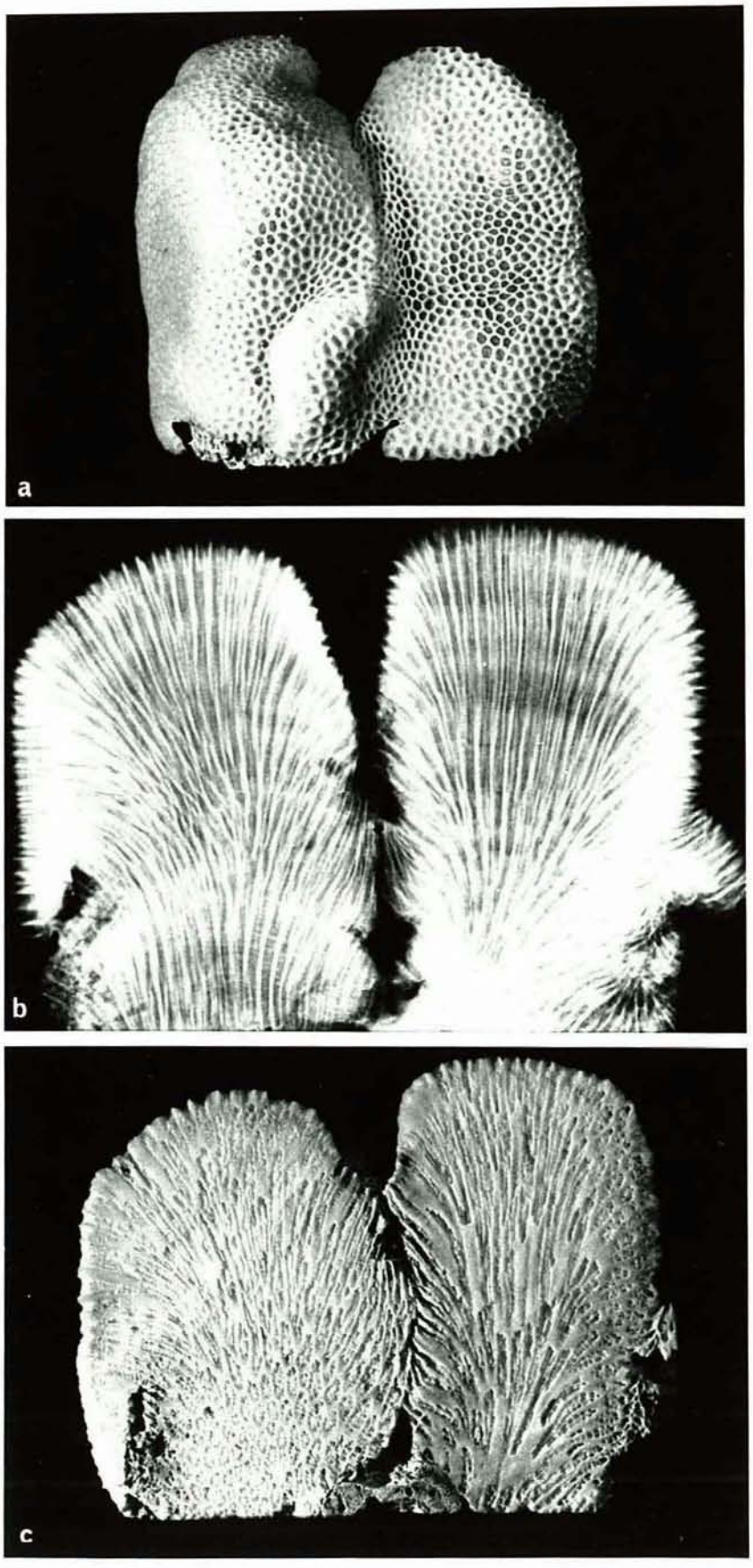

a
b
c

FIGURE 47

Goniastrea allogeneic interactions.

(a) A small inset allogeneic growth on the crown of a colony. This specimen is further analysed in Figure 48. Magnification x 0.7

(b) An allogeneic interaction showing the convoluted junction that may occur in the contact region. Such junctions give the impression of equally balanced growth pressures on either side of the graft. Magnification x 0.5

(c) Under-surface of the above colony, showing the discrete early settlement and growth centres. Magnification x 0.5

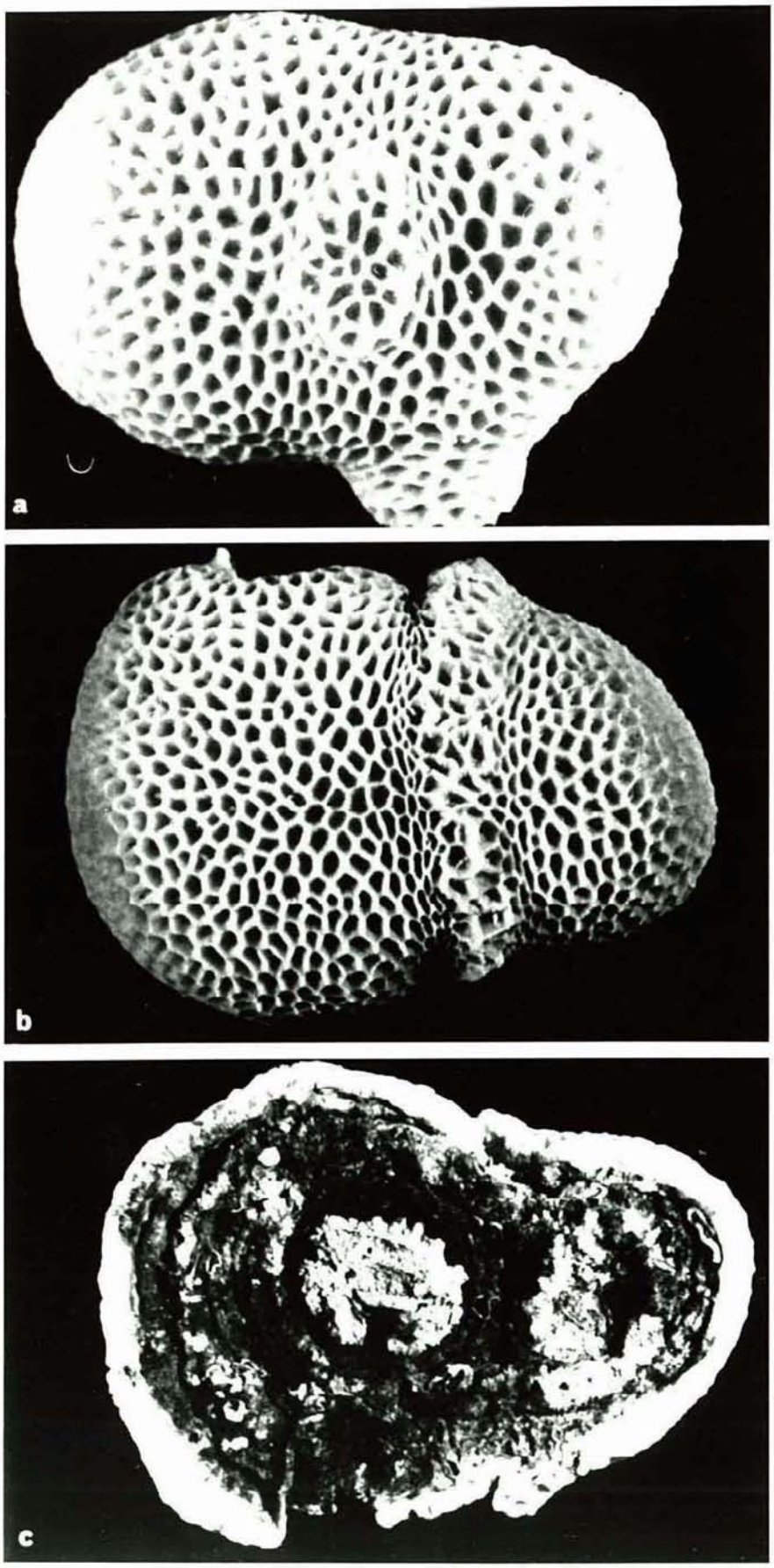

a
b
c

FIGURE 48

A Xerographic reproduction of a slice taken along the major axis of the specimen of Goniastrea aspera illustrated in Figure 47a.

The overlay illustrates the main morphological features shown by this specimen.

1-1 = first damaged surface

2-2 = second damaged surface

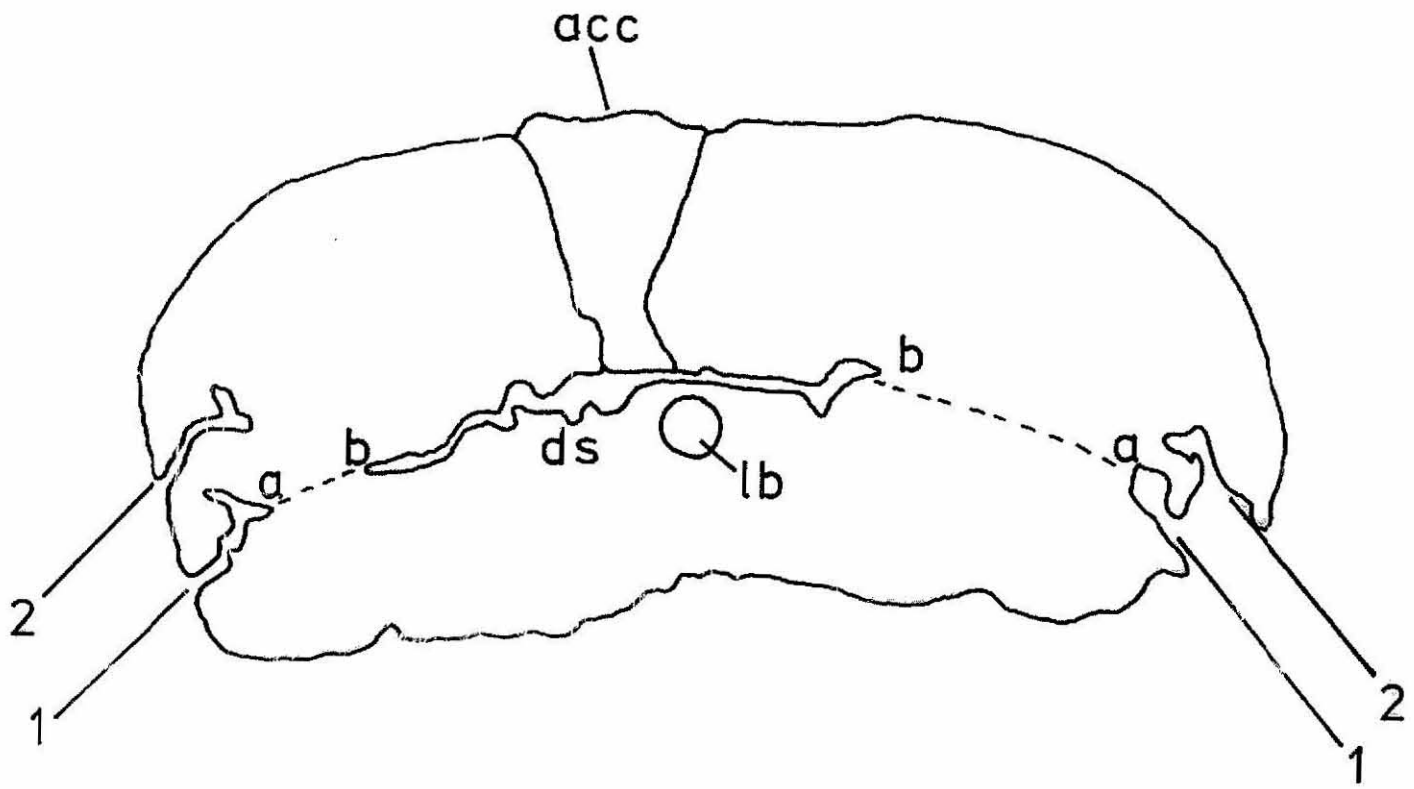
b-b and a-a indicate the living annulus of tissue remaining after the 1-1 killing

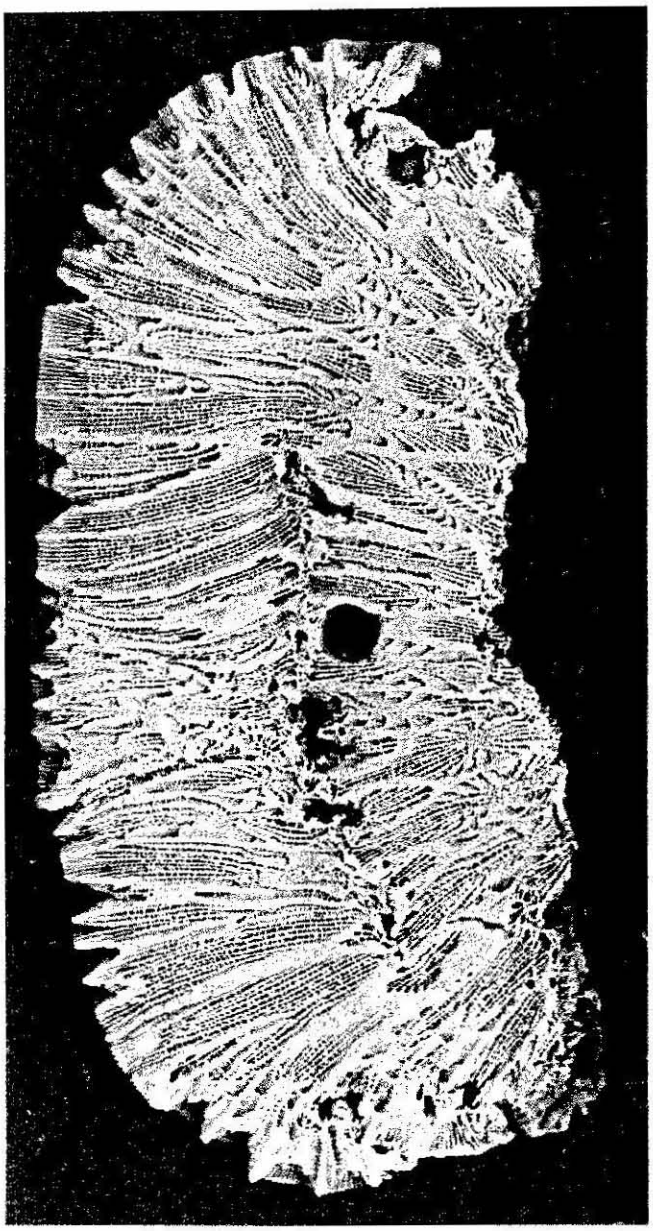
ds = the dead surface left after the first killing

acc = allogeneic colony insert

lb = Lithophaga burrow

Magnification x 1


acc
b
a
b
ds
lb
a
2
1
2
1



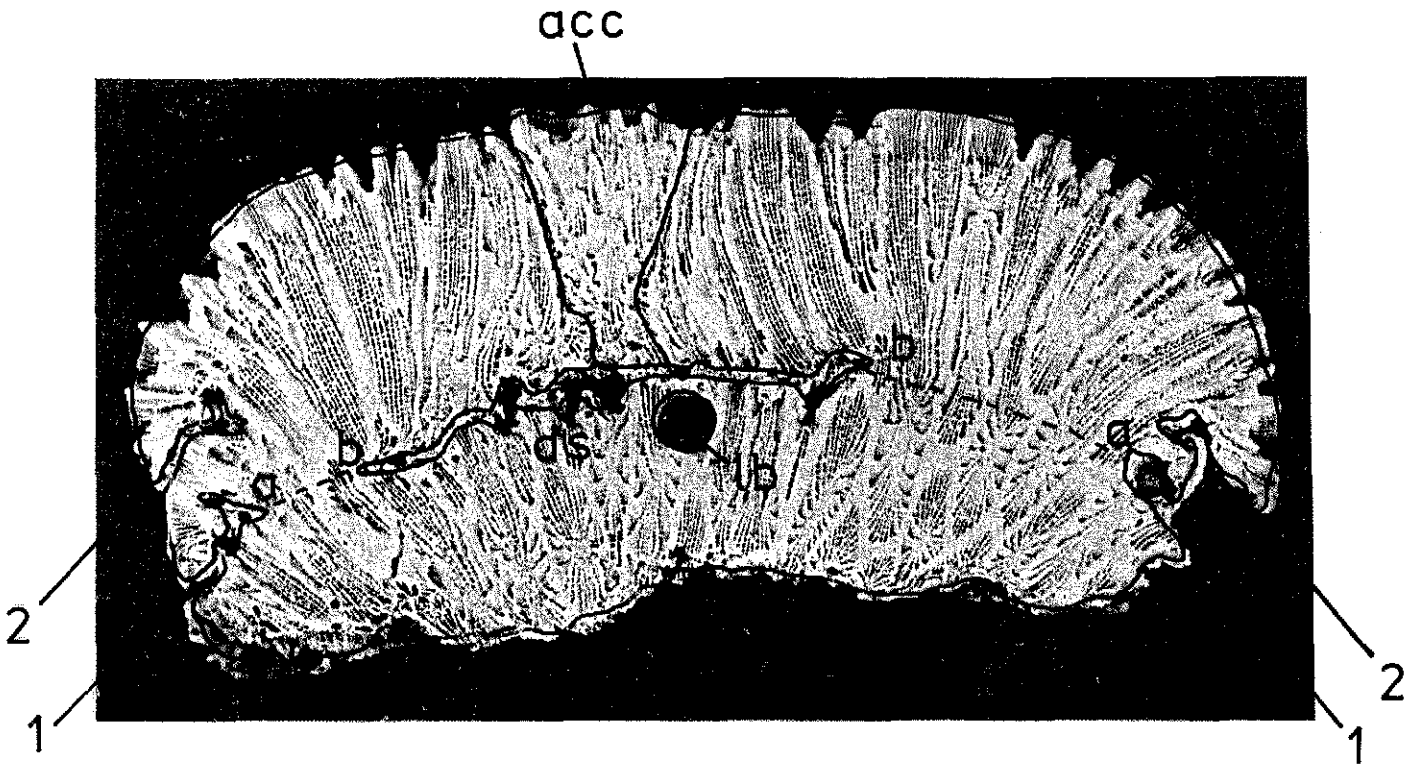

acc
2
1
2
1

FIGURE 49

Acropora sp. neoplasm.

(a) Vertical view of the neoplasm and Acropora sp. colony. The specimen was sliced prior to the photograph. Magnification x 0.5

(b) Close-up view of the surface of the neoplasm. The lack of polyps and the undifferentiated coenosteum are evident. Magnification x 1.8

(c) The cut surface of the neoplasm, showing the concentric growth bands, originating on the same level as the tips of the damaged branches. Magnification x 1.3

a
b
c

FIGURE 50

Histological detail of the Hydroid - Acropora formosa specimen. Stained in Haematoxylin-Eosin, and (a) photographed by Nomarski phase.

Hydroid tissues can be seen ramifying through the coral structure. The tissues pass between the calicoblastic epiderm and the skeleton. The hydroid is therefore an ectoparasite.

The cavity occupied by the skeleton is cross hatched in (b). In this 'external' position, the mesenterial filaments cannot be used as a defense mechanism. Magnification (a) x 180
(b) x 90

c = coelenteric cavities

s = cavities that contained skeleton

h = hydroid tissue of the 'internal' stolon

hp = hydroid polyp

m = mesogloea

g = gastroderm

e = epiderm

z = zooxanthella

mf = mesenterial filaments

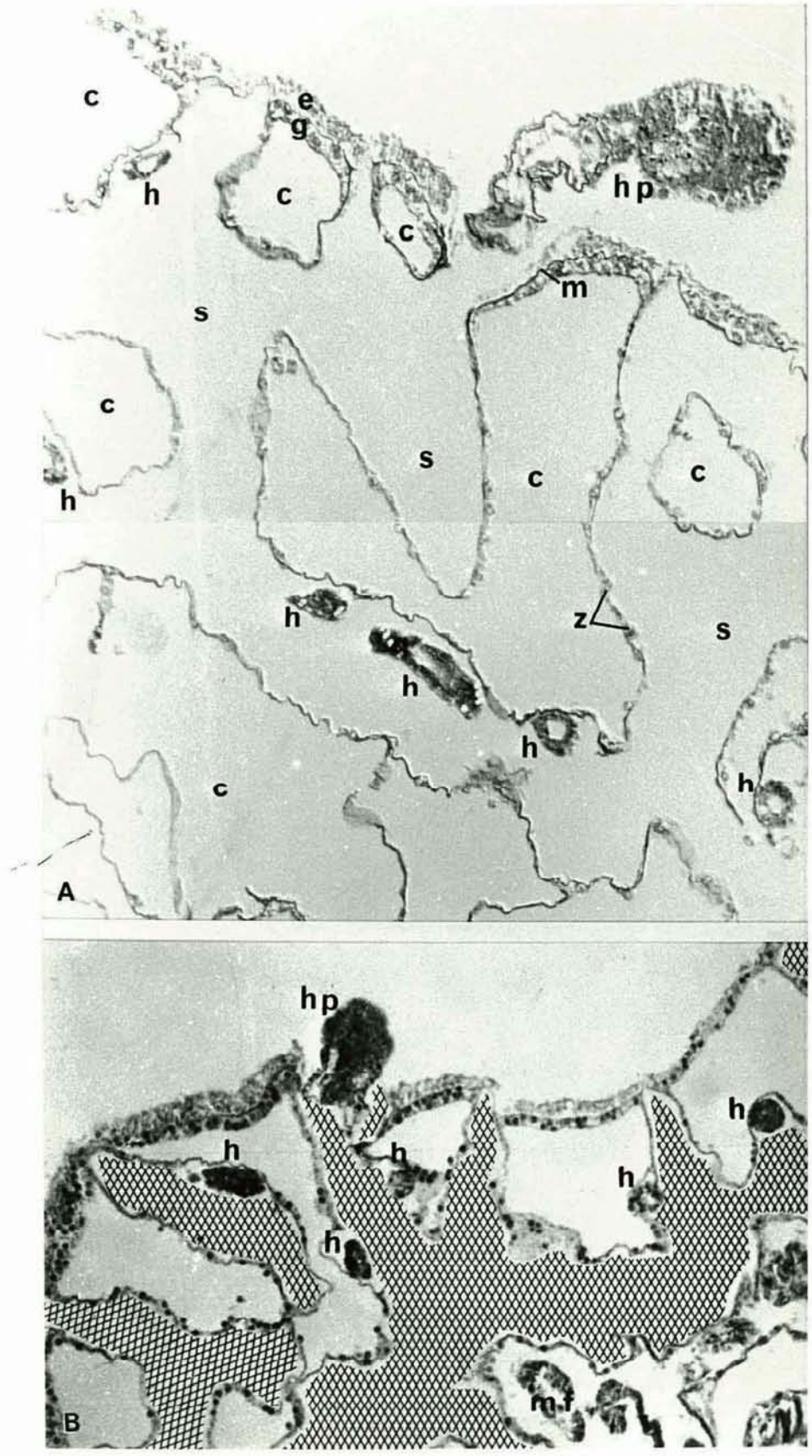

c
e
g
h
c
c
hp
m
s
c
s
c
c
h
h
z
s
h
h
h
c
h
A
hp
h
h
h
h
h
h
m
B

FIGURE 51

Fungia fungites, an isogeneic specimen.

This specimen is unusually composed of two polyps.

(a) shows the two distinct mouth structures, while

(b) indicates the two origins of this specimen.

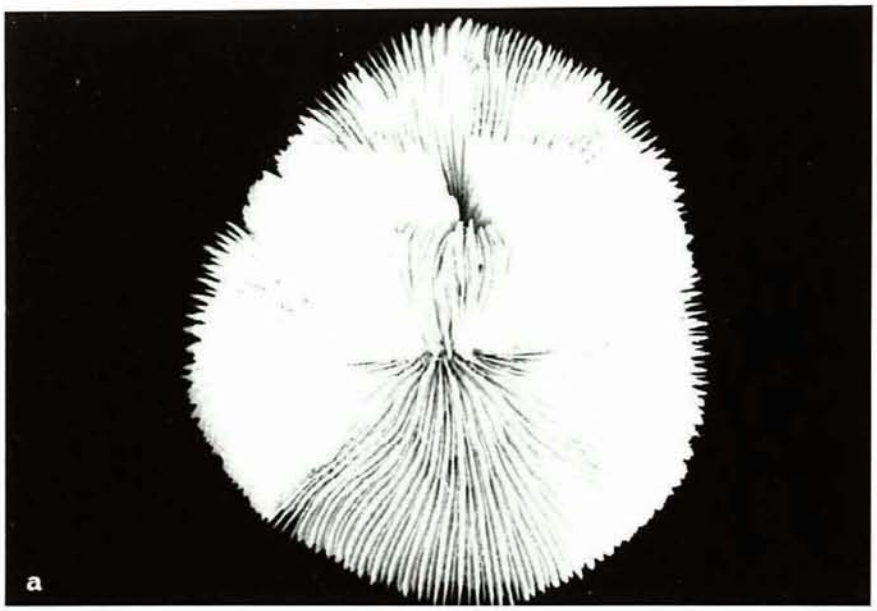
(c) shows the fused nature of the septa.

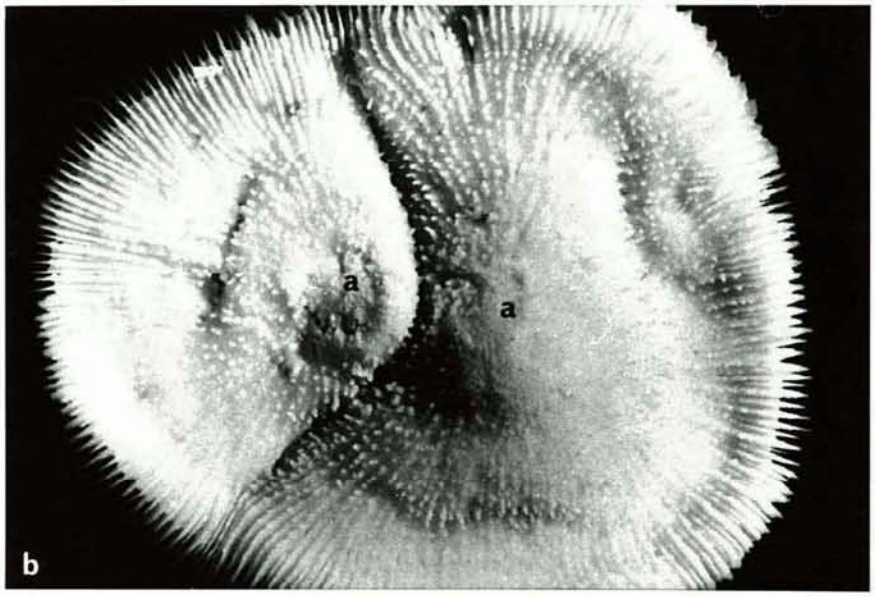
a = the attachment points on the anthocyathus of the anthocaulus

Magnification (a) x 1

(b) x 1.3, and

(c) x 4


a


a
a
b


c

CHAPTER VII GENERAL CONCLUSIONS

General Conclusions

Other than coral buried by movements of reef debris or silt, the mechanical effects of a cyclone are minimal on reef corals. The associated high rainfall is singly the most damaging factor of cyclones, but even the lowered salinity that it produces is mainly restricted to the shallower reef regions by hydrographic constraints associated with the concomittant density changes. Short period, high intensity rainfall produces swifter runoff and hence stronger salinity gradients inshore. Such high rainfall is also associated with the monsoonal rains.

The mechanical distribution of corals over the reef by cyclone induced waves places corals into unselected sites as opposed to the selected settlement of planulae. Thus many intercolony contacts are promoted, both immediately and as fragments grow. This is especially evident with the more susceptible branching corals. Massive colony interaction, such as that shown by Goniastrea is a result of the close settlement of planulae.

A method has been devised to allow the analysis of experimental interactions made by grafting coral branches together. Experimental grafts show that corals are able to distinguish between 'self' and 'non-self', and that this discrimination allows isogeneic tissues to fuse, while 'non-self' reactions proceed through allogeneic indifference to active rejection shown by xenografts. Extracoelenteric digestion appears to be a form of xenogeneic interaction that has made use of feeding mechanisms rather than cytological reactions.

Although allogeneic tissues may appear to fuse in the initial stages of a graft, they remain functionally, as revealed by the non-

transmission of nerve impulses across the graft junction, and histologically not fused. These observations were aided by introducing an embedding stage before decalcification, the interrelation of soft tissues are maintained during processing for histological examination. Electron microscopical examination of such close tissue contacts would clarify this distinction between 'close' and 'fused'. There are slow responses that cause regression of an opposing tissue in allografts and xenografts that can only be interpreted as immunological in nature. The specificity and memory characteristics of interactions show that a relatively advanced recognition system exists at the coelenterate level of phylogeny.

Grafting has shown that while growth polarities and senility barriers do not exist for the coenosarc, polyp tissue appears highly individualistic and will not fuse even with isogeneic tissues. This has a functional significance as polyp tissue is the only actively moving part of a coral and as such will come into frequent contact with other isogeneic polyps of the colony.

New terminology has been introduced to define the fused and aggregated colonies observed by other workers, and to align their work with the findings of this study. The observation that planulae settle gregariously to form one colony appears to contravine the known tissue interactions, in that only isogeneic tissue should fuse. If, however, they were derived from one common parent then they may fuse to form one colony, as has been observed in some hydroids with one haplotype in common. Only further breeding experiments will clarify these observations.

The outcome of coral interactions is wholly dependant upon the

genetic constitution of the interacting tissues. The earlier hopes of using such tissue reactions as a taxonomic tool have been thwarted by the exquisite sensitivity of the grafting technique. However, such specificity will allow the genetic mapping of reef coral colonies and be invaluable in coral population studies.

With a knowledge of the type of damage that cyclones cause, it is expected that asexual reproduction will be found to be a significant factor in the maintenance of inshore reefs.

GLOSSARY

Acontia - the aboral continuation of the mesenterial filament free from the supporting mesentery.

Aggregated colony - colony formed by the growing together of originally distinct individuals to form a composite growth. There is no functional union of tissues, which remain mutually indifferent to each other.

Allograft - a graft formed between allogeneic tissues, i.e. different genetic constitution, but of the same species.

Anamnesis - Immunological memory, q.v.

Anthocaulus - the sessile stage of a Fungia produced from the settlement of a planula. This sequentially produces numerous buds asexually.

Anthocyathus - neanic stage in Fungia ontogeny, after separation from the anthocaulus.

Coenenchyme - collective term for both coenosarc and coenosteum.

Coenosarc - common soft tissue connecting polyps in a colony.

Coenosteum - skeletal deposits formed between the corallites of a colony.

Epitheca - rim of skeletal tissue laterally surrounding the free edge of the coenosarc. An extension of the basal plate.

Fused colonies - aggregated colonies where the soft tissues are in fused contact so that the coelenteric canals are in communication. The whole responds functionally as one unit.

Holdfast - the formation of a pad of undifferentiated coenenchyme in response to contact with a substratum so as to form an attachment point. Such pads necessitate the formation of a basal plate and an epitheca.

Immunological memory - the ability to respond to a second antigenic contact with a quickened response that is specific.

Isograft - a graft formed between isogeneic tissues, i.e. of identical genetic constitution, derived from the same colony.

Mesenterial filament - the thickened inner free margin of a mesentery, composed of a cnidoglandular and absorptive-excretory region.

Protocneme - one of the first 12 mesenteries developed in the early ontogeny of the polyp. Additional mesenteries are termed metacnemes.

Stereome - calcareous skeletal material deposited as a secondary layer on the initial skeleton.

Xenograft - a graft between xenogeneic tissues, i.e. tissues derived from different species of animals, and hence genetically dissimilar.

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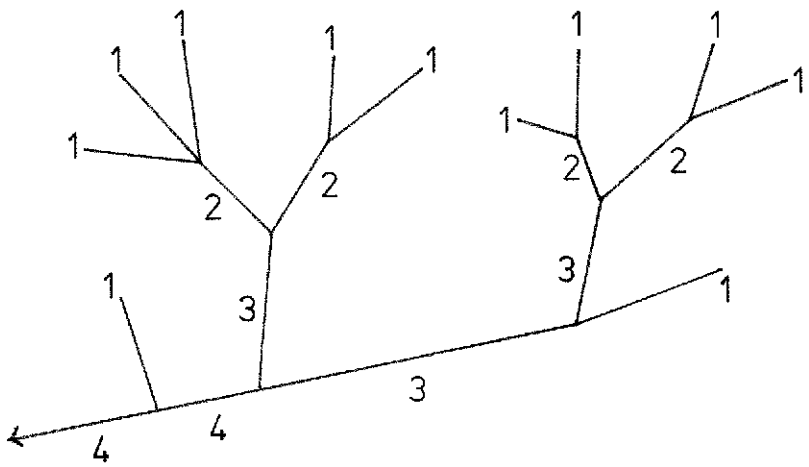
APPENDIX 1

DIAMETER TO LENGTH ALLOMETRIES FOR ACROPORA FORMOSA

The relationship of length to diameter for branches of Acropora formosa was determined by measurement with vernier calipers. For any one environment type, it is possible to show an allometric relationship between length and diameter exists. Working from the tips towards the base, the branches are allocated order numbers, dependent upon the number of times they receive other branches. Therefore terminal branches will always be (1). If two branches of equal number unite, then the next highest order number is allocated to the common stem, i.e. (2). Distance is measured from the furthest tip. The diameter of a branch is assumed to be related to the amount of material that is cantilevered from it. As the shape of a coral skeleton is an historic integration of all past calcium deposition, it is possible to use the length to diameter allometries in a predictive way. This method is as yet in its infancy and requires considerably more development, especially in the way in which allometries change with species and environment.



LINE DIAGRAM OF BRANCH ORDER TERMINOLOGY

A tentative relationship of diameter to length for eight colonies at Nelly Bay showed the following:

$$D = 0.8 (L)^{0.23}$$

where D = diameter (cm) and L = Length (cm)

Example: Diameter to length relationship for Acropora formosa from Feather Reef, near Innisfail, Queensland (Specimen illustrated in Figure 38).

L (cm) from Tip	First order branch D (cm)				
	a	b	c	d	e
1	0.85	0.95	1.15	0.80	0.80
2	0.90	1.00	1.20	0.85	1.10
3	1.00	1.10	1.30	0.90	1.10
4	1.00	1.15	-	0.95	1.05
5	-	-	-	1.10	-
	Second order branch D (cm)				
5	1.35	1.20			
6	-	1.25			
7	1.50	1.20			
8	1.50	1.20			
9	1.55	1.35			
	Third order branch D (cm)				
10	1.60				
11	-				
12	1.80				

The relationship for this coral appeared to be linear. A regression analysis showed the relationship to be:

$$D = 0.07\ L + 0.83$$

where D = diameter (cm) and L = length (cm)

Figures 38 and 39 show a branch of Acropora formosa that has been encircled by A. hyacinthus.

If the allometries were the same when the branch was encircled by the Acropora hyacinthus, the increase in diameter from 1.2 to 1.6 cm is the equivalent to an increase in height of 5.7 cm. It is interesting to note that the D - L relationship for this coral from the main Barrier Reef was linear, while it is closer to a power curve fit for A. formosa from Nelly Bay. The sample size may have been too small to show a true relationship in the former coral.