REPRODUCTIVE PATTERNS OF SCLERACTINIAN CORALS ON SINGAPORE'S REEFS

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Summary

In this study, patterns of coral reproduction were investigated on fringing reefs south of mainland Singapore (1° 10' N), an equatorial location that is typically considered to have little environmental seasonality. The gametogenic cycles of two common scleractinian corals, *Porites lutea* (a gonochoric broadcaster) and a morphospecies of *Platygyra* (a hermaphroditic broadcaster), were investigated at three sites from March 2001 to April 2002. Both species had very similar, strongly seasonal patterns of gametogenesis, with maturation of gametes and spawning occurring primarily in April. A second, smaller peak in reproductive activity occurred in September/October for *P. lutea* and October/November for *Platygyra* sp., suggesting that some colonies also spawn at this time. Distinct and predictable seasonal patterns of sea surface temperature (SST), salinity and rainfall do occur as a result of the Southeast Asian Monsoon system. Sunshine fluctuates seasonally, but a distinct pattern can only be seen when data are averaged over a number of years, as this parameter is highly variable over short time scales. For both species, increases in average gamete sizes and numbers coincided with a rise in SST following the Northeast monsoon, indicating that this factor may provide the seasonal cue for gamete maturation and spawning in Singapore. Mass synchronous spawning among reef corals, is one of the most remarkable of reproductive phenomena. Synchronous spawning within species is understandable because it increases the chances of fertilisation success, however, what drives many unrelated coral species to spawn synchronously is less clear. A possible advantage of this strategy is that opportunistic predators are satiated during spawning periods. Considering that most mass spawning species are congeneric, the most plausible explanation is that species have responded similarly, but independently

to environmental cues to maximize fertilisation success within species, resulting in many species releasing gametes during discreet "spawning periods". Similarly, selective pressures (ultimate factors) may have caused many species to spawn at the time of the year when environmental conditions are most suitable for successful fertilisation, larval survival and recruitment. In equatorial regions, in the absence of large environmental fluctuations, where conditions for breeding are suitable year round, it was predicted that the extent of reproductive seasonality and spawning synchrony would be low, i.e. species would tend to spawn at different times to avoid gamete wastage, the production of non-viable inter-species hybrids and competition between larvae for settlement space. Sampling of an assemblage of *Acropora* in March 2002 and April 2003 revealed that a high proportion of species contained mature gametes at the same time (68%, n = 19 and 79%, n= 14), although, within species, the proportions of colonies that contained mature gametes varied considerably. Corals were observed spawning *in situ* between the 3rd and the 6th nights after the full moons of March 2002 and April 2003. Twenty-four scleractinian species were observed releasing gametes and at least twelve species spawned simultaneously on one night, within a 2 hr period. Sampling of corals prior to the spawning events revealed that as many as 50 species may release gametes during these spawning periods. Spawning occurred while sea temperature was rising following the northeast monsoon. Spawning consistently began 1 - 1.5 hrs after sunset, during the second low tide of the day, at a time when tidal mixing was negligible. These observations suggest that there is sufficient variation in annual sea temperature to provide a strong seasonal cue for gamete maturation, while diel, lunar and tidal cycles may play a role in determining the time for coral spawning to occur. The finding that many species spawn together, during a discreet period in Singapore, shows that multi-species coral

spawning can indeed be a feature of equatorial coral reefs. Furthermore, these findings lend support to the hypothesis that multi-species spawning in reef corals evolved because species respond similarly, but independently to environmental cues to maximise fertilisation success within species. In the final chapter, replicate fragments of the corals *Goniopora columna*, *G. lobata* and *G. fruticosa* were removed from large colonies on the upper reef slope at Pulau Hantu. These fragments were transplanted within the same site and depth at Pulau Hantu, to a more disturbed site closer to mainland Singapore (Cyrene reef) and to greater depth at both sites. Control fragments were also transplanted a few meters away from the parent colonies. Fragments were left for approximately one year then harvested just prior to the predicted spawning month. Fecundity (average number of oocytes per polyp), reproductive effort (average oocyte size) and polyp diameter were compared between experimental fragments, controls and parent colonies. Fecundity and reproductive effort and polyp diameter between controls and transplants to the shallow site at Pulau Hantu were not significantly affected, indicating that fragmentation and transplantation in these species had no negative impact on reproduction. However, fragments transplanted to depth and to a more disturbed site closer to mainland Singapore had significantly less oocytes and smaller oocytes. Elevated turbidity and sedimentation at the more disturbed site may have reduced the amount of energy available to corals for reproduction. While these factors are indeed causes of stress and mortality in corals, two other factors may have been important: competition with macro-algae, (which flourishes at Cyrene) and/or exposure to elevated nutrients.

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The most erroneous stories are those we think we know best – and therefore never scrutinize or question.

Stephen Jay Gould (1941 –2002)

I love deadlines. I like the whooshing sound they make as they fly by.

Douglas Adams (1952 – 2001)

Chapter 1

General Introduction

1.1 Introduction

In recent years, there has been a great deal of scientific interest in the processes that structure and change coral reef communities, both in space and in time. This interest is driven partly because of the great intrinsic value that reefs represent to human society, and partly because coral reefs are changing and declining at an unprecedented rate. Coral reefs and their associated fauna provide goods and services worth hundreds of billions of dollars to tropical maritime nations, particularly in developing countries, where the majority of reefs are found (Bryant et al. 1998). It is thought that some 30% of reefs worldwide are severely damaged, and many may be destroyed within the next few decades (Wilkinson 2002). Much of this decline has been attributed to a combination of: land-based anthropogenic impacts; over-harvesting of fish and other marine organisms; and more recently, the impacts of global climate change on the frequency of coral diseases and coral bleaching events (Jackson et al. 2001; Hughes et al. 2003). While coral reefs have survived and flourished over millions of years, in spite of great fluctuations in climate and sea level, the increasing kinds and scales of human impact on coral reefs have raised fears of their widespread loss, and consequently the loss of the many benefits that reefs provide for society, if prompt action is not taken to protect them from human destruction (Pandolfi et al. 2003).

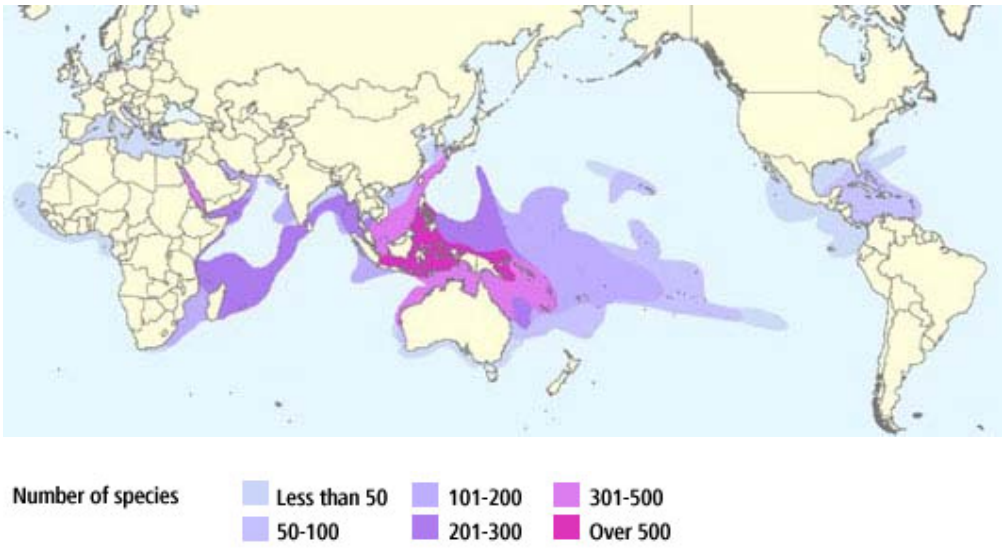


Fig.1.1 Map of World showing areas of coral diversity. Source Reefs at Risk in Southeast Asia (Burke et al. 2002). Used with permission.

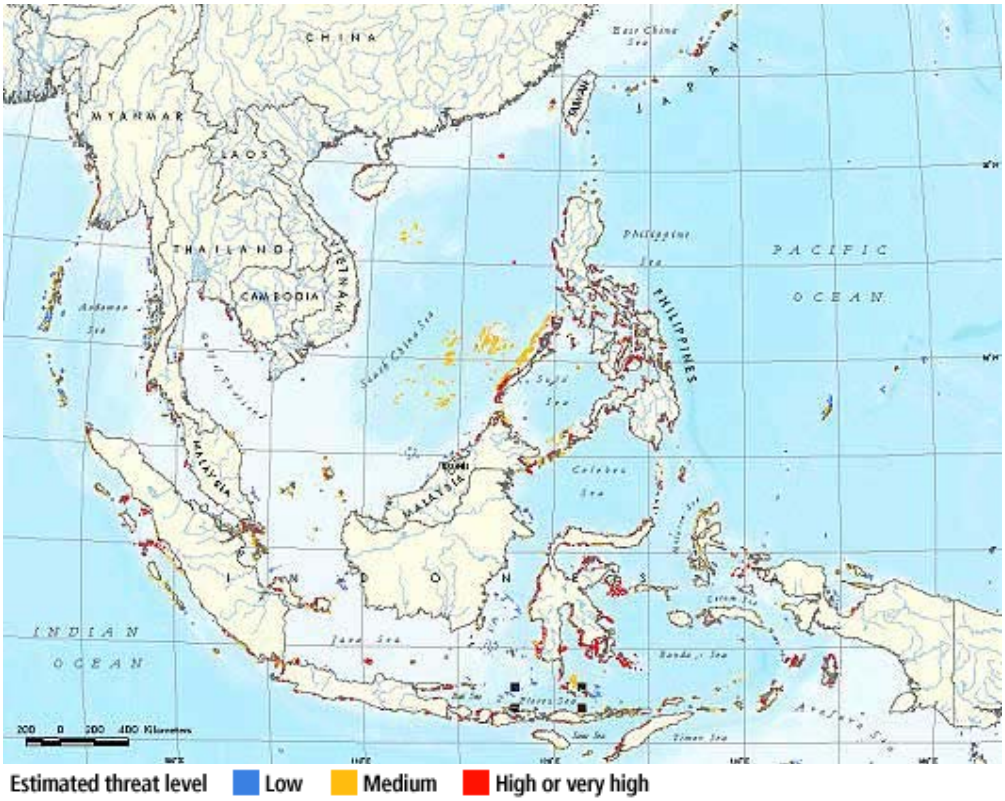


Fig 1.2 Map of Southeast Asia showing areas of threatened reefs. Source Reefs at Risk in Southeast Asia (Burke et al. 2002). Used with permission.

1.2 A justification for further research on coral reproduction

Sexual reproductive patterns in scleractinian corals have received scientific attention for some two centuries; and many advances in knowledge have been made, particularly in the last two decades (Harrison and Wallace 1990). However, due to the structural simplicity and remarkable phenotypic plasticity of cnidarians, it has been difficult to make many robust generalizations about coral reproduction (Fautin 1997; Kinzie 1999). The patterns of reproduction in scleractinian corals influence many aspects of coral reef ecology and evolution (Campbell 1974; Harrison and Wallace 1990; Veron 1995). Consequently, a better understanding of these patterns are of great interest if we hope to understand how corals will adapt to increasing anthropogenic impacts and changes in global climate (Kinzie 1999). Furthermore, because the patterns of connection between coral populations are largely determined by water currents, the timing of coral spawning influences larval dispersal, which in turn influences the potential for reefs to persist, and recover from disturbances (Glynn et al. 1991; Richmond 1997). Therefore, knowing the timing of reproduction in corals could improve understanding of these patterns of connection, and assist in the identification of reefs in need of higher levels of protection. Improved knowledge about the timing, seasonality and synchronicity of coral reproduction can also have a number of useful applications for the scientific study, management and conservation of coral reefs. Knowing the timing of spawning of a coral population is a prerequisite for conducting many types of experimental work, including studies of evolution (Kenyon 1997), fertilisation, hybridisation (Willis et al. 1997, van Oppen et al. 2002), fecundity (Ward and Harrison 2000), larval biology (Hayashibara et al. 1997), settlement (Heyward and Negri 1999, Baird et al. 2003) recruitment (Hughes et al.

1999) and systematics (Babcock et al. 2003). Furthermore, gametes collected from spawning colonies provide an excellent source of DNA for molecular studies (Lopez et al. 1999). Information on spawning times could be of importance for minimising the effects of coastal development on reefs, if destructive practices such as dredging could be temporarily halted during spawning periods, as is the case on parts of the Great Barrier Reef (GBR) in Australia (A. Baird, personal communication). Coral spawning events can be used to attract local media attention, thus helping to raise the profile of coral reefs among the general public (Chang 2002). Knowledge of coral spawning times could help in the restoration of degraded reefs that have limited natural sources of larvae if spawn slicks - which can contain billions of coral embryos - are maintained until they are competent and seeded onto denuded reefs, in controlled densities, to artificially enhance recruitment levels (Heyward et al. 2002; Nonaka et al. 2003). Another method for restoring damaged reefs is to transplant artificially fragmented pieces of coral (Clark and Edwards 1995, 1998). However, more research is needed to understand the effects on reproductive success of fragments used in coral transplantation, if the aim of restoration is to create coral communities that are reproductively viable (Yap et al. 1998; Yap 2003). Finally, measurements of reproductive output can be used as an indicator of pollution and environmental stress, as previous studies have shown that corals experience reductions in fecundity and reproductive effort when subjected to reductions in water quality (e.g. elevated turbidity, sedimentation and nutrients) (Kojis and Quinn 1984; Tomascik and Sander 1987; Ward and Harrison 2000). In short, an understanding of sexual reproduction in corals is central to the study of coral evolution, ecology and biology and is essential if efforts to prevent global coral reef degradation are to succeed.

1.3 Coral reproduction in Southeast Asia: the need for more research

Studying coral reproductive cycles can be time consuming, and may involve repeated field trips to remote reefs, in developing countries, where expertise and facilities are lacking. Hence, for many coral reef regions the timing of coral spawning is not yet known. In particular data are lacking for South Asia, The Southern Indian Ocean, Eastern Africa, and many of the Pacific Islands (Harrison and Wallace 1990; Richmond and Hunter 1990). Virtually nothing is known of the timing of coral reproduction in Southeast (SE) Asia (Bermas and Alino 1994), a region that contains more than 30% of the world's reef area and is home to 600 of the almost 800 scleractinian species (Burke et al. 2002, Fig 1.1). This lack of basic information is particularly worrying as an estimated 88% of SE Asia's reefs are threatened by human activities (Burke et al. 2002, Fig 1.2). Prior to the study presented here, detailed accounts of coral reproduction and spawning in SE Asia were limited to the Philippines (Bermas et al. 1992) and Indonesia (Central Java Sea) Edinger et al. (in Tomascik et al. 1997) (Table 1.1). While for most parts of Indonesia and West Malaysia only anecdotal accounts of coral spawning are available (Table 1.1), and to my knowledge, no reports exist from Vietnam, Cambodia, or the island of Borneo.

1.4 The aim of this study

Singapore is an interesting place to carry out studies on coral reproduction for two reasons: a) it is an equatorial location that is typically considered to have little seasonal environmental variation; and b) it is a densely populated and industrialised island nation where relatively diverse coral reefs exist, in a heavily impacted

environment. Thus, there are two themes to this work: the main theme, and the subject of three chapters, is a study of the patterns of reproductive and environmental seasonality and the extent of reproductive synchrony, both within and between species. The second theme is examined in the final chapter through a field experiment, where reproductive effort and fecundity were examined in coral fragments which were removed from *Goniopora* colonies from one site and transplanted within the same site; to a more disturbed site closer to the mainland; and to a greater depth at both sites.

Table 1.1 A summary of published and anecdotal observations of coral spawning times from Indonesia, Malaysia and the Philippines

Country and location	Observation and species	Dates and years	Source
Indonesia			
Karimunjawa Islands, Java Sea	Mass spawning of 29 scleractinian species	Oct & Nov 1995	Edinger et al in (Tomascik et al. 1997)
	Coral spawning (species unknown)	4 days after full moon Oct 1997	D. Browne (pers. comm)
Anambas Island	Spawning of *Pachyseris* spp.	May 1996	D. Lane (pers. comm)
Komodo	Release of egg bundles from Acroporid species	6 nights after October full moon 1998	H Fox (pers. comm)
North Mollucas	Observation of Acroporid species with mature gonads	Sept 1996	G. Llewellyn (pers.comm)
Manado	Synchronous spawning of Acroporid species	Oct 2001 & Mar 2002	M. Erdmann (pers. comm)
West Malaysia			
Redang Island	Spawn slicks observed on water surface	Full moon Sept 2000	D. Fenner (pers. comm)
Philippines			
Bolinao, Puerto Galera	25 scleractinian species exhibiting 'ripe' gonads	April-May 1991 & 1992	Bermas et al. (1992)

1.5 Overview of chapters

The remainder of this chapter will be a general introduction to coral reproduction, focusing on the aspects that are relevant to the thesis. Excellent reviews on this subject already exist (Fadlallah 1983; Harrison and Wallace 1990; Richmond and Hunter 1990), so I have not attempted to re-write an extensive review. Instead the subjects that are most salient to the thesis are briefly covered. **Chapter 2** introduces the study sites and briefly describes Singapore's marine environment, climate and coral reefs. **Chapter 3** examines the gametogenic cycles of some common coral species found on Singapore's reefs, and attempts to relate these cycles to the seasonal environmental patterns. **Chapter 4** investigates the extent of reproductive synchrony both within and between species in an assemblage of *Acropora*. **Chapter 5** describes the timing and species participation of spawning events on Singapore's reefs, to look at the extent of multi-species synchrony and to examine the ultimate and proximate factors that may be involved in controlling the timing of coral spawning events. **Chapter 6** describes an experiment to look at the effects of fragmentation and transplantation on fecundity and reproductive effort in *Goniopora* corals. And finally, in the general discussion in **Chapter 7** the main findings are summarized and future directions for research are proposed. Although there are clearly general themes that run throughout the thesis, each chapter has been written essentially as a separate paper, complete with methods, results and discussion. The introductions to each chapter have been kept short because most of the relevant introductory material is presented here in **Chapter 1**.

1.6 Introduction to coral reproduction

1.6.1 Reproductive strategies of scleractinian corals

There are 4 main patterns of sexual reproduction in corals: hermaphroditism, where individuals contain both male and female gametes; gonochorism, where individuals are either male or female; broadcasting, where gametes are released for external fertilisation; and brooding, where fertilisation occurs within the polyp (Harrison and Wallace 1990). However, considering the remarkable plasticity of cnidarians, this 2 by 2 scheme is almost certainly a simplification, and quite a number of different developmental pathways and sexual modes may exist (Fautin 1991; Wasson and Newberry 1997). Some variations have already been identified, for example *Galaxea fascicularis* is classified as pseudo-gynodioecious, as 'male' colonies produce testes bundled with sterile, un-pigmented eggs (which act as a buoyancy device for the testes) (Harrison 1988). Reproductive data are available for around one third of the total number of identified scleractinian species, and of these, more than 60% are hermaphroditic broadcasters, indicating that this is the predominant mode for scleractinian corals (Richmond and Hunter 1990). Patterns of sexuality are generally thought to be consistent within scleractinian families. Hermaphroditism is found in the families Acroporidae, Faviidae, Merulinidae, Mussidae, Pectiniidae and Pocilloporidae, whereas gonochorism is found in the Agariciidae, Caryophylliidae, Dendrophylliidae, Flabellidae, Fungiidae and Siderastreidae (Harrison and Wallace 1990). The family Poritidae contains both gonochoric and hermaphroditic species. As with most modular organisms, corals are capable of a vast array of asexual reproductive techniques, including fragmentation (Highsmith 1982), polyp "bail out"

(Sammarco 1982), and the dramatically named "phoenix effect" in mushroom corals (Krupp et al. 1992).

1.6.2 Gametogenesis

Corals do not have true sex organs, however it is acceptable to refer to the sites of gamete development as gonads (Campbell 1974), and throughout this work I will use the terms ovaries and testes when referring to sites of female and male gamete development. Histological examination of corals show that primordial germ cells begin development in the endodermal cell layer, in the mesenteries adjacent to the mesoglea, and then migrate into, and are eventually enveloped by mesoglea (Campbell 1974; Szmant-Froelich et al. 1980). In most species studied to date, gonads continue development within the mesenteries, however the arrangement of gonads varies depending on family (Harrison 1985). In hermaphroditic species, ovaries and testes may be intermingled on the same mesentery; they may develop separately, but adjacent on the same mesentery; or they may develop on different mesenteries. The length of the gametogenic cycle in broadcasting species is usually less than 12 months, allowing for one or possibly two annual gametogenic cycles (Harrison and Wallace 1990).

1.6.3 Oogenesis

In *Astrangia danae*, *Favia fragum* and other scleractinians that have been examined histologically, the earliest recognizable stages of oogenesis are what appear to be primordial oogonia present in the gastrodermis of the mesenteries, adjacent to the

mesoglea (Szmant-Froelich et al. 1980; Szmant-Froelich et al. 1985). At this stage the cells usually have large nuclei, little cytoplasm and are solitary rather than in aggregations as is the case with early spermatogonia. These cells migrate in to the mesoglea around the same time that vitellogenesis (production of yolk) begins. As oocyte development continues the ratio of cytoplasmic to nuclear volume increases. Oocytes often form irregular shapes due to the spatial constraints within the corallite. When oocytes are close to maturity a vitelline membrane forms beneath the plasma membrane and the nuclei often migrate towards the periphery of the cell (Szmant-Froelich et al. 1980; Szmant-Froelich et al. 1985; Glynn et al. 1991; Glynn et al. 1994). Degeneration, resorbtion and fusion of oocytes have been reported in a number of species, and it would appear that this phenomenon is a unique feature of cnidarians (Campbell 1974; Harrison and Wallace 1990). Oocytes often become highly pigmented a few weeks prior to spawning and can be pink, red, yellow, orange, gray, green, aquamarine or purple in colour (Harrison et al. 1984, Babcock et al. 1986). Mature eggs are thought to be rich in lipids, which may act as storage and as a buoyancy device (Harrison and Wallace 1990). In some coral species, zooxanthellae migrate in to the eggs prior to fertilisation or release (Kojis and Quinn 1981a; Heyward 1986; Tomascik and Sander 1987), but in most cases, larvae acquire zooxanthellae post fertilisation. Mature egg sizes vary depending on family, but maximum diameters generally range between 100μm and 800μm (Harrison and Wallace 1990).

1.6.4 Spermatogenesis

The first recognizable signs of spermatogenesis in scleractinians are the appearance of small aggregations of cells in the endodermis of the mesenteries, adjacent to the mesoglea. These cells enter, or are engulfed by mesoglea and testes develop as a result of new cells being added, either by migration of more cells from the gastrodermis or by mitosis of the existing cells. These spermatogonia then differentiate into primary spermatocytes, which undergo meiosis to form secondary spermatocytes and then spermatids. Spermatids then undergo spermiogenesis and develop the features of mature spermatozoa such as a flagellum and organelles of the sperm midpiece. In the lead up to spawning, sperm development may proceed very rapidly (Campbell 1974; Szmant-Froelich et al. 1980; Szmant-Froelich et al. 1985; Harrison and Wallace 1990; Glynn et al. 1991; Glynn et al. 1994). The motility of flagellae can be used to assess the level of sperm maturity prior to spawning by observing live sperm smears under the compound microscope (Harrison et al. 1984, Babcock et al. 1986).

1.6.5 Gametogenic cycles and spawning

Like many marine invertebrates, corals generally have cyclic reproductive patterns that are based on annual, bi-annual or monthly rhythms (Campbell 1974). Many broadcasting corals have just one or two annual spawning events, whereas most brooding corals have a monthly cycle of spawning and complete a number of gametogenic cycles each year (Harrison and Wallace 1990). For many broadcasting corals, spawning consists of a short, intense event, occurring over one or a few nights,

whereas brooders may release planulae over a number of days. Spawning involves the release of gametes, either separately in the case of gonochoric species, or as egg-sperm bundles in the case of hermaphroditic species (Richmond 1997; Richmond and Hunter 1990). For many species, gamete bundles float to the surface where fertilisation takes place in the two-dimensional environment of the sea surface. During 'mass' spawning events (see below, Harrison et al. 1984) these aggregations of gametes appear as slicks on the surface, and can be a few kilometers in length, making them visible from the air (Oliver and Willis 1987). For 19 broadcasting species on the Great Barrier Reef (GBR), cleavage of fertilised eggs began two hours after spawning, and planula larvae were fully developed and mobile within 36-48 hrs after spawning (Babcock and Heyward 1986). Scleractinian planulae are often spheroidal or cigar shaped and ciliated so that they can swim throughout the water column (Harrison and Wallace 1990). Larvae are often fully developed within four to seven days, after which they begin benthic-searching behaviour, settle and metamorphose into juvenile polyps, complete with mesenteries, tentacles and primordial skeleton (Babcock and Heyward 1986; Harrison and Wallace 1990). After metamorphosis, polyps then divide by asexual budding and grow to form coral colonies. Colony growth continues by both polyp division and skeletal accretion. While polyp size is determined genetically, colony size is probably limited more by environmental constraints (Hughes et al. 1992). Therefore colonies may grow to several meters in diameter by simply adding more polyps and secreting skeleton. It is assumed that entire colonies are clonal (i.e. all polyps are genetically identical), however this has not been tested sufficiently, and it is possible that somatic mutation or other mechanisms may produce colonies which exhibit a genetic mosaicism (Fautin 1997; Hughes et al. 1992). Colonies often experience partial mortality and

fragmentation so that new colonies are formed; indeed asexual fragmentation is probably the most important mode of reproduction in many corals (Highsmith 1982). The coral can be seen at three levels therefore: the polyp, the colony and the genet (defined as all the polyps, fragments and colonies that are derived from the same zygote) (Hughes et al. 1992). The age at which colonies reach sexual maturity, varies between species and between brooders and broadcasters, with brooders maturing earlier (about 1-2 years) compared to broadcasters (about 4 years) (Harrison and Wallace 1990). Although in *Goniastrea favulus* sexual maturity is not simply a function of polyp age, rather a function of the interaction between polyp age and colony size (Kojis and Quinn 1985).

1.6.6 Fecundity, reproductive effort and stress

Reproductive success in corals is often measured in terms of fecundity (number of gametes or planulae produced) and reproductive effort (size or biomass of gametes or planulae). Kojis and Quinn (1984) suggested that coral fecundity could be useful as a biological indicator, if this paramenter is depressed by environmental stressors such as elevated turbidity and sedmentation. Corals need sufficient light for their symbiotic algae to carry out photosynthesis, although the energetics of reproduction in corals is still poorly understood, at least one study has shown that corals use photosynthetically derived materials for the production of gametes (Rinkevich 1989). As water turbidity increases, less light is available for photosynthesis, and under conditions of high sedimentation, photosynthetically derived materials may be diverted away from other essential functions and in to mucus production to help dispel sediment from the coral's feeding mechanisms (Rogers 1990). Therefore, when water turbidity and

sedimentation are high, reproductive output could be depressed because the amount of energy that is available from photosynthesis is reduced. Some field studies comparing fecundity and reproductive effort in populations of brooding corals between sites, that experience different levels of turbidity and sedimentation, have indicated that this may be the case. For example, in Papua New Guinea, fecundity in the brooding coral *Acropora palifera* was negatively correlated with depth, turbidity and sedimentation (Kojis and Quinn 1984). Similarly, in Barbados, Tomascik and Sander (1987) found in the gonochoric brooder, *Porites porites*, that colonies exposed to elevated turbidity and eutrophication, contained lower numbers of larvae compared to colonies on a less disturbed reef. The role of sediment as a stressor on corals is not well understood, as diverse reefs are found in the presence of high sediment levels and corals are capable of ingesting (thus benefitting from) fine suspended sediments in the water column (Anthony 1999; 2000). Suprisingly, few comparative fecundity studies have been conducted on broadcasting species, despite the fact that this is the dominant mode of reproduction in scleractinians (Villinski 2003). Light availability and sedimentation are not the only factors that can affect reproduction in corals, exposure to elevated nutrients alone may be enough to depress reproductive effort. During the ENCORE experiment on the GBR Ward and Harrison (2000) found that colonies of *Acropora aspera* and *A. longicyathus* produced significantly smaller and fewer eggs and contained less testes material when exposed to elevated nitrogen. Similarly, Cox and Ward (2002) found that planulation ceased in colonies of *Pocillopora damicornis* following 4 months of exposure to elevated ammonium levels.

Fragmentation is an important mode of asexual reproduction in many corals (Highsmith 1982), however, repeated physical disturbances causing colony

fragmentation can reduce fecundity in some corals and may even result in colony mortality (Szmant-Froelich 1985). In a study of *Pocillopora damicornis*, experimental removal of fragments delayed the onset of planulation, and most fragments died within 18 days, releasing few planulae (Zakai et al. 2000). As a result, sexual reproductive output of whole colonies was reduced, because of the reduction in tissue volume (Zakai et al. 2000). In the Caribbean broadcaster, *Montastrea annularis*, Van Veghel and Bak (1994) found that small superficial surface wounds on the colony surface reduced the fecundity of polyps within 2cm of the lesions, compared to polyps 20cm away from lesions. Similarly, in colonies of *Favia favus* in the Red Sea, Oren et al. (2001) found that small lesions ($<1cm^{-2}$) repeated monthly, caused localised reductions in fecundity. Whereas large lesions ($2 - 3cm^{-2}$) caused reductions in fecundity up to 15 cm away from the site of injury. Fragments of *Goniastrea favulus* removed from colonies 6 months prior to spawning had lower fecundity than equivalent fragments that had been separated for 2 years (Kojis and Quinn 1981b; 1985). The effects of coral bleaching have been investigated in the soft coral *Lobophytum compactum* (Michelak-Wagner and Willis 2001). Egg size and polyp fecundity were significantly reduced for 2 annual breeding seasons following experimental bleaching, despite the fact that corals recovered their zooxanthellae within 10 to 18 weeks. Similarly, following a natural bleaching event on the GBR, Baird and Marshall (2002) found a substantial decrease in the reproductive output of *Acropora* populations, partly because of colony mortality, and partly because fewer of the surving colonies were gravid compared to previous years when bleaching did not occur.

1.6.7 Environmental cues regulating timing of reproduction

Breeding synchrony is a common strategy in marine invertebrates, and is presumably necessary to ensure fertilisation success among populations of sessile marine invertebrates (Campbell 1974). To understand the environmental causes of reproductive timing and synchrony, it is useful to distinguish between ultimate and proximate factors. Proximate factors are those that act as cues or constraints to regulate gametogenic cycles, the onset of gamete maturation and the timing of spawning. Proximate factors can be further divided into necessary environmental conditions (e.g. cellular activities necessary to reproduction only occur within certain environmental limits), and specific environmental signals (i.e. cues that control the timing of reproduction) (Olive 1995). Ultimate factors, on the other hand, are evolutionary selective pressures that have caused the timing of reproduction. In other words, spawning at one time, rather than another increases fitness in some way, for example by maximising fertilisation success, larval survival, larval settlement or post-settlement survival (Oliver et al. 1988; Olive 1995) (Fig 1.3).

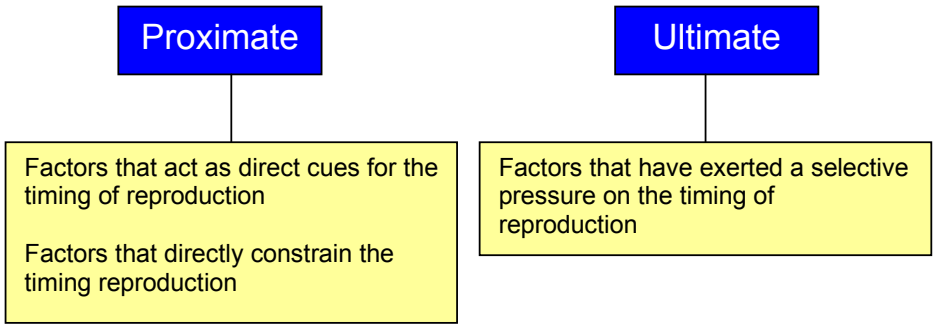


Fig. 1.3 The three ways in which proximate and ultimate factors can affect the timing of reproduction

At a proximate level, environmental factors such as sea surface temperature (SST), irradiance, photoperiod, lunar and diel cycles have been suggested as important cues for reproduction in corals (Harrison and Wallace 1990). Sea temperature is postulated to be the most important environmental factor regulating breeding cycles in marine invertebrates (Orton 1920). For broadcasting corals, there is now a reasonable body of correlative data to suggest that changes in SSTs provide a seasonal cue for reproduction (Babcock et al. 1986; Richmond 1997; Richmond and Hunter 1990; Mendes and Woodley 2002), and it is plausible that a physiological basis for such a causal relationship exists (Olive 1995). However, it is important to be aware that a causal link cannot be established based purely on observations of concurrence (Olive 1995). Only one example of a manipulative experiment testing the relationship between temperature and spawning time in broadcasting corals exists in the literature. Hunter (1988) showed that spawning was delayed in colonies of *Montipora verrucosa* and *M. dilatata* maintained at winter seawater temperatures during the normal spawning period for these species. Broadcasting corals can spawn when sea temperatures are rising (i.e. late spring/early summer), when SSTs are falling (i.e. late summer/early autumn), or during the interim period in the warmest months of the year (i.e. during summer). On the Great Barrier Reef (GBR), the majority of broadcasting corals spawn in late spring/early summer (October and November), coinciding with a period of rapidly rising SSTs prior to the summer maximum (Harrison et al. 1984; Willis et al. 1985; Babcock et al. 1986). However, some *Acropora* species spawn one to three months after the main spawning season (Wolstenholme 2003); spawning at some inshore reefs occurs one month earlier than offshore reefs; and three species of *Montipora* also spawn in late Summer/early Autumn (March and April) after the summer maximum (Stobart et al. 1992). In Western Australia (WA) the main

spawning season for broadcasting species is 5 months apart from that on the GBR (March and April), although many corals spawn bi-annually on Scott Reef (S 14°, E 121°) with the second spawning season occurring at the same time as the main spawning season on the GBR (J. Gilmour, personal communication). The seasonal reversal is interesting between the west and east coasts of Australia, because both coasts experience similar seasonal patterns of SSTs (Simpson 1985). In Hawaii, five species of *Montipora* spawn synchronously, predominantly in summer (July), at the time of maximum SSTs and solar irradiance (Heyward 1986). Similarly, in the Caribbean (Curacao) and Gulf of Mexico at least seven broadcasting species spawn in August and September during the period of maximum SSTs (Hagman et al. 1998; Van Veghel 1993; Van Veghel 1994). At Akajima Island, Okinawa (Japan), spawning of at least 85 broadcasting scleractinians occurs over a period of 5 months. Most *Acropora* spawn during a period when SSTs are rapidly rising. Whereas most Faviids spawn during the warmest months of the year when SSTs are stable (Heyward et al. 1987; Hayashibara et al. 1993). Similarly, in Eilat (northern Red Sea), spawning of 22 broadcasting scleractinians occurs over a few months, as SSTs are rising and during the period of maximum SSTs (Shlesinger and Loya 1984; Shlesinger et al. 1998). In Southern Taiwan, at least 36 species spawn while SSTs are rising (April/May), whereas in Northern Taiwan at least 20 broadcasting corals spawn during Summer when SSTs are at the annual maximum (Dai et al. 1992). So the relationship between spawning times and changes in annual SSTs is not completely consistent, suggesting that another factor or factors such as photoperiod or irradiance may be important. Furthermore, in some regions (such as equatorial reefs) annual sea temperature variation may be too small to act as a reliable cue (Olive 1995). Babcock et al. (1994) suggest that photoperiod may be more important than sea temperature as a proximate

cue for spawning, because the majority of spawning occurs in the same month in tropical (Ningaloo) and temperate (Houtman-Abrolhos) reefs on the West coast of Australia, despite more than two months difference in the timing of seasonal temperature minima between the two regions, while the seasonal photoperiod patterns are similar (with respect to the timing of seasonal maxima and minima) (Babcock et al. 1994). More recently, Penland et al. (2004) questioned the role of sea temperature in timing of spawning, and suggested that solar insolation maxima (the electromagnetic energy incident on the earth's surface), which also varies seasonally even at the equator, is a better predictor of spawning times in the Western Pacific (Penland et al. 2004). Corals may possess cells that are photosensitive, light may act directly on gametes or seasonal changes in solar irradiance may be perceived indirectly through the photosynthetic activities of the symbiotic zooxanthellae (Harrison and Wallace 1990).

While temperature, photoperiod or irradiance may provide seasonal cues for reproduction for broadcasting corals, lunar and diel cycles are putative factors in determining the night and time of coral spawning. Most of the broadcasting corals studied to date appear to follow a lunar pattern, with gamete release usually occurring on or shortly after the full or new moon. Lunar phase has experimentally been shown to be an important cue for spawning in *Montipora verrucosa* and *M. dilitata*, and planulating in the brooder *Pocillopora damicornis* in Hawaii (Jokiel et al. 1985; Hunter 1988). And so far, one study has shown that scleractinians can detect the light of equivalent intensity and wavelength to that of moonlight (Gorbunov and Falkowski 2002). Gamete release usually occurs just after sunset, although spawning can occur earlier in the evening or at dawn, and experimental manipulation of the light/dark

cycle revealed that the onset of darkness acts as a cue for spawning in *Goniastrea aspera*, *M. verrucosa* and *M. dilitata* (Babcock 1984; Hunter 1988).

The ultimate evolutionary causes of spawning in corals are not well understood. Temperature could play an ultimate role in the timing of spawning, if spawning when sea temperatures are at or close to the annual maxima increases the rate of larval development, growth and survival (Olive 1995). Sunlight might also be an important factor, because corals derive most of their energy from photosynthetic symbiotic algae. Based on a meta-analysis of spawning times and climatic conditions at 20 geographical locations, Mendes and Woodley (2002) concluded that rainfall has had an ultimate role in the timing of coral spawning, at two possible levels. Firstly, rainfall increases river runoff and reduces salinity in surface waters, so heavy rainfall during spawning could greatly reduce fertilisation success and larval survival. Secondly, spawning just prior to the heaviest rainfall months would benefit larvae and newly settled polyps because increased nutrients might stimulate phytoplankton blooms and provide a source of food for larvae and new settlers (Mendes and Woodley 2002).

On reefs in the GBR and in Western Australia, corals spawn during a period of slack water and low tide, Babcock et al. (1986) proposed that spawning at this time might enhance fertilisation success because of reduced dispersal and dilution. Thus tides may have played an ultimate role in selecting the night and time of spawning (Oliver et al. 1988). There may be a selective advantage to spawning at night, as this strategy could reduce predation on eggs and reduce the risk of photo-damage during embryo development.

Finally, Simpson (1985) considers genetic legacy to be the underlying factor behind spawning timing, because corals on the east and west coasts of Australia spawn at different times, despite experiencing the same seasonal pattern of sea temperature. Simpson (1985) proposed that the timing of spawning was set in the corals ancestral environment, and that the observed differences in spawning patterns are as a result of changes in current regimes (because these control the transport of coral larvae from more equatorial reefs). However, this does not explain how the pattern first evolved in the ancestral populations (Oliver et al. 1988). Furthermore, recent studies show that corals in Western Australia also spawn at the same time as those on the GBR (J. Gilmour personal communication). In the absence of rigorous experimental evidence, hypotheses about ultimate causes for the timing of reproduction in broadcasting corals should be viewed with a degree of caution (possibly even profound skepticism). The fact that many of the seasonal parameters that may influence spawning patterns are not independent of each other (e.g. SSTs, solar insolation, rainfall, cloud cover and photoperiod), adds a further complication to improving understanding of the relationship between environmental parameters and spawning cycles. Further multi-disciplinary studies are needed to better understand the causal relationship between environmental patterns and spawning seasonality and synchrony, including biogeographical comparisons, manipulative experiments and physiological and molecular level investigations of the biological basis for a causal relationship (Olive 1995).

1.6.8 Reproductive seasonality and synchrony

One of the most remarkable features of coral communities is the phenomenon of multi-species synchronous spawning, or 'mass spawning' (Harrison et al. 1984; Willis et al. 1985; Babcock et al. 1986). This was first documented on the GBR in Australia in the early 1980's. There, over a period of eight nights in late spring, at least 133 different coral species release their gametes for external fertilisation and as many as 31 species have been found to participate in spawning on the same night on one reef (Babcock et al. 1986, Willis et al. 1985). Twenty years on from the first mass spawning observations and it is still not clearly understood what causes this remarkable reproductive phenomenon to occur. Within-species spawning synchrony is understandable as it increases the chances of successful fertilisation. However, the reason why many unrelated species might spawn together are less clear – particularly when one considers the potential disadvantages of such a strategy, for example, the production of non-viable inter-species hybrids and competition between larvae for settlement space (Shlesinger and Loya 1984). A possible advantage of this strategy is that opportunistic predators are satiated during spawning periods. One possible explanation is that timing of reproduction in most species is constrained by some environmental factor to occur only at one time of the year when conditions are suitable. Another possible explanation is that selective pressures (ultimate factors) may have caused many species to spawn at the time of the year when environmental conditions are optimal for successful fertilisation, larval survival and recruitment (Oliver et al. 1988). Considering that most mass spawning species are congeneric, the most plausible explanation is that species have responded similarly, but independently

to seasonal environmental cues to maximize fertilisation success within species, resulting in many species releasing gametes during discreet 'spawning periods'.

1.6.9 Geographical and latitudinal variations in spawning synchrony

One way to gain a better understanding of the 'ultimate' causes of multi-specific spawning synchrony, is to compare the "results" of natural, long-term experiments, i.e. to investigate and compare the timing of coral spawning in different regions, that exhibit a range of seasonal and environmental conditions (Oliver et al. 1988). Early examples of such comparisons showed that multi-species reproductive synchrony was not a characteristic of all coral communities (Richmond and Hunter 1990). In particular, studies in the Red Sea and the Caribbean found the corals there tended to be asynchronous (Shlesinger and Loya 1984, Szmant 1986). The lack of synchrony in these regions was mostly attributed to a distinct narrowing in the range of environmental parameters, such as annual sea surface temperature and tidal amplitude (Oliver et al. 1988; Richmond and Hunter 1990). In the Gulf of Eilat, Israel (Northern Red Sea), spawning is reported to occur over 3 to 4 months in the summer, apparently, with no overlap in timing between species, although spawning within species is synchronous and highly predictable (Shlesinger and Loya 1984; Shlesinger et al. 1998). Shlesinger and Loya (1984) suggested that in the Eilat, where most environmental parameters remain constant for three to four months during the summer, corals would tend to partition their spawning times temporally as a strategy to avoid unsuccessful hybridization and competition for settlement space between larvae.

In equatorial regions where sea temperature and tidal ranges are often small, it was thought that little reproductive synchrony among species would occur (Harrison and Wallace 1990). Indeed, Oliver et al. (1988) found a reduction in spawning synchrony in three scleractinian species studied at five locations along a latitudinal gradient ranging from the southern GBR (Heron Island, 23 º S) and the northern coast of Papua New Guinea (PNG) (Madang, 5º S). Similarly, Kojis (1986) found that the brooding species *Acropora palifera* spawned only once a year on the southern GBR, but spawned year round in the northern GBR and in PNG. However, more recent research has shown that high levels of multi-specific reproductive synchrony do occur in some coral communities that had previously been thought to be asynchronous. In the Caribbean, at least seven scleractinian species have been documented spawning synchronously between the seventh and tenth nights following the full moons in August and September (Gittings et al. 1994; Boland 1998; Hagman et al. 1998). In the Solomon Islands (8º N) where there is little fluctuation in annual temperature or tidal amplitude, Baird et al. (2001) found that 28 of 41 *Acropora* species contained mature eggs in the week prior to the full moon in November 1999. In the Karimunjawa Islands (central Java Sea) where sea temperature ranges between 27.5°C and 29°C, (Edinger et al. in Tomascik et al. 1997) observed 22 scleractinian species spawning over three nights following the full moon in October 1995. More recently, in Palau (7° 30'N), Penland et al. (2004) observed that the majority of spawning in broadcasting corals in 2002 was after the full moon in April, although multi-species spawnings were also observed after the full moons of May, August and September 2002 and February 2003 (Penland et al. 2004). These observations indicate that multi-species spawning synchrony may indeed be a feature of equatorial reefs, however further observations are needed to establish the extent of synchrony and to understand what

factors have caused synchrony to occur in the absence of large environmental fluctuations.

Chapter 2

Singapore's marine environment, coral reefs and a description of the study sites

2.1 Introduction

Singapore is a small, heavily populated island nation situated at the southern most tip of Peninsula Malaysia, approximately 137 km north of the Equator, between latitudes 1º 09'N and 1º 29'N, and longitudes 103º 36'E and 104º 25'E (Fig. 2.1). The country consists of one large, main island and some 63 small offshore islands, most of which lie to the south of the main island (Fig 2.2). The total land area including the offshore islands is approximately 659.9 km^2 and the surrounding territorial waters have an almost equal area of 630 km^2 (Chou and Goh 1996; Ministry of Information and the Arts 2000). Despite the nation's small size, Singapore has developed rapidly in the last few decades, to become one of the most economically successful countries in Asia. As Singapore is a maritime nation, the coast and territorial waters are seen as being vital to the country's continuing development. The port currently ranks as one world's busiest and many of the islands have been transformed to provide space for offshore oil refineries, military training areas and landscaped recreational areas (Chou and Goh 1996). Extensive land reclamation has been carried out since 1963 around all of mainland Singapore and many of the southern islands, causing the loss of more than 65% of Singapore's coral reef area (Hilton and Manning 1995; Chou 1996; Chou et al. 1994). Apart from the direct loss of coastal habitats caused by reclamation, Singapore's marine ecosystems are exposed to a range of anthropogenic stressors

including: high levels of sedimentation and turbidity, dredging of shipping lanes and dumping of earth spoils (Lane 1991; Low and Chou 1994; Chou and Goh 1996); high levels of coastal eutrophication (Gin et al. 2000); and marine pollution from occasional oil and chemical spills (e.g. Chong and Chiang 2001).

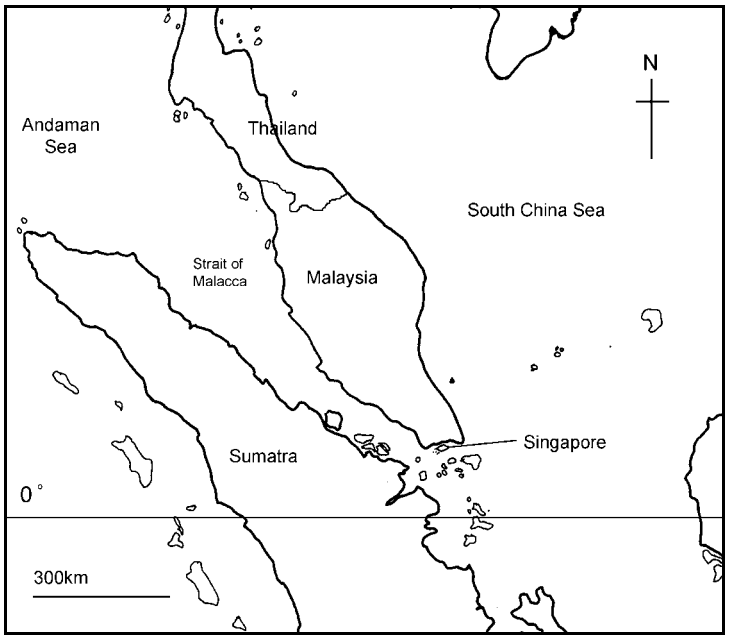


Fig 2.1 Map of South East Asia showing the proximity of Singapore to the equator.

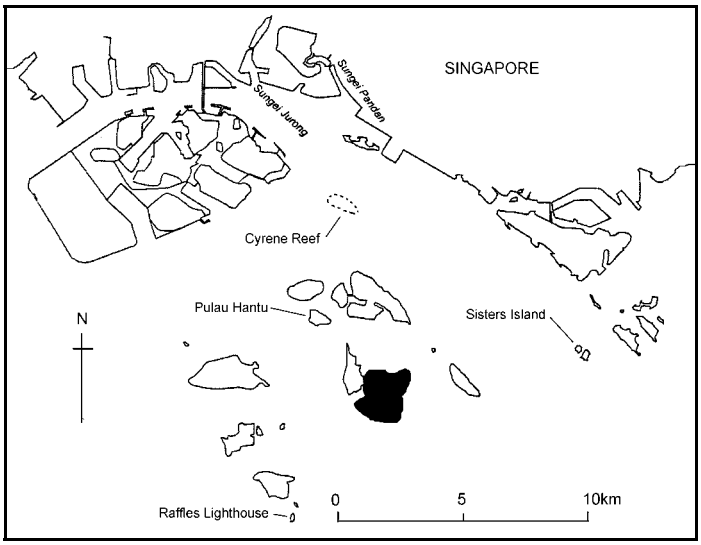


Fig 2.2 Map of Singapore's southern islands showing the relation to the main island and the location of the four study sites, the blackened area is a reclaimed landfill site attached to Pulau Semakau.

2.2 Coral reefs of Singapore

Little is known about the status of Singapore's reefs prior to 1960s, when major land reclamation began. Admiralty charts from 1953 show that the distribution and total area of coral reefs has been greatly reduced in the last 50 years by reclamation (Hilton and Manning 1995; Chou and Goh 1996; Hilton and Chou 1999). Unfortunately, since major coastal development began in the early 1960's, the Government has shown very little interest in conserving Singapore's marine ecosystems; consequently the local coral reefs enjoy little formal protection (Hilton and Manning 1995; Frances 2001). Sadly, awareness about local marine ecosystems is also lacking among the general populace, with most people being unaware of the existence of diverse coral reefs in Singapore's waters (Chang 2002; Frances 2003).

Fringing and patch reefs are found around most of Singapore's southern islands. The reefs are described as having wide reef flats and steep reef slopes, but lacking lagoons or distinctive reef crests (Chuang 1977; Chou and Wong 1985; Goh et al. 1994; Hilton and Chou 1999). Despite the unfavourable environmental conditions, coral diversity is relatively high. Chou et al. (1994) reported that 197 scleractinian coral species belonging to 55 genera have been recorded during baseline surveys. Coral cover is variable, but can be as high as 75% on the upper reef slope of certain reefs (Chou 1996). The fringing reef on the west side of Raffles Lighthouse is probably similar in terms of coral diversity to some of the inner reefs of Australia's Great Barrier Reef (e.g. Magnetic Island, A. Baird personal communication). The scleractinian communities found on Singapore's reefs are dominated by foliose, massive and encrusting growth forms, while branching and digitate growth forms are

less common (Goh et al. 1994). Scleractinian corals are restricted to the reef flat and the upper margins of the reef slope (between 0 and 8 meters depth) (Chua and Chou 1991), and it seems likely that the relatively poor water transparency is the primary factor preventing corals from surviving deeper on the reef slope (Chou 1996).

2.3 The marine environment and seasonality

2.3.1 Water quality

Average underwater visibility around the southern islands decreased from an average of 10 m in the mid 1980s, to an average of 2 m a decade later (Chou 1996). In over 300 scuba dives in Singapore's waters since 1999, I have seen horizontal underwater visibility ranging anywhere from 0.5 m to around 5 m (personal observation). It seems likely that water clarity was not always this poor. In one anecdotal account from the 1850s of a day trip to one of the southern islands, Benjamin Cook reported "The sea here is most perfect for swimming. By any shore where there is not mangroves, the water is calm, without currents and beautifully clear" (in Marshall 2004). Rogers (1990) considered sedimentation rates between 1 and 10 mg cm^{-2} day^{-1} to be normal for un-impacted reefs. In a study carried out between 1989 and 1993, Low and Chou (1994) found sedimentation rates ranging from 5 – 45 mg cm^{-2} day^{-1}. Mean sedimentation rates were higher at the site closest to mainland Singapore (Cyrene, 14.64 mg cm^{-2} day^{-1}) than at sites further away (Pulau Hantu, 9.90 mg cm^{-2} day^{-1}) and (Raffles Lighthouse, 7.5mg cm^{-2} day^{-1}). Furthermore, sedimentation rates at Cyrene sometimes exceeded 44 mg cm^{-2} day^{-1} compared to the other sites, which never exceeded 13 mg cm^{-2} day^{-1}. Similarly, Todd et al. (2004) found that average levels of

total suspended solids (TSS) and sedimentation were higher at Cyrene than at Pulau Hantu and Raffles Lighthouse, indicating that there is a general pattern of decreasing TSS and sedimentation with increasing distance from the mainland.

2.3.2 Tidal patterns

Singapore experiences a semi-diurnal tidal pattern i.e. there are two high tides and two low tides each day. Tidal range or tidal amplitude, defined as the difference between mean high water spring and mean low water springs, is 2.4 m at Raffles Lighthouse (Maritime and Port Authority of Singapore 2002, 2003). Tidal streams are variable but can be as high as 3.5 knots. In this study, diving work was conducted during slack water, or when the diving site was situated in the lee of the direction of water movement. Most fieldwork was done on the western sides of the study sites; therefore diving work was conducted during the flood tide when water movement was predominantly in a westerly direction.

2.3.3 Environmental seasonality

Singapore has a climate that is typical of many equatorial, maritime locations, i.e. temperature and rainfall levels are relatively high and constant throughout the year (Nieuwolt 1973). Because of the uniformity of conditions, Singapore's environment is typically considered to be 'aseasonal'. However, distinct seasonal variation is observed, particularly in the marine environment, and this seasonality is controlled by the Southeast Asian monsoon system (Tham 1973). Singapore experiences two monsoon seasons and two inter-monsoons each year. The Northeast (NE) monsoon

usually begins in November or December and usually ends in late February or early March. Slightly lower air temperatures and heavier rainfall in Singapore typify the NE monsoon period. The Southwest (SW) monsoon occurs between May and August and brings slightly higher temperatures and occasionally heavy southwesterly storms (known locally as 'Sumatras'). However, Sumatras tend to be restricted to the west coast of Peninsular Malaysia, therefore rainfall in Singapore is not usually higher at this time. The first inter-monsoon usually occurs in March or April, and the second inter-monsoon usually occurs in September or October. The inter-monsoon periods are characterised by low wind speeds and lower rainfall. Air temperature varies little with the changing monsoons, however rainfall, wind direction, prevailing water currents, salinity and seawater temperature (SST) are affected (Nieuwolt 1973). These changes result in the 'seasonality', (at least in terms of temperature) being more pronounced in the marine environment than on land.

2.3.4 Sea surface temperature (SST) and salinity

Around Singapore's southern islands SSTs are lowest during the NE monsoon (December - March) (Fig 2.4). Between the end of the NE monsoon and the beginning of the SE monsoon (March – May) SSTs increase from around 27 - 28 ºC to 30 – 31 ºC, and the annual peak in SST usually occurs in May and June (Fig 2.4). A second smaller temperature peak occurs during the second inter-monsoon (October – November) just prior to the onset of the NE monsoon (Tham 1973) (Fig 2.4). Similarly, air temperatures dip at the start of the NE monsoon, and they peak at the start of the SE monsoon. However, the annual variation in air temperature is less than that of sea temperature (Tham 1973, Fig 2.4). Gin et al. (2000) found salinity values

ranging from 28.7 ppt to 32.2 ppt annually (Fig 2.5). Maximum values (30 – 31.5 ppt) occur during the NE monsoon and the first inter-monsoon (between October and May), whereas lowered salinity (28.5 – 29.9 ppt) is found during the SW monsoon from June to September (Fig 2.5). During the height of the NE monsoon from December to January there is also a slight drop when values range from 30.1 – 30.7 ppt (Tham 1973) (Fig 2.5). The annual changes in SST and salinity in the Singapore Straits are a combined result of rainfall, solar radiation, wind and the movement of water masses from the surrounding seas (Tham 1973). During the NE monsoon there is a net transport of water from the South China Sea bringing lower temperatures but higher salinity. However, heavy rainfall around Singapore and along the east coast of Peninsular Malaysia during the height of the NE monsoon (December and January), dilute the water entering the Singapore Strait. During the SW monsoon, there is a net transport of water from the Java Sea and Malacca Strait bringing higher temperatures but lower salinity (Tham 1973).

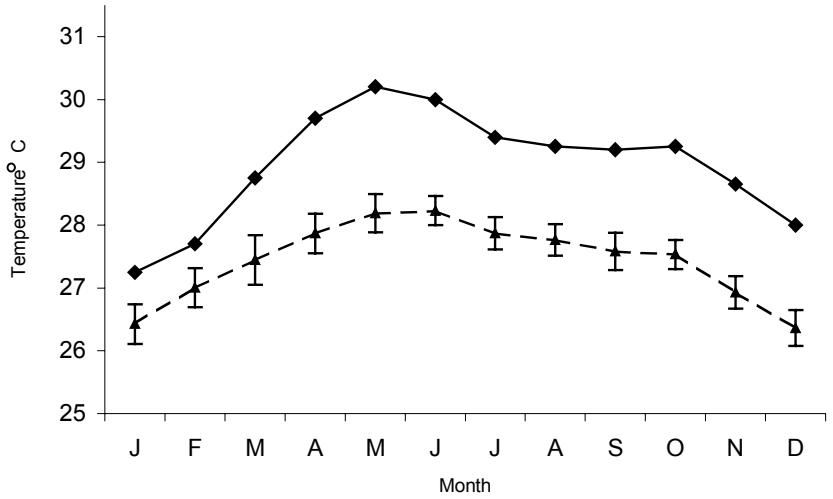


Fig. 2.3. The pattern of annual change in sea surface temperature in the Singapore Straits compared with air temperature. The solid line is mean monthly sea temperatures adapted from Tham (1973), and the dashed line is mean monthly air temperatures taken between 1982 and 2002 (Meteorological Service Singapore). Error bars are standard deviations.

2.3.5 Rainfall

Singapore does not have a true dry season, as even during the inter-monsoons, total monthly rainfall is usually in excess of 150 mm (Fig 2.6). Although it is wet throughout the year and rainfall levels are highly variable, there is a marked increase in precipitation for a period of 1 to 2 months during the northeast monsoon, usually between November and January (Fig 2.6), although this period of maximum precipitation can begin as early as October and as late as the end of January (Nieuwolt 1973).

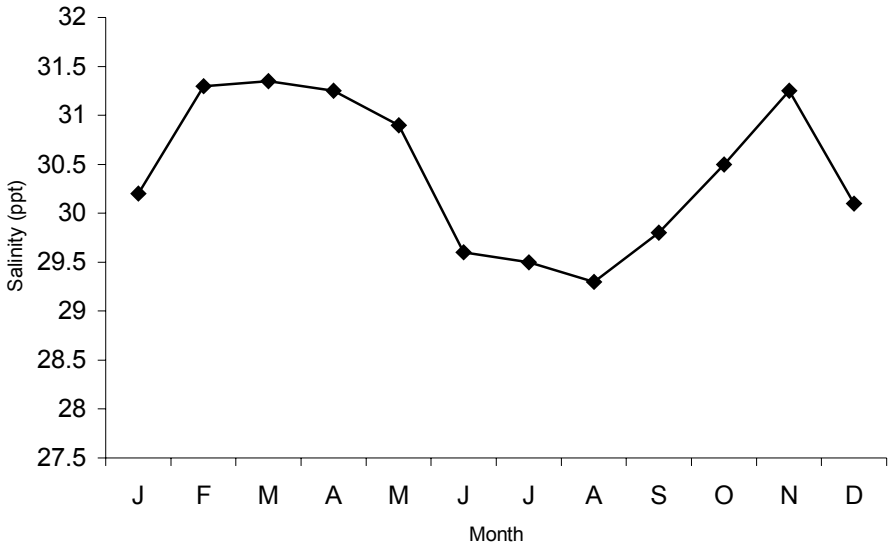


Fig 2.4. Seasonal pattern of salinity in parts per thousand in the Singapore Straits, from Tham (1973).

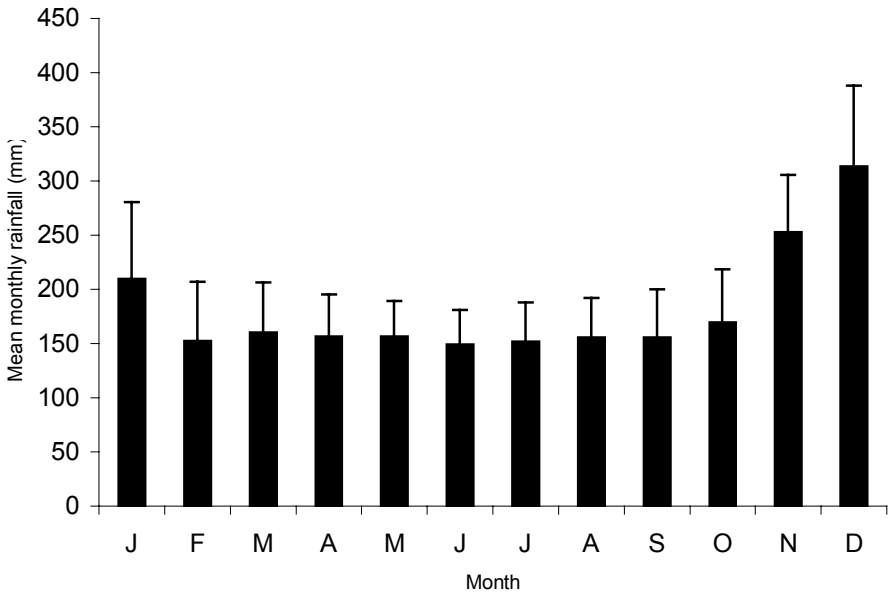


Fig. 2.5. Mean monthly rainfall (mm) at stations around Singapore from 1951 to 2000 (Singapore Meteorological Office). Error bars are standard deviations.

2.3.6 Solar radiation and photoperiod

The length of day (photoperiod) in Singapore is almost constant throughout the year, with the difference between the longest and the shortest day being only nine minutes (Nieuwolt 1973). However, the amount of solar radiation (or solar insolation) does vary seasonally at the equator, partly as a result of annual changes in cloud cover (i.e. there are more rain clouds during the NE monsoon which hinders the penetration of sunlight) (Nieuwolt 1973), and partly because the geometric center of the Sun's disc crosses the equator twice a year, at the vernal and autumnal equinoxes (around the end of March and September respectively in the Northern hemisphere), causing two annual maxima in solar insolation (Penland et al. 2004).

2.4 Site descriptions

2.4.1 Raffles Lighthouse (1˚ 10'N, 103˚ 45'E)

Situated 15km south of the mainland (Fig 2.2), Raffles Lighthouse (Pulau Satumu) is the southernmost coral reef site in Singapore's territorial waters. Reclamation work in 1976 increased the island's size and affected much of the reef flat of the surrounding fringing reefs (Leng et al. 1990, Fig 2.7). Water turbidity and sedimentation is generally lower here than at other reef sites in Singapore (Low and Chou 1994; Todd et al. 2001), however waves created by passing tankers intermittently stir up sediments and can reduce underwater visibility to almost zero. Raffles Lighthouse has one of the few coral reefs in Singapore where an assemblage of the Genus *Acropora* can be found (personal observation). The healthy coral assemblages and reasonable underwater visibility, make this a popular site for recreational divers (Leng et al. 1990). However in 2003, access to this site was restricted by the Maritime Port Authority of Singapore (MPA) due to security concerns.

2.4.2 Pulau Hantu (1° 13'N, 103° 45'E)

Pulau Hantu (meaning Ghost Island in Malay language) is a designated recreational island located 8 km south of mainland Singapore (Fig 2.2). It actually consists of two islands (Pulau Hantu Kecil and Pulau Hantu Besar) (Fig 2.7). In 1974 and 1975 extensive reclamation was carried out to transform the islands into an area designed for family outings and recreational boating. The combined land area was increased from 240 m^2 to 1.22 km^2. Much of the reef flat surrounding the islands was covered

with sand, and a rock bund was constructed to prevent coastal erosion. Although much of the reef flat was destroyed, the upper reef slope remained relatively untouched (Chou 1988). Leng et al. (1990) surveyed the reef community of the patch reef just to the west of Pulau Hantu and found a well-established and diverse scleractinian community. Water conditions are generally calm at this site as the western patch reef acts as a barrier protecting the fringing reef from incoming wave action. Water visibility is lower, but not significantly different, from that at Raffles Lighthouse (Low and Chou 1994).

2.4.3 Cyrene Reef (1˚ 15'N, 103˚ 45'E)

Situated about 4 km south of mainland Singapore (Fig 2.2), Cyrene consists of three patch reefs (Terumbu Pandan, Pandan Beacon and South Cyrene Beacon), all of which are completely submerged except during low spring tides (Fig 2.7). Cyrene is the closest coral reef site to mainland Singapore, and sits in the middle of a fairway for ships travelling to and from the port, petro-chemical and industrial facilities. A survey by Chua (1990) found the cover of live coral at 3 m depth to be the lowest of all reefs surveyed in Singapore at that time (including Raffles Lighthouse, Pulau Semakau, Pulau Hantu and Hantu West). Goh and Chou (1992) found that average live coral cover was much lower at Cyrene compared to Raffles Lighthouse, although generic diversity was similar at both sites. Conditions at Cyrene do not appear to have improved in the last decade and much of the reef is now covered in dead coral rubble (personal observation). Thick growths of macro-algae, particularly *Sargassum* spp. and *Enteromorpha* spp. cover large areas of the reef flat to the upper edge of the reef slope seasonally between October and January (personal observation).

2.4.4 Sisters Island West (1° 12.5' N, 103° 50' E)

The Sisters are two islands, situated approximately 5.7 km south-east of mainland Singapore (Fig 2.2) that have been partially reclaimed and converted into recreational islands (Fig 2.7). No studies have previously been carried out on the reefs surrounding the Sisters Islands. However, fringing reefs with relatively high scleractinian abundance and diversity (equivalent to Raffles Lighthouse and Pulau Hantu) are found at this site despite the proximity to the mainland (personal observation). The islands are exposed to the open sea on all sides and experience strong tidal currents.

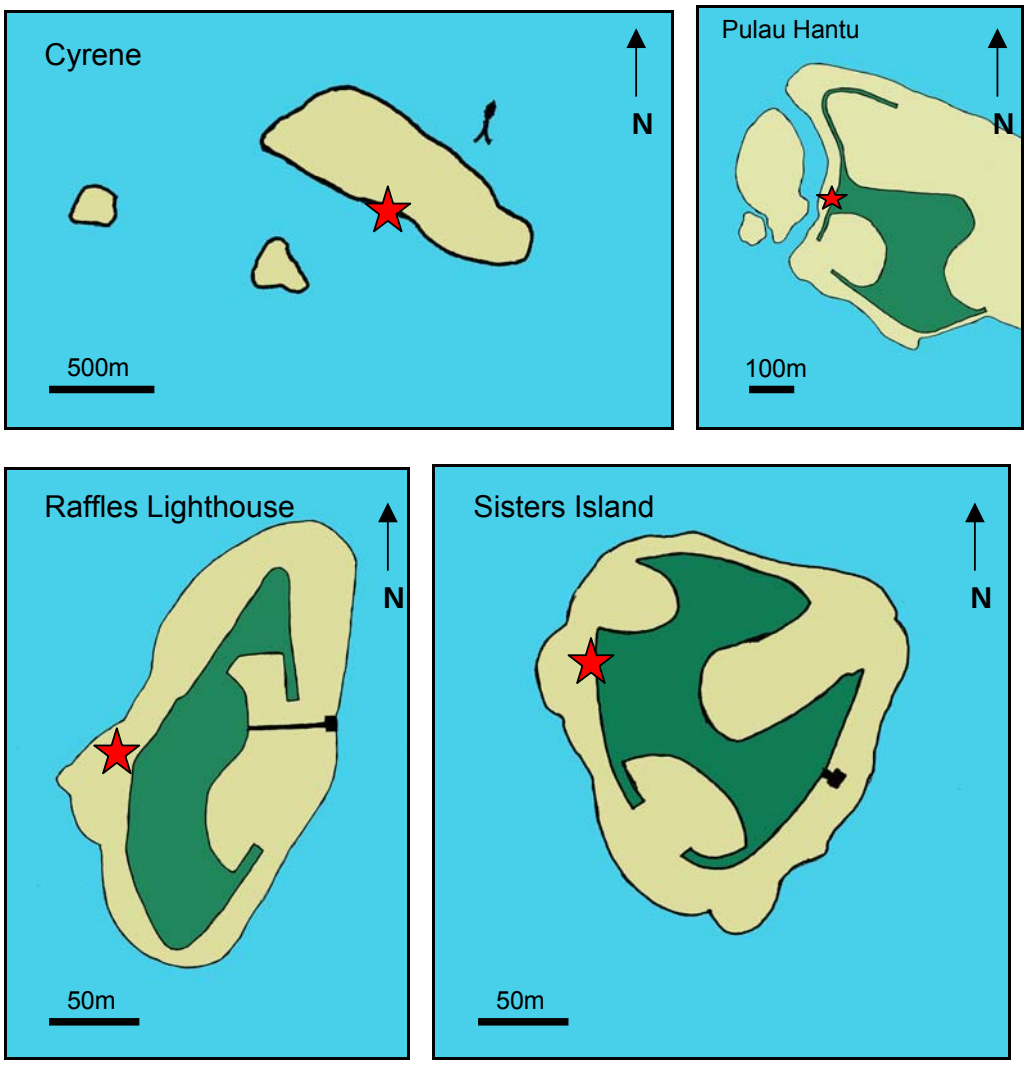


Fig 2.6. Maps of study sites, clockwise from top left: Cyrene reef, Pulau Hantu (west), Raffles Lighthouse and Sisters Island (west). The pale shaded areas are coral reef, red stars indicate locations of study and sampling sites.

Chapter 3

The relationship between gametogenic cycles of scleractinian corals and seasonal environmental patterns in Singapore

Abstract

The gametogenic cycles of two common scleractinian corals, *Porites lutea* (a gonochoric broadcaster) and a morphospecies of *Platygyra* (a hermaphroditic broadcaster), were investigated at three sites from March 2001 to April 2002. Both species had very similar, strongly seasonal patterns of gametogenesis, with maturation of gametes and spawning occurring primarily in April. A second, smaller peak in reproductive activity occurred in September/October for *P. lutea* and October/November for *Platygyra* sp., suggesting that some colonies also spawn at this time. Singapore is typically considered to be 'aseasonal', with little annual variation in environmental parameters. However, distinct and predictable seasonal patterns of sea surface temperature, salinity and rainfall do occur as a result of the Southeast Asian Monsoon system. Sunshine fluctuates seasonally, but a distinct pattern can only be seen when data are averaged over a number of years, as this parameter is highly variable over short time scales. For both species in this study, average increases in gamete size and numbers coincided with a rise in sea surface temperatures following the Northeast monsoon, indicating that this factor may provide the seasonal cue for gamete maturation and spawning in Singapore.

3.1 Introduction

This chapter focuses on the reproductive cycles of two scleractinian species from reefs around Singapore's southern islands. The species selected for this study were *Porites lutea* (Poritidae, Scleractinia) (Milne Edwards and Haime, 1851) and a morphological species (morphospecies) of the genus *Platygyra* (Faviidae, Scleractinia) (Ehrenberg, 1834). These corals were chosen because, a) they have contrasting reproductive strategies (*P. lutea* is typically gonochoric and *Platygyra* species are usually hermaphroditic), and b) they are abundant on most of Singapore's offshore reefs. The aims of this study were as follows: a) to describe the annual gametogenic cycles of *Porites lutea* and the *Platygyra* morphospecies using histological techniques; b) to compare the reproductive patterns of these species in Singapore with other geographical regions, based on existing studies; c) to examine and compare the reproductive seasonality and levels of intra-specific synchrony of these species; and d) to examine the relationship between the gametogenic cycles and the ambient seasonal environmental patterns.

3.1.1 *Porites lutea*

Porites lutea forms large, smooth, hemi-spherical or helmet shaped colonies that are creamy-brown in colour (Veron 2000). As the corallites are small (i.e. <2 mm in diameter), *P. lutea* can only be distinguished from other similar species by microscopic examination of skeletal characteristics. Corallites of *P. lutea* have a distinctive septation: the dorsal directive septum is usually shorter than the lateral pairs, and the ventral triplet of septa is usually fused (Veron and Pichon 1982). In

Singapore, colonies are common on the reef flats and upper reef slopes at most reef sites, and most colonies are between 0.5 m and 2 m in diameter (Chou 1988; and personal observation). The abundance of this species and its large colony size make it an ideal study species and one suitable for sequential sampling of individual colonies.

Detailed studies of reproduction of *P. lutea* have been done on the Great Barrier Reef (GBR) at Lizard Island, (14° 41'S) (Harriot 1983), at Heron Island (23° 27'S) (Kojis and Quinn 1981a) and in the Northern Red Sea at Eilat (29°30'N) (Shlesinger et al. 1998) (Table 3.1). In all of these studies, *P. lutea* was gonochoric and a broadcast spawner, although Shlesinger et al. (1998) found a few male polyps in one predominantly female colony. In these previous studies, it was found that the gametogenic cycle ranged in length from four to six months for oogenesis, and one to three months for spermatogenesis (Table 3.1). Observations of spawning *in situ* are rare, but are thought to occur over short periods of about a week in Heron Island (Kojis and Quinn 1981a) and after the full moon in November at Magnetic Island (GBR) (Babcock et al. 1986); and after the full moon in June and July at Akajima Island, Okinawa (Japan) (Hayashibara et al. 1993) (Table 3.1).

3.1.2 *Platygyra* morphospecies

Colonies of the genus *Platygyra* are abundant on the reef flats at most sites around Singapore's southern islands (Chou 1988; and personal observation). Colonies are massive and are usually dome shaped or sometimes flattened. Corallites are usually meandroid, forming valleys of varying length, with a number of polyps in each valley. In appearance, *Platygyra* is most similar to the genus *Goniastrea* but is differentiated

by a lack of paliform lobes and collumellae (Veron 2000). The genus is usually divided into species that have very short or monocentric valleys and those that have longer, or meandroid valleys (Veron et al. 1977; Veron 2000;). Miller and Benzie (1997) described seven species from the GBR that could be distinguished morphologically, however there was a great deal of overlap in characteristics among these species. Furthermore, there appeared to be few genetic differences between morphological species (termed morphospecies) (Miller and Benzie 1997), and no obvious temporal or gametic level reproductive barriers were found (Miller and Babcock 1997). Electrophoretic screening of the seven morphospecies suggested that they shared a common gene pool, although mating was not entirely random according to Hardy-Weinberg equilibria (Miller and Benzie 1997). In trials, it has been shown that fertilisation between morphospecies can occur at rates equivalent to within morphospecies fertilisations (Miller and Babcock 1997). *Platygyra* species including *P. lamellina*, *P. pini*, *P. sinensis*, *P. daedala*, *P. ryukyuensis* and *P. contorta* have been documented participating in coral spawning events on the GBR (Willis et al. 1985; Babcock et al. 1986; Richmond and Hunter 1990); in Western Australia (WA) (Babcock et al. 1994); in the Central Pacific (i.e. Guam, Marshall Islands and Palau) (Heyward 1988; Richmond and Hunter 1990); Japan (Okinawa) (Heyward et al. 1987; Hayashibara et al. 1993); and in the Red Sea (Shlesinger and Loya 1984; Shlesinger et al. 1998). The existing studies show all *Platygyra* species to be hermaphroditic broadcasters. However, to my knowledge, there are no detailed descriptions of the length and pattern of gametogenic cycles of any *Platygyra* species. Despite some of the taxonomic uncertainties inherent in this genus, it was chosen for this study because of its relative abundance at all reef sites around Singapore.

3.2 Methods and Materials

3.2.1 Study sites

Samples were collected each month, for a period of 14 months, between March 2001 and April 2002, from three sites around Singapore's southern islands: Raffles Lighthouse, Pulau Hantu and Sisters Island (Chapter 2, Fig 2.1, 2.7). Whenever possible, the three sites were visited on the same day or on consecutive days, however due to logistical difficulties (e.g. boat availability, strong currents), on some occasions sampling days were spread over a period of one week. Three sites were chosen primarily because the number of coral colonies at any one site was not sufficient for the proposed study. Furthermore, the aim of the study was to establish the pattern of gametogenesis for corals around Singapore's southern islands, so the selection of three sites was considered to be more representative. Each of the three sites has experienced extensive land reclamation of the reef flat area, and they are similar in terms of their coral communities and environmental conditions (see Chapter 2 for site descriptions).

3.2.2 Sample collection

All sampling was done using SCUBA and samples were removed from colonies using a hammer and chisel. Small samples of approximately 3 - 4 cm^2 were collected and placed in separate pre-labeled, plastic Ziploc bags.

3.2.3 *Porites lutea*

Distinguishing *P. lutea* from other massive *Porites* species is not possible in the field. To identify colonies, small pieces of skeleton were removed, cleaned of tissue using household bleach, rinsed in water and dried in air before examination under a dissecting microscope (for description of corallite detail see above). In February 2001 samples were collected from a total of 30 *Porites* colonies and examined in this way. From these, eleven colonies of *P. lutea* (4 from each Pulau Hantu and Sisters and 3 from Raffles Lighthouse) were identified and permanently tagged prior to the start of the monitoring in March 2001. The colonies were labeled (RL1-3, PH1-4 and SIS1-4), and one sample (3 - 4 cm^2) was collected monthly from each of the 11 colonies (n = 154). To permanently mark the location of the colonies, two angle iron stakes were hammered in to the substrate and bolted together, next to the colony. A rope and buoy were tied on as a surface marker, and a rope was tied between colonies on the reef, making it easy to find the colonies even in poor visibility. All colonies were greater than 75 cm in diameter, and monthly sequential samples were taken at least 15 cm away from each other to avoid causing localized stress and negative impacts on fecundity or reproductive effort (Oren et al. 2001). Sequential sampling was chosen for this species partly because of the difficulties in identifying *Porites* spp. *in situ*, but also because following reproductive changes in individuals make it possible to correctly ascertain the reproductive strategy (i.e. hermaphroditism or gonochorism) (Giese and Pearse 1974).

3.2.4 *Platygyra* morphospecies

The genus *Platygyra* was chosen, as it is one of the most dominant genera found on Singapore's reefs (personal observation). The abundance of colonies meant that a random collection could be carried out over a 14-month period, without the need to take multiple samples from individual colonies. On each sampling occasion, four colonies were sampled at each of the three sites, so a total of twelve colonies were sampled each month (n = 168). Although sampled colonies were not tagged, it was easy to tell if a colony had previously been sampled by the presence of a sample scar. Colonies of *Platygyra* do not lend themselves well to sequential sampling because of the small size, the difficulty in removing small samples (sample removal tends to be more destructive than for *Porites* species) and their inability to recover quickly from lesions caused by the sampling. Sample scars were obvious on colonies for months after sampling, suggesting that healing of lesions was very slow.

A visual survey was conducted at the three study sites to identify the most common morphospecies of *Platygyra* on Singapore's reefs. Only one morphospecies was identified as being sufficiently abundant to conduct a 14-month study, without repeat sampling of colonies. Colonies were massive and usually dome shaped, valleys were usually short or monocentric, and walls ranged in width from 2 - 4 mm. However, examination of samples post-collection revealed that they could be divided in to two *possible* morphospecies. Separate analysis of the data from the two potential morphospecies was conducted and no significant difference was found in the reproductive patterns (see appendix 2 for full description), so subsequently, data for all samples were pooled, and treated as one morphospecies. The morphospecies used

in this study most closely resembled *Platygyra pini* and *P. verweyi* although some samples had more meandering valleys and narrower walls, resembling *P. ryukyuensis* (Veron 2000). Due to the taxonomic uncertainty I will refer to the morphospecies as *Platygyra* sp. throughout the thesis.

3.2.5 Sample processing

The sample processing procedure was the same for both *Platygyra* and *P. lutea* and followed the method used by Szmant-Froelich et al (1980) and Glynn et al. (1991). Coral samples were fixed immediately after collection in a seawater-Zenker's solution (50g zinc chloride, 25g of potassium dichromate per liter of seawater) with 5% formaldehyde for 18 to 24 hrs. Samples were fixed in separate, labeled, disposable 200 ml plastic pots, filled with solution. Following fixation, samples were rinsed for 18 to 24 hrs in running tap water and stored in 70% ethanol. The ethanol was changed when necessary to remove dissolved pigments and other precipitates. Samples were decalcified in a 10% solution of HCl with 0.7g EDTA, 0.008g sodium potassium tartrate and 0.14g sodium tartrate per liter of solution. Decalcification generally took about one week, with regular changes of acid. Samples were rinsed in tap water and stored in 70% alcohol until histological processing

3.2.6 Histological technique

Tissue samples of around $1cm^2$ were processed for histological sectioning. Pieces of coral tissue were placed in perforated, labeled plastic containers designed for wax embedding of large tissue samples (Cole-Palmer). The coral tissue samples were

dehydrated in an alcohol series, cleared using Histoclear (National Diagnostics) and embedded in paraffin wax (BDH Laboratory Supplies) using a tissue processor (Reichert-Jung Histokinette 2000). Samples were orientated in cross section and cut with a microtome (Reichert-Jung 2030) at 6 - 8μm thickness and stained with a modified Heidenhain's Aniline Blue (Luna 1968) with azocarmine G (Glynn et al. 1991). Cover slips were mounted with DPX (BDH Laboratory Supplies). The approximate location of the gonads within the polyp was established, by taking cross sections at regular spatial intervals (approximately every 100μm) through mature polyps and by examination of longitudinal sections of mature polyps. At least three slides were made from each sample at intervals of approximately 250μm for *P. lutea* and 300μm for *Platygyra* sp. throughout the reproductive region of the polyp, each slide consisted of three to four serial sections.

3.2.7 Microscopy

Slides were analysed under a binocular compound microscope (Olympus BH2) at magnifications of x40 to x1000. A square of area 0.25cm^2 was placed over the slide and the geometric diameters and gametogenic stages of all gonads within the square were recorded. The maximum gonad diameter (d1) and a second measurement perpendicular to the maximum (d2) were measured with a calibrated ocular micrometer. The geometric diameter (d) of gonads was the square root of d1 multiplied by d2:

$$d = \sqrt{(d1 \times d2)}$$

These data were used in all subsequent analyses. Gonad classification followed the widely used system developed by Szmant-Froelich et al. (1985). Four gametogenic stages of oogenesis and spermatogenesis were identified for each species and these are described in detail in the results section.

3.2.8 Environmental parameters

The seasonal patterns of sea surface temperature (SST, ºC), salinity (ppt), rainfall (mm) and daily sunshine hours were examined during the study period. Seawater temperature measurements were taken twice-monthly at all three sites between March 2001 and November 2001 with a multi-parameter probe (YSI Inc.) at a depth of approximately 0.5 m. From December 2001 until the completion of the study (May 2003) all temperature measurements were taken using an underwater continuous temperature logger (Hobo Tidbit). The logger was placed at approximately 0.5 m (at spring low tide) on the reef flat at Raffles Lighthouse (Fig 2.1) and was preset to take temperature readings every hour. Monthly mean temperature values were from the mean of all temperature measurements taken during the month, whether from the logger or from the point samples. Salinity (ppt) was measured at all sites twice monthly with a multi-parameter probe (YSI Inc.) and values used in analyses were monthly means. Data on rainfall and sunshine were obtained from the Singapore Meteorological Service. Total daily rainfall (mm) was from the rain station at Sentosa, which is the closest station to the southern islands (1° 15'N, 104° 50'E). Daily sunshine hours were collected using a sunshine recorder. This is an optical instrument that estimates the amount (in hours) of sunshine each day, by focusing sunlight on to a piece of card, which then is burnt by bright sunlight. Although this is not a direct

measurement of the amount of irradiance or photosynthetically active radiation (PAR), it gives an idea as to whether there is a seasonal pattern for sunlight. Daily sunshine hours were averaged to give a monthly mean for daily sunshine hours.

3.2.9 Data analysis

The gametogenic stage data were used to test for reproductive seasonality. A chi-square analysis was performed to determine if the proportion of colonies with mature eggs (Stage IV) compared to the proportion of colonies without mature eggs was independent of seasons. The year was divided into 4 seasons based on the Southeast Asian Monsoon as follows: Northeast (NE) Monsoon (November – February); SW Monsoon (May-August); first inter-monsoon (March-April); and second inter-monsoon (September-October) (modified from Nieuwolt, 1973). Data were pooled for each season and a contingency table was constructed with 5 rows (representing the 5 seasons covered during the study), and 2 columns (representing number of colonies with mature eggs and number colonies without mature eggs).

All of the data for gonad size, gonad numbers, sea surface temperature (SST), salinity, rainfall and sunshine were tested for normality and homogeneity of variance (by examination of plots and the Kolmogorov-Smirnov test) (SPSS v9). Only the environmental data were normally distributed. One-way ANOVA (SPSS v9) was used to compare the mean temperatures of the three study sites, i.e. to see if the readings taken at Raffles Lighthouse were representative of all study sites. Data transformation failed to normalize the reproductive data; so non-parametric correlation tests (Kendall's Tau, SPSS v9) were used to compare reproductive patterns between

species. However, correlations between the environmental parameters were carried out using a parametric test (Pearson product-moment, SPSS v9). The data for gonad size were used to compare the seasonal reproductive cycles of the two species (*P. lutea* and *Platygyra* sp.). Reproductive cycles and spawning times of *P. lutea* and *Platygyra* sp. were compared with the seasonal environmental cycles of SST, salinity, rainfall and sunshine hours. No attempt was made to statistically correlate the gametogenic cycles with the seasonal environmental parameters, because some of the monthly values for gonad size and numbers were zero (i.e. for at least 2-3 months post spawning the size and number of gonads would be independent of any environmental factors).

3.3 Results

3.3.1 *Porites lutea*

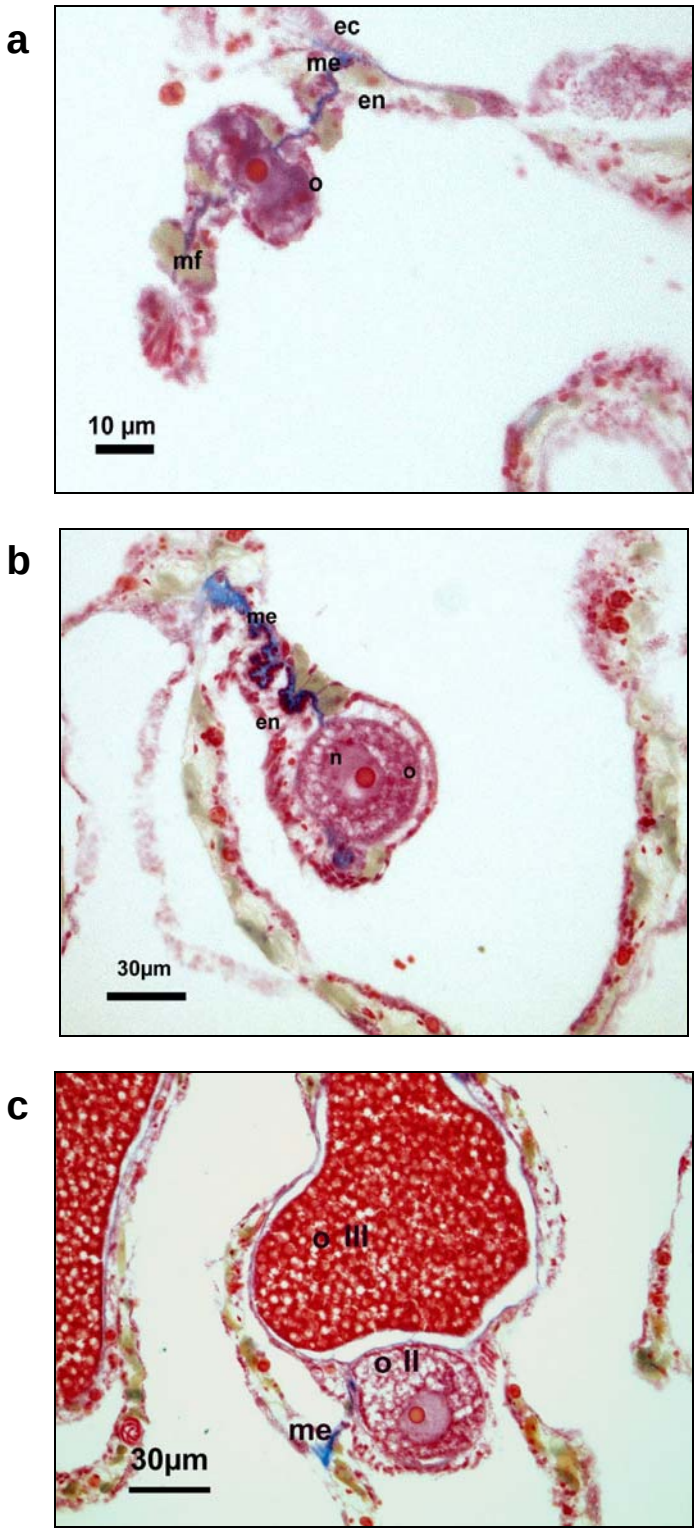
3.3.1.1 Gametogenesis

Histology indicated that one gonad developed within each mesentery, so each polyp contained 12 gonads in total. Gonads were arranged in vertical columns running orally-aborally, beginning in the sub-oral region of the polyp. Oogenesis lasted for 4 to 6 months and spermatogenesis lasted for 1 to 3 months. Four gametogenic stages were recognized of both oocytes and testes based on histological characteristics and relative size (Szmant-Froelich et al. 1980).

Table 3.1 Geographical and latitudinal comparison of reproductive patterns in *P. lutea* from existing studies and present study.

Location	Reproductive strategy	Length of gametogenic cycle (months)	Month of spawning	Reference
Eilat, Israel, N. Red Sea, (29 °N)	Gonochoric, some male polyps found in predominantly female colony	♀ 5, ♂ 2	July	Schlesinger et al. 1998
Akajima Island, Okinawa, Japan, (26 °N)	Gonochoric	not studied	Jun & Jul 5-6 nights after full moon	Hayashibara et al. 1993
Guam, (12 °N)	Gonochoric	not studied	Unknown, but probably between Jul & Sept	Heyward 1988
Singapore, (1 °N)	Gonochoric	♀ 4-6, ♂ 1-3	Predominantly Apr, but may also spawn in Mar, May, Sep and Oct	Present study
Lizard Island, GBR, (14 °S)	Gonochoric	♀ 4-6, ♂ 1-2	Nov – Jan	Harriott 1983
Magnetic Island, GBR, (19 °S)	Gonochoric	not studied	Disappearance of gametes in Nov, 3 – 7 days after full moon	Babcock et al. 1986
Heron Island, GBR, (23 °S)	Gonochoric	♀♂ approx 4	mid-Jan – early Feb	Kojis and Quinn 1981a

3.3.1.2 Oogenesis



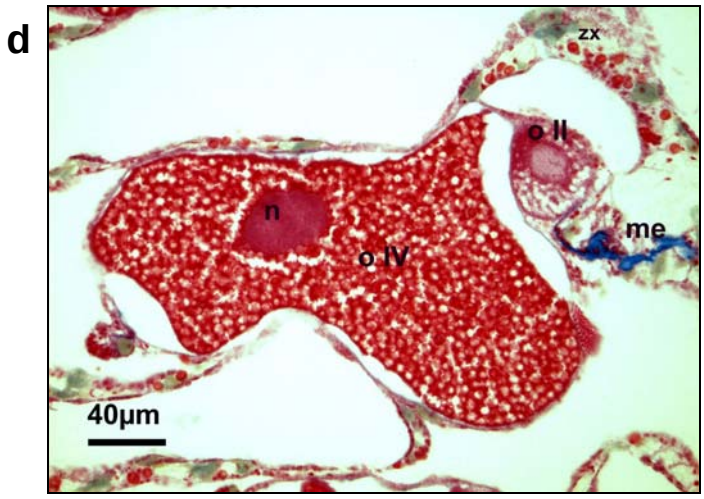


Fig 3.1 Oogenesis in *Porites lutea*. a) Stage I oocyte in mesenterial filament of endoderm, b) stage II oocyte, c) stage II and III oocytes, d) stage II and IV oocytes. Labels: ec = ectoderm, en = endoderm, me = mesoglea, mf = mesenterial filament, n = nucleus, o = oocyte, o II = stage II oocyte, o III = stage 3 oocyte, o IV = stage IV oocyte, zx = zooxanthellae.

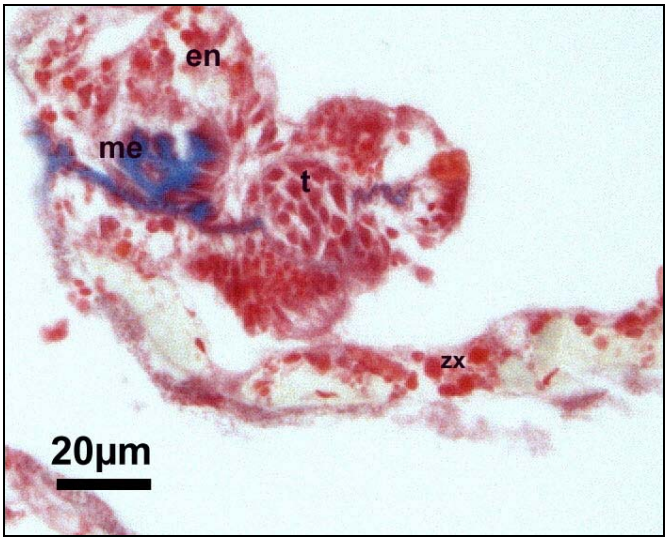
Stage I oocytes were first identifiable in the mesoglea or endodermal tissue adjacent to mesoglea in the mesenteries (Fig 3.1 a). They appeared as oval nuclei with a thin layer of cytoplasm. Maximum diameters ranged from 10 – 40µm. Stage I oocytes stained gray-blue to purple, the nucleus was reddish to pink and the nucleolus often stained bright red. Stage II oocytes had maximum diameters between 40 and 90µm (Fig 3.1 b). They were completely enveloped in mesoglea and surrounded by a layer of cytoplasm that had a grainy appearance. Staining properties were similar to that of Stage I with cytoplasm ranging from blue-gray to purple-red in colour. In stage III oocytes, vitellogenesis increased the amount of cytoplasm (extensive yolk formation), and cytoplasm was distinctly pinkish-red in colour (Fig 3.1 c). Oocytes ranged in size from 90 to 150µm (max diameter) and were round, oval or irregularly shaped. A patch of fine-grained, gray-blue staining cytoplasm was associated with the nucleus. Zooxanthellae were present in the endodermal tissue adjacent to the oocytes but had not entered the oocytes. Stage IV oocytes were large and irregularly shaped and

appeared as if they were squashed together (Fig 3.1 d). Maximum diameters generally ranged from 150 to 300µm, although some were as large as 350µm. Oocytes were characterized by the presence of zooxanthellae in the cytoplasm. The nucleus had migrated close to periphery of egg and become saddle shaped. Cytoplasm appeared to have 'pulled away' from the nucleus leaving an empty halo around the nucleus.

3.3.1.3 Spermatogenesis

Stage I testes appeared as small bundles of 5 - 10 cells (each approx 4µm in diameter) adjacent to or in the process of being engulfed by mesoglea (Fig 3.2 a). The cells stained dark purple. Stage II testes ranged in diameter from 20 to 70µm and were totally surrounded by mesoglea (Fig 3.2 b). The enlargement of stage II testes appeared to be a result of migration of primary spermatocytes from endodermal tissue or by division of cells. Staining properties and size were similar to those of stage I. Stage III testes were generally between 70 and 150µm in diameter (Fig 3.2 b). Cells proliferated and migrated to the periphery of testes so a lumen formed in the center. Size and staining properties were similar to those of Stages I and II. In stage IV testes, the spermatocytes had divided and so were much smaller than in stage III (Fig 3.2c, d). Testes were between 100 and 300µm in diameter. Cells stained dark magenta because of the condensation of nuclei, and tails stained light pink. Testes were oval, teardrop shaped or irregular. Spermatozoa were arranged with tails pointing in one direction to give the impression of a bouquet (Fig 3.2c, d).

a



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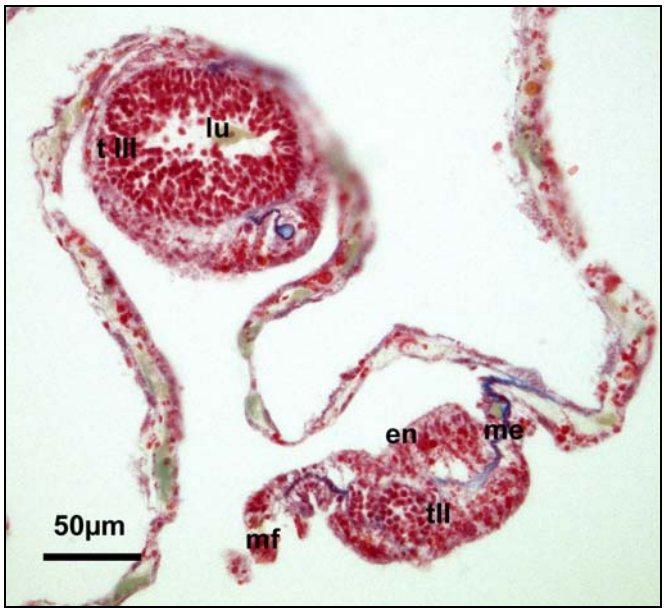

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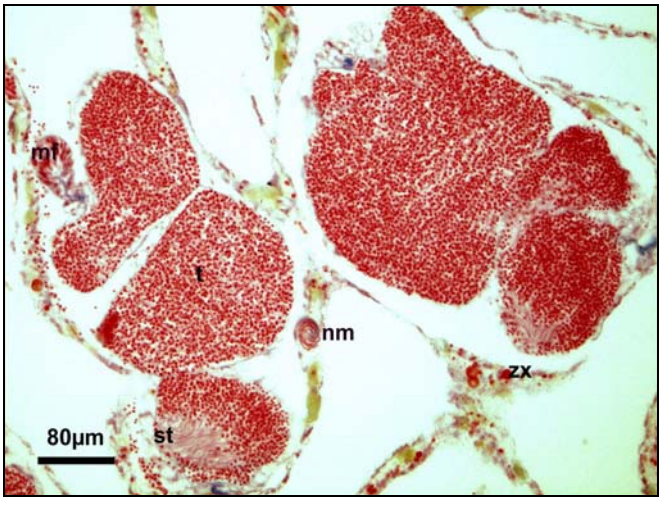


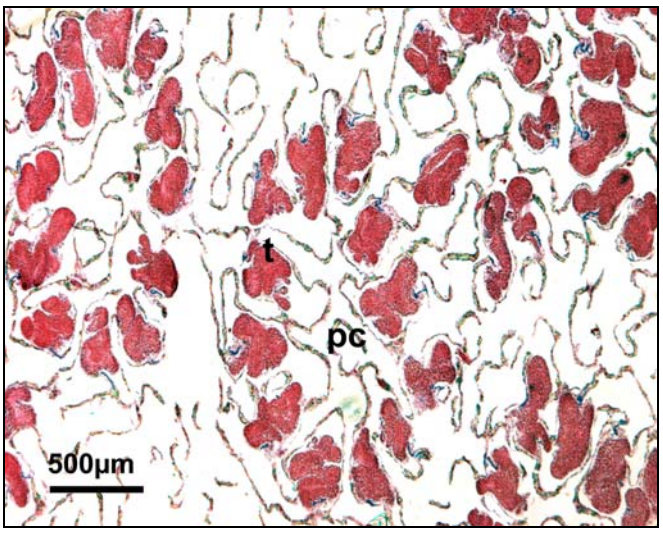


Fig 3.2. Spermatogenesis in *Porites lutea*. a) stage I testes in endodermal mesentery, b) stage II and stage III testes, c) stage IV testes, d) cross section of mature polyp containing stage IV testes. Labels: ec = ectoderm, en = endoderm, lu = lumen, me = mesoglea, mf = mesenterial filament, nm = nematocyst, pc = polyp center, st = spermatozoa tails, t = testes, t I = stage I testes, t II = stage II testes, zx = zooxanthellae.

3.3.1.4 Reproductive strategy

The sequential sampling of colonies made it possible to examine the strategies of the individual colonies. Of the eleven colonies studied, eight colonies were females (i.e. containing only eggs) (Fig 3.3) and three colonies were males (i.e. containing only testes) (Fig 3.4) during the 14 months of sampling. All of the colonies showed the same pattern of sex in both years suggesting that this species is not a sequential or alternating hermaphrodite. No incidences of hermaphroditism were observed, indicating that *P. lutea* is gonochoric in Singapore. The eight female colonies were: RLPL3, SISPL1, SISPL2, SISP3, SISPL4, PHPL2, PHPL3 and PHPL4 (Fig 3.3); and 3 the three male colonies were RLPL1, RLPL4 and PHPL1 (Fig 3.4).

3.3.1.5 Females

The peak of reproductive activity in the eight female colonies was in March (in terms of gamete numbers) and in April (in terms of gamete size), although one colony (PHPL3) contained large numbers of stage IV oocytes in May (Fig 3.3). Five of the colonies (PHPL3, PHPL4, SISPL4, RLPL3 & SISPL1) had a second smaller peak in oogenesis between August and November (Fig 3.3). However, only one of these colonies (PHPL3) contained stage IV oocytes and numbers of oocytes equivalent to those in March and April (Fig 3.3). These data indicate that some colonies of *P. lutea* undergo oogenesis and spawn bi-annually, although not all colonies complete this second cycle.

3.3.1.6 Males

Mean testes size, mean number of testes and the relative proportions of different testes stages were considerably different between the three male colonies (Fig 3.4). Large stage IV testes were only found in one colony (RLPL4) and these were abundant in March, April and May 2001 and March and April 2002 (Fig 3.4b and e). Whereas stage IV testes were never found in RLPL1 or PHPL1 (Fig 3.4a and c), indicating that these colonies did not spawn, or had very short spermatogenic cycles. Large numbers of stage III testes were found in RLPL1 in Mar 2002, but these were not followed by stage IV testes the next month (Fig 3.4a). PHPL1 was the least 'fecund' colony out of the three male colonies, and only contained a few small testes (stages I and II) in March 2001 and 2002 (Fig 3.4c and f).

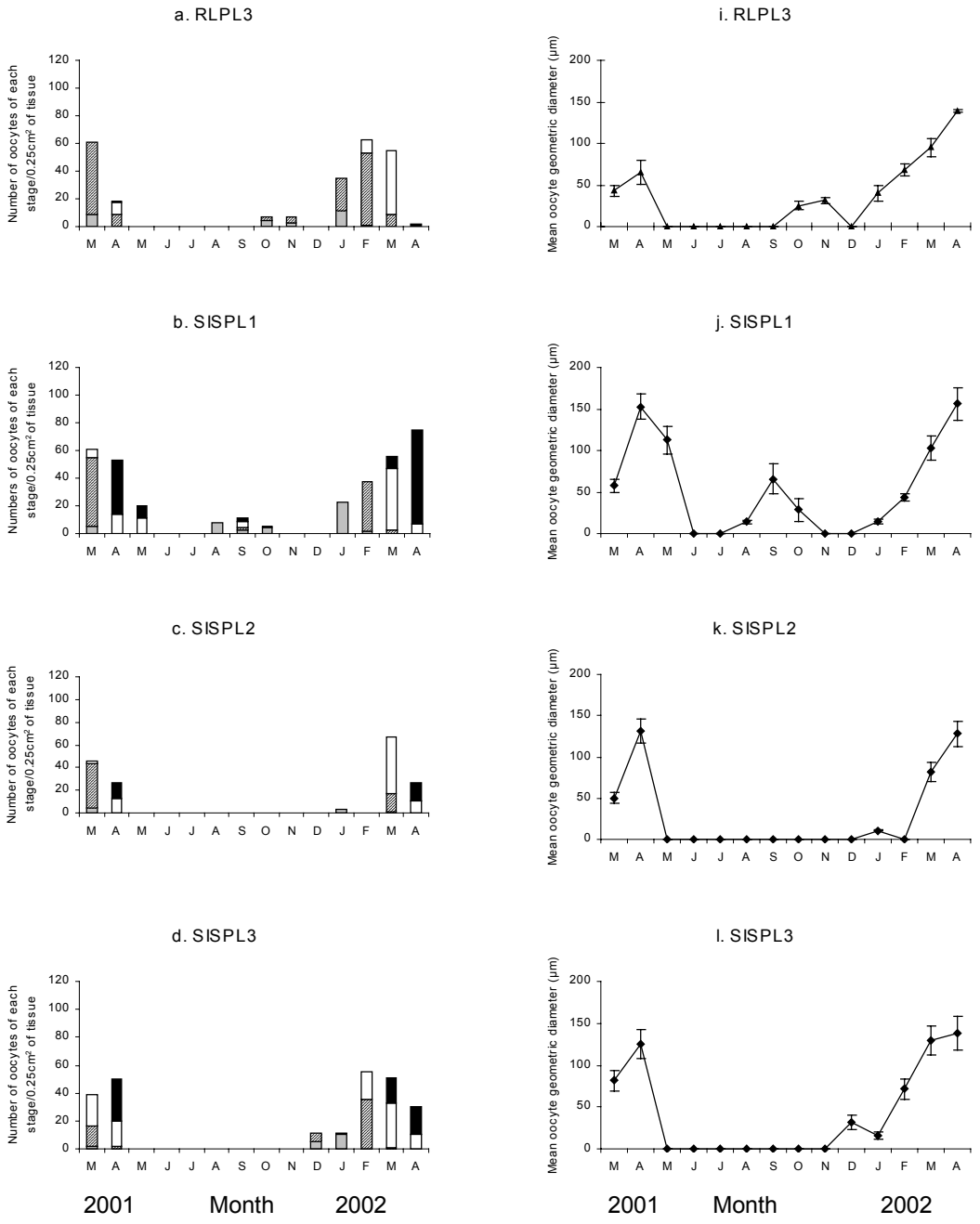
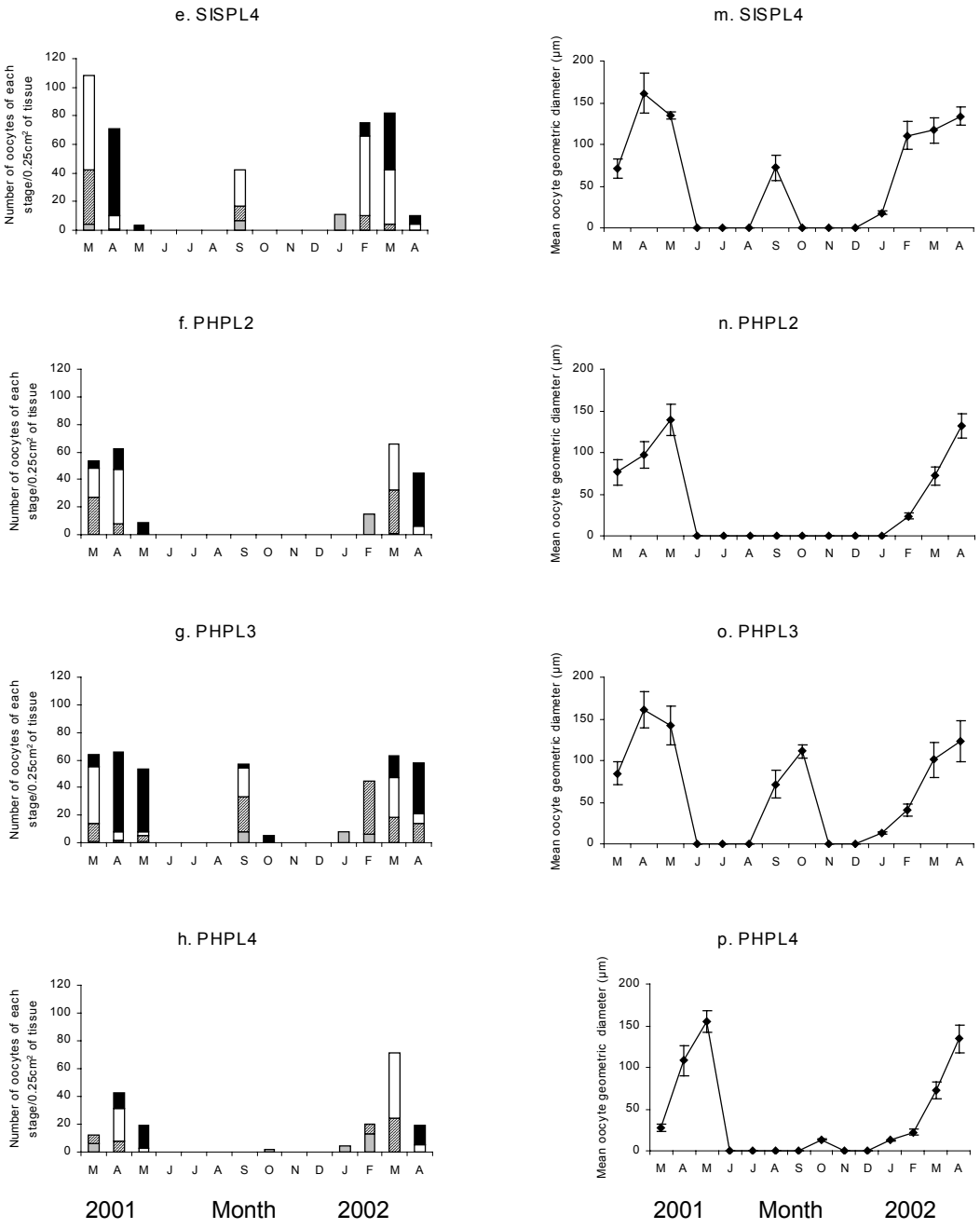


Fig. 3.3 Individual reproductive phenologies of *Porites lutea* female colonies. Graphs a) – h) are oocyte numbers/0.25cm^2 of tissue, showing the different proportions of each stage (gray = stage I, diagonal stripes = stage II, white = stage III, black = stage IV). Graphs i) – p) are mean oocyte geometric diameter (µm), error bars are standard deviations.



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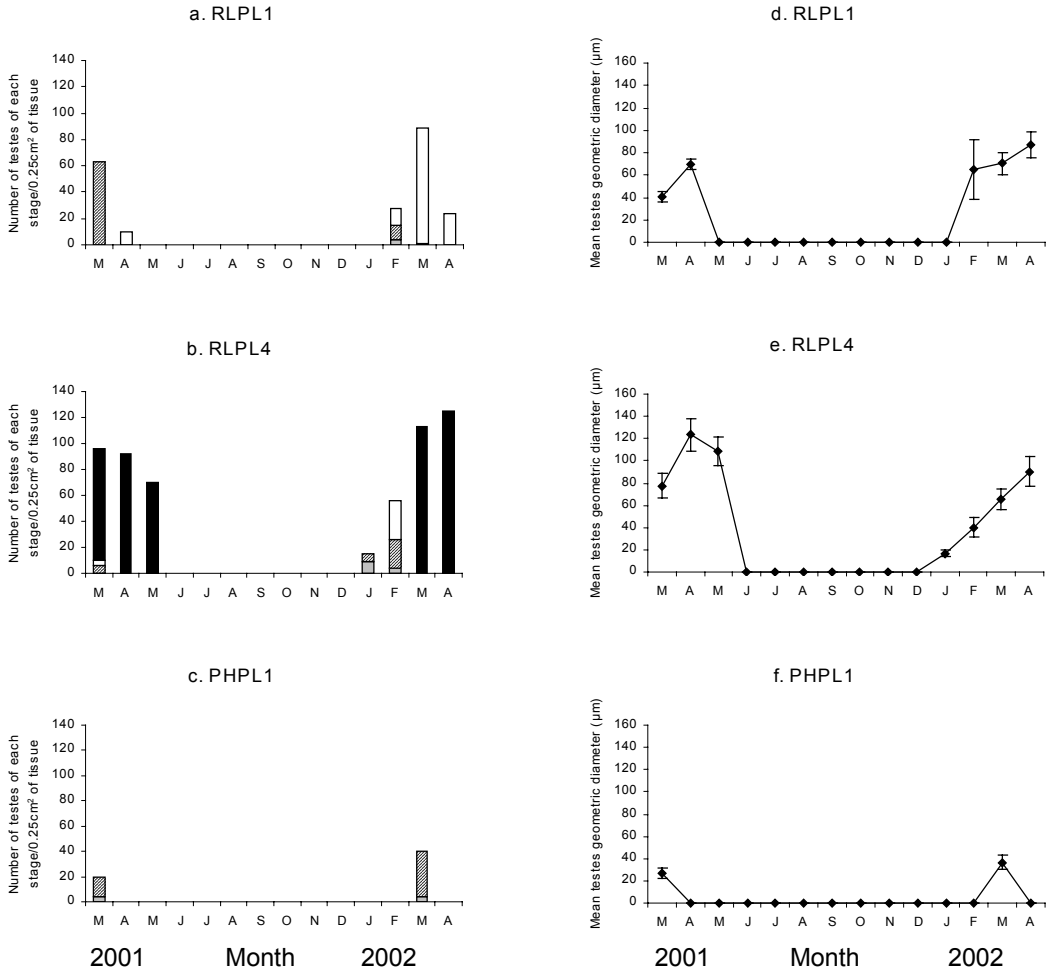


Fig. 3.4 Reproductive phenologies of individual male colonies of *Porites lutea*. Graphs a) – c) are numbers of testes/0.25cm^2 of tissue, showing the relative proportions of each gametogenic stage (gray = stage I, diagonal stripes = stage II, white = stage III, black = stage IV). Graphs d) – f) are mean geometric testes diameters (µm), error bars are standard deviations.

3.3.2 *Platygyra* sp.

3.3.2.1 Gametogenic cycle and reproductive strategy

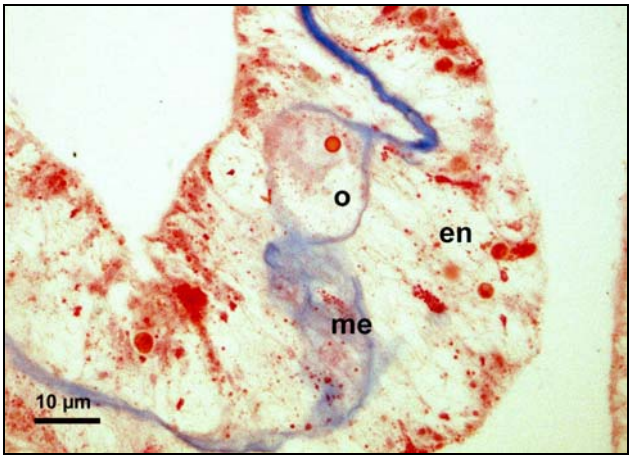
Because sampling of *Platygyra* sp. was random each month (i.e. colonies were not sequentially sampled), it was not possible to follow individual colonies through successive breeding seasons (as it was with *P. lutea*). However, all of the colonies sampled during the breeding season were simultaneous hermaphrodites, suggesting that this is the reproductive strategy for *Platygyra* sp. in Singapore. In mature colonies, oocytes and testes were intermingled on the same mesenteries and gamete development occurred in the sub-oral region. In mature colonies each gonad usually contained around six oocytes. Oogenesis lasted between six and nine months, whereas spermatogenesis lasted between two and three months. Four gametogenic stages were defined for both oocytes and testes based on histological characteristics and relative size. Many aspects of the gametogenic cycle were similar to that of *P. lutea*.

3.3.2.2 Oogenesis

Stage I oocytes were first identifiable in the endodermal tissue adjacent to mesoglea in the mesenteries and maximum diameters ranged between 20 and 50μm. They appeared as oval nuclei with a thin layer of cytoplasm. Stage I oocytes stained light purple, the nucleus was gray-blue and the nucleolus often stained bright red (Fig 3.5a). Stage II oocytes were 60 to 140μm in diameter. They were completely enveloped in mesoglea and surrounded by a layer of cytoplasm that had a grainy appearance. Staining properties were similar to that of Stage I with cytoplasm ranging from blue/gray to purple/red in colour (Fig 3.5b). In stage III oocytes vitellogenesis

increased the amount of cytoplasm (extensive yolk formation), and cytoplasm was distinctly pinkish-purple in colour (Fig 3.5c). Oocytes ranged in diameter from 100 – 250µm and were round or oval. By stage III a distinct magenta membrane could be seen around oocytes. Cytoplasm appeared denser in the center of oocytes around the nucleus. A patch of fine grained, light pink staining cytoplasm was associated with the nucleus (Fig 3.5c). Stage IV oocytes were large and oval or teardrop shaped.

a



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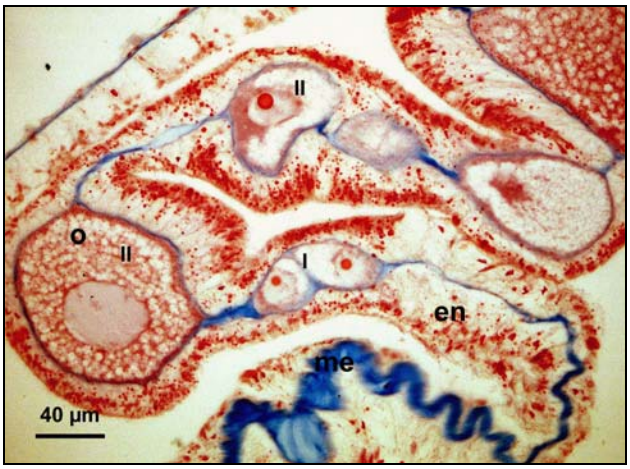

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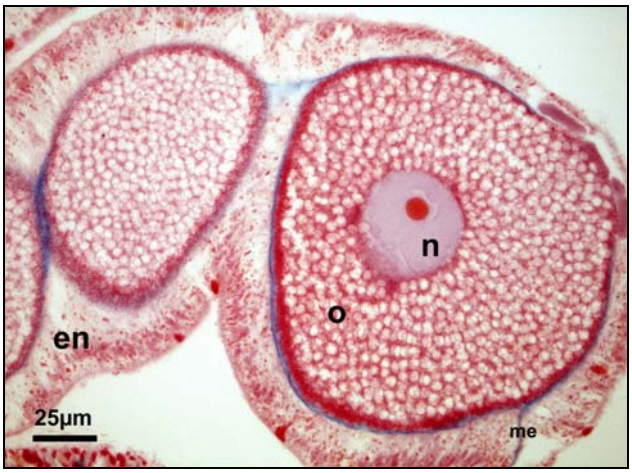

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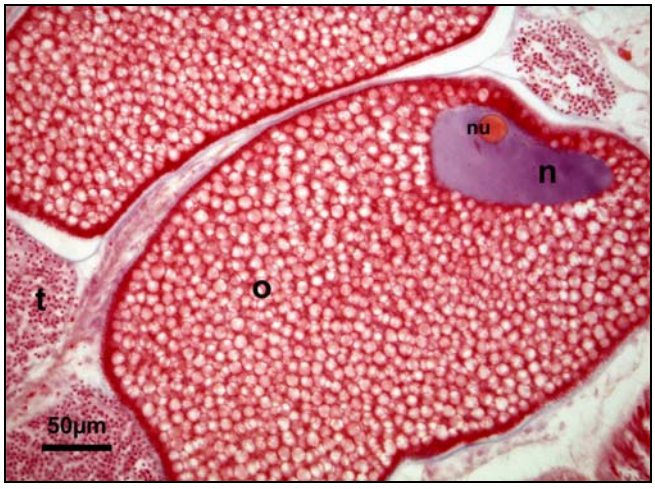


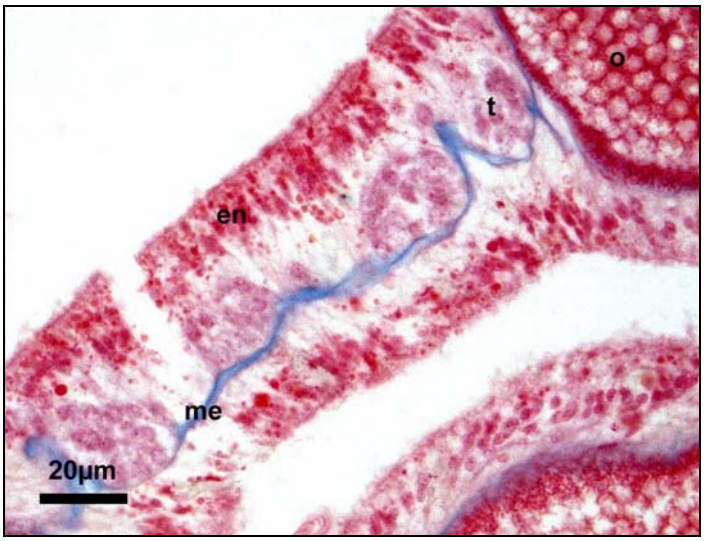
Fig 3.5 Oogenesis in *Platygyra* sp. a) Stage I oocyte entering mesenterial mesoglea, b) stage I and II oocytes together in mesenterial mesoglea, c) Stage III oocyte, d) stage IV oocyte. Labels: en = endoderm, me = mesoglea, n = nucleus, nu = nucleolus, o = oocyte, t = testes.

Maximum diameters ranged between 200 – 600µm. In contrast with *P. lutea*, oocytes did not contain zooxanthellae (Fig 3.5d). The nuclei had migrated close to the periphery of the egg and were round, oval or saddle shaped. In some cases an indentation appeared in the oocytes at the site of the nucleus (Fig 3.5d).

3.3.2.3 Spermatogenesis

Stage I testes appeared as small bundles of 5 - 10 cells (each approx 4μm in diameter) adjacent to or in the process of being engulfed by mesoglea. The cells stained light to navy blue (Fig 3.6a). Stage II testes were totally surrounded by mesoglea, and maximum diameters ranged between 30 and 100μm. The enlargement of stage II testes appeared to occur as a result of migration of primary spermatocytes from endodermal tissue or by division of cells. Staining properties and spermatocyte size were similar to those of stage I (Fig 3.6b). Stage III testes were 70 – 200μm in diameter. Cells proliferated and migrated to the periphery of testes so a lumen formed in the center. Size and staining properties were similar to those of Stages I and II (Fig 3.6c). In stage IV testes, the spermatocytes had divided and so were much smaller than in stage III. Maximum diameters of stage IV testes were generally between 100 and 300μm. Cells stained dark magenta because of the condensation of nuclei, tails were evident and stained golden. Testes were oval, teardrop shaped or irregular. Spermatozoa were arranged with tails pointing in one direction to give the impression of a bouquet (Fig 3.6d).

a



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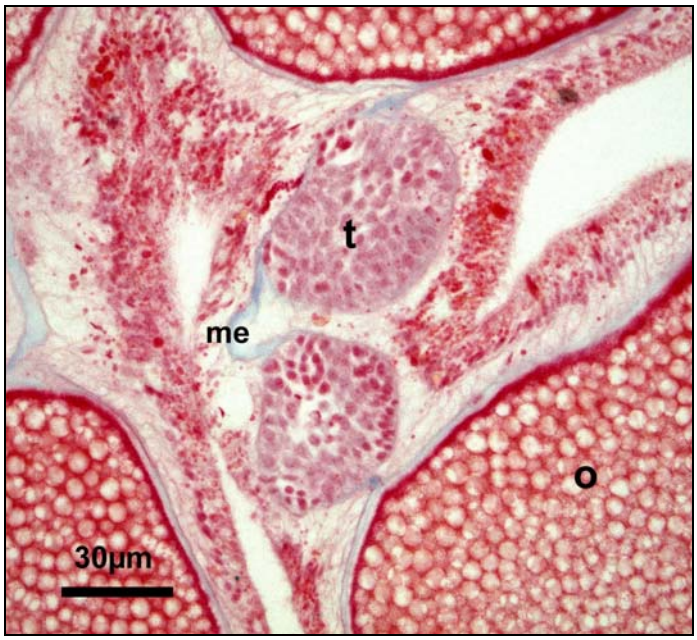

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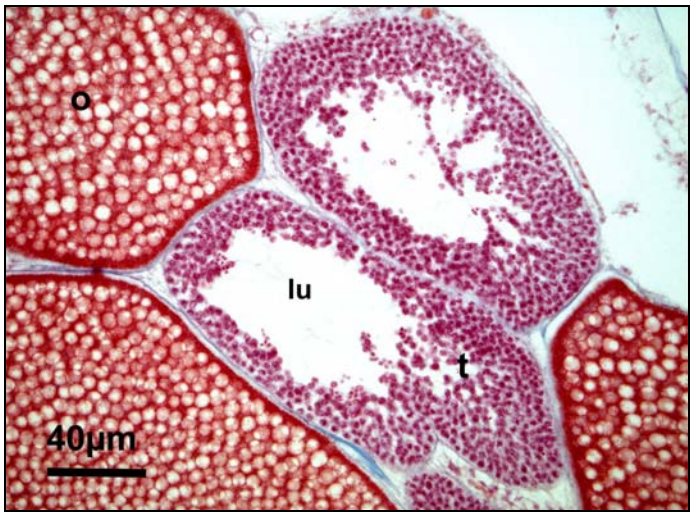

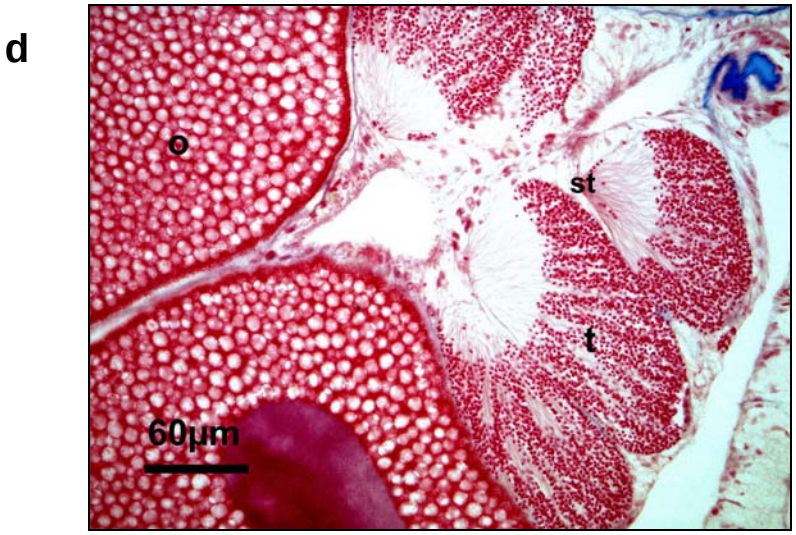

Fig 3.6 Spermatogenesis in *Platygyra* sp. a) Stage I testes entering mesenterial mesoglea, b) Stage II testes, c) Stage III testes with lumen, d) Stage IV testes. Labels: en = endoderm, lu = lumen, me = mesoglea, o = oocyte, st = spermatozoa tails, t = testes.

3.3.3 Comparison of gametogenic cycles and reproductive seasonality of *Porites lutea* and *Platygyra* sp.

Comparisons of the reproductive cycles of *Platygyra* sp. and *P. lutea* revealed strong significant correlations in mean monthly gonad size (oocytes: $\gamma = 0.69$, $p < 0.001$; testes: $\gamma = 0.66$, $p < 0.002$, $n = 14$) and mean monthly gonad numbers (oocytes: $\gamma = 0.55$, $p < 0.01$; testes: $\gamma = 0.58$, $p < 0.01$, $n = 14$), indicating that these unrelated species had very similar reproductive cycles (Table 3.2). Gametes of different sizes and stages were found at the same time in many of the colonies sampled, however the reproductive cycle was clearly seasonal for both *Platygyra* sp. and *P. lutea*. Chi square analysis showed that presence of mature oocytes and mature testes (stage IV)

Table 3.2. Correlations (Kendall's Tau) between *Platygyra* sp. and *P. lutea* monthly mean gonad geometric diameter (μm); and monthly mean gonad numbers (0.25 cm^{-2}) over 14-month period of study.

Platygyra sp. v *P. lutea*			
	df	Test statistic (γ)	P
Oocyte size	14	0.69	0.001
Testes size	14	0.66	0.001
Oocyte numbers	14	0.55	0.01
Testes numbers	14	0.58	0.01

Table 3.3. Results of Chi-square test to look for association between presence of mature gametes and seasons. Contingency table consisted of two columns and 5 rows. N = number of colonies sampled, df = degrees of freedom.

Species	N	df	Test statistic	P
Platygyra sp. oocytes	12	4	99.3	p<0.001
Platygyra sp. testes	12	4	41.4	p<0.001
P. lutea oocytes	8	4	20.1	p<0.01

was highly significantly associated with seasons for *Platygyra* sp. (df = 4, test statistic = 99.3, $p < 0.0001$; df = 4, test statistic = 41.4, $p < 0.001$) and for mature oocytes (stage IV) in *P. lutea* (df = 4, test statistic = 20.1, $p < 0.01$) (Table 3.3). The majority of stage IV gametes were present during the first inter-monsoon season (March and April) indicating that spawning occurs mostly during these months.

3.3.4 Overall pattern of gametogenesis of *Porites lutea* in Singapore

Gametogenesis showed a clear seasonal pattern when data from all colonies and sites were combined. The highest proportion of colonies containing stage IV oocytes was in April in both years (100 % of colonies sampled, n = 8) (Fig 3.7). However, stage IV eggs were also found in some of the colonies in March, May, September, October 2001 and February and March 2002. Similarly, in both years, mean egg size was highest in April and egg numbers were highest in March (Fig 3.8a, c). No gonads

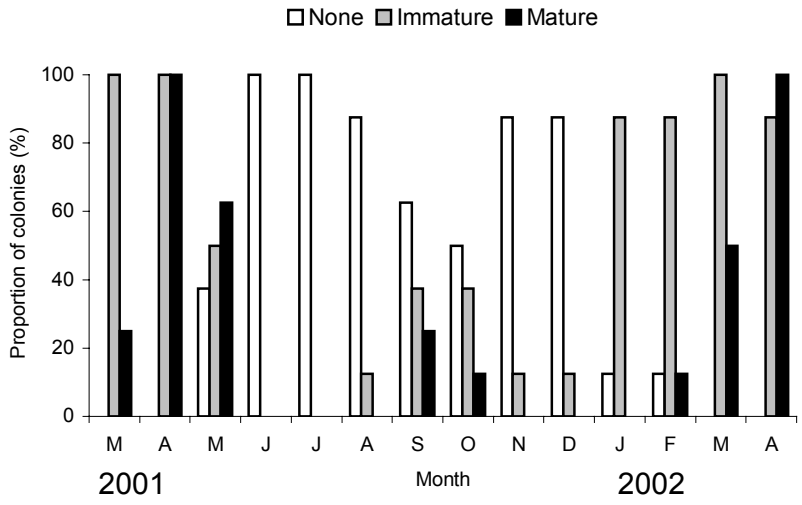

Fig 3.7 Proportion (%) of all female colonies of *P. lutea* (n = 8) that contained mature oocytes (stage IV), immature oocytes (stages I –III) or were empty each month. Totals are >100% in some months because colonies contained oocytes of different stages at the same time.

were found in either June or July and only small numbers of predominantly early stage oocytes were found between August and December (Fig 3.8c), except in one colony (PHPL3) where large numbers of oocytes, including stage IV gametes, were found in September and October 2001 (Fig 3.3g, o). These data indicate that the majority of spawning occurred in April, but some colonies may have spawned partially or fully in other months, or 'split' spawned over more than one month. It is harder to make generalizations about the seasonality of spermatogenesis because only three male colonies were studied and, despite the fact that two of the colonies were from the same site, there were large differences between colonies in testes sizes, testes numbers and the relative proportions of stages (Fig 3.4). However, there was still a clear seasonal pattern. None of the three colonies contained gonads between June and December, and mean testes diameters and numbers showed a similar pattern to that of

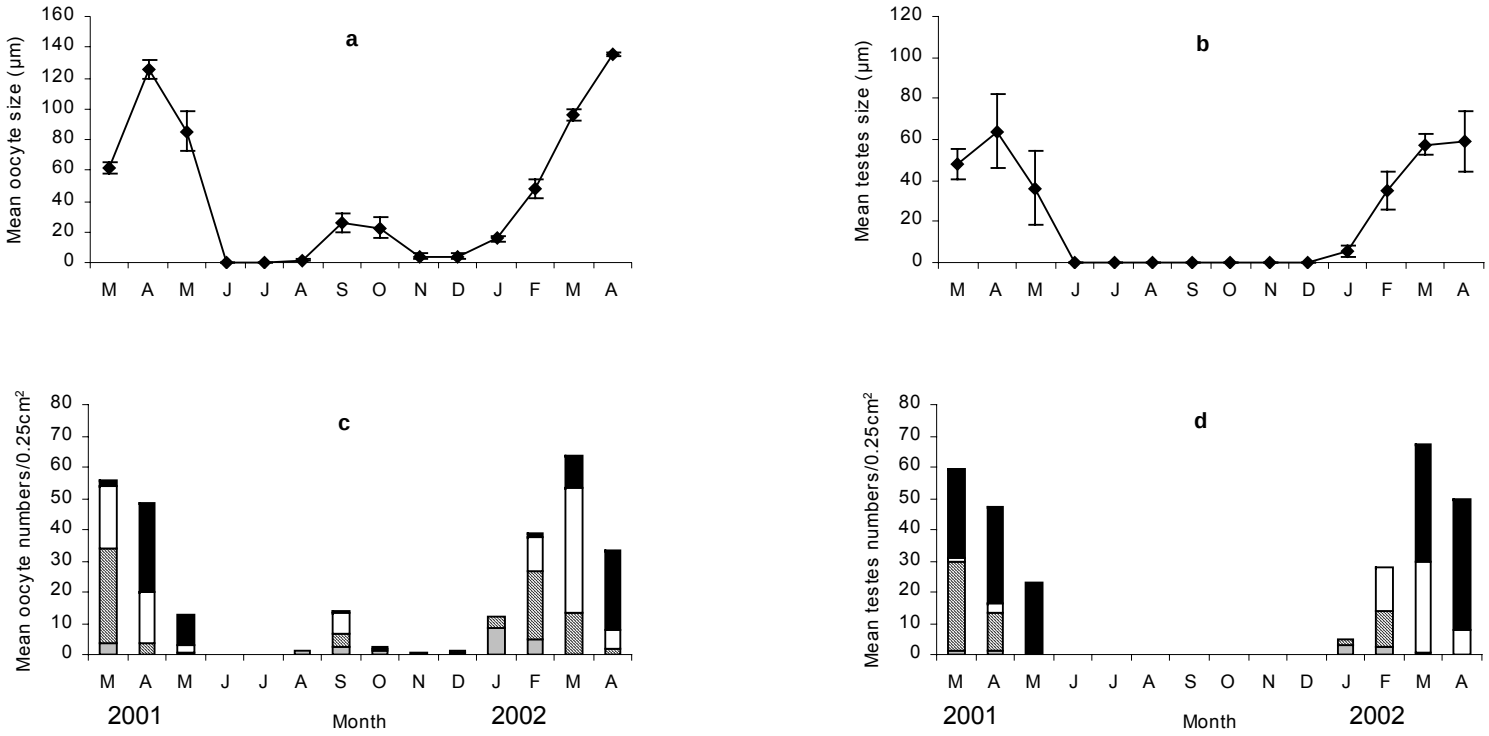

Fig 3.8 Overall reproductive phenology of *Porites lutea* in Singapore between March 2001 and April 2002 a) mean oocyte geometric diameter (µm) (n = 8), b) mean testes geometric diameter (µm) (n = 3), error bars are standard errors; c) mean number of oocytes/0.25cm^2 of tissue (n = 8) and d) mean number of testes/0.25cm^2 of tissue (n = 3), showing proportions of each gametogenic stage (gray = stage I, diagonal stripes = stage II, white = stage III, black = stage IV).

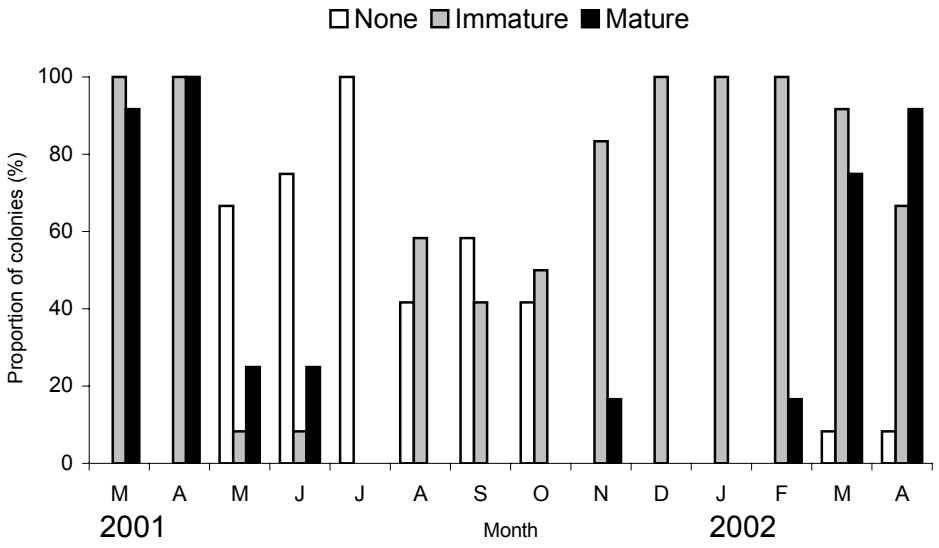

Fig 3.9 Proportion of all sampled colonies (%) of *Platygyra* sp. that contained mature oocytes (stage IV), immature oocytes (stages I – III) or were empty each month (n = 12). Totals are > 100% in some months because colonies contained both mature and immature oocytes simultaneously.

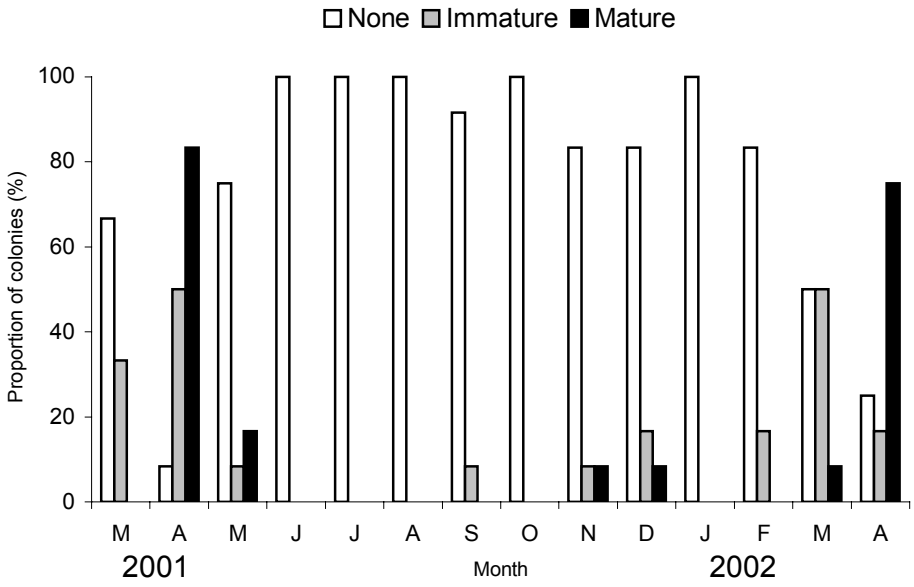

Fig 3.10 Proportion of all sampled colonies (%) of *Platygyra* sp. that contained mature (stage IV), immature (stages I – III), or no testes each month (n = 12). Totals are > 100% in some months because colonies contained both mature and immature oocytes simultaneously.

egg size, with a peak in size in April in both years (Fig 3.8b, d). Similarly, average proportions of stage IV testes were high in March and April 2001 and March, April and May 2002 (Fig 3.8d). However, this is as a result of the overall high proportion of stage IV testes of only one colony (RLPL4).

3.3.5 Overall pattern of gametogenesis of *Platygyra* sp. in Singapore

In 2001 and 2002, large mature oocytes and testes were found predominantly in April. The proportion of colonies containing mature (stage IV) oocytes and testes was highest in April in both years (oocytes, 100% and 92%; testes 83% and 75% n = 12) (Fig 3.9 and 3.10). Oocyte development began in August 2001, indicating that the gametogenic cycle may be as long as 8 months (Fig 3.11a, c). Few oocytes appeared in samples in May and June 2001 and none were present in July 2001, whereas few testes were found in May 2001 and none were present from June to August 2001 (Fig 3.11a - c). Some (very few) testes were present between September and December 2001 but none were present in January 2001 (Fig 3.11b, d). In 2002, spermatogenesis began in February and a high proportion of large mature testes were present in April 2002, indicating that the spermatogenic cycle was 2 to 3 months long (Fig 3.11b, d). The presence of mature gametes in March and April and the disappearance of most gametes in May suggests that spawning of *Platygyra* sp. occurs in both months. However, mature testes were predominantly found in April, indicating that most spawning occurs in this month. Some mature oocytes were present in November and in some colonies, these were accompanied by mature testes, indicating that some colonies spawn at this time, however it is not clear whether individual colonies have two annual gametogenic cycles or whether populations 'split' spawn.

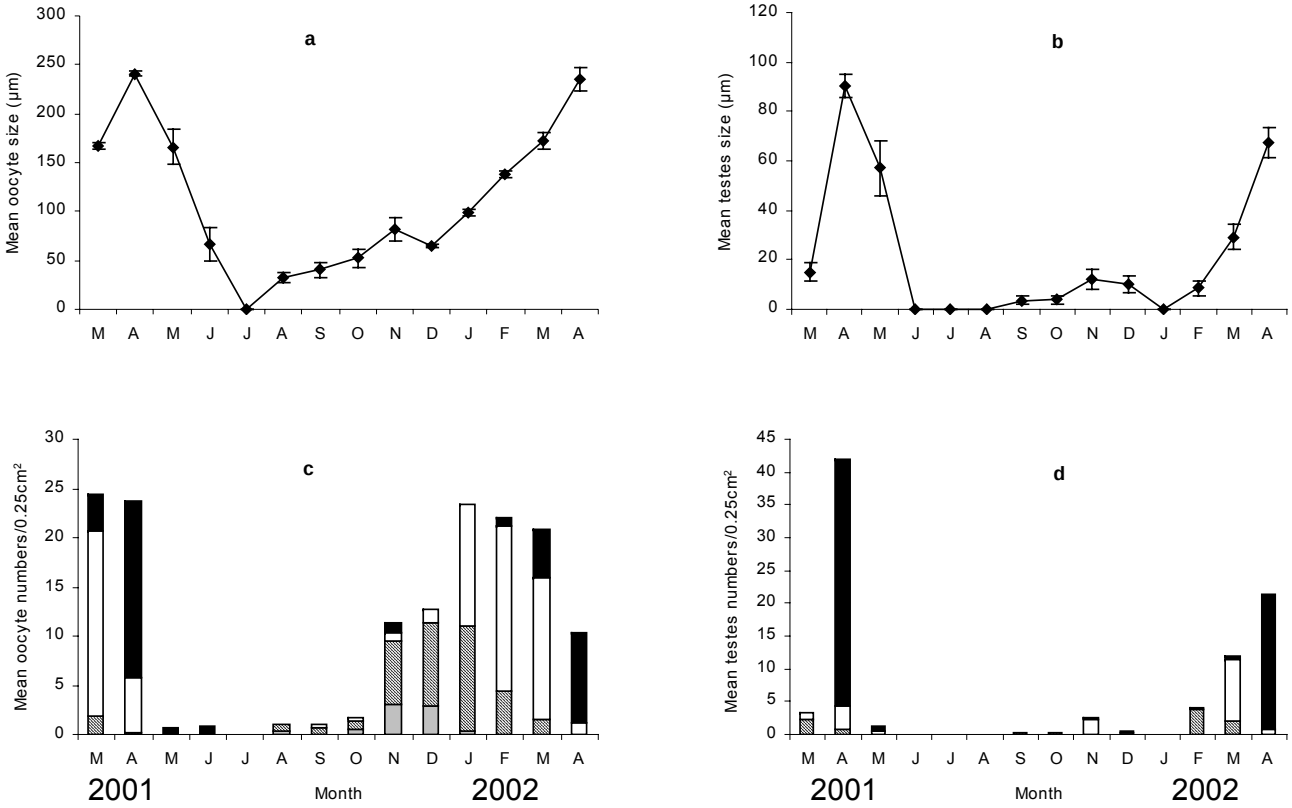

Fig 3.11 Overall reproductive phenology of *Platygyra* sp. in Singapore between March 2001 and April 2002 (n = 12). a) mean oocyte geometric diameter (µm); b) mean testes geometric diameter (µm), bars are standard errors; c) mean number of oocytes/0.25 cm^2 tissue; and d) mean number of testes/0.25 cm^2 tissue showing proportions of each stage (gray = stage 1, diagonal stripes = stage 2, white = stage 3, black = stage 4).

3.3.6 Relationship between gametogenesis and seasonal environmental parameters

Sea surface temperature (SST) was not significantly different between the three study sites ($df = 44$, $f = 0.002$, $p < 0.998$) (Table 3.4), so the average monthly values from the temperature logger at Raffles Lighthouse were assumed to be representative for all of the study sites. SST, salinity and to a lesser extent rainfall showed distinct seasonal patterns (Fig 3.12). Mean monthly SSTs during the study period were between 27.45 °C (+/-0.16 SD) in Jan 2002 to 30.55 °C (+/-0.35 SD) in May 2001, an annual variation of 3.1 °C (Fig 3.2a). SST was lowest in January (the height of the NE monsoon). There was a marked increase in SST following the NE monsoon. SST peaked in May (the end of the first inter-monsoon), but gradually declined until a second smaller peak in October and November (around the second inter-monsoon) (Fig 3.12a). The pattern for salinity was also distinctly seasonal. Salinity was highest in March (31.20 ppt ± 0.12 SD). From March, salinity declined and was lowest between July and October, before gradually increasing again until March, and declining again in April (Fig 3.12b). The predicted spawning times for both *P. lutea* and *Platygyra* sp. (based on the presence and subsequent disappearance of mature gametes), coincided with an increase in SST, and a decline in salinity, following the NE monsoon (Fig 3.12a, b). Between January and April 2002, mean SST increased from 27.45 °C (± 0.16 SD) to 29.65 °C (± 0.37 SD) (an increase of 2.2 °C) (Fig 3.11a). The rise in SST between January and April coincided with an increase in gamete size for both species (Fig 3.12a). Between January and April 2002 mean oocyte diameter increased from 15.63µm (± 5.5 SE) to 135.44µm (± 3.43 SE) for *P. lutea*, and from 98.5µm (± 6.14 SE) to 235.28µm (± 22.59 SE) for *Platygyra* sp. (Figs 3.8a & 3.11a). Whereas, mean testes diameter increased from 5.50 µm (± 5.49 SE) to

Table 3.4. Results of one-way ANOVA to test for differences in average sea temperature (°C) between three study sites.

	SS	Df	s^2	F	P
Between	0.00311	2	0.001556	0.002	0.998
Within	29.227	42	0.696		
Total	29.230	44			

Table 3.5. Contingency table showing correlations (Pearson product-moment) between seasonal environmental factors, SST (ºC), salinity (ppt), total monthly rainfall (mm) and sunshine hours, df = 14, **= p <0.01.

	SST	Salinity	Rainfall	Sunshine
SST		r = -0.12	r = -0.29	r = -0.15
Salinity			r = -0.03	r = 0.08
Rainfall				r = -0.67**

59.13μm (± 29.58 SE) for *P. lutea*, and from 0 to 67.34μm (± 11.80 SE) for *Platygyra* sp. (Figs 3.8b & 3.11b). A second peak in reproductive activity for both species coincided with a period of decreasing SST following the SW monsoon, prior to the second annual peak in SST before the onset of the NE monsoon (Fig 3.12a). There was also a distinct seasonal pattern for rainfall (Fig 3.12c), total monthly rainfall amounts were between 360.8 mm (December) and 54.2 mm (May 2001) (Fig 3.12c). As might be expected, total monthly rainfall was highest during the NE monsoon, and remained lower throughout the SW monsoon and the second inter-monsoon (Fig 3.12c). However, high rainfall levels were also recorded in March 2001 (309.3 mm), the month that is typically considered to be the start of the first inter-monsoon. The main spawnings for both species occurred after the period of heaviest rainfall (Fig 3.12c). Similarly, mean daily sunshine was highly variable (Fig 3.12d), and no clear seasonal pattern could be discerned. However, mean daily sunshine hours were lower during the NE monsoon, and there was a significant negative correlation between sunshine hours and total rainfall amounts ($\gamma = -0.41$, $p < 0.05$, $n = 14$) (Table 3.5), presumably because more rain clouds during certain months

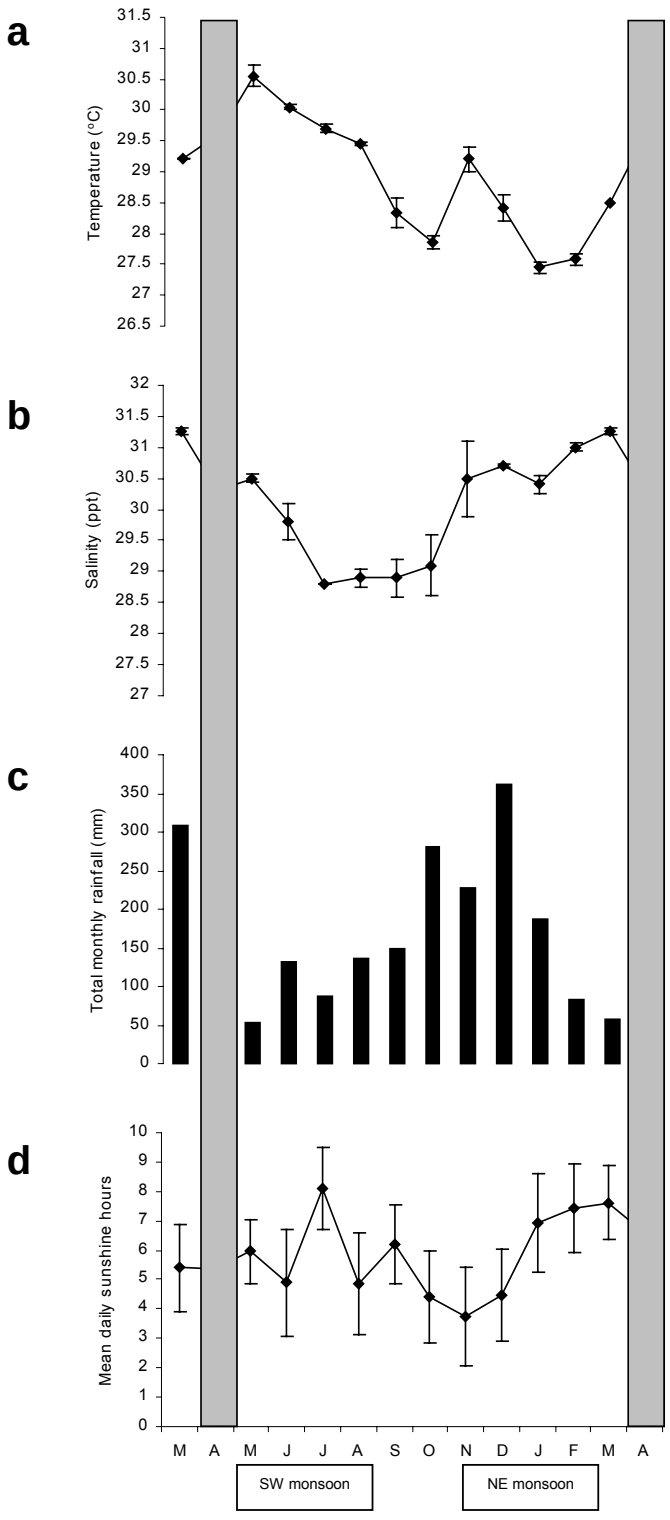

Fig 3.12 Monthly averages of environmental parameters from March 2001 to April 2002, a) sea surface temperature (°C), b) salinity (ppt), c) rainfall (mm) and d) daily sunshine hours. Gray bars indicate the main spawning months for *P. lutea* and *Platygyra* sp.

(e.g. November and December 2001) reduced sunlight penetration (Fig 3.12d). The lack of a distinct seasonal pattern for this parameter meant that there was no obvious relationship between reproduction and sunshine hours.

3.4 Discussion

3.4.1 Gametogenesis and reproductive strategies

Reproductive characteristics, such as the arrangement of gonads, the length of the gametogenic cycle and the reproductive strategy of in this study, were similar to those found for *Porites lutea* on the Great Barrier Reef (Kojis and Quinn 1981a; Harriot 1983) and in the Red Sea (Shlesinger et al. 1998) (Table 3.1). No incidences of hermaphroditism were observed among the eleven colonies of *P. lutea*, all of which were followed through at least two successive breeding seasons. In Bermuda Tomascik and Sander (1987) found that the normally gonochoric coral *Porites porites*, reverted to hermaphroditism on polluted reefs. Despite the relatively poor water quality conditions in Singapore (Chou 1996; Gin et al. 2000), a similar pattern was not found here. It seems reasonable to conclude that *P. lutea* is gonochoric in Singapore, and that the strategy is stable for this species (Kojis and Quinn 1981a). The sex ratio among *P. lutea* was skewed, with more female than male colonies (8:3). This may have been a result of the small number of colonies sampled (n = 11), however skewed sex ratios have been found among other populations of gonochoric corals. For example Kojis and Quinn (1981a) found that 41 out of 44 colonies of *P. lutea* at Heron Island (Great Barrier Reef) were female.

It is thought that most broadcasting corals only complete one annual gametogenic cycle (Harrison and Wallace 1990). While all of the eight female colonies of *P. lutea* had one major gametogenic cycle, five of these colonies also had a second annual peak in reproductive output. This second peak was variable between colonies, and in only one of the colonies (PHPL3), was the fecundity equivalent to the levels reached during the main spawning months (in March and April). Hence, it is not clear whether this second annual gametogenic cycle led to spawning or to the gametes being re-absorbed (Harrison and Wallace 1990). Histological analysis of samples of *Porites* spp. and *Goniopora* spp. corals collected from Singapore in 1999 and 2001 also found some colonies with mature gametes in October/November (Guest et al. 2000). Furthermore, bi-annual spawning of certain coral species has been reported in the Great Barrier Reef (GBR) (for 3 species of *Montipora*) and Western Australia (WA) (Stobart et al. 1992; J. Gilmour, personal communication), although bi-annual spawning has not previously been documented for *P. lutea*. In both the GBR and in WA the second annual spawning event involves fewer species, fewer colonies within populations and is more variable in extent from year to year, compared to the main spawning event (Stobart et al. 1992; J. Gilmour, personal communication). For a colony that spawns in March or April, there should be sufficient time to complete a second gametogenic cycle and spawn again in October or November (providing a resting period post-spawning is not needed before another gametogenic cycle begins). It is not clear therefore, why some colonies begin, but do not complete a second gametogenic cycle. One possibility is that the second gametogenic cycle terminates because of a lack of appropriate environmental cues (proximate cues), another possibility is that there is not sufficient energy available to complete the second cycle (i.e. reproduction is constrained by some environmental factor). In either case, the

success of the second spawning may be highly variable due to the variability in extent and timing of seasonal environmental factors from year to year. In Singapore, the environmental changes that occur during the NE monsoon (i.e. lower SSTs, changes in salinity and reduced PAR) may account for the failure of most corals to complete a second gametogenic cycle. Stobart et al. (1992) found that *Montipora digitata*, *M. aequituberculata* and *M. peltiformis* spawned bi-annually at Magnetic Island (GBR), but the second spawning varied considerably over two years of observation. In the year when egg production and spawning were less successful there was higher than average rainfall in the period between spawning events, which resulted in increased turbidity, reduced salinity (and presumably lower irradiance due to increased cloud cover) (Stobart et al. 1992). Monitoring individuals or populations of corals over successive breeding seasons, in conjunction with long-term monitoring of the seasonal environmental patterns is required to see whether the success of the second cycle correlates with fluctuations in the timing and the strength of weather conditions during the NE monsoon. For example, in years when the NE monsoon is particularly mild, or the onset is delayed, there might be sufficient time and energy available for most colonies to complete a second gametogenic cycle, resulting in a second spawning in October and November in Singapore.

It is not clear why there was such a marked difference in reproductive output between the three male colonies of *P. lutea* in this study. The seasonal cycle was similar among the three colonies, however stage IV testes were only found in samples collected from one colony (RLPL4). One possibility is that the spermatogenic cycle was very short. As each colony was only sampled once each month, the apparent differences in 'fecundity' may be a result of sampling colonies when they were at

different points during a short (i.e. 4 – 6 week) spermatogenic cycle. However, this does not explain the similar patterns seen for both years in all three colonies both in terms of gonad size and the relative abundance of different stages (Fig 3.4). In a study of nine sympatric hermaphroditic *Acropora* species, Wallace (1985) found a significant variation in polyp fecundity (numbers of eggs per polyp) between colonies of eight of the species. Otherwise, few studies to date have documented variation in polyp fecundity among reproductively mature corals within populations (Harrison and Wallace 1990), and this subject deserves further investigation. These findings show that, when possible, following individual colonies through successive breeding seasons can be more illuminating than randomly sampling colonies (Giese and Pearse 1974).

All of the *Platygyra* sp. colonies sampled during the spawning season were hermaphroditic, with oocytes and testes intermingled within the mesenteries. This strongly suggests that the *Platygyra* species in this study were simultaneous hermaphrodites with a broadcasting mode of gamete release. This reproductive strategy is shared by the majority of Faviid species studied to date (Harrison 1985) and broadcast spawning of *Platygyra* species has been documented elsewhere (Richmond and Hunter 1990). In the present study, oocytes appeared in some colonies as early as August, but were low in abundance until November, indicating that the oogenic cycle may last between six and nine months, whereas spermatogenesis lasted between two and three months. A similar pattern has been recorded for a number of other Faviids, with gametogenic cycles ranging from five to nine months for oogenesis and one to three months for spermatogenesis (Harrison and Wallace 1990).

In the present study, there appeared to be a second smaller peak in reproductive activity between September and December. It is hard to tell whether any colonies underwent a full second annual gametogenic cycle because individual *Platygyra* colonies were not sequentially sampled. Some of the *P. lutea* colonies also had two annual gametogenic cycles, similarly in *Platygyra* it is not known if any of this early gamete development led to spawning. However, why some colonies would expend energy by beginning, but not completing a second gametogenic cycle is not clear (see discussion above). In this study, the finding that both *P. lutea* and *Platygyra* sp. had a second annual peak in reproductive activity around October/November, indicates that this pattern may be common among scleractinians in Singapore.

3.4.2 The relationship between gametogenesis and environmental seasonality

It is probably no coincidence that the seasonality of the reproductive cycles was significantly correlated between these unrelated species. Spawning occurred primarily in April, although some colonies may have spawned in March and/or May. In both species there appeared to be a second peak in reproductive activity with the possibility that some colonies spawned in September, October and/or November. In April 2002 sampling was carried out 2 days after the full moon. Some of the colonies of *Platygyra* sp. appeared to have spawned partially or fully and this was reflected in the lower numbers of gametes in April 2002 compared to April 2001 (Fig 3.11c, d). These findings indicate that spawning may occur around the full moon. Therefore, the mature gametes in samples in May 2001 may not have spawned and would eventually be re-absorbed (Harrison and Wallace 1990). Mass spawning among an assemblage of

Platygyra and many other species (but not *P. lutea*) was observed following the full moons of March 2002 and April 2003 (Discussed in Chapter 5).

In both species there was clearly a strong seasonality, with the peak of reproductive activity occurring during the first inter-monsoon (March - April). This indicates that environmental factors related to the monsoon seasons may be involved in driving the annual patterns of reproduction. Temperature is considered to be one of the most important factors regulating gametogenic cycles in marine invertebrates (Orton 1920; Giese and Pearse 1974). However, global comparisons of coral spawning times and SSTs show that there are a number of inconsistencies in this relationship (see Chapter 1 for discussion). Furthermore, it was generally predicted that temperature variation in equatorial locations would be too small to act as a reliable cue for synchronising reproduction within populations (Giese and Pearse 1974; Richmond and Hunter 1990; Olive 1995). Despite Singapore's equatorial location, the annual pattern of SST was distinctly seasonal (Fig 3.12a). Logging of SST at Raffles Lighthouse until May 2003 revealed that a similar seasonal SST pattern occurred in 2002 and 2003 (see Chapter 5, Fig 5). Furthermore, the pattern is consistent with that found by Tham (1973), indicating that changes in SST around Singapore's southern islands are predictable and markedly seasonal each year. A dramatic increase in the average size of both oocytes and testes in both species, over a 3 month period from January to April in 2002, coincided with a rise in mean monthly SST of 2.2 °C following the NE Monsoon (Figs 3.8, 3.11 & 3.12a), therefore the main spawning times occurred when SSTs were rising or at their annual peak (Fig 3.12a). A second, smaller annual peak in reproductive activity in both species, coincided with a period of decreasing SSTs following the SW monsoon, prior to the second annual peak in SST before the onset

of the NE monsoon (Figs 3.8, 3.11 & 3.12a). These findings are consistent with those from other regions, where the final maturation of gametes and spawning coincide with the period of annual maximum SSTs, or when SSTs are changing (either warming or cooling) (Harrison and Wallace 1990; Richmond and Hunter 1990). These data lend support to the hypothesis that gamete maturation in corals is regulated by a rapid change in SSTs (Babcock et al. 1986). While there is a predictable pattern of change, and the annual range of SSTs is more than 3 °C, SSTs remain relatively warm throughout the year (Fig 3.12a). Orton's rule predicts that breeding in marine invertebrates should be continuous above some critical temperature (Orton 1920). However, breeding in these two species was distinctly seasonal, despite the fact that SSTs did not drop below 27 °C, a temperature that is adequate for gamete maturation and spawning to occur in other locations (e.g. Hayashibara et al. 1993). While it is true that some colonies did undergo a second gametogenic cycle, it is not clear whether this led to spawning, furthermore bi-annual spawning of appears to be a feature of broadcasting scleractinians in higher latitude locations that experience more marked seasonal SST changes (e.g. GBR, Stobart et al. 1992; and WA, J. Gilmour personal communication).

Similarly, for salinity there was a seasonal pattern related to the monsoon seasons, and the pattern was consistent with that found by Tham (1973) (Fig 3.10b). However, it is hard to think of any plausible biological basis for salinity as a seasonal reproductive cue in corals. Furthermore, predictability may be an important factor when an environmental cue is regulating reproductive seasonality. Mean monthly salinity was considerably more variable than mean monthly SSTs (Fig. 3.11b). Heavy

rainfall may locally alter salinity, particularly in shallow water, meaning that this factor would be unreliable as a seasonal cue for reproduction.

There was also a distinct seasonal pattern for rainfall during the study, with rainfall levels being higher during the NE monsoon season, particularly in December. It has been suggested that rainfall has acted as an ultimate factor in determining the seasonal timing of spawning in corals (Mendes and Woodley 2002). Rainfall decreases salinity, particularly in surface waters, and fertilisation rates are negatively affected by sudden reductions in salinity (Richmond 1997). Therefore it is possible that spawning during heavy rain showers could negatively impact fertilisation success. There is some evidence to support this, as Harrison et al. (1984), noted during the mass spawning at Magnetic Island in November 1981 that the majority of larvae and gametes were destroyed by heavy rainfall. Furthermore, Mendes and Woodley (2002) found that meta-analysis of 19 geographical locations revealed the majority of spawning occurs in months without heavy rainfall when temperatures are warmest. They predicted that in equatorial locations where temperature variations are small, spawning generally occurs before the months of heaviest rainfall. The possible advantage of this strategy is that increased rainfall may elevate nutrient levels (because of river run-off), thus benefiting newly settled larvae. In the present study it was found that the majority of spawning occurred just *after* the period when rainfall is typically highest, not before (Fig 3.12c). While it is tempting to believe that rainfall has exerted an ultimate control on the timing of spawning in corals, particularly in locations that experience little annual variation in SSTs, there are a number of flaws in this hypothesis. Rainfall levels may be different between offshore islands or reefs and the mainland (where rainfall measurements are usually taken). Furthermore,

higher total monthly rainfall amounts would not necessarily increase the chances of spawning coinciding with a rain shower, particularly if rain showers occur mostly at one time of the day (i.e. early morning). Therefore frequency, duration and timing of rainfall events would be more appropriate in any analysis of the relationship between these factors. In their meta analysis of 19 geographical locations, Mendes and Woodley (2002) found that temperature alone was not sufficient to explain timing of spawning, however average air temperatures (not SSTs), were used. In the present study it can be seen that variations in SST were not dependent on air temperatures (because SSTs are controlled more by changes in prevailing water currents than by ambient air temperatures) (Tham 1973; Gin et al. 2000). SSTs showed a more distinct seasonal pattern than air temperature and the annual range of mean monthly SST is at least 2 ºC greater than that of mean monthly air temperature in Singapore (Chapter 2, Fig 2.4), indicating that air temperature should not be used to predict the timing of spawning.

The present study shows that even in equatorial locations such as Singapore, there is sufficient annual seasonality (in terms of SST) to provide a cue for corals to synchronise spawning within populations. Nonetheless, the inconsistencies in the relationship between SSTs and spawning times suggests that some other factor, in addition to SSTs, is important in regulating the timing of spawning in broadcasting corals. Solar radiation has previously been suggested as one possible factor (Heyward 1986; Harrison and Wallace 1990), but surprisingly few investigations have been done to look at the relationship between solar radiation and reproduction in corals. The products of photosynthesis by the symbiotic zooxanthellae have been shown to be important in contributing to reproduction (Rinkevich 1989), suggesting that a causal

link may exist. Penland et al. (2004) found solar insolation to be a better predictor of spawning times than SSTs for corals in the Western Pacific. Similarly, Mendes and Woodley (2002) found a strong correlation between light intensity and gonad size in *Montastrea annularis* in Jamaica. In this study, mean daily sunshine hours were extremely variable, and no obvious seasonal pattern was discernible. Direct underwater measurements of PAR would have been more appropriate, however collecting such data in Singapore was not feasible. Underwater sensors measuring PAR need to remain clear of any obstructions, however Singapore's waters experience very high levels of sedimentation and biofouling (Gin et al. 2000), meaning that sensors would need to be cleaned frequently. Alternatively, PAR could be measured in air using sensors located close to the field site, and an estimate of actual PAR received by corals could be computed, providing the average light attenuation for seawater at the site was known (see Brown et al. 1999).

While it is possible to infer relationships between reproductive patterns and seasonality, it should be noted that any concurrence does not imply a causal relationship (Olive 1995). Further studies should involve manipulative experiments to see what effects environmental factors such as SST and PAR have on the reproductive cycles of these species, combined with investigations into the underlying molecular and genetic basis for a causal relationship (Olive 1995).

3.5 Conclusion

Even in equatorial location such as Singapore, there is probably sufficient seasonal variation for corals to use factors such as sea surface temperatures as 'cues' for

reproduction. The finding that two scleractinian species, from different families (Poritidae and Faviidae), have almost identical gametogenic cycles, indicates that these two broadcast spawning species have responded similarly, but independently, to the available annual environmental signals (Babcock et al. 1986; Olive 1995). It seems unlikely that any coastal location is truly 'aseasonal'. This means that reproductive synchrony within populations of broadcast spawners (at least in terms of gamete maturation) is just as likely to occur in an equatorial region with modest (but nonetheless distinct) annual environmental variation as it is in a high latitude location with large variations in environmental seasonality. Environmental conditions are suitable year round for breeding in Singapore, so it seems unlikely that the timing of reproduction in *P. lutea* or *Platygyra* sp. is constrained by any environmental factor. Although it is possible that these species may gain some 'ultimate' selective advantage by spawning when environmental conditions are optimal for fertilisation, larval survival, settlement and recruitment.

The fact that two unrelated species had almost identical seasonal reproductive patterns indicates that there may also be a high degree of multi-species reproductive seasonality and synchrony (i.e. other scleractinian species may respond similarly to seasonal cues), both in terms of gamete maturation and in terms of spawning. The next two chapters will examine this in further detail: in **Chapter 4**, I examine the degree of reproductive synchrony and seasonality in an assemblage of the widespread scleractinian genus *Acropora*; and in **Chapter 5,** I examine the timing and species participation of multi-species synchronous spawning periods in Singapore.

Chapter 4

Reproductive seasonality in an equatorial assemblage of *Acropora*

Abstract

The extent of reproductive seasonality and synchrony among Singapore's corals was investigated in an assemblage of the widespread scleractinian coral genus *Acropora*. Sampling of corals over 14 months between March 2002 and May 2003 revealed that a high proportion of the species in the assemblage contained mature gametes at the same time, although within species the proportions of colonies that contained mature gametes varied considerably. The great majority of reproductive activity was concentrated in the first inter-monsoon (March and April), however a small number of colonies were also fecund in May, October and November. A high proportion of the colonies sampled in March 2002 and April 2003 contained mature gametes (48.5% n = 113 & 47.4% n = 98, respectively). The proportions of species containing mature gametes in the same month were also high in both years (68%, n = 19 & 79%, n = 14, respectively). The findings demonstrate that distinct reproductive seasonality is a characteristic of equatorial coral assemblages.

4.1 Introduction

The few available latitudinal comparisons of coral reproductive patterns appear to indicate that spawning seasonality and synchrony are less pronounced among equatorial assemblages of scleractinian corals (Oliver et al. 1988). However, in Chapter 3 it was shown that two unrelated scleractinian species exhibited almost identical seasonal reproductive patterns. The aim of the study in this chapter was to examine the extent of reproductive seasonality and synchrony, both among and within species, in an assemblage of the genus *Acropora*. *Acropora* was chosen because it is the dominant genus in both area cover and numbers in most reef habitats throughout the central Indo-Pacific, and exhibits high levels of reproductive synchrony among species (Wallace 1985).

4.2 Methods and Materials

Sampling was carried out on the fringing reef on the west side of Raffles Lighthouse (Fig. 2.2, Chapter 2) monthly, from March 2002 until May 2003 (except in June and August 2002 when no sampling was done). This site was chosen, as it is one of the few reefs around Singapore that has a suitable assemblage of *Acropora* corals. Sampling trips were carried out each month between one and eight days before the full moon. On each occasion, I made two surveys using SCUBA, by swimming parallel to the reef for a distance of approximately 200m, once along the reef flat and once along the top of the reef slope (1 – 5m depth, approx area 20 x 200m). Each sampling generally took 2 to 3 hours depending on water visibility and currents. Any *Acropora* colony encountered during the survey was sampled, except for small

individuals (i.e. <20 cm in diameter). Due to the relatively low abundance of *Acropora* on Singapore's reefs, it was inevitable that some colonies were sampled more than once throughout the 14 months, however the searching pattern meant that no colony was sampled more than once on each occasion. Identification to species level was only made in the months of March 2002 and March, April and May 2003. When species identification could not be done *in situ*, colonies were photographed with a Sony digital camera in an Ikelite underwater housing. No voucher specimens were taken, as this would have further depleted the number of colonies in the assemblage. Between 70 and 113 colonies were checked on each sampling occasion (Fig 4.1). The reproductive state of *Acropora* species can be gauged easily by breaking off a branch below the expected sterile zone (Wallace 1985) and noting the presence or absence of eggs. Mature eggs in *Acropora* are pigmented (usually red, pink or orange in colour) and large enough to be visible to the naked eye (i.e. 600 – 700 μm diameter, Wallace 1985) (see Fig 4.2). The available evidence indicates that colonies that contain visible, pigmented eggs are likely to spawn on or shortly after the subsequent full moon; colonies with eggs that are visible but un-pigmented (white) are likely to spawn within one to three months; and colonies with no visible eggs have either just spawned or are unlikely to spawn for at least three months (Harrison et al. 1984; Baird et al. 2002). The sampling procedure followed Baird et al. (2002) and involved removing up to 3 individual branches from each colony and noting one of the 3 reproductive conditions (presence of pigmented eggs, presence of visible white eggs or no visible eggs). Colonies were only scored as empty if all 3 branches were found to be empty. This method permitted the examination of large numbers of colonies with the minimum amount of time and effort. However, it did not provide any details about the size of mature oocytes, the length of the gametogenic

cycle or the exact night and hour of spawning. No colony mortality was observed as a result of this sampling method and branch stumps could be seen re-growing on sampled colonies within one month. A chi-square analysis was performed to determine if the proportion of colonies with mature eggs was independent of seasons. The year was divided into 4 monsoon seasons as in Chapter 3 (NE Monsoon, November – February; SW Monsoon, May-August; first inter-monsoon, March-April; and second inter-monsoon, September-October) (Nieuwolt, 1973). Data were pooled for each season and a contingency table was constructed with 5 rows (representing the 5 seasons covered during the study), and 2 columns (representing number of colonies with mature eggs and number of empty colonies).

4.2 Results and Discussion

The assemblage of *Acropora* at Raffles Lighthouse showed a high degree of inter-specific reproductive seasonality, with the majority of spawning concentrated in March and April (Fig. 4.1). Mature eggs were present in colonies in March, April, May, October and November. However, the great majority of mature colonies was found in March and April 2002 (48.5 and 23 % respectively of colonies sampled) and April 2003 (47.4% of colonies sampled) (Fig. 4.1). In October, November (2002), March and May (2003) 2.1, 3.8, 7 and 1.3% of colonies were mature respectively (Fig. 4.1). Large numbers of colonies contained visible, immature (white) oocytes one to two months before the main spawning periods in March and April 2003 (February 2003 45.5%, and March 2003 55% of the colonies sampled) (Fig. 4.1). Chi-square analysis indicated a highly significant association between the season and the presence of mature gametes ($df = 4$, test statistic = 252, $p < 0.001$), with the majority

of mature eggs being present during the first inter-monsoon season (March – April).

Very low numbers of colonies containing immature white eggs were found in almost

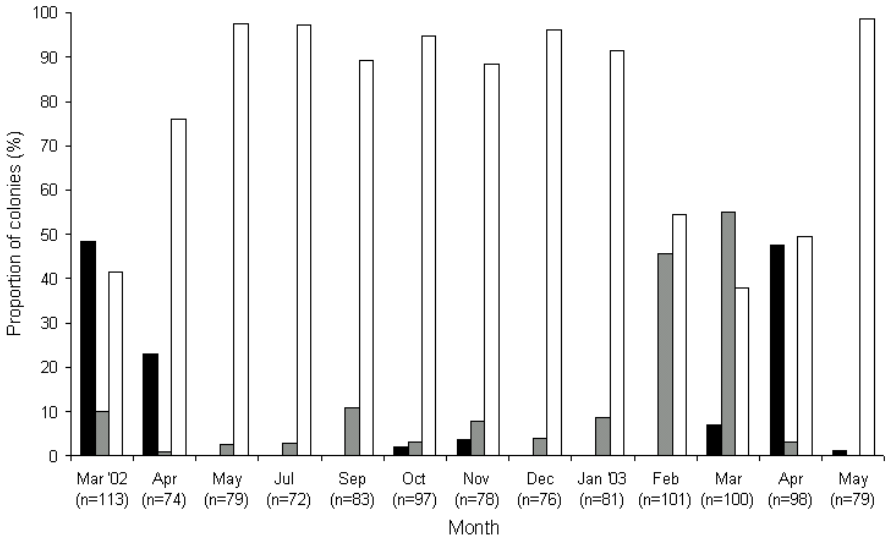

Fig 4.1 The overall proportion of colonies of *Acropora* species. at Raffles Lighthouse that contained mature eggs (black bars), immature eggs (gray bars) or no visible eggs (white bars) between March 2002 and May 2003 (n = total number of colonies sampled).

Fig 4.2 A broken branch on a colony of *Acropora intermedia* revealing brightly pigmented eggs in March 2002 (Photo by author).

every month (Fig 4.1), however, it is not clear if these colonies spawned outside of the main spawning season or retained immature eggs for some months prior to spawning, because they were not tagged and sequentially sampled. The most probable explanation for their presence is that large mesenterial filaments were mistaken for white eggs.

Some colonies of *Acropora digitifera* and *A. hyacinthus* contained mature oocytes in both March and April in 2003 (Table 4.1), indicating that spawning in these populations was "split" over two months (Willis et al. 1985; Wolstenholme 2003). Split spawning of populations over consecutive months has been reported elsewhere e.g. Japan (Shimoike et al. 1992) and Australia (Willis et al. 1985; Baird et al. 2002; Wolstenholme 2003; J. Gilmour personal communication), and is thought to be a result of individuals within a population differing in their responses to the timing of the full moon. Split spawning within populations in Singapore appeared to be more prevalent when the full moon fell at the end of the month i.e. in March (29^{th}) and April (27^{th}) 2002 (Fig 4.1), however further observations are needed to establish whether this pattern is consistent. In October and November only some colonies of *A. humilis* had mature eggs. Two fecund colonies were tagged and found to contain mature gametes again in April 2003, indicating that some colonies of this species spawn twice a year. The finding that bi-annual spawning occurs in some *Acropora* here is consistent with the findings for *Porites lutea* and *Platygyra* sp. in Singapore (Chapter 3) and for reef corals on the GBR and in WA (Stobart et al. 1992; J. Gilmour personal communication) (for discussion see Chapter 3).

In total, 23 *Acropora* species were sampled in March 2002 and March, April & May 2003. Due to the haphazard nature of the sampling, not all 23 species were represented in each sampling month (Table 4.1). In both years, a high proportion of the species encountered during the surveys, had at least one colony with mature eggs. Of the 19 species sampled in March 2002, 13 (68%) had at least one colony with mature eggs, and of the 14 species sampled in April 2003, 11 (79%) had at least one colony with mature eggs (Table 4.1). Few generalisations can be made about within-species synchrony of gamete maturation, due to the lack of species identification for many of the samples and the relative rarity of many species in the assemblage (Table 4.1). However populations of some species appeared to be quite synchronous (in terms of gamete maturation), for example 86% of *A. humilis* (n = 7) and 75% of *A. digitifera* (n = 16) colonies sampled were mature just prior to the full moon in April 2003 (Table 4.1). Other species had moderate or low levels of population synchrony, such as *A. hyacinthus*, where 42% of colonies sampled were mature in April 2003 (n = 12), and *A. tenuis*, where only 12% of the colonies sampled were mature in April 2003 (n = 8) (Table 4.1). Colonies of *A. austera* were checked in March, April and May 2003 (n = 9, 10 and 5 respectively), however mature eggs were never observed (Table 4.1). It is not clear why certain species lacked fecund colonies during the main spawning periods. It is possible that some individuals spawn at other times of the year, or not at all in some years (i.e. some colonies "skip" a year altogether) (Hughes et al. 2000). Reproductive output may also be reduced by environmental stressors, such as chronic fragmentation (Wallace 1985), bleaching (Baird and Marshall 2002) or decreased water quality (Ward and Harrison 2000). Long term studies following individual colonies and populations through time are required to understand the reproductive patterns of these species on Singapore's reefs.

	March 2002				March 2003				April 2003				May 2003			
Species	mature	immature	empty	n	mature	immature	empty	n	mature	immature	empty	n	mature	immature	empty	n
Acropora austera	0	0	100	4	0	0	100	9	0	0	100	10	0	0	100	5
A. cerealis	67	0	33	6	ns	ns	ns	0	ns	ns	ns	0	ns	ns	ns	0
A. digitifera	62	9	29	21	7	93	0	14	75	0	25	16	0	0	100	12
A. donei	0	0	100	1	ns	ns	ns	0	ns	ns	ns	0	ns	ns	ns	0
A. florida	0	0	100	2	0	50	50	2	ns	ns	ns	0	0	0	100	1
A. grandis	0	0	100	2	ns	ns	ns	0	ns	ns.	ns	0	ns	ns	ns	0
A. granulosa	ns	ns	ns	0	0	100	0	4	100	0	0	4	0	0	100	4
A. humilis	60	20	20	5	0	100	0	5	86	0	14	7	0	0	100	4
A. hyacinthus	48	4	48	25	23	59	18	22	42	0	58	12	0	0	100	13
A. intermedia	33	0	67	3	0	0	100	2	0	0	100	3	0	0	100	1
A. latistella	38	12	50	8	0	0	100	12	20	20	60	10	0	0	100	11
A. lianae	ns	ns	ns	0	0	100	0	2	ns	ns	ns	0	ns	ns	ns	0
A. loripes	100	0	0	1	0	100	0	5	71	0	29	7	0	0	100	4
A. microclados	100	0	0	1	50	50	0	2	ns	ns	ns	0	ns	ns	ns	0
A. millepora	33	67	0	3	0	82	18	11	57	14	29	7	0	0	100	8
A. muricata	0	33	67	6	0	0	100	1	ns	ns	ns	0	0	0	100	2
A. nasuta	57	29	14	7	ns	ns	ns	0	100	0	0	1	ns	ns	ns	0
A. samoensis	0	0	100	1	ns	ns	ns	0	0	0	100	3	0	0	100	1
A. secale	100	0	0	5	ns	ns	ns	0	75	0	25	8	11	0	89	9
A. selago	86	0	14	7	ns	ns	ns	0	ns	ns	ns	0	ns	ns	ns	0
A. tenuis	20	0	80	5	0	25	75	8	12	0	88	8	0	0	100	1
A. verweyi	ns	ns	ns	0	0	100	0	1	50	0	50	2	0	0	100	3
Totals	48.5	10	41.5	113	7	55	38	100	47.4	3.1	49.5	98	1.3	0	98.7	79

Table 4.1 The proportion of *Acropora* colonies (%) in each species that contained mature eggs, immature eggs or no visible eggs for March 2002 and March, April and May 2003. n = number of colonies sampled of each species; ns = not sampled.

Clearly, marked seasonality in reproductive activity is a feature of the *Acropora* assemblage at Raffles Lighthouse. The finding that gamete maturation is distinctly seasonal and synchronous among the *Acropora*, *Porites lutea* and *Platygyra* sp. indicates that these corals may participate in multi-species spawning periods (or 'mass spawnings') (Willis et al. 1985). In fact, multi-species synchronous spawning events were documented in 2002 and 2003 and will be discussed in the next chapter. On Singapore's reefs, the annual range in sea surface temperature is only 3 - 4º C (Tham 1973), which is relatively small compared to coral reefs at higher latitudes (e.g. Magnetic Island, GBR, which experiences an annual variation of 12º C (Babcock et al. 1986), suggesting that a large range in annual sea surface temperature is not a pre-requisite for reproductive seasonality or multi-species spawning synchrony. It may be significant that Singapore has a semi-diurnal tide and a relatively large tidal amplitude of 2.4 m (Raffles Lighthouse, Maritime Port Authority of Singapore 2002) in comparison to other low latitude reefs (e.g. Solomon Islands, 0.8m, Baird et al. 2002; and Madang, PNG, 1m, Oliver et al. 1988). While, it is conceivable that tides play an ultimate role in selecting for the nights and time of spawning (because mass spawning during extended periods of slack water may increase fertilisation success, (Babcock et al. 1986)), it is not clear how large tidal amplitudes could affect the extent of reproductive seasonality. Furthermore, synchronous spawning of corals does occur on reefs that experience small tidal amplitudes e.g. the Solomon Islands (Baird et al. 2001), and the Houtman-Abrolhos Islands (Western Australia) (Babcock et al. 1994) suggesting that this factor is not an ultimate cause of multi-specific synchrony.

Chapter 5

Patterns of coral spawning in Singapore

Abstract

Five field trips were conducted between October 2001 and April 2003 to document multi-species coral spawning events in Singapore. Corals were only observed spawning during three of the five weeklong trips, following the full moons of March 2002, March and April 2003. The timing of spawning appeared to be related to the rise in sea temperature following the NE monsoon. Spawning consistently began 1-1.5 hrs after sunset, suggesting that onset of darkness was the final proximate cue for spawning, in species that were observed to release gametes. In both 2002 and 2003, corals spawned between the third and sixth nights after the full moon, during the second low tide of the day, at a time when tidal mixing was negligible. These observations suggest that the moon and/or tidal rhythms may play a role in determining the timing of coral spawning in Singapore. Twenty-four scleractinian species were observed spawning *in situ* and at least 12 species spawned simultaneously on one night at Raffles Lighthouse. A further 27 species were found to contain mature eggs just prior to the spawning periods, indicating that more than 50 coral species may participate in multi-species spawning events in Singapore.

5.1 Methods and Materials

5.1.1 Spawning observations

Field trips to observe coral spawning were carried out in the weeks following the full moons of October and November 2001, March 2002 and March and April 2003 (Table 5.1). Most of the available evidence indicates that coral spawning occurs on, or shortly after the full moon (Harrison and Wallace 1990). Therefore, field observations began on the night of the full moon, and continued until the fifth or sixth night after (Table 5.1). All of the field trips involved camping at the field site (Appendix 4, Fig. A4.1). In 2001 and 2002 observations were done at Raffles Lighthouse (Fig 2.1), but in 2003 landing at Raffles Lighthouse was prohibited by the Maritime Port Authority, so spawning observations were carried out on the fringing and patch reefs on the western side of Pulau Hantu Besar (Fig. 2.1). Water conditions were excellent in March/April 2002 with visibility in excess of 5 meters. However, conditions were much poorer in 2003 at Pulau Hantu, and visibility ranged between 1 and 2 meters (personal observation).

Month and year	Location	Date of full moon	Dates of field trip
October 2001	Raffles Lighthouse	October 2nd	2nd - 6th
November 2001	Raffles Lighthouse	November 1st	1st – 4th
March/April 2002	Raffles Lighthouse	March 29th	30th – 4th
March 2003	Pulau Hantu	March 18th	18th, 21st – 23rd
April 2003	Pulau Hantu	April 17th	17th – 23rd

Table. 5.1 Dates of field trips to observe coral spawning showing the locations and dates of the full moons.

To ascertain the number of species that were mature prior to the spawning events, polyps were fractured from colonies underwater, and the presence or absence of visible pigmented eggs was recorded (method described in previous chapter, and see

Fig 5.1). In 2002 only the genus *Acropora* and the species *Diploastrea heliopora* were sampled; but in April 2003 a total of 5 families (including *Acropora*) and 29 species were sampled in this way.

To determine what time corals began spawning; loose coral colonies of mixed species were collected from the reef and put in large, aerated fiberglass tanks on the shore to be monitored at intervals. The tanks were emptied and refilled with seawater every six hours using a submersible pump fixed to the seabed with an angle iron and cable ties. Netting was used to cover the tanks during the day to protect the corals from excessive sunlight (Fig A4.1). To document coral spawning I patrolled an area of reef parallel to the shore including reef flat and upper reef slope (approximately 100m of shoreline), by snorkeling or scuba diving. Any colonies observed spawning were photographed with a Nikonos V and a 1:3 close up lens (Ocean Optics, London).

During the April 2003 trip, all spawning colonies were tagged to monitor split spawning within colonies. Due to the random search pattern, some species might have spawned but not been documented. This qualitative method was used to gain an overall picture of timing and species participation, over an area of reef in a short space of time, with limited manpower (usually only 2 people). Many reports show that spawning often begins at or just after sunset (Harrison and Wallace 1990), so the patrols began at sunset (approximately 1900 local time) and continued until around 2200 or until spawning activities, if any, had ended.

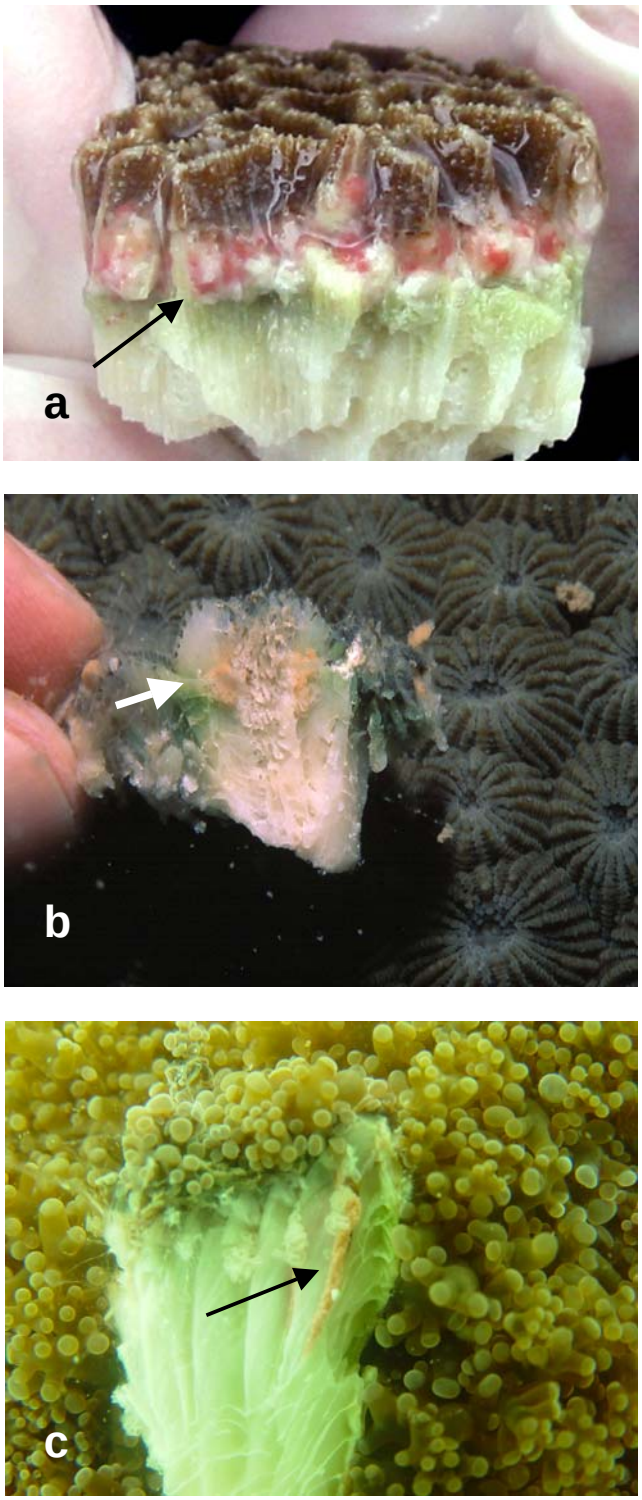

Fig. 5.1. Pigmented mature oocytes in fractured polyps of a) *Platygyra* sp., b) *Diploastrea heliopora* and c) *Euphyllia divisa* indicated by arrow.

5.1.2 Environmental data

The methods used to collect the data on sea temperature and tidal cycles are described in Chapter 3. A correlation (Spearman's product-moment, SPSS v 9) was conducted to compare the sea temperature patterns leading up to and during the spawning periods (March 1st and May 5th) in 2002 and 2003. All temperature readings were taken on the reef at Pulau Hantu during the March and April 2003 field trips. However, a comparison of temperature measurements taken at Raffles Lighthouse and Pulau Hantu in 2002 showed no significant difference in temperatures between sites (Chapter 3, Table 3.4) indicating that the temperature patterns at the two sites are the same.

5.2 Results

5.2.1 Timing of spawning

Spawning was observed during the field trips carried out in March 2002, March 2003 and April 2003 (Tables 5.2 & 5.3). No coral spawning was observed in October and November 2001, and only one colony of *Echinopora lamellosa* was observed spawning in March 2003 on the fourth night after full moon (March 22nd). Discharged gametes were seen for the first time in the tanks at around 2030 (local time), approximately 1.5 hrs after sunset, on the third night after the full moon in March 2002 (April 1st). Immediately after this, I observed corals spawning on the reef. Sunset was at approximately 1900 every day. Day length in Singapore is approximately 12 hours, varying by only 9 minutes annually, so sunset is at

approximately the same time throughout the year (Nieuwolt 1973). Spawning activity took place between approximately 1930 and 2230 on each night, but was mostly concentrated between 2000 and 2200 hours.

5.2.2 Species participation

A total of 19 scleractinian species were observed spawning in 2002 (Table 5.2) and 13 species in 2003 (Table 5.3). In all, 24 scleractinian species were documented spawning *in situ* over the successive seasons. Including *Acropora* (results described in previous chapter), a further 27 scleractinian species contained mature eggs prior to the spawning, but were not observed to spawn (Table 5.4). These coral species were from 17 genera and 7 families. On any one night, at least 5 to 12 species spawned simultaneously (Tables 5.2, 5.3). The highest number of 12 species spawning simultaneously was observed on the fourth night after full moon of March 2002 (April 2nd, Table 5.2).

Twenty-one of the species that spawned were hermaphroditic broadcasters, releasing egg/sperm bundles. One species was gonochoric (*Euphyllia ancora*) and only female colonies were seen releasing eggs. Two species (*Galaxea fascicularis*, *G. astreata*) were functional hermaphrodites Harrison 1988) and both male and female colonies spawned (Tables 5.2, 5.3).

In both years, spawning was dominated by certain species or genera. In 2002 spawning was dominated (in terms of the number of colonies) by *Acropora* spp., *Favites* spp., *Goniastrea* spp., *Platygyra* spp., and *Galaxea fascicularis* (Table 5.2);

and in 2003 by *Euphyllia ancora*, *G. fascicularis*, *Merulina ampliata* and *Pectinia* sp. (Table 5.3). Although other species spawned during the spawning periods, in most cases only 1 to 2 colonies were witnessed releasing, suggesting that some colonies within populations may not spawn at all or may spawn at other times during the year (Tables 5.2, 5.3).

Table 5.2 Record of species observed spawning, number of colonies (n) and spawning nights for March/April 2002 at Raffles Lighthouse.

Family and species	n	Spawning nights (number of nights after March full moon)
Family Acroporidae		
Acropora millepora	3	3 - 5
A. hyacinthus	2	5
Acropora sp. 1	?	3 - 5
Family Faviidae		
Echinopora sp.	1	4
Favia sp.	1	4
Favites halicora	1	3
Favites sp. 1	>5	3
Favites sp. 2	1	4
Goniastrea minuta	3	3 - 5
G. pectinata	1	3
Leptoria phrygia	1	3
Platygyra ryukyuensis	>5	3 - 4
P. sinensis	>5	3 - 4
Platygyra sp.	>5	3 - 5
Family Merulinidae		
Merulina ampliata	1	5
Oculinidae		
Galaxea fascicularis	>5	4 - 5
G. astreata	1	4
Pectiniidae		
Echinophyllia sp.	1	5
Pectinia paeonia	1	4

In both years, the majority of the species observed to spawn were from the family Faviidae. Mass synchronous spawning among the Faviidae occurred on the third and fourth nights after the full moon in March 2002 (1st and 2nd April) (Table 5.2).

However, a similar pattern was not detected in 2003 and spawning among the Faviidae was distinctly patchy (Table 5.3). Among the *Acropora*, spawning occurred over at least three nights in 2002 at Raffles Lighthouse. Due to the paucity of colonies at Pulau Hantu, it was not possible to make further observations of the timing of spawning in *Acropora* in 2003.

Table 5.3 Species participation, number of colonies (n) and spawning nights for April 2003 at Pulau Hantu.

Family and species	n	Spawning nights (number of nights after March full moon)
Family Faviidae		
Echinopora lamellosa	1	5
Echinopora sp.	1	1
Favites sp.	1	5
Goniastrea minuta	1	3
Oulophyllia benettae	1	3
Oulophyllia sp.	1	3
Platygyra ryukyuensis	2	3 – 5
Platygyra sp. 1	1	6
Family Merulinidae		
Merulina ampliata	>5	3 – 6
Family Oculinidae		
Galaxea fascicularis	>5	3 – 6
Family Pectiniidae		
Pectinia lactuca	2	4 – 5
P. paeonia	>5	3 – 6
Family Euphyllidae		
Euphyllia ancora	3	4 - 6

'Split' spawning over consecutive nights was observed in coral populations of species from all of the families (Tables. 5.2, 5.3.). Split spawning was also observed over two consecutive nights in April 2003 in some individual colonies of *Pectinia paeonia* and *Galaxea fascicularis*.

5.2.3 Relationship with temperature, lunar and tidal cycles

Continuous hourly logging of SST at Raffles Lighthouse from December 2001 until May 2003 revealed a marked seasonal pattern (Fig 5.3, also see Chapter 3). Annual peaks occurred in April/May and in October/November, and an annual low occurred in December/January, during the NE monsoon (Fig 5.3). The main spawning season for corals occurs at a time when the seawater temperature is rising, following the NE monsoon (Fig 5.3). Between the end of February and the beginning of May a rise of approximately 3°C was recorded (Fig. 5.4). There was a highly significant association between years, in hourly sea temperature values measured between March 1st and May 5th, when the temperature logger was removed ($r = 0.89$, df. = 1559, $p<0.0001$) (Fig 5.4). Mean daily sea temperatures during the spawning periods ranged from 29°C to 29.9 °C (Table. 5.5), and no marked changes in temperature were recorded on the days when spawning was observed at Raffles Lighthouse in March/April 2002 (Fig 5.5).

Table. 5.4. Coral species, number of colonies sampled and egg colours from fractured polyps in March 2002 and April 2003 prior to the full moons of those months. All *Acropora* sampled contained red, pink or orange eggs. The first number in *n* represents sample from March 2002 and the second is from April 2003. Only *Acropora* and *D. heliopora* were sampled in March 2002.

Family and species	n	Egg colour
Family Acroporidae		
A. cerealis	4, 0	Red, pink or orange
A. digitifera	13, 12	-
A. granulosa	0, 4	-
A. humilis	3, 5	-
A. intermedia	1, 0	-
A. latistella	3, 4	-
A. loripes	1, 5	-
A. microclados	1, 0	-
A. nasuta	3, 1	-

A. prostrata	0, 1	-
A. samoensis	0, 0	-
A. secale	5, 2	-
A. selago	6, 1	-
A. tenuis	1, 2	-
A. valida	1, 0	-
A. verweyi	0, 1	-
Montipora sp.	1	Green
Family Faviidae		
Diploastrea heliopora	6, 2	Red/orange
Favia sp. 1	2	Blue/green
Favia sp. 2	1	Blue/green
Favia sp. 3	1	Blue/green
Favites abdita	3	Orange
Favites complanata	1	Blue/green
Favites halicora	2	Red
Platygyra pini	1	Red
Platygyra sinensis	2	Red
Family Merulinidae		
Merulina scabricula	1	Orange
Family Mussidae		
Lobophyllia hemprichii	2	Orange
Symphyllia agaricia	1	Orange
Symphyllia radians	1	Orange
Symphyllia recta	2	Orange
Family Euphyllidae		
Euphyllia divisa	1	Red

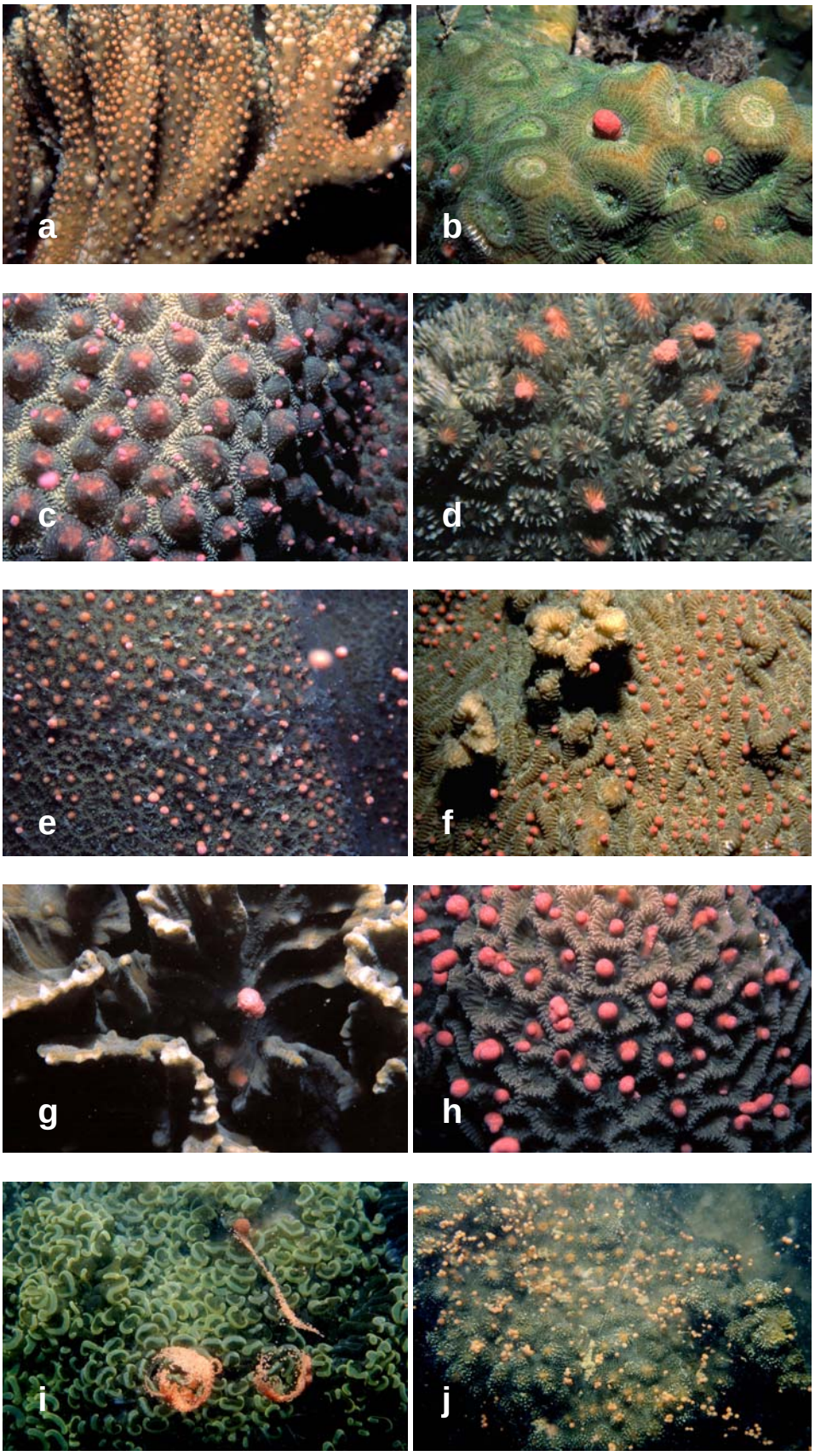
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Fig 5.2 Corals spawning at night in Singapore a) *Acropora* sp. with egg sperm bundles 'set' just prior to spawning, b) *Favites halicora*, c) *Favites* sp., d) *Galaxea fascicularis*, e) *Goniastrea minuta*, f) *Merulina ampliata*, g) *Pectinia paeonia*, h) *Platygyra* sp., i) *Euphyllia ancora*, j) *Echinopora lamellosa*.

Table 5.5. Mean sea temperature on days of spawning (°C ± SD), and number of species that were observed to spawn.

Date	1st Apr 2002	2nd Apr 2002	3rd Apr 2002	22nd Mar 2003	18th Apr 2003	20th Apr 2003	21st Apr 2003	22nd Apr 2003	23rd Apr 2003
Mean sea temperature (°C)	29.15 ± 0.14	29.18 ± 0.15	29.25 ± 0.18	29.01 ± 0.12	29.74 ± 0.13	29.85 ± 0.21	29.85 ± 0.14	29.86 ± 0.13	29.85 ± 0.1
No. of species observed spawning	10	12	8	1	1	7	6	8	5

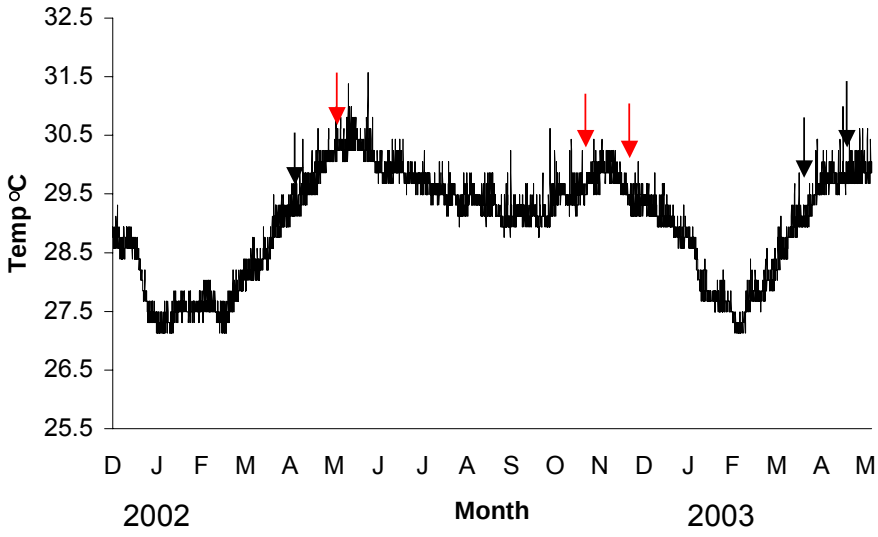


Fig. 5.3 Hourly measurements of sea temperature from December 2001 to May 2003 with times of observed (black arrows) and predicted coral spawning (red arrows).

In both March 2002 and April 2003, the majority of spawning was observed from the third to the sixth nights after the full moon. However the field trips ended after the fifth night in 2002 and after the sixth night in 2003, so spawning may have continued for a number of nights after observations had finished. What appeared to be small patches of spawned eggs were observed floating on the water's surface between the full moon night and the first and second nights after full moon in April 2003, however no corals were actually witnessed releasing gametes on those nights. Presumably these were from coral colonies that release one or two days prior to the main spawning nights. In March 2002 the full moon fell at the end of the month (29^{th}) so spawning actually occurred in the first week of April (Guest et al. 2002). No field trips were conducted after the April full moon in 2002, however evidence from *in situ* fracturing of polyps and histological sampling suggest corals also spawned at this time, indicating split spawning over two months in 2002 (Chapter 4, Fig. 4.1). In 2003 the full moons of March and April fell mid-month so spawning occurred during the third week of the month, however most of the spawning was concentrated after the April full moon with only one colony observed to spawn in March (Table 5.3).

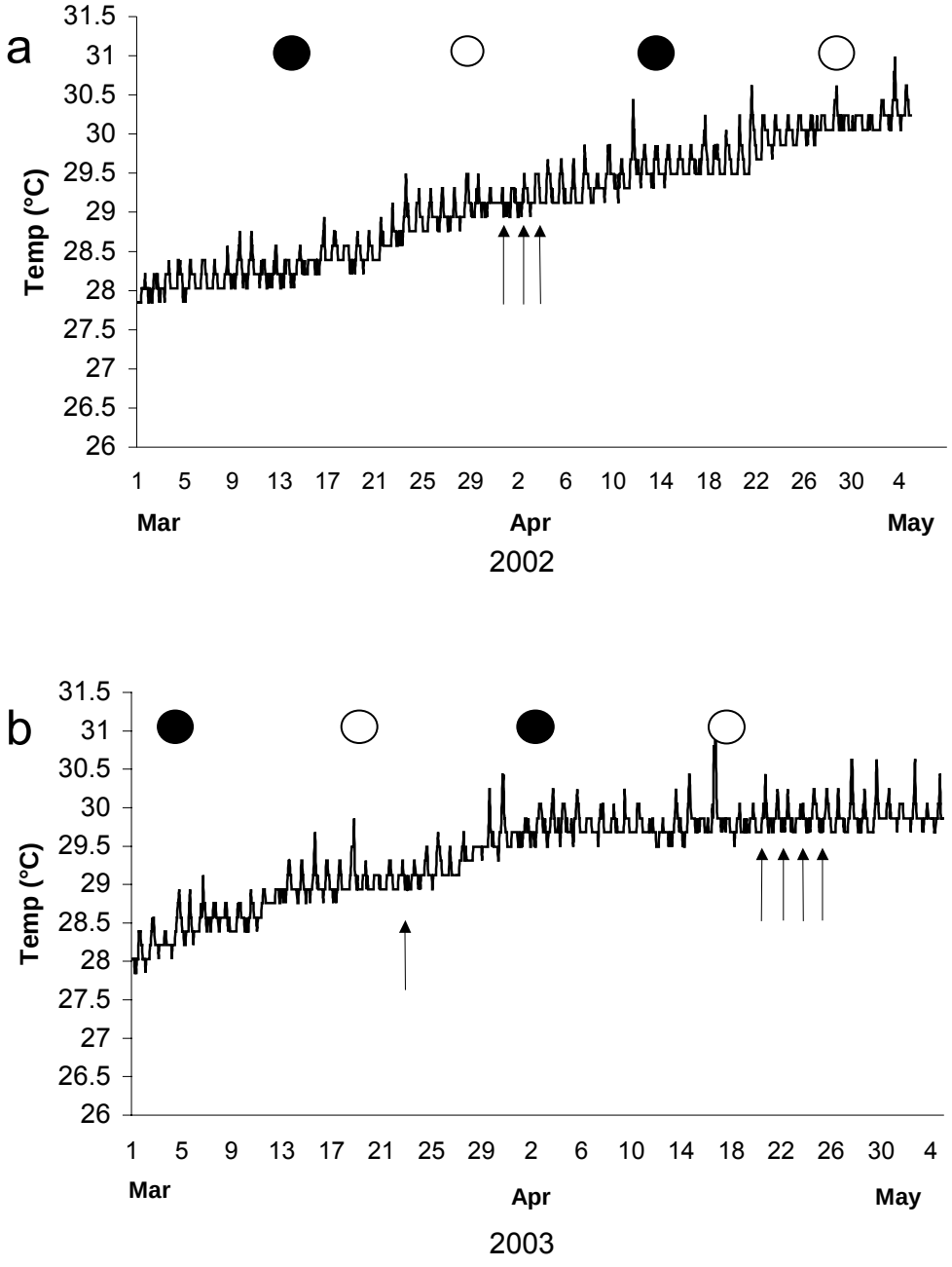


Fig. 5.4 Sea surface temperature at Raffles Lighthouse and lunar patterns between March 1st and May 5th in a) 2002 and b) 2003. Open circles = full moon; filled circles = new moon; arrows indicate nights that spawning was observed.

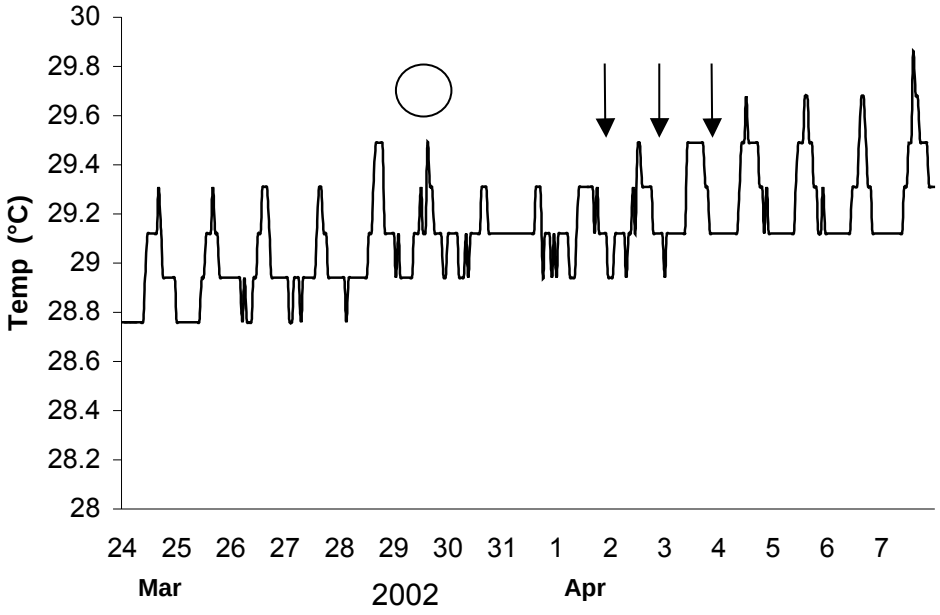
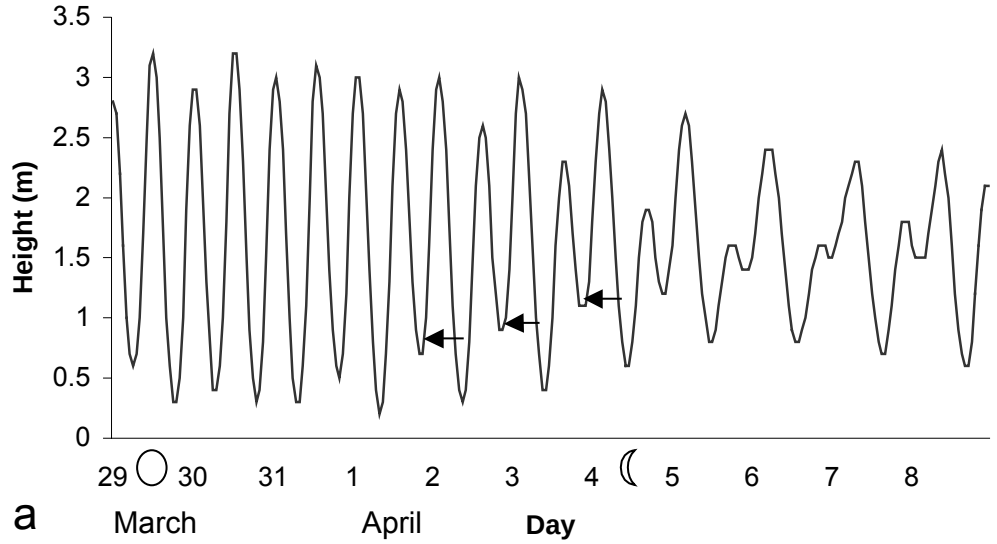


Fig. 5.5 Hourly sea temperature from March 24th until April 7th at Raffles Lighthouse in 2002. Empty circle = full moon; arrows = nights spawning was observed.

In Singapore the spring tides occur, either on, or one to two days after the full and new moon, whereas neap tides occur just after the last quarter moon. In both years, spawning nights coincided with the end of the spring tides and the beginning of the neap tides (Fig 5.6). During the week following the full moon in any month, the second low tide of the day occurs between the hours of 1900 and 2100 at Raffles Lighthouse and at Bukom (the closest tidal station to P. Hantu) (Maritime and Port Authority of Singapore 2002, 2003). Spawning consistently began close to the time of the second low tide of the day (Fig 5.6), at a time when tidal mixing was minimal (Fig 5.7). The difference in tidal height in meters between the hours of 2000 and 2200 ranged from 0 – 0.4 m and current speed was negligible (Fig 5.7)



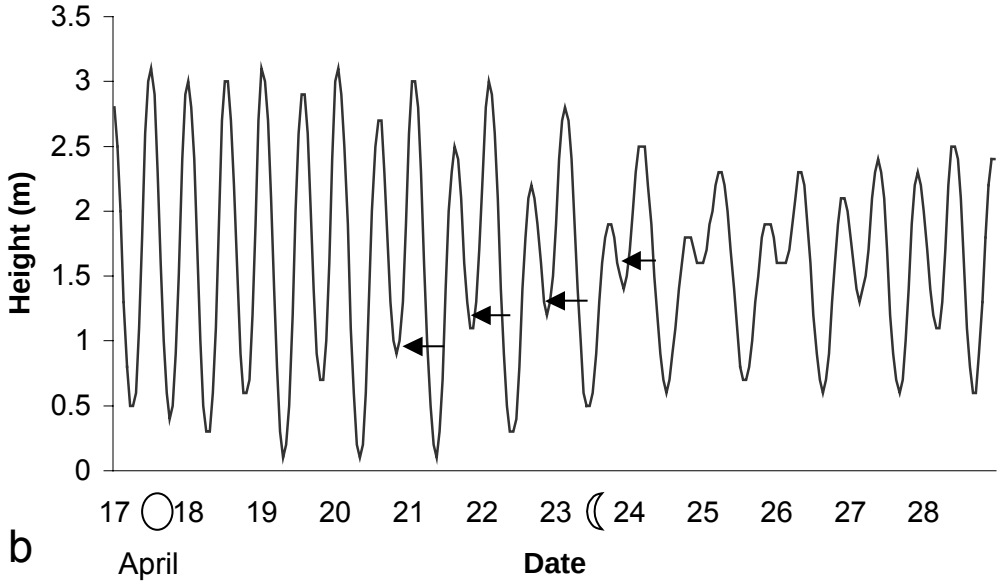


Fig. 5.6 Tidal rhythms for the ten days following the full moons in a) March 2002 at Raffles Lighthouse; and b) April 2003 at Bukom (the closest tidal station to Pulau Hantu). The date of the full moon and last quarter moon are indicated. Arrows point to the approximate start time of observed coral spawning.

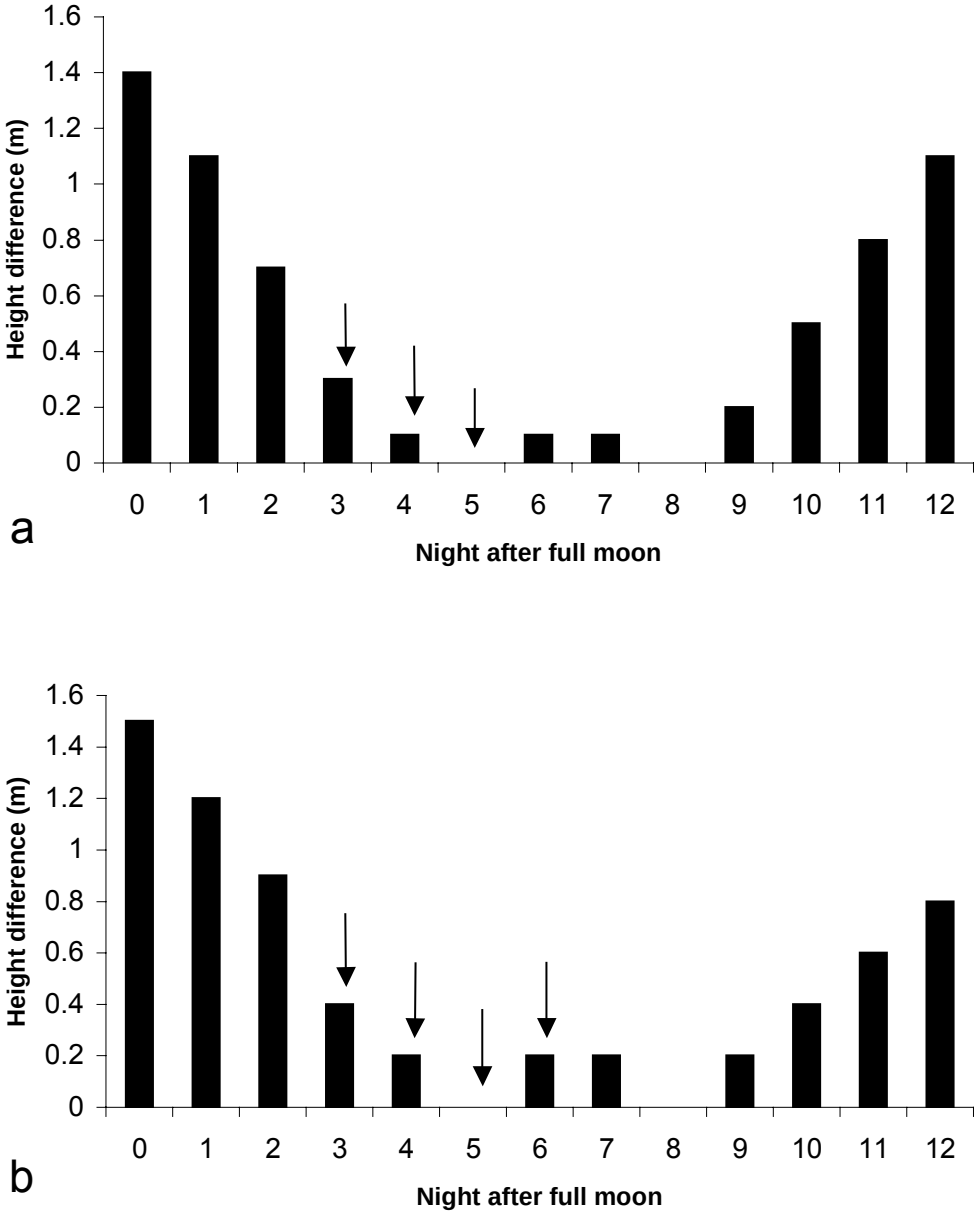


Fig. 5.7 The difference in tidal height (m) between the hours of 2000 and 2200 (the approximate time of spawning) following the full moons of a) March 2002 at Raffles Lighthouse; and b) April 2003 at Bukom. Arrows indicate the nights that spawning was observed.

5.3 Discussion

5.3.1 Timing of spawning

In Singapore the great majority of coral spawning occurs in March and April (Tables 5.2, 5.3, and see Chapters 3 and 4). Spawning was concentrated during a discreet period beginning on the third night after the full moon, continuing until at least the fifth or sixth nights after full moon. Some colonies of *Acropora*, *Platygyra* sp. and *Porites lutea* probably spawn in May and between September and November, (see Chapters 3 and 4). However no coral spawning was observed during the field trips carried out in October and November in 2001, suggesting that spawning was minimal, or spawning occurred at other times of the day or night (i.e. at dusk) and was missed during the observation periods. Spawning consistently began 1 – 1.5 hrs after sunset, suggesting that the onset of darkness acts as a final proximate cue for spawning. This observation is consistent with reports from other regions (Harrison and Wallace 1990) and with experimental studies (e.g. *Goniastrea aspera*, Babcock 1984). Spawning at night may have adaptive significance, as predation by planktivorous organisms that rely heavily on vision may be reduced (Babcock et al. 1986). Furthermore, nocturnal spawning may reduce the damaging effects of UV radiation during the initial period of embryo development (Jeffry 1990 a,b).

5.3.2 Species participation and mass synchronous spawning in Singapore

This is the first detailed account of multi-specific coral spawning in Singapore, or for a coral reef so close to the equator. Twenty-four scleractinian species were observed

spawning *in situ*, a further 27 species contained mature eggs prior to the spawning periods (Tables 5.2, 5.3 and 5.4), and at least 12 species spawned simultaneously on one night at Raffles Lighthouse (Table 5.2). These species represent almost one third of all the scleractinian species found in Singapore waters (Chou et al. 1994). The patterns observed in Singapore are consistent with the definition of a 'mass' coral spawning described for parts of the Great Barrier Reef, Western Australia and other regions (Harrison et al. 1984; Simpson 1985; Willis et al. 1985; Babcock et al. 1986; Babcock et al. 1994; Hayashibara et al. 1993; Richmond and Hunter 1990; Tomascik et al. 1997; Baird et al. 2001). All of the species that were observed spawning in Singapore have been observed spawning in other locations (Babcock et al. 1986; Richmond and Hunter 1990; Hayashibara et al. 1993). Since representatives of all coral species found on Singapore's reefs were not tagged and monitored, and as most of the observations were carried out *in situ* in poor visibility, it is likely that a number of other species spawned, but were not observed and documented. Therefore, the actual number of species that participate in the mass spawning period may be considerably higher. Furthermore, some colonies or species may spawn at other times, i.e. during the day, later in the night or at dusk. This has been documented in populations of *Goniastrea favulus*, which spawn during late afternoon, on some reefs on the Great Barrier Reef (Babcock et al. 1986). Similarly in Okinawa, Japan, six *Acropora* species spawn 1.5 - 3 hrs earlier than other mass spawning acroporids (Fukami et al. 2003).

The sexual characteristics observed in Singapore's corals are consistent with reports from elsewhere (Fadlallah 1983; Harrison 1985; Harrison and Wallace 1990; Richmond and Hunter 1990), with the majority of species being hermaphroditic

broadcasters and fewer being gonochoric broadcasters. No brooding corals were observed releasing planulae. The brooding coral *Pocillopora damicornis* is known to spawn larvae around the new moon in Singapore (unpublished data). It is possible therefore that other brooders and broadcasters spawn at this time, however no observations were carried out around new moon to confirm this.

5.3.3 Relationship with temperature, lunar and tidal cycles

Around Singapore's southern islands, sea temperature varies by 3 to 4 °C annually, with peaks occurring in April/May and in October/November and an annual low in December/January, during the NE monsoon (Fig 5.3). The main spawning occurs at a time when the seawater temperature is rising, following the NE monsoon (Fig 5.3). Temperature rose gradually between the end of February and the beginning of May, and spawning nights did not appear to correlate with any temperature peaks (Figs. 5.4 & 5.5). In contrast, at Akajima Island, Japan, Hayashibara et al. (1993) found that spawning nights coincided with marked changes in seawater temperature (as well as a drop in salinity and increase in current strength) with spawning happening 6 to 12hrs after the first peak of temperature on each day. It seems likely therefore, that the rise in sea temperature following the NE monsoon acts as a seasonal cue in Singapore, but not as a final trigger for the night of spawning. This is consistent with findings from a number of other regions, where it is typical for coral spawning to occur during the annual warming, or during the warmest months of the year (Harrison and Wallace 1990; Richmond and Hunter 1990).

Spawning was observed in both years following the full moon, although no observations were made after the new moon, so it is possible that some species or individuals spawned at this, or at other times. Spawning began three nights after the full moon and continued until at least the fifth or sixth night. In 2002 the majority of spawning was after the March full moon, whereas in 2003 it was after the April full moon. However, in both years some corals spawned in both months. This pattern of 'split' spawning between months is a common feature of coral communities elsewhere (Willis et al. 1985, see Chapter 4 for discussion).

There was a strong correlation between years, in the pattern of sea temperature rise prior to spawning, whereas the main spawning events happened at different times and appeared to be related to the timing of the full moon (Fig 5.4). Many coral species exhibit a lunar pattern of gametogenesis and spawning (Harrison and Wallace 1990), and experimental studies indicate that the moon is involved in cueing planulation in brooding corals and spawning in some broadcasters (Jokiel et al. 1985; Hunter 1988). It is not completely clear whether the spawning rhythms are regulated by moonlight, or by some other factor related to the lunar cycle. However, experimental evidence demonstrates that corals can sense the low levels of light from the moon. Gorbunov and Falkowski (2002) found that the Caribbean corals *Eusmilia fastigiata*, *Montastrea cavernosa*, *Manicina areolata* and *Diploria labyrinthiformis* show extraordinarily sensitive photoreception in the blue region of the spectrum indicating that moonlight itself could act as a proximate cue for spawning. It is not known whether elevated levels of turbidity affect the ability of corals in Singapore to spawn successfully, as it may hamper their ability to detect moonlight. It is interesting to note that fewer species and colonies were seen spawning in 2003 compared to 2002 (Tables 5.2, 5.3)

and water turbidity was much higher in 2003 compared and with 2002 (personal observation). However, this could be coincidence, differences in sites or just due to natural variation in fecundity (Hughes et al. 2000). Further studies should be conducted to look at the levels of nocturnal irradiance that can be detected underwater during different lunar phases, and what effect (if any), elevated turbidity has on spawning success.

The corals that spawned in Singapore in March/April 2002 and 2003 released gametes during the second low tide of the day at a time when tidal mixing was minimal (Figs 5.6, 5.7). In Singapore, a similar tidal pattern occurs in all months, i.e. from the third to the sixth nights after full moon, the second low tide of the day is at approximately the same time as sunset, and the difference in tidal height (hence tidal mixing) is minimal (i.e. < 0.4m) between the hours of 2000 and 2200 when most of the corals spawned (Maritime and Port Authority of Singapore 2002, 2003). These findings suggest that tides may have played an ultimate role in determining the night and time, but not the month of spawning. Babcock et al. (1986) proposed that spawning during a period of slack water and low tide on the Great Barrier Reef, might enhance fertilisation success because of reduced dispersal and dilution. However, this pattern has not evolved in all locations. At Akajima Island, Japan, corals spawn during the high tide when currents are strong (Hayashibara et al. 1993). At Akajima, there may be a stronger selective pressure for dispersal (Hayashibara et al. 1993), however it seems more plausible that natural selection operates on fertilisation success (Hughes et al. 2000). In temperate (Houtman-Abrolhos) and tropical (Ningaloo) reefs in Western Australia, corals spawn on the same nights, despite large differences in tidal phase and tidal amplitude. These observed differences might result from a genetic

legacy from ancestral corals, where timing has been set by tidal rhythms (Babcock et al. 1994). The original adaptive value may have been lost in the descendants, however the inherited spawning rhythm will remain the same, providing that the new tidal regime does not exert a selective pressure to counteract the genetic legacy (Babcock et al. 1994). Furthermore, if the genetic connection between reefs remains high, this may inhibit the ability of the population to adapt to the new conditions.

5.3.4 Mass spawning of corals on an equatorial coral reef

The finding that many scleractinian species spawn together, during a discreet spawning period in Singapore, refutes the hypothesis that mass coral spawning is *not* characteristic of equatorial coral reefs (Oliver et al. 1988; Richmond and Hunter 1990) (see discussion Chapter 4). Multi-species spawning events have now been documented in almost all of the coral reef regions of the world (e.g. Great Barrier Reef, Harrison et al. 1984; Willis et al. 1985; Babcock et al. 1986); Western Australia, (Babcock et al. 1994; Simpson 1985); Caribbean, (Hagman et al. 1998; Van Veghel 1993); Japan, (Heyward et al. 1987; Hayashibara et al. 1993); Indonesia, (Tomascik et al. 1997; M. Erdman personal communication); Taiwan, (Dai et al. 1992); Maldives, (personal observation); Central Pacific, (Richmond and Hunter 1990); Solomon Islands, (Baird et al. 2001); and Palau (Penland et al. 2004). While marked reproductive seasonality and synchrony appear to be a feature of most (perhaps all) coral communities, the extent of synchrony within and between species may vary between regions, and between years (Harrison and Wallace 1990; Richmond and Hunter 1990). It is not clear what causes the observed regional variations. Typically, annual ranges in environmental parameters (e.g. sea

temperature) are implicated (Oliver et al. 1988; Richmond and Hunter 1990). However, the finding that multi-species synchrony occurs in equatorial locations, in the absence of large annual, environmental fluctuations and possible seasonal constraints on the timing of spawning (Baird et al. 2001; Tomascik et al. 1997; and present study), indicates that there is no simple relationship between, either latitude *or* the annual range of environmental parameters and the extent of spawning synchrony. Indeed in some higher latitude locations, e.g. Japan and the Red Sea, where the annual sea temperature range is about three times that of Singapore, the patterns of reproductive seasonality are reported to be more protracted (Schlesinger and Loya 1984; Hayashibara et al. 1993).

While many corals do spawn in synchrony during mass spawning periods, many do not; both in Singapore and on other coral reefs. Even on the Great Barrier Reef, where more than 130 species spawn during discreet periods following the full moons in October and November (Willis et al. 1985), many species and colonies spawn outside of these periods or not at all in some years. For example (Kojis and Quinn 1981a; Harriot 1983) found spawning over at least four months between November and February in broadcasting *Porites* species at Heron Island (23° 27‘S) and Lizard Island (14° 39’S). In a two year study of nine sympatric *Acropora* species at Big Broadhurst Reef, (18° 55’S) Wallace (1985) found that colonies of *Acropora granulosa* spawned in late summer (i.e. approximately 4 months after the main spawning period), colonies of *A. sarmentosa* showed little synchrony between colonies, and colonies of *A. horrida* were never found to contain mature gametes. In a study to compare latitudinal variation in spawning synchrony among the *Acropora*, Baird et al. (2002) found evidence of asynchrony between colonies for most of the *Acropora* species studied at

Lizard Island, Pelorus Island (18° 40'S) and Lady Elliot Island (23° 45'S). Similarly, in a study of five species and seven morphospecies from the *Acropora humilis* group at Lizard Island, Wolstenholme (2003) found that many colonies spawned one, two and three months after the main mass spawning period. Hughes et al. (2000) found that some colonies of *A. hyacinthus*, *A. cytherea* and *A. millepora* did not release eggs in one year in both the northern and southern GBR, and van Oppen et al. (2002) found that, in two out of four years, gametes were absent prior to mass spawning episodes from at least some colonies of *A. aspera* at Orpheus Island (18° 34'S).

The present study shows, that even in an equatorial location such as Singapore, there is probably sufficient annual seasonal environmental variation to provide suitable cues for reproductive synchrony in corals. The findings of this study lend support to what is probably the most plausible hypothesis explaining multi-species spawning: considering that most mass spawning species are congeneric, multi-species reproductive synchrony evolved because species respond independently, but similarly to the seasonal, lunar and diel environmental cues, to maximise fertilisation success (Babcock et al. 1986). Multi-species spawning may even increases fitness, despite potential disadvantages such as gamete wastage and competition for settlement space, if egg-eating predators are satiated. Spawning at a time when environmental conditions are most favourable for successful fertilisation, larval survival, settlement and recruitment may also be a factor, although it seems unlikely that there are any seasonal environmental constraints in a location such as Singapore. This means that reproductive synchrony (in terms of gamete maturation *and* spawning) is just as likely to occur in an equatorial region with more modest (nonetheless distinct) seasonality,

as it is in a high latitude location that experiences large seasonal variations in environmental parameters.

Chapter 6

Experimental fragmentation and transplantation of *Goniopora* corals

Abstract

Replicate fragments of the corals *Goniopora columna* (Dana, 1846), *G. lobata* (Milne Edwards and Haime, 1860) and *G. fruticosa* (Saville-Kent, 1893 in: Veron and Pichon, 1982) were removed from large 'parent' colonies on the upper reef slope at Pulau Hantu. These fragments were transplanted and attached to underwater frames at the same depth as the parent colonies at Pulau Hantu, to a more disturbed site closer to mainland Singapore (Cyrene reef) and to greater depth at both sites. Control fragments were also transplanted a few meters away from the parent colonies. Fragments were left for approximately one year then harvested just prior to the predicted spawning month. Fecundity (average number of oocytes per polyp), reproductive effort (average oocyte size) and polyp diameter were compared between clonal experimental fragments, controls and parent colonies. Fragments transplanted to the same depth at Pulau Hantu were not significantly affected, indicating that fragmentation and transplantation in these species had no negative impact on reproduction. However, clonal fragments transplanted to greater depth, and to the more disturbed site closer to mainland Singapore had significantly less oocytes, smaller oocytes and smaller polyps after one year. Elevated turbidity and sedimentation at the more disturbed site may have reduced the amount of energy

available to corals for reproduction. While these factors are indeed causes of stress and mortality in corals, another important factor, may have been competition with macro-algae, or exposure to elevated nutrients at Cyrene.

6.1 Introduction

Transplantation of coral fragments is one commonly used method for restoring damaged reefs, particularly in areas where natural recruitment of coral larvae is absent (Clark and Edwards 1995, 1998). One of the main aims of coral reef restoration is to create coral communities that are reproductively viable (Yap 2003). However, very little is known about the reproductive success of fragments used in coral transplantation for reef restoration (Yap et al. 1998). While fragmentation is a normal asexual reproductive strategy in corals, it can negatively impact the health and fecundity of the parent colony and the fragments. A number of studies have revealed that fragmentation can cause reductions in reproductive effort and even mortality in some corals (Highsmith 1982; Zakai et al. 2000) (see Chapter 1 for discussion). It has been suggested that coral reproduction can be used as an indicator of stress on coral reefs, as some studies have shown that corals suffer reductions in fecundity and reproductive effort when exposed to reduced water quality (e.g. elevated turbidity, sedimentation and nutrients) (Kojis and Quinn 1984; Tomascik and Sander 1987; Ward and Harrison 2000). In this chapter, fragmentation of colonies of the scleractinian genus *Goniopora*, and subsequent transplantation of the fragments was conducted to test whether removal from the parent colony affects the health of the fragments, and whether transplantation to a more disturbed reef has a negative impact on fecundity or reproductive effort.

6.1.2 Goniopora

Species in the genus *Goniopora* (Blainville, 1830 in: Veron and Pichon 1982) are easily recognisable because of their large, fleshy polyps, which are extended both day and night (Fig. 6.1a). Polyps always have 24 tentacles, and both polyp detail and colour can be used for identification to species. Indeed, many species are easier to identify *in situ*, than from dried skeletons (Veron 2000). *Goniopora* are usually gonochoric broadcast spawners (Harrison 1985; Richmond and Hunter 1990). Eight *Goniopora* species have been recorded releasing gametes during the mass coral spawning on the Great Barrier Reef (Willis et al. 1985; Babcock et al. 1986). One exception is *G. queenslandiae decima*, which is reported to be a gonochoric brooder in Okinawa (Fadlallah 1983). As with other scleractinians, *Goniopora* colonies reproduce asexually by fragmentation (Highsmith 1982). It is a general assumption that columns within a 'colony' are genetically identical clones, however it is possible that mutations occur during somatic growth in long-lived modular organisms (i.e. somatic mutation) (Hughes et al. 1992), however this has not been demonstrated so far in a coral.

Large columnar colonies (i.e. 2 – 6 m in diameter) of the genus *Goniopora* are found on some of Singapore's reefs (Fig 6.1b). Particularly notable are a number of large colonies, on the upper reef slope of the western fringing reef at Pulau Hantu (Fig 2.1). Columnar colonies of the genus *Goniopora* are unusual, because the 'colonies' are actually made of a number of individual columns, with tissue concentrated at the top of the columns (Fig 6.1c). The spatial segregation of tissue on each column from the tissue of other columns means that each column may be biologically 'independent' of

the rest of the colony, indeed the columns may even compete for resources such as light and food. Because of the physical separation of tissue between columns, it is possible that fragmentation (removal) of columns from the parent colony, may have no negative impact on the fecundity or reproductive effort of the columns or the parent colonies.

The aim of this chapter was to carry out a field transplant experiment using fragments of *Goniopora* corals to see how a) fragmentation; and b) changes in water quality and depth affect fecundity and reproductive effort. The project had three main aims:

1) To look at the effect that colony fragmentation has on reproductive effort and fecundity. Does colony fragmentation have any effect on reproduction in a coral colony that has a columnar structure, such as *Goniopora*? i.e. can individual columns be considered as independent colonies.

2) To study the effects on coral reproductive effort and fecundity by transplanting fragments to a more disturbed reef site.

3) To study the effects of reduced irradiance on reproductive effort and fecundity by transplanting fragments to greater depth at the two study sites.

Throughout this chapter, fecundity is used to refer to the average number of gametes per polyp and reproductive effort refers to the average size of gametes.

6.2 Materials and methods

6.2.1 Study sites

Pulau Hantu and Cyrene were chosen as study sites as they differ in their communities of corals, their relative environmental conditions and their respective distances from mainland Singapore (Chapter 2, Fig 2.1). Previous studies have established that sedimentation rates are higher at Cyrene than at other Southern Island reefs, including Pulau Hantu. Low and Chou (1994) found mean sedimentation rates of 14.64 mg cm^{-2} day^{-1} at Cyrene, compared to 9.90 mg cm^{-2} day^{-1} at Pulau Hantu. Furthermore sedimentation rates at Cyrene sometimes exceeded 44 mg cm^{-2} day^{-1} compared to the other sites, which never exceeded 13 mg cm^{-2} day^{-1} (Low and Chou 1994). Similarly, Todd et al. (2004) found that average levels of total suspended solids (TSS) and sedimentation were higher at Cyrene compared to Pulau Hantu, indicating that there is a general pattern of decreasing TSS and sedimentation with increasing distance from mainland Singapore. Chua (1990) found the cover of live coral at 3 m depth at Cyrene to be the lowest of all reefs surveyed in Singapore at that time (including Raffles Lighthouse, Pulau Semakau, Pulau Hantu and Hantu West). Even though some scleractinians still survive at Cyrene reef, relatively few healthy colonies now remain (personal observation) (see Chapter 2 and Fig 2.1).

6.2.2 Environmental measurements

The water quality parameters secchi depth (m), temperature (°C), salinity (ppt), and dissolved oxygen (mg l^{-1}) were measured at Pulau Hantu and Cyrene reef on a twice

monthly basis during the study. Temperature, dissolved oxygen and salinity were measured *in situ* on each sampling occasion with a multi-parameter probe (YSI Instruments Inc.) at approximately 3 m depth. Previous studies around Singapore's southern islands revealed that the water column is well mixed, therefore shallow and deep were not compared and all samples were taken from 3 m depth (Gin et al. 2000). It was assumed therefore that the main factor that differed between the deep and shallow sites was irradiance. Todd et al. (2004) found that irradiance was inversely related to depth at Pulau Hantu and Cyrene, with <0.6% of surface photosynthetically active radiation (PAR) reaching the deepest site, compared to > 20% reaching the shallowest sites.

6.2.3 Selection of parent colonies

Goniopora lent itself well to the proposed study as the 'parent' colonies are very large (2 – 6 m in diameter), so a number of clonal fragments could be extracted easily without causing extensive damage to the parent colony. A search was conducted using SCUBA along the reef crest at Pulau Hantu. Twelve colonies of *Goniopora* spp. were found and tagged using angle iron stakes hammered into the substrate, and aluminium labels (labeled GO1-12). These twelve colonies make up the entire population of large *Goniopora* colonies on the fringing reef on the west coast of Pulau Hantu Besar (Fig 2.1, Chapter 2). All of the colonies were found on the upper reef slope. Colonies were identified to species level by examining features of the living polyps, skeletal characteristics and colony morphology (Veron and Pichon 1982; Veron 2000). Photographs were taken of polyps *in situ* with a Nikonos V and close up kit (Ocean Optics, London), and small skeleton samples were removed using a hammer and

chisel. Coral tissue was removed from skeleton by immersing samples in dilute household bleach (20 - 50%) for 24 to 48 hrs, and drying in air. The twelve colonies consisted of the following species: six *G. columna* (GO1, GO4, GO6, GO8, GO11 and GO12), 3 *G. lobata* (GO2, GO5 & GO9), two *G. fruticosa* (GO7 and GO10) and one colony that could not be reliably identified to species level (GO3).

It has been shown in previous chapters that spawning of many corals around Singapore occurs in March and April, however it was not known whether *Goniopora* species also participate in spawning at this time. To establish which month these colonies spawn, samples were taken at monthly intervals between January 2002 and May 2002. Samples were processed using standard histological techniques (see Chapter 3 for details). Histological slides were examined under the compound microscope (Olympus BH2). Each slide was examined for presence or absence of gonads and the approximate size and maturity of gonads was estimated visually to establish the approximate spawning month. Histological examination revealed that five of the colonies were male (GO2, GO3, GO5, GO8 and GO9) and seven colonies were female (GO1, GO4, GO6, GO7, GO10, GO11 and GO12), with no indication of hermaphroditism in any of the colonies. All twelve colonies contained gametes in February 2002, whereas in mid-March 2002 four colonies were empty (GO5, GO9, GO10 and GO12), indicting that they had spawned. By the end of April 2002, all colonies except one (GO4) were empty. These findings strongly indicate that *Goniopora* also spawns in March and/or April in Singapore. The transplantations were carried out in early February 2002 over a period of one week. All of the fragments and controls were left for one year, and harvesting was done over three

consecutive days in early February 2003, at least one month before the predicted spawning time.

6.2.4 Experimental setup

Two replicate fragments from each parent colony were transplanted to each of four sites. At each site, a sheet of stainless steel wire mesh was attached to an aluminium frame, which was anchored firmly to the substrate using steel angle iron bars. The frame was raised approximately 50 cm above the substrate. Fragments were mounted in durable plastic perforated cups using cable ties and were attached randomly, but evenly spaced on the frame. To avoid damaging the living coral tissue at the tops of the columns, care was taken to wrap cable ties around the bases of the coral columns (Fig 6.1d).

Transplantations were made to a shallow site at Pulau Hantu (PHS), located on the upper reef slope at the same depth as the parent colonies (depth 2.3 m); from the shallow upper reef slope to the deeper reef slope on the same reef at Pulau Hantu (PHD) (depth 8.9 m); between Pulau Hantu (offshore) and Cyrene reef (nearshore) upper reef slope (CYS) (depth 2.2 m); and lower reef slope (CYD) (depth 7.6 m) and finally a set of control fragments (C) were placed a few meters away from each parent colony. These were set up to determine if there was any effect of putting many fragments together on the frames. This resulted in four sites (two at each reef) and five sets of coral fragments in all (including controls). So ten fragments were removed from each parent colony (two fragments to each of five sites).

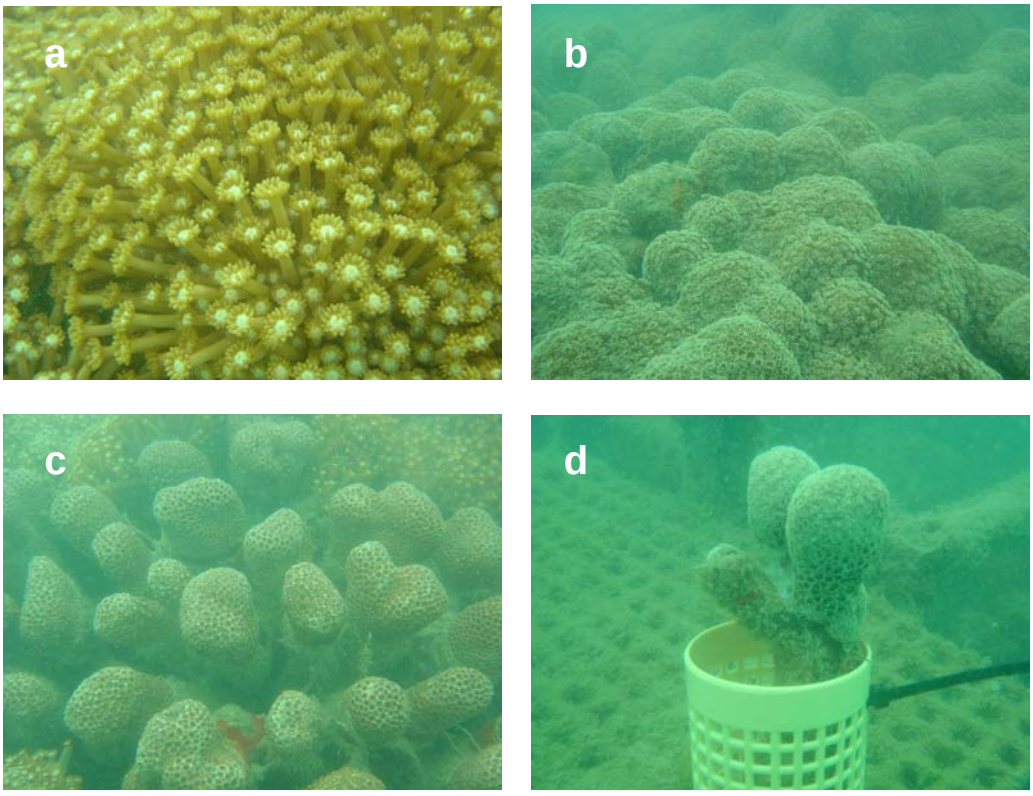


Fig 6.1. a) Polyp detail of *Goniopora* sp., b) a large 'colony' of *Goniopora columna*, c) individual columns are not connected by tissue in these *Goniopora* 'colonies', d) an individual *Goniopora* fragment in its experimental pot, attached to the frame at Pulau Hantu shallow.

Harvesting of the fragments was done by removing the entire fragment (including cup and label) underwater and placing in separate ziploc bags. Pieces were removed from each fragment (approx 3 – 4 cm^2 of coral) and fixed immediately on the boat. At the time of harvesting, two replicate samples were also taken from the parent colonies (P) to compare with controls. So in total, two samples from each of the twelve colonies were harvested from six places (P, C, PHS, CYS, PHD and CYD), making a total of 144 samples. Samples were fixed in 10% seawater-buffered formalin for 24 hrs, rinsed in running tap water, decalcified in a solution of 5 - 10% buffered HCl until only soft tissue remained, placed in 70% ethanol and examined immediately, as once pigments have dissolved it is harder to discern gonad material from surrounding coral

tissue.

6.2.5 Reproductive measurements

Fecundity and reproductive effort of the fragments was examined by dissecting polyps, and measuring and counting gonads directly under a dissecting microscope (Zeiss). This method is considered to be better than examination of histological sections, as gametes from whole polyps are measured rather than thin tissue sections (Ward and Harrison 2000). Ten polyps were randomly selected from each sample and gently dissected using fine forceps and tissue dissecting scissors. The presence or absence of gonads was noted for each polyp, all oocytes were counted from three gravid polyps and ten randomly selected oocytes were measured. Unfortunately, examination of fragments from the male colonies revealed that testes were too small to be reliably measured in this way, so only the female colonies were examined and used in subsequent analysis. The maximum diameter and a second measurement perpendicular to that were taken to calculate the oocyte size. Oocyte size was calculated as the mean of the two measurements (Ward and Harrison 2000). The diameter of five polyps from each sample was also measured under the dissecting microscope to see if transplantation had affected the size of the polyps.

6.2.6 Data analysis

Differences in environmental parameters between the two sites was tested using *t*-tests after testing for equality of variances (Levene's test, $p<0.05$, SPSS 9.0). Wilcoxon's signed ranks test was used to test for differences in number of oocytes per

polyp (fecundity); oocyte size (reproductive effort); and polyp diameter between clonal fragments. Tests were conducted between a) parent colonies and control fragments (P - C) (to examine the effects of fragmentation); b) control fragments and Pulau Hantu shallow fragments (C - PHS), (to examine the effects of transferring to the frames); c) Pulau Hantu shallow fragments and Cyrene shallow fragments (PHS - CYS) (effects of transplanting to a more disturbed site). No test was conducted to compare the shallow and deep frames because mortality of the deep fragments was very high (see Results, Fig 6.2a). A matched pairs test was used because fragments at the different sites were not independent (i.e. they were clones of each other) and the sample sizes was relatively small (n = 7). Wilcoxon's matched pairs test only requires a sample size of 6, and makes few assumptions about the shape of the data (Dytham 1999).

6.3 Results

6.3.1 Environmental differences between sites

Only secchi depth was significantly different between Pulau Hantu (2.15 m ± 0.99 SD) and Cyrene (1.56 m ± 0.74 SE) ($t = 2.058$, $p<0.05$) (Table 6.1). Dissolved oxygen was higher at Cyrene (6.29 mg l^{-1} ± 0.09 SE) compared to Pulau Hantu (6.09 mg l^{-1} ± 0.07 SE), probably because this site is more exposed and experiences stronger wave action than Pulau Hantu (Table 6.1). There was no difference in sea temperature or salinity between sites (Table 6.1).

6.3.2 Reproduction

Testes in the male colonies were too small for reliable measurements to be made, so only female colonies were measured and analysed. This meant that only 7 of the 12 colonies samples were analysed (GO1, GO4, GO6, GO7, GO10, GO11 & GO12). Most of the parent colonies contained large oocytes indicating that colonies were close to spawning. Samples from one parent colony (GO1) were completely empty, indicating that this colony had already spawned. However, all of the transplanted fragments from GO1 still contained large mature oocytes, indicating that the transplanted fragments did not spawn at the same time as the parent colony. Therefore, for the analysis between parent colonies and controls GO1 was excluded, however as transplanted fragments had not spawned GO1 was included in all other analyses (i.e. comparisons between transplanted fragments). Only a few relatively small oocytes were found in the parent colony and the transplants of GO10, but it is not clear if this colony had already spawned, leaving behind oocytes that would subsequently be re-absorbed (Harrison and Wallace 1990), or whether development was at an earlier stage in this colony. Nonetheless, because of the uncertainty regarding the reproductive state of colony GO10, it was excluded from subsequent statistical analysis.

6.3.3 Mortality

Mortality among the female fragments was highest at the deep sites, where only 28.5% (n = 14) of the fragments survived at both sites. At the shallow sites, mortality only occurred at Cyrene, where one of the fragments died during the experiment

(GO1, 7 %, n = 14). No mortality was recorded at Pulau Hantu shallow. As mortality was extremely high at the deep sites, no attempt was made to include the deep sites in the statistical analyses (Fig 6.2a).

Table 6.1. Results of t-tests comparing environmental parameters between Pulau Hantu and Cyrene. Values are means ± 1 SD. * = p <0.05.

	Site		*T*-test		
	P. Hantu	Cyrene	Statistic	df	P
Temperature (°C)	29.5 ± 0.85	29.35 ± 0.94	0.412	24	0.684
Salinity (ppt)	30.24 ± 0.98	30.20 ± 1.03	0.131	36	0.896
DO (mg l^{-1})	6.09 ± 0.27	6.29 ± 0.28	1.794	23	0.086
Secchi depth (m)	2.15 ± 0.99	1.56 ± 0.74	2.058	35	0.047*

Table 6.2. Results of Wilcoxon's matched pairs tests between sites for mean oocyte numbers per polyp. P = parent colonies, C = controls, PHS = Pulau Hantu shallow, CYS = Cyrene shallow. * = p <0.05.

	P	C	PHS	CYS
Means ±1SE	29.28 ± 17.62	27.31 ± 19.45	30.33 ± 22.61	20.94 ± 12.10
	Between sites			
	P - C	C - PHS	PHS - CYS	
N	5	6	6	
Test statistic (*W*)	-	0.734	1.992	
P	-	0.463	0.046*	

Table 6.3. Results of Wilcoxon's matched pairs test between sites for mean oocyte diameter (μm). * = p <0.05.

	P	C	PHS	CYS
Means ±1SE	335.49 ± 29.49	325.68 ± 36.32	333.68 ± 41.82	252.85 ± 44.00
	Between sites			
	P - C	C - PHS	PHS – CYS	
N	5	6	6	
Test statistic (*W*)	-	0.105	2.201	
P	-	0.917	0.028*	

Table 6.4. Results of Wilcoxon's matched pairs test between sites for mean polyp diameter (mm). * = p <0.05.

	P	C	PHS	CYS
Means ±1SE	2.60 ± 0.15	2.63 ± 0.29	2.66 ± 0.27	2.00 ± 0.28
	Between sites			
	P - C	C - PHS	PHS – CYS	
N	7	7	7	
Test statistic (*W*)	0.169	0.676	2.371	
P	0.866	0.499	0.018*	

6.3.4 Reproductive effort and fecundity

At the two deep sites - of the fragments that survived - the majority of polyps were empty. At PHD only 10%, and at CYD only 15% of polyps contained visible gonads (n = 10) (Fig 6.1.b). At the shallow sites, the proportions of polyps containing gonads were similar and ranged from 75.38% at CYS to 86.43% at PHS (n = 10). The three shallow 'sites' at Pulau Hantu (P, C & PHS) had similar proportions and the fragments at Cyrene had a slightly lower proportion of polyps containing oocytes (Fig 6.2b) The proportion of fecund polyps was slightly lower among the samples from the parent colonies (P) because GO1 (the colony that had spawned) was included (Fig 6.2b).

Average number of oocytes per polyp ranged from 20.94 (± 12.10 SE) at CYS to 30.33 (± 22.61 SE) at PHS (Table 6.2, Fig 6.3a) and oocyte size ranged from 252.85µm (± 44.00 SE) at CYS to 335.49µm (± 29.49 SE) in the samples from the parent colonies (Table 6.3, Fig 6.b). It was not possible to test statistically the difference between the parent colonies and controls because only five parent colonies were included in the analyses (Wilcoxon's matched pairs test requires a minimum sample size of six) (Dytham 1999). Average oocyte size and number of oocytes per

polyp was slightly less in the controls than in the parent colonies. There was no significant difference in oocyte numbers or oocyte size between controls (C) or transplants to Pulau Hantu shallow (PHS) indicating that moving the fragments away from the parent colonies had no effect on the health of fragments (Table 6.2 and 6.3). In all of the fragments except one (GO7) transplanted to Cyrene, fecundity and oocyte size was slightly lower than those at Pulau Hantu shallow. Fragments at CYS had significantly less oocytes and significantly smaller oocytes on average (Table 6.2 and 6.3) than those at PHS. The average number of oocytes and oocyte size was very different between shallow and deep sites, with deeper sites containing far, fewer and smaller oocytes (Fig 6.3 and 6.4). Polyp diameters ranged from 2.6mm (± 0.07 SE) to 2.62mm (0.14 SE) among the PHS transplants and the parent colonies. Mean polyp diameter at CYS was 2mm (± 0.142 SE) and was significantly smaller than those at PHS. Polyps at PHD and CYD were smallest at 1.78mm (± 0.21 SE) and 1.82mm (± 0.12 SE) respectively.

6.4 Discussion

Although fragmentation is an important asexual reproductive mechanism in corals (Highsmith 1982), it can lead to reductions in fecundity, and in some cases mortality (Zakai et al. 2000). In a study of fragmentation in the brooding coral *Pocillopora damicornis*, it was found that most fragments removed from colonies died within 18 days, and released few planulae. Furthermore, release of planulae was significantly lower in damaged versus undamaged colonies (Zakai et al. 2000). Due to the columnar structure of the *Goniopora* colonies in this study, fragmentation had little or no effect on fecundity or reproductive effort of the fragments, and did not appear to

negatively affect the parent colonies. Removal of fragments from the parent colonies may have reduced competition with neighbouring fragments for light and food. However, mean oocyte numbers and oocyte size were only slightly higher among fragments that were transplanted to the frame at PHS, indicating that there was no significant energetic benefit gained by being separated from neighbouring columns (Table 6.2, Fig 6.3).

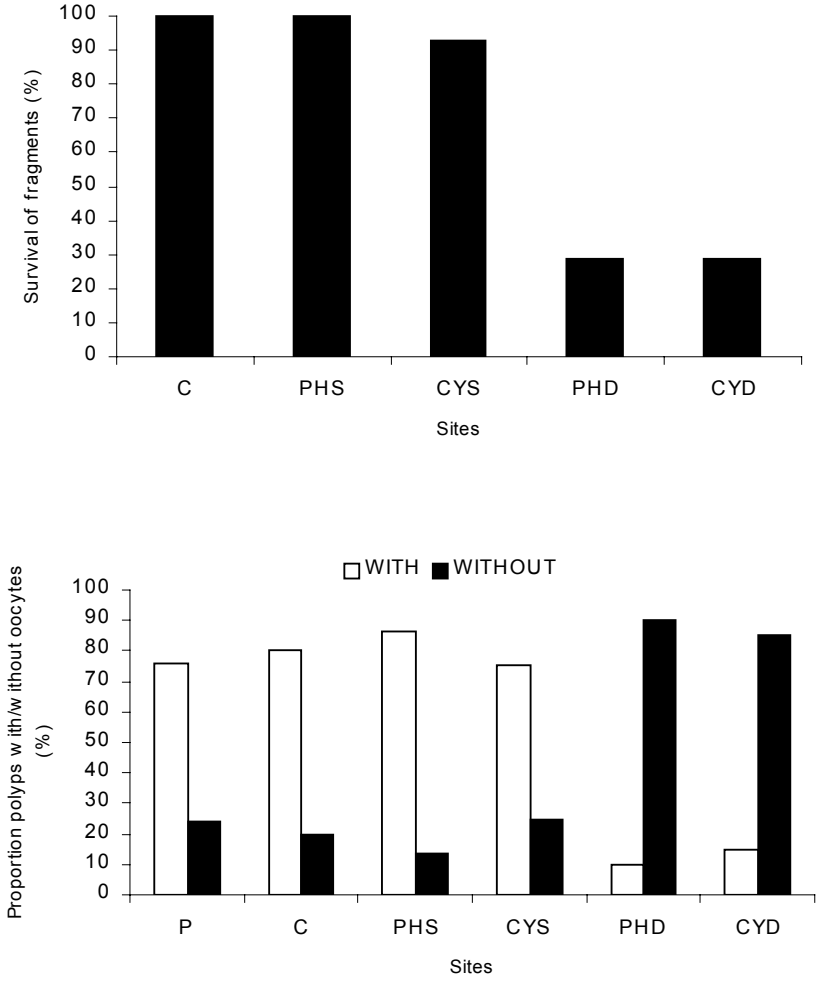


Fig 6.2 a) Proportion of fragments that had survived (%) at the six sites after approximately 12 months after transplantation, and b) the proportion of polyps from each sample that contained oocytes compared to those that did not (n = 10) at the six sites. P = parent colonies, C = controls, PHS = Pulau Hantu shallow, CYS = Cyrene shallow, PhD = Pulau Hantu deep, CYD = Cyrene deep.

In most coral colonies, polyps are connected by living tissue, and there is the possibility of transfer of resources throughout the colony. In these *Goniopora* 'colonies' it seems unlikely that resources can be transferred between columns, as they are not physically connected by living tissue (which is one of the defining features of a true colony). In reality, *Goniopora* 'colonies' consist of many individual colonies connected only by skeleton, but not by living coral tissue. This colony structure may have a number of advantages. For example, the advantage gained by individual columns being aggregated may be to provide stability, because solitary corals or fragments can easily be dislodged, broken off and turned over by strong waves. Asexual reproduction by fragmentation may occur readily with little expense to the parent colony in terms of colony function. Lastly, 'colonies' may be able to withstand partial mortality as a result of disease, as pathogens infecting one part of the colony would not be able to spread to other parts of the colony via contiguous tissue.

In the case of one colony (GO1), the 'parent' colony had apparently spawned, however all of the transplanted fragments (including controls) still contained large mature oocytes. Interestingly, this colony was the closest of all the colonies to the frame at PHS, and the control fragments were placed not more than 1m from the parent colony. This finding indicates that being part of the colony may be important for spawning synchronicity within colonies. Chemical signals are known to be important in triggering spawning at the colony level (Coll et al. 1990), and it is possible that spawning is synchronized within a colony because release of hormones or gametes from some columns induces spawning in other columns.

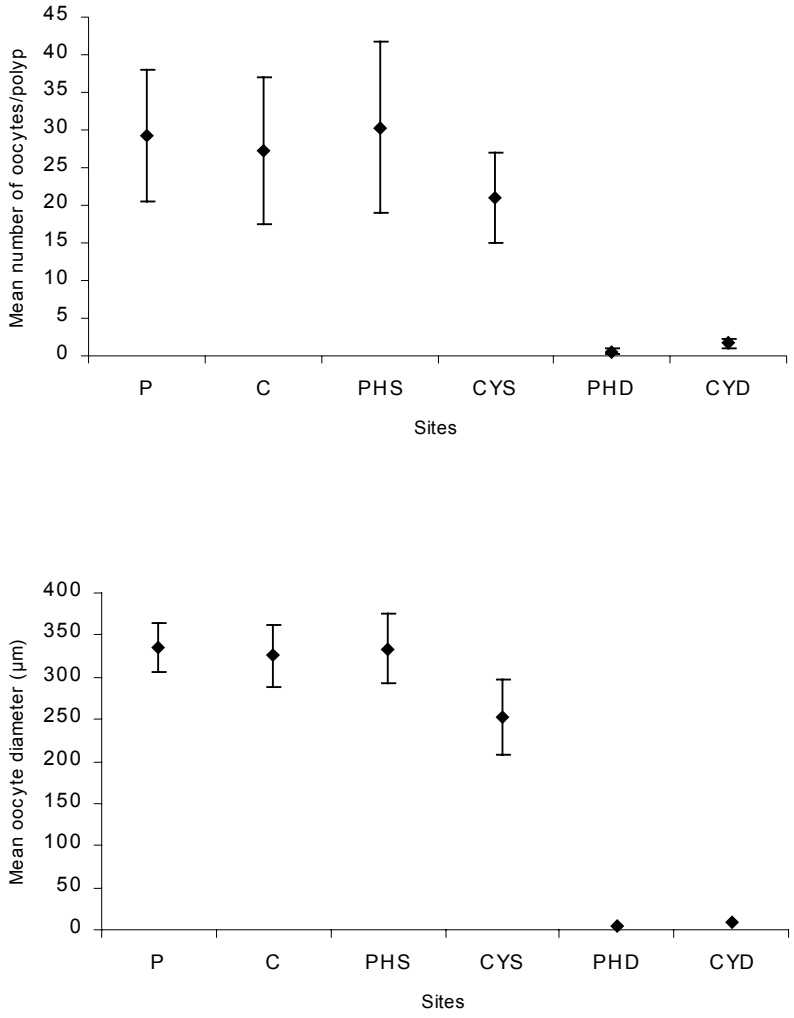


Fig. 6.3 a) Average numbers of oocytes per polyp, and b) average oocyte diameter (µm) from the six sites, P = parent colonies, C = controls, PHS = Pulau Hantu shallow, CYS = Cyrene shallow, PHD = Pulau Hantu deep, CYD = Cyrene deep. Error bars are 1 SE of the mean.

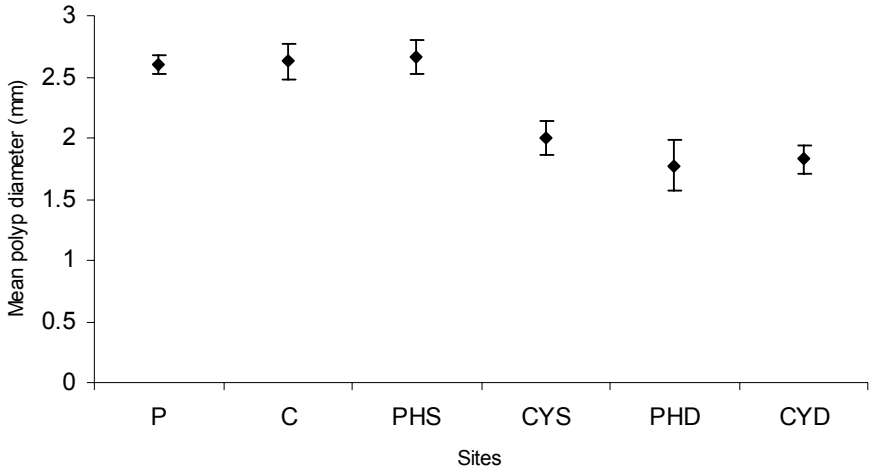


Fig 6.4 Average polyp diameters (mm) of fragments transplanted to 5 sites compared to parent colonies (P). Error bars are 1 SE of mean. C = controls, PHS = P. Hantu shallow frames, CYS = Cyrene shallow frames, PHD = Pulau Hantu deep frames, CYD = Cyrene deep frames.

It was not possible to examine and measure the testes of the male colonies reliably under the dissecting microscope. This could be done by histological examination and subsequent examination of samples of the male colonies, however there was not sufficient time to do this during the project. It would be of interest to do this at a later stage to see if the male colonies responded in a similar way to the females. Furthermore, comparing the histological features of the coral tissue and the state of the gonads from the fragments at Pulau Hantu and Cyrene would be of interest to examine the cytological effects from the stress of being transplanted to a more disturbed site and a deeper (darker) site.

Many reef rehabilitation projects involve fragmentation and transplantation of coral fragments (Clark and Edwards 1995; Yap 2003). However, little is known about how fragmentation affects fecundity of either the fragments or the donor colonies (Yap et

al. 1998; Zakai et al. 2000). The results of the present study indicate that these species may be useful in reef rehabilitation projects that involve transplantation of coral fragments, as the health of fragments and donor colonies did not appear to be affected, when fragments were transplanted to a site with suitable conditions. Furthermore, the attractiveness of these species may mean that they would quickly restore aesthetic value to small areas of damaged reef. Although fecundity was not affected by fragmentation and transplantation, it is not clear whether transplanted fragments spawned simultaneously with the parent colonies. This factor is an important consideration, if the aim of the project is to create coral reefs with reproductively viable populations of corals.

Fragments transplanted to Cyrene (a more disturbed site) had on average significantly fewer oocytes, smaller oocytes and smaller polyps after one year (Table 6.2). The observed reductions in fecundity and polyp size at Cyrene could have been due to a combination of factors including: reduced irradiance due to increased turbidity (so less energy from photosynthesis), increased energy needed for removal of sediment, or other factors related to the elevated levels of suspended sediments at the more disturbed site (Kojis and Quinn 1984; Tomascik and Sander 1987; Rogers 1990; Ward and Harrison 2000). A number of previous studies have found reductions in fecundity and reproductive effort in corals exposed to reduced water quality. Kojis and Quinn (1984) found a negative correlation between fecundity of *A. palifera* and 3 factors: depth, turbidity and sedimentation rate in Papua New Guinea. Similarly, Tomascik and Sander (1987) found that the gonad index of *Porites porites* was significantly lower at a site that experienced eutrophication, compared to unaffected sites in Barbados. In this study, one factor that was not quantitatively measured was the

abundance of macro-algae at the two sites. During many of the monthly checks, the fragments at Cyrene reef had become overgrown by macro-algae such as *Enteromorpha* sp. and *Sargassum* sp., whereas no macro-algal overgrowth was found on the frames at Pulau Hantu. It is not clear why macro-algae flourishes at Cyrene, but it may be due to: increased nutrients; a lack of herbivores; a lack of competition for space from hard corals; or a combination of all of these factors (Goh and Chou 1992). Hence, the reductions in average oocyte size, oocyte numbers per polyp and polyp diameter at Cyrene may have been due to competition with algae for light. Increased growth of macro-algae is often associated with increased nutrients (which were not measured during this study). During the ENCORE experiment on the Great Barrier Reef, (Ward and Harrison 2000) found that colonies of *Acropora aspera* and *A. longicyathus* produced significantly smaller and fewer eggs and contained less testes material when exposed to elevated nitrogen. Cox and Ward (2002) found that planulation ceased in colonies of *Pocillopora damicornis* following four months of exposure to ammonium. Previous studies have measured nutrients levels at sites around the Southern Islands and found no significant differences between offshore and nearshore sites (Gin et al. 2000), however nutrients may vary a great deal from day to day because of rainfall events and river run-off, meaning that irregular point samples may not be sufficient to show differences between sites. It is assumed that the major factors that have caused mortality of corals around Singapore's coast are sedimentation and turbidity (Low and Chou 1994). While these factors are indeed causes of mortality in corals (Rogers 1990), other important factors, particularly in nearshore reefs such as Cyrene, may be competition with macro-algae (which may flourish as a result of increased nutrients, lack of competition for space by hard corals and/or lack of herbivorous grazing) (Goh and Chou 1992) and/or exposure to elevated

nutrients. Fragments transplanted to depth suffered high levels of mortality following bleaching, presumably as a result of reduced light levels during episodes of high turbidity. In the fragments that survived, eggs were much smaller and fewer than in shallow fragments. It seems likely that the reduced reproductive output occurred because fragments suffered severe stress as a result of dark-induced bleaching, and such a response has been found in other studies (Michelak-Wagner and Willis 2001; Baird and Marshall 2002). The deep fragments had survived well until episodes of high turbidity occurred approximately three to four months after the study began. This made it difficult to assess the role of reduced irradiance, as corals probably suffered more as a result of severe stress. Although the depth of the fragments (7 – 9 m) is considered normal for many coral reefs, it may have been too deep for Singapore's reefs that experience such high turbidity levels and low light penetration. These findings support the conclusion that the depth limit of corals in Singapore is determined by light as a result of high levels of turbidity (Chou et al. 1994).

Chapter 7

Conclusions

7.1 Reproductive patterns of Singapore's scleractinian corals

Despite Singapore's proximity to the equator, the patterns of sea surface temperature, salinity, and total monthly rainfall are markedly seasonal around Singapore's southern islands. Monitoring of the gametogenic cycles of two scleractinian species (*Porites lutea* and a morphospecies of *Platygyra*) over 14 months between March 2001 and April 2002, revealed marked reproductive seasonality and within-species synchrony (in terms of gamete maturation). Both species exhibited a very similar seasonal pattern, indicating that these unrelated species have responded similarly to exogenous cues to synchronise spawning within species. Gamete maturation coincided with the annual rise in sea surface temperatures following the NE monsoon, indicating that this factor may play a role in the seasonal timing of spawning in Singapore. Sampling of an assemblage of *Acropora* over 14 months between March 2002 and May 2003 revealed a high proportion of species containing mature gametes in the same months (March/April), although within species the proportions of colonies that contained mature gametes varied considerably. These data indicated that many broadcast spawning scleractinian corals reproduce during the first inter-monsoon (predominantly in April) in Singapore. However, some colonies probably spawn in other months including March and May, and some colonies have a second, smaller peak in reproductive activity between September and November, although no corals were observed to spawn during field trips carried out in October and November 2001. Coral spawning was documented *in situ* during discreet periods, following the full

moons of March 2002 and March and April 2003. At least 24 species were observed to spawn *in situ* and up to 12 species spawned on the same night on one reef. The presence of mature (pigmented) gametes in fractured polyps prior to the spawning periods, indicate that as many as 50 species may participate in these spawning periods. Most spawning was observed three to six nights after the full moon, one to two hours after dusk, at low tide, when the amount tidal mixing was minimal. These findings indicate that temperature may be a seasonal cue for gamete maturation, while lunar cycles and the onset of darkness are the most likely cues for the nights of spawning.

Clearly, marked reproductive seasonality and multi-species spawning of broadcasting corals is not just a characteristic of reefs at higher latitudes. Even in an equatorial location such as Singapore, there is probably sufficient annual variation in sea surface temperatures, and other environmental factors, to act as cues for the timing of reproduction in broadcast spawning marine invertebrates. Environmental conditions are suitable for reproduction of scleractinians throughout the year in Singapore, thus it seems unlikely that the timing of reproduction is constrained by any factor, although it is possible that evolutionary selective pressures have caused broadcasting to occur at a time when conditions are optimal for fertilisation, larval survival, settlement or recruitment. I suggest that the findings of this study lend support to the hypothesis, that multi-species spawning of broadcasting corals evolved because congeneric species respond similarly, but independently, to environmental signals to synchronise spawning within species, as this maximises fertilisation success.

7.2 Experimental fragmentation of *Goniopora* corals

Experimental transplantation of fragments of two species of *Goniopora* revealed that fragmentation and transplantation had no effect on fecundity and reproductive effort, although it may have affected some of the fragment's ability to spawn in synchrony with parent colonies. However, fragments transplanted to a more disturbed site closer to mainland Singapore and to greater depth had, on average, fewer and smaller eggs. Fragments transplanted to greater depth suffered high levels of mortality following bleaching, presumably as a result of reduced light levels during episodes of high turbidity. The more disturbed site (Cyrene) had significantly higher turbidity levels than the donor site (Pulau Hantu) during the study period, and previous studies have shown significantly higher sedimentation rates at Cyrene compared to Pulau Hantu. Elevated turbidity and sedimentation at the more disturbed site may have reduced the amount of energy available to corals for reproduction. While these factors are indeed causes of stress and mortality in corals, other important factors may have been competition with macro-algae (which flourishes at Cyrene) or exposure to elevated nutrients.

7.3 Relevance and limitations of this study

When I started this project, the scope was very broad and I could have worked on almost any aspect of reproduction in corals. However, I realised quickly that I was in a unique position. While reproductive patterns of corals in certain regions (e.g. the Great Barrier Reef and the Caribbean) have received a great deal of study for the past two decades, almost nothing was know about the patterns of reproduction of

Southeast Asia's reef corals, or for corals on equatorial reefs. This is remarkable when one considers that Southeast Asia is home to some of the most diverse and most threatened coral reefs in the world. Furthermore, for almost two decades a dogma has remained entrenched in the literature that equatorial reef corals exhibit little reproductive seasonality and spawning synchrony. My primary aim therefore was to improve understanding of the timing and patterns of spawning of reef corals in Southeast Asia; to examine what drives coral reproduction on equatorial reefs, in the absence of large seasonal environmental fluctuations; and to shed further light on the probable causes of multi-species spawning among assemblages of broadcasting scleractinian corals.

Observation of concurrence of reproduction with a seasonal change in environmental factors is not sufficient to imply cause. Therefore, it would have been good to follow the studies described in chapters 3, 4 and 5 with a series of manipulative experiments to examine the causal relationship between environmental factors (e.g. sea surface temperatures) and reproduction. Unfortunately, a suitable aquarium facility was not available to conduct such studies, which is why I shifted my focus in the final chapter and conducted a manipulative field experiment to examine the effects of fragmentation and transplantation on fecundity and reproductive effort. It is becoming increasingly common to attempt to restore damaged reefs by transplanting fragments of coral colonies. However, little is known about how fragmentation and transplantation affects reproductive effort and spawning of the fragments, or the donor colonies. There were a number of aspects of this experiment that were not completely satisfactory, for example the inability to reliably measure testes in the male fragments (which reduced the overall sample size) and the high mortality among the deep

fragments, making it impossible to satisfactorily examine the effect of reduced irradiance alone, on fecundity and reproductive effort. Nonetheless, the experiment was successful in establishing that *Goniopora* colonies could be fragmented with little or no negative impact on gametogenesis (although it is possible that spawning may be delayed), demonstrating that this genus may be useful in experiments that require many clonal fragments, and as a potential candidate for coral transplantation and reef restoration projects.

7.4 Future directions for research in coral reproduction

1. *Continued research to compare geographical and regional patterns of the timing of spawning in relation to seasonal environmental patterns.* Previously, these studies have been hindered by of the inaccessibility of many sites and an insistence on traditional, labour intensive methods for studying timing of reproduction (see Appendix 3 for further discussion). The approach used for sampling *Acropora* in this project was reliable for estimating timing of spawning, and was considerably less time consuming than the more traditional technique of histological processing.

2. *Manipulative experiments to examine the effects of seasonal environmental factors on reproductive timing.* These require suitable outdoor, flow-through aquaria or *in situ* experimental set ups, where environmental conditions such as irradiance and water temperature can be reliably controlled. These experiments should be done in locations where the natural reproductive patterns are well established, and where there is an abundance of appropriate species.

3. Further studies into the physiological and molecular basis for a causal relationship between environmental factors and reproduction in corals. Studies should look at which genes are transcribed in association with gametocyte proliferation, and how subsequent gamete development is regulated by endocrine systems within corals (Olive 1995).

4. *Further studies to identify coral species that lend themselves well to fragmentation and transplantation, combined with studies into the effects of transplantation on reproductive success.* Reproductive strategies, fecundity and other life history characteristics should be key considerations when choosing species or colonies for coral transplantation and reef restoration, if the aim is to produce populations and communities of corals that will become reproductively viable.

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Appendix 1

Study sites

Fig A1.1 a) Pulau Hantu at low tide showing the channel between Pulau Hantu Kechil and Pulau Hantu Besar, the Shell oil refinery in the background is on neighbouring Pulau Bukom; b) the fringing reef and rock bunds on the west side of Pulau Hantu Besar where the field sampling and experimental work was conducted; c) low tide at Raffles Lighthouse; d) the location of the fringing reef on the west side of Raffles Lighthouse where all collections and spawning observations were conducted; e) the navigational beacon at Cyrene reef showing mainland Singapore in the background; f) Sisters island west where sample collection was carried out.

Appendix 2

Gametogenic cycles

A2.1 Field collections

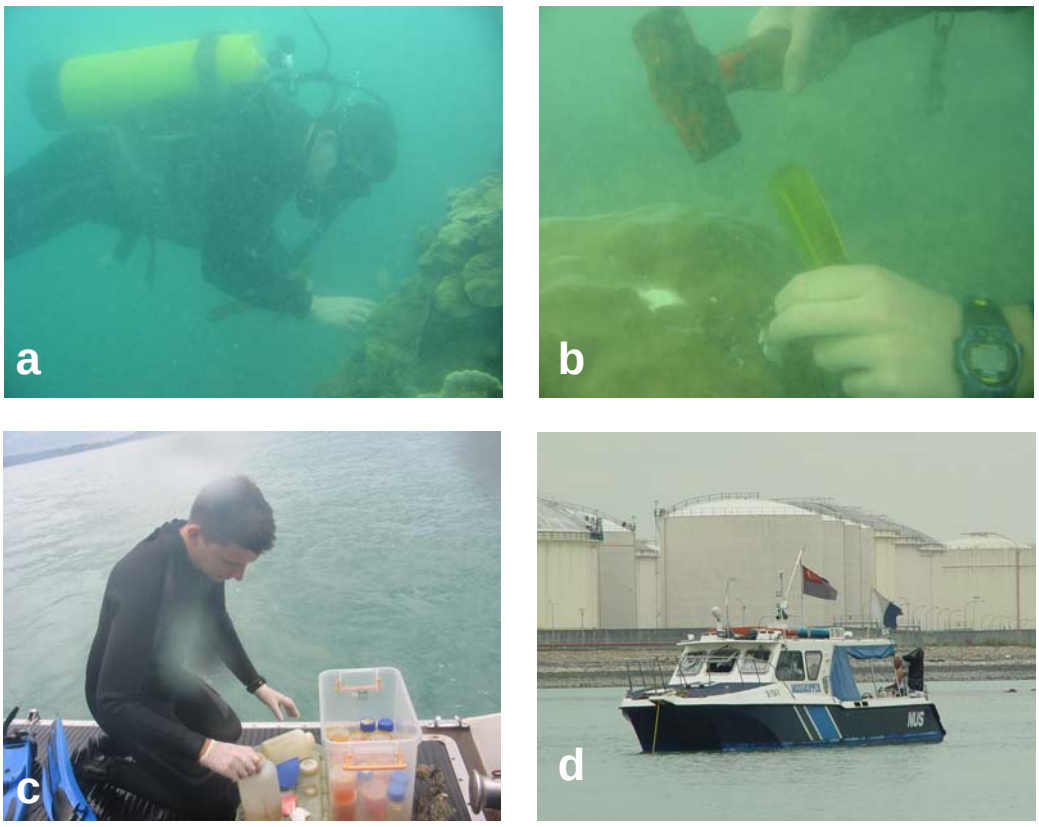


Fig A2.1 a) Collecting coral samples on the reef at Sisters Island, note the turbid water conditions, horizontal visibility ranged between 0.5 m and 5m; b) coral samples were removed from colonies with hammer and chisel; c) samples were fixed immediately on the back deck of the boat; d) 'The Mudskipper', the boat used for most of the field work.

A2.2 Comparison of *Platygyra* morphospecies

Many of the sample collections were conducted in poor visibility (i.e. <1m), and occasionally strong currents. As a result, it was sometimes difficult to distinguish between very similar morphospecies at the time of collection. Post-collection, samples were re-examined and divided into two possible morphospecies (*a* and *b*) based on valley width and wall width (measured with Vernier calipers). Morphospecies *a* had

valleys <4 mm and walls <2 mm. Morphospecies *b* had valleys >4 mm and walls ≥2 mm. The gametogenic cycles of the two possible morphospecies were compared to see if the separation was justified, at least in terms of reproductive timing. Monthly mean oocyte diameter and oocyte numbers were significantly correlated between the two morphospecies of *Platygyra* ($\gamma = 0.92$, $p < 0.001$, $\gamma = 0.89$, $p < 0.001$, $n = 13$)(Table A2.1), indicating that their annual reproductive patterns were indistinguishable (Fig A2.1, A2.2). It was assumed therefore that the data for the *Platygyra* species could be pooled for all subsequent analysis. Considering that *Platygyra* species are highly morphologically variable (Veron et al. 1977; Miller and Babcock 1997; Miller and Benzie 1997; Veron 2000), it is not clear whether the distinction that I made between *a* and *b* was real, or a result of intra-specific morphological plasticity. Miller and Benzie (1997) found that although seven *Platygyra* morphospecies spawned simultaneously on the Great Barrier Reef, and that cross fertilisation was successful, mating was not completely random according to Hardy-Weinberg equilibria, suggesting that reproductive barriers may exist at finer temporal scales. Nonetheless, the pooling of the morphospecies in this study is still valid, because the seasonal patterns were statistically indistinguishable.

Table A2.1 Correlations (Kendall's Tau, SPSS v9) between two *Platygyra* morphospecies monthly mean oocyte geometric diameter (μm) and monthly mean oocyte numbers (0.25 cm^{-2})

Platygyra morphospecies	df	Test statistic (γ)	P
Oocyte size	13	0.92	0.001
Oocyte numbers	13	0.89	0.001

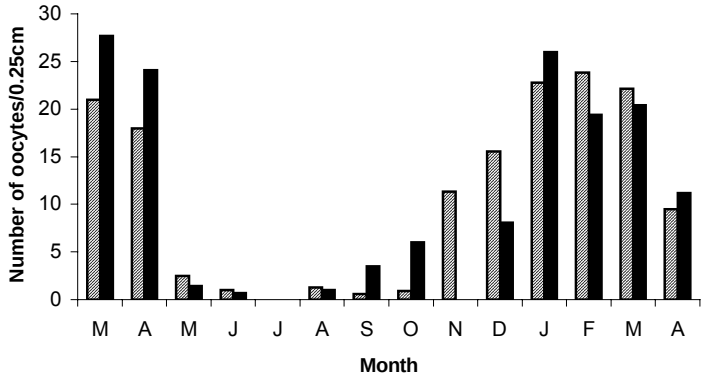


Fig A2.2. Comparison of two morphospecies of *Platygyra* showing number of oocytes/0.25cm^2 tissue, diagonal stripes = *Platygyra* sp. *a*, and black = *Platygyra* sp. *b*. No colonies of *Platygyra* sp. *b* were found in Nov 2001 sample.

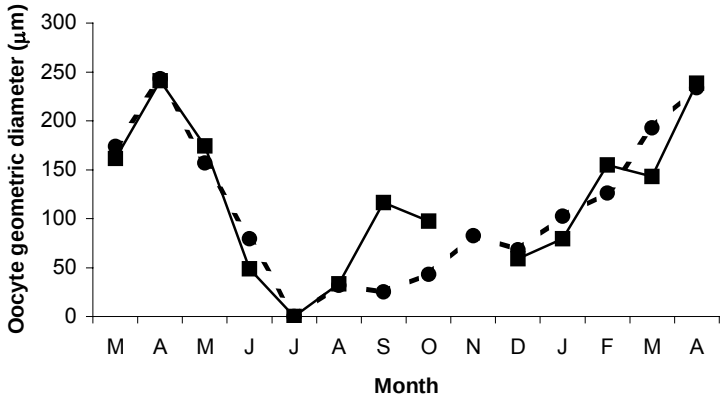


Fig A2.3 Comparison of two morphospecies of *Platygyra*, showing mean monthly oocyte diameter (µm), broken line = *Platygyra* sp. a, solid line = *Platygyra* sp. b. No colonies of *Platygyra* sp. *b* were found in the Nov 2001 sample.

Appendix 3

Acropora seasonality

A3.1 Publications

Reproductive seasonality in an equatorial assemblage of scleractinian corals

Guest JR, Baird AH, Goh BPL and Chou LM. *Coral Reefs* (in press)

Accepted: 31st January 2004

Key words: Reproductive seasonality, *Acropora*, Synchronous spawning, Singapore, Equatorial, Coral Reef

Introduction

Multi-specific, synchronous spawning of scleractinian corals was first documented on Australia's Great Barrier Reef (GBR) in the early 1980's (Harrison et al. 1984). There, over a period of 8 nights in late spring, at least 133 coral species release their gametes for external fertilisation and more than 30 species spawn on the same night on one reef (Willis et al. 1985; Babcock et al. 1986). However, the causal factors responsible for this remarkable phenomenon are still not clearly understood (see review in Harrison and Wallace 1990). Comparisons of reproductive patterns - from sites at a variety of latitudes, with contrasting seasonal and environmental

conditions - can help to elucidate the "ultimate" causes of reproductive seasonality and synchrony (Oliver et al. 1988). Early examples of such comparisons showed that multi-species reproductive synchrony is not a characteristic of all coral communities (Richmond and Hunter 1990). In particular, studies in parts of the Red Sea and the Caribbean found that corals at those sites tended to spawn asynchronously (Shlesinger and Loya 1984; Szmant 1986). This lack of synchrony was attributed to a reduction in environmental seasonality and a narrowing in the ranges of certain environmental parameters, in particular annual sea surface temperatures (Richmond and Hunter 1990), and tidal amplitudes (the difference between mean low water springs and mean high water springs) (Oliver et al 1988).

In equatorial regions where sea temperature range and tidal amplitude are often small, it was predicted that reproductive seasonality and synchrony between species would be reduced (Richmond and Hunter 1990). Indeed, the findings of some studies seemed to lend support to this hypothesis. For example, Oliver et al. (1988) reported a reduction in spawning synchrony in three scleractinian species studied at five locations along a latitudinal gradient ranging from the southern GBR (Heron Island, 23º S) to the northern coast of Papua New Guinea (PNG) (Madang, 5º S). However, more recent studies have found that multi-specific reproductive synchrony does occur in some coral communities, which previously had been thought to be asynchronous. In the Caribbean (Gulf of Mexico), at least seven scleractinian species have been documented spawning synchronously between the 7^{th} and 10^{th} nights following the full moons in August and September (Hagman et al. 1998). In the Solomon Islands (8º N) where there is little fluctuation in annual temperature or tidal amplitude, Baird et al. (2001) found that 28 of 41 *Acropora* species contained mature eggs in the week prior to the full moon in November 1999. In the Karimunjawa

Islands (central Java Sea) where sea temperature ranges between 27.5 ºC and 29 ºC, Edinger et al. (in Tomascik et al. 1997) observed 22 scleractinian species spawning over 3 nights following the full moon in October 1995.

Remarkably, virtually nothing is known of the timing of coral reproduction in Southeast Asia, a region that contains more than 30% of the world's reef area and is home to 600 of the almost 800 scleractinian species (Burke et al. 2002). This lack of basic information is particularly worrying as an estimated 88% of Southeast Asia's reefs are threatened by human activities (Burke et al. 2002). Singapore is a small, heavily populated island nation situated at the southern most tip of Peninsula Malaysia, approximately 137 km north of the equator (Fig. 1a). In spite of Singapore's proximity to the equator, multi-specific synchronous coral spawning was documented in 2002 occurring after the March full moon (Guest et al. 2002). This study began a week prior to the spawning, on an assemblage of *Acropora* on the fringing reef at one of Singapore's southern islands. The aim of this study was to determine what time of the year the *Acropora* spawn in Singapore, and to examine the extent of reproductive seasonality and synchrony both among and within species.

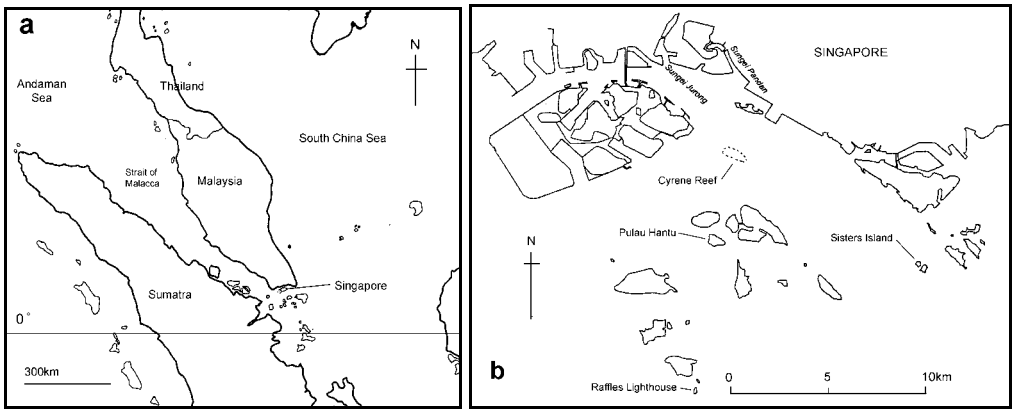


Fig.1. a) Map showing location of Singapore in South East Asia and proximity to the equator; and b) map of Singapore's southern islands in relation to the main island, showing the study site, Raffles Lighthouse.

Methods and materials

Sampling of *Acropora* species was carried out on the fringing reef on the west side of Raffles Lighthouse (Pulau Satumu, 1˚ 10'N, 103˚ 45'E) monthly, from March 2002 until May 2003 (except in June and August 2002 when no sampling was done) (Fig. 1b). Sampling trips were carried out each month between 1 and 8 days before the full moon. The sampling protocol involved a scuba diver making two surveys by swimming parallel to the reef for a distance of approximately 200m, once along the reef flat and once along the top of the reef slope (1 – 5m depth, approx area 20 x 200m). Any *Acropora* colony encountered during the survey was sampled, except for small (i.e. <20cm in diameter), sexually immature individuals. Between 72 and 113 colonies were sampled on each occasion (Fig. 2). Due to the relatively low abundance of *Acropora* on Singapore's reefs, it was inevitable that some colonies were sampled more than once throughout the 14 months, however the searching pattern meant that no colony was sampled more than once on each occasion. Identification to species level was only made in the months of March 2002 and March, April and May 2003. The reproductive state of *Acropora* species can be gauged easily by breaking off a branch below the expected sterile zone (Wallace 1985) and noting the presence or absence of eggs. Mature eggs in *Acropora* are pigmented (usually red, pink or orange in colour) and large enough to be visible to the naked eye (i.e. 600 – 700 μm diameter, Wallace 1985; and see Guest et al 2002). The available evidence indicates that colonies that contain visible, pigmented eggs are likely to spawn on or shortly after the subsequent full moon; colonies with eggs that are visible but un-pigmented (white) are likely to spawn within 1 to 3 months; and colonies with no visible eggs have either just spawned or are unlikely to spawn for at least three months (Harrison et al. 1984; Baird et al. 2002). The sampling procedure followed Baird et al (2002)

and involved removing up to 3 individual branches from each colony and noting one of the 3 reproductive conditions (presence of pigmented eggs, presence of visible white eggs or no visible eggs). Colonies were only scored as empty if all 3 branches were found to be empty. This method permitted the examination of large numbers of colonies with the minimum amount of time and effort. However, it did not provide any details about the size of mature oocytes, the length of the gametogenic cycle or the exact night and hour of spawning.

A chi-square analysis was performed to determine if the proportion of colonies with mature eggs was independent of seasons. Singapore experiences a monsoonal climate, so the year was divided into 4 seasons based on the monsoon system as follows: Northeast Monsoon (November – February); Southwest Monsoon (May-August); first inter-monsoon (March-April); and second inter-monsoon (September-October) (Nieuwolt, 1973). Data were pooled for each season and a contingency table was constructed with 5 rows (representing the 5 seasons covered during the study), and 2 columns (representing number of colonies with mature eggs and number of empty colonies).

Results and Discussion

The assemblage of *Acropora* at Raffles Lighthouse showed a high degree of inter-specific reproductive seasonality, with the majority of spawning concentrated in March and April (Fig. 2). Mature eggs were present in colonies in March, April, May, October and November. However, the great majority of mature colonies were found in March and April 2002 (48.5% and 23 % respectively of colonies sampled) and April 2003 (47.4% of colonies sampled) (Fig. 2). In October, November (2002),

March and May (2003) 2.1%, 3.8%, 7% and 1.3% of colonies were mature respectively (Fig. 2). Large numbers of colonies contained visible, immature (white) oocytes one to two months before the main spawning periods in March and April 2003 (Feb 2003 45.5%, and Mar 2003 55% of the colonies sampled) (Fig. 2). Chi-square analysis indicated a highly significant association between the season and the presence of mature gametes (df = 4, test statistic = 252, $p < 0.001$), with the majority of mature eggs being present during the first inter-monsoon season (March – April).

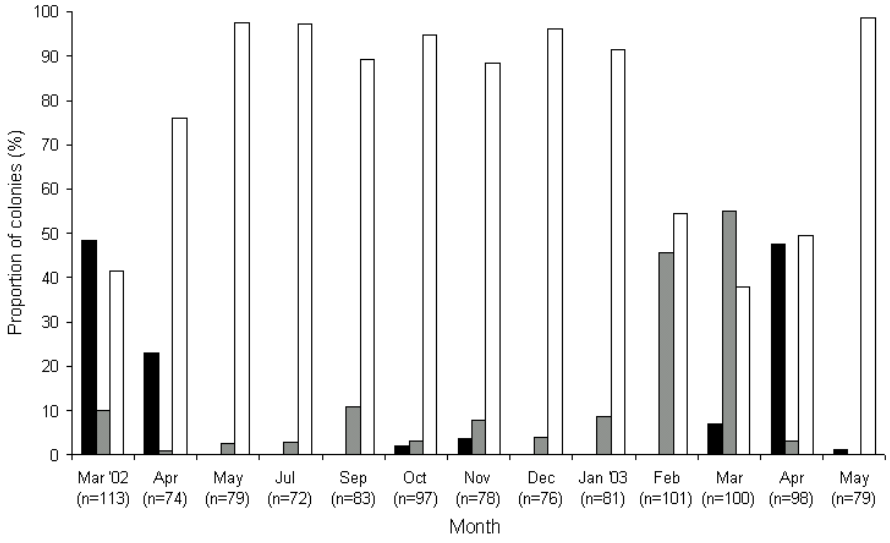


Fig 2. The overall proportion of colonies (%) of *Acropora* spp. at Raffles Lighthouse that contained mature eggs (black bars), immature eggs (gray bars) or no visible eggs (white bars) between March 2002 and May 2003 (n = total number of colonies sampled).

Some colonies of *Acropora digitifera* and *A. hyacinthus* contained mature oocytes in both March and April in 2003 (Table 1), indicating that spawning in these populations was "split" over two months (Willis et al 1985; Wolstenholme 2003). In October and November only some colonies of *A. humilis* had mature eggs. Two fecund colonies were tagged and found to contain mature gametes again in April 2003, indicating that

some colonies of this species spawn twice a year. This finding is consistent with reports of bi-annual spawning of some species from the GBR and Western Australia (WA). At both of those locations the second spawning event is smaller and highly variable from year to year (Simpson 1985; Stobart et al. 1992; J. Gilmour pers. comm).

In total, 23 *Acropora* species were sampled in March 2002 and March, April & May 2003. Due to the haphazard nature of the sampling, not all 23 species were represented in each sampling month (Table 1). In both years, a high proportion of the species encountered during the surveys, had at least one colony with mature eggs. Of the 19 species sampled in March 2002, 13 (68%) had at least one colony with mature eggs, and of the 14 species sampled in April 2003, 11 (79%) had at least one colony with mature eggs (Table 1). Few generalisations can be made about within-species synchrony of gamete maturation, due to the lack of species identification for many of the samples and the relative rarity of many species in the assemblage (Table 1). However populations of some species appeared to be quite synchronous (in terms of gamete maturation), for example 86% of *A. humilis* (n = 7) and 75% of *A. digitifera* (n = 16) colonies sampled were mature just prior to the full moon in April 2003 (Table 1). Other species had moderate or low levels of population synchrony, such as *A. hyacinthus*, where 42% of colonies sampled were mature in April 2003 (n = 12), and *A. tenuis*, where only 12% of the colonies sampled were mature in April 2003 (n = 8) (Table 1). Colonies of *A. austera* were checked in Mar, Apr and May 2003 (n = 9, 10 and 5 respectively), however mature eggs were never observed (Table 1). It is not clear why certain species lacked fecund colonies during the main spawning periods. Some individuals may spawn at other times of the year, or not at all in some years (possibly as a result of environmental stressors), (e.g. see Wallace 1985; Hughes et al

2000; Baird and Marshall 2002). Long term studies following individual colonies and populations through time are required to understand the reproductive patterns of these species on Singapore's reefs.

Spawning of at least 18 scleractinian species from 5 families (Acroporidae, Faviidae, Merulinidae, Oculinidae and Pectiniidae) was documented at Raffles Lighthouse between the 3rd and the 5th nights after the full moon in March 2002 (Guest et al 2002). It was not possible to visit Raffles Lighthouse at night in 2003, however night-time field trips to a nearby reef in April 2003 (Pulau Hantu, 1° 13'N, 103° 45'E), revealed synchronous spawning of at least 13 scleractinian species from 5 families (Euphyllidae, Faviidae, Merulinidae, Oculinidae and Pectiniidae) between the 3rd and 6th nights after the full moon (unpublished data). In both years, spawning took place between the hours of 2000-2200, and between 5 and 12 species were witnessed spawning simultaneously on each night. These observations strongly indicate that many species of *Acropora*, along with other scleractinian genera release gametes during a distinct "mass-spawning period", following the full moons of March and April, similar to that described by Willis et al (1985) on the GBR. Additional studies on Singapore's coral reefs are needed before further generalizations can be made about the duration of the spawning periods, the number of species that participate and the extent of spawning synchrony within and between species.

Clearly, marked seasonality in reproductive activity is a feature of the *Acropora* assemblage at Raffles Lighthouse. Further investigations are needed to understand what factors have caused this pattern in an equatorial location that is typically considered to have little annual variation in environmental parameters. On Singapore's reefs, the annual range in sea surface temperature is only 3 - 4º C (Tham 1973), which is relatively small compared to coral reefs at higher latitudes (e.g.

Magnetic Island, GBR, which experiences an annual variation of 12º C, Babcock et al. 1986), suggesting that a large range in annual sea surface temperature is not a pre-requisite for reproductive seasonality or multi-species spawning synchrony. It may be significant that Singapore has a semi-diurnal tide and a relatively large tidal amplitude of 2.4 m (Raffles Lighthouse, Maritime Port Authority of Singapore 2002) in comparison to other low latitude reefs (e.g. Solomon Islands, 0.8m, Baird et al 2002; and Madang, PNG, 1m, Oliver et al 1988). While, it is conceivable that tides play an ultimate role in selecting for the nights and time of spawning (because mass spawning during extended periods of slack water may increase fertilisation success, Babcock et al. 1986), it is not clear how large tidal amplitudes could affect the extent of reproductive seasonality. Furthermore, synchronous spawning of corals does occur on reefs that experience small tidal amplitudes e.g. the Solomon Islands (Baird et al. 2001), and the Houtman-Abrolhos Islands (WA) (Babcock et al. 1994) suggesting that this factor is not an ultimate cause of multi-specific synchrony.

	March 2002				March 2003				April 2003				May 2003			
Species	mature	immature	empty	n	mature	immature	empty	n	mature	immature	empty	n	mature	immature	empty	n
Acropora austera	0	0	100	4	0	0	100	9	0	0	100	10	0	0	100	5
A. cerealis	67	0	33	6	ns	ns	ns	0	ns	ns	ns	0	ns	ns	ns	0
A. digitifera	62	9	29	21	7	93	0	14	75	0	25	16	0	0	100	12
A. donei	0	0	100	1	ns	ns	ns	0	ns	ns	ns	0	ns	ns	ns	0
A. florida	0	0	100	2	0	50	50	2	ns	ns	ns	0	0	0	100	1
A. grandis	0	0	100	2	ns	ns	ns	0	ns	ns.	ns	0	ns	ns	ns	0
A. granulosa	ns	ns	ns	0	0	100	0	4	100	0	0	4	0	0	100	4
A. humilis	60	20	20	5	0	100	0	5	86	0	14	7	0	0	100	4
A. hyacinthus	48	4	48	25	23	59	18	22	42	0	58	12	0	0	100	13
A. intermedia	33	0	67	3	0	0	100	2	0	0	100	3	0	0	100	1
A. latistella	38	12	50	8	0	0	100	12	20	20	60	10	0	0	100	11
A. lianae	ns	ns	ns	0	0	100	0	2	ns	ns	ns	0	ns	ns	ns	0
A. loripes	100	0	0	1	0	100	0	5	71	0	29	7	0	0	100	4
A. microclados	100	0	0	1	50	50	0	2	ns	ns	ns	0	ns	ns	ns	0
A. millepora	33	67	0	3	0	82	18	11	57	14	29	7	0	0	100	8
A. muricata	0	33	67	6	0	0	100	1	ns	ns	ns	0	0	0	100	2
A. nasuta	57	29	14	7	ns	ns	ns	0	100	0	0	1	ns	ns	ns	0
A. samoensis	0	0	100	1	ns	ns	ns	0	0	0	100	3	0	0	100	1
A. secale	100	0	0	5	ns	ns	ns	0	75	0	25	8	11	0	89	9
A. selago	86	0	14	7	ns	ns	ns	0	ns	ns	ns	0	ns	ns	ns	0
A. tenuis	20	0	80	5	0	25	75	8	12	0	88	8	0	0	100	1
A. verweyi	ns	ns	ns	0	0	100	0	1	50	0	50	2	0	0	100	3
Totals	48.5	10	41.5	113	7	55	38	100	47.4	3.1	49.5	98	1.3	0	98.7	79

Table 1. The proportion of *Acropora* colonies (%) in each species that contained mature eggs, immature eggs or no visible eggs for March 2002 and March, April and May 2003. n = number of colonies sampled of each species; ns = not sampled.

A3.2 Reply to reviewers: Guest et al. Reproductive seasonality in an equatorial assemblage of scleractinian corals

This manuscript, when first submitted to *Coral Reefs* was criticised by one of the anonymous reviewers for a variety of reasons. We believed that most of the major criticisms of the manuscript stemmed from a failure to clearly describe: our objectives; our methods and the assumption behind them; what we documented, and what patterns can be inferred from the data. I thought it would be informative to readers to include our response to some of the reviewer's comments here, as they are pertinent to the thesis as a whole.

Objectives of the study

The aim of this study was to see if the corals in an *Acropora* assemblage at Raffles Lighthouse in Singapore are predominantly reproductively active in the same month, over a period of a few months, or spread out over the year. From our samples "on the fly" (Reviewer 2) we have established, in a single year of sampling, that reproductive activity in the *Acropora* in Singapore is concentrated in two months (March and April), with some (very few) corals reproductively active in May & Oct/Nov. This is in contrast to the widely accepted belief that equatorial assemblages of broadcast spawning scleractinian corals exhibit low levels of reproductive seasonality.

The methods used

We do not accept that our data are "circumscribed" by either the procedures or the rarity of the species. We believe that the procedures used for this study (i.e. visually assessing maturity of gametes *in situ*) are entirely appropriate. It is no longer necessary to observe gamete release on every occasion to infer spawning in corals. The available empirical evidence shows that if a colony contains mature (pigmented) eggs, it is likely to spawn on or shortly after the next full moon (see Wolstenholme 2003, Baird et al 2002, Guest et al 2002). The histological/microscopical approach allows for detailed descriptions of the reproductive patterns of a few species, whereas our approach allowed for less detailed descriptions of many species. Microscopic examination, *in situ* spawning observations and frequent sampling during the spawning periods provide more detail (i.e. length of gametogenic cycle, exact time and night of spawning) - but at the expense of massive amounts of extra time, effort, money and manpower. We were not interested in determining the length of the gametogenic cycles; consequently, histological or microscopical examination of gonad development would have provided no additional relevant information, in this study of seasonality and synchrony of gamete maturation. Similarly, we consider histological examination of spermatogenesis unnecessary because in the *Acropora* both sperm and eggs mature at the same time and are released together, packaged in egg/sperm bundles. We believe that an insistence on such traditional methods has hindered the documentation of spawning patterns in many coral assemblages for years.

To make it clear that we are aware of some of the limitations of the method, we have added of the following lines to the methods and materials: "This method permitted the examination of large numbers of colonies with the minimum amount of time and effort. However, it did not provide any details about the size of mature oocytes, the length of the gametogenic cycle or the exact night and hour of spawning."

Synchrony/seasonality

The main criticism of this manuscript was that our data are not sufficiently detailed to argue for reproductive "synchrony". This is a fair comment, as we have not made detailed observations of the exact night and time that each *Acropora* species spawns. We have changed "synchrony" to "seasonality", in the title and in most cases throughout the text. However we feel it would be wrong to "totally drop the idea of synchrony" (reviewer 2) from the manuscript. Our argument that synchronous spawning occurs in Singapore is based on sound techniques, good data and solid inductive reasoning. These data establish that the patterns of spawning in this equatorial assemblage of *Acropora* are not substantially different to patterns of spawning on the GBR and elsewhere (in particular see Baird et al 2002 and Wolstenholme 2003). We have done this convincingly, with the minimum of time and effort. The presence of mature (pigmented) gametes, tell us which colonies and which species are likely to spawn during that month. When the data are combined with our *in situ* observations at this site (Guest et al 2002) where colonies of at least 18 species from 5 families spawned over a period of 3 days (the 3rd to the 5th nights after full moon between the hours of 2000 and 2200) and at least 12 species spawned on one night, and all other published work on spawning behavior in the *Acropora*, we believe

we are justified in concluding that the majority of spawning of colonies with mature eggs will be occur during a "spawning period" (as described by Willis et al 1985), following the next full moon. In 2003 we were refused permission to land at Raffles Lighthouse at night by the local port authorities, however spawning observation trips were done at a nearby site (Pulau Hantu) following the full moons in March and April 2003. Unfortunately, this site has very few *Acropora* colonies, however in April 2003 I observed other scleractinian corals spawning from the 3rd to the 6th night after the full moon. At least 13 species from 5 families were involved and as many as 8 species spawned on one night within a period of 2 hours (between 2000 and 2200).

To clarify this a more detailed description of our spawning observations has been included in the results and discussion section (see paragraph 2 page 10)

Rarity of certain species and generalisations about inter and intra specific variation

Some of the species were rare, however we do not see how this affects our conclusions with respect to multi-species synchrony/seasonality. If there is only one colony of a given species and it contains mature eggs, empirical evidence indicates it will spawn following the next full moon (see refs above), and we therefore include the species in our tally of the proportion of the *Acropora* participating in spawning in that month. If anything, the rarity of many of the species sampled suggests that species participation is probably higher than we estimate, because the probability of finding at least one colony with mature gametes will increase as the abundance of that species increases. We do accept that rarity, and the fact that we did not carry out species identification on every sampling occasion, make it difficult to make many

generalisations about intra-specific synchrony/seasonality. Hence, we have limited our discussion of intra-specific variation to species and months with adequate sample sizes (*A. humilis, A. hyacinthus, A. tenuis, A. digitifera* and *A. austera*).

We do not agree that our data are "sketchy", they provide a detailed description of the annual reproductive patterns of the *Acropora* at this site, equivalent to that of many other well studied *Acropora* assemblages elsewhere. Furthermore, our results are entirely novel, being the first substantial detailed description of seasonal spawning patterns in an equatorial coral assemblage, and they are the first to challenge a dogma that has remained entrenched since the end of the 1980's: namely that there is a breakdown in reproductive seasonality and synchrony in broadcasting coral assemblages close to the equator.

Appendix 4

Coral spawning

A4.1 Spawning field trips

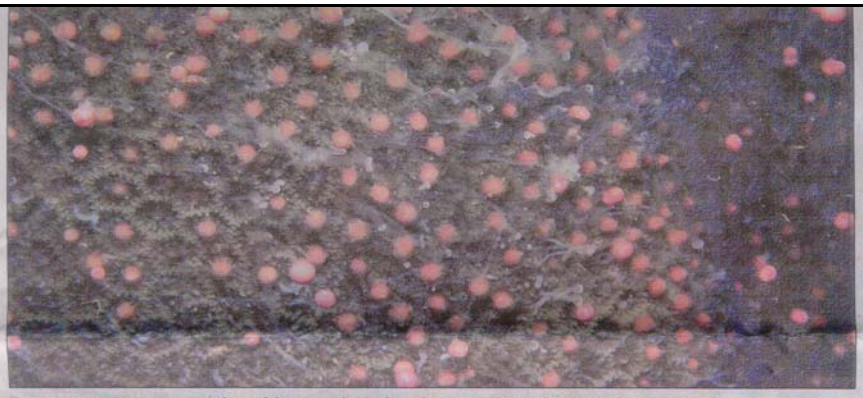
Fig. A4.1 The coral spawning field trips: a) the reef at Raffles Lighthouse where night snorkels and dives were carried out and loose coral colonies were collected; b) these were stored in large tubs on the shore and replenished with sea water approx every 6 hours via a submersible pump; c) diver clambering down the rock bunds at sunset to look for coral spawning activity; d) the camp site at Pulau Hantu used for the spawning trips in 2003.

A4.2 Dissemination of research

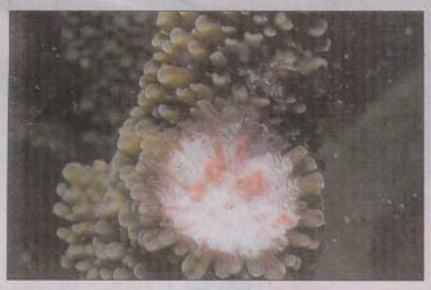
Sadly, few people are aware that diverse coral reefs can be found around Singapore's coastline. Mass coral spawning attracts media attention and can be used as a way of raising awareness about coral reefs. Therefore I tried to disseminate the results of the spawning studies research, not only within the scientific community, but also to the general public. I was fortunate to have the opportunity to publicise my research at scientific symposia and in the local media. I presented aspects of my PhD work, including the coral spawning observations at four international scientific conferences:

- The Ninth International Coral Reef Symposium, Bali, Indonesia, October 2000
- Asia-Pacific Congress on Marine Science and Technology, Kuala Lumpur, Malaysia, May 2002
- Summer Meeting of the American Society of Limnology and Oceanography, Victoria, Canada, June 2002
- The European Meeting of the International Society for Reef Studies, Cambridge, England, September 2002

Two articles about the coral spawning were published in local newspapers (Figs A4.1 & A4.2) and a short item was broadcast on the Straits Times television news after the spawning in March/April 2002.



Egg and sperm bundles were released from corals, such as this goniastrea or brain coral, here last week.



Eggs nestle inside the corals (above) waiting to be released when the moon and tides are just right.

'Tis the season for coral spawning

By **CHANG AI-LIEN**
SCIENCE CORRESPONDENT

LAST week's combination of full moon and warm sea currents proved to be potent factors, resulting in Singapore waters coming alive with millions of tiny egg and sperm parcels.

The occasion — the corals' spawning season, an event so rarely witnessed it has never been documented before.

But this time, two researchers captured on video and in photographs those magical moments as they occurred in the waters around Raffles Lighthouse.

The spawning is a good omen for reefs here, said National University of Singapore doctoral student James Guest, who documented the event with researcher Karenne Tun of the Tropical Marine Science Institute.

"There were at least 30 species spawning," said Mr Guest, who is doing his PhD thesis on coral reproduction. "The fact that they're doing so indicates that the reefs here are still alive and well. They could even spread if the waters surrounding them are cleaned up a bit."

He gauged the time that the spawning would occur after three years of studying reefs here.

He said it probably happened simultaneously at other reefs here and around the region, as different species of coral in the same region tend to spawn at the same time, after months of gestation.

The mass event was the coral equivalent of a foam party, and filled the waters around the island with miniature pink balls of egg and sperm on three nights last week.

Each tiny parcel which is not eaten by fish and crabs floats to the water's surface, where it hopefully gets fertilised. If it is, a microscopic larva forms.

The larva settles days later on a hard surface, like a rock, metamorphosing into a sedentary coral polyp, which multiplies and forms a colony. It could take hundreds of years for a colony to grow to the size of a small car.

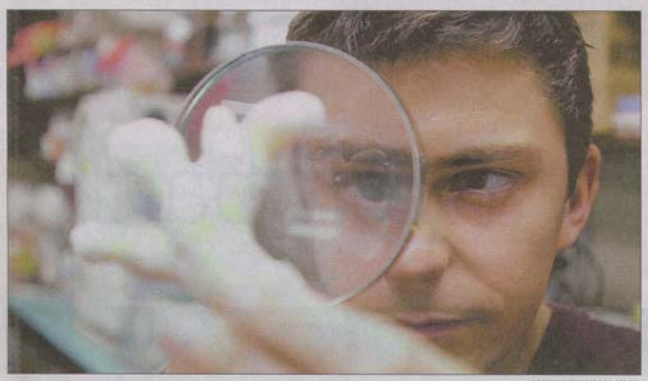
Only one in thousands of eggs makes it through this process.

Nearly 100,000 sq km of coral reefs — one-third of the world's total — can be found in South-east Asia, home to more than 600 of the world's 800 coral species and the global hub of marine life. Singapore has about 150 of the species.

Yet coral spawning in the region, which typically happens once a year when the moon and tides are just right, has been documented only in the Philippines and Indonesia.

Mr Guest added that since spawning happens so rarely, knowing when it will happen means that industrial activity, such as dredging, could be stopped so that the event is not disrupted.

He said: "Many people are surprised to find out there's coral in Singapore. Though visibility in the waters here is poorer, coral life here is as good as parts of Australia's Great Barrier Reef."



HOW HWEE YOUNG

One of a lucky few, Mr Guest caught the coral birth on camera. He gauged when it would occur after three years of studying the reefs here.

Fig. A4.2 Article published in the straits Times after the 2002 coral spawning (Chang 2002). The bottom picture is of me looking at a coral skeleton through a magnifier - something that I hardly ever did during my PhD! But the Straits Times photographer thought it made me look more like a scientist!

TODAY • WEDNESDAY • MARCH 26, 2003

HOME NEWS

Wealth under the waves

Watch rare coral spawning off Pulau Hantu

by Joy Frances
joy@newstoday.com.sg

FEW but the most ardent of scuba divers would spend time studying coral reefs around Singapore when they could visit pristine reefs in nearby Malaysia, Indonesia and the Philippines. But, last weekend, that's exactly what a handful of divers did.

Led by marine biologist James Guest, the six divers headed to Pulau Hantu on Friday hoping to view coral spawning. This happens only once a year over about a month, when coral species release millions of eggs and sperm.

At Australia's Great Barrier Reef, the spawn looks like a technicolour snowstorm heading for the surface – a big and bright streak.

Mr Guest, a Briton who has been in Singapore for the last four years, said the waters here have lower visibility, but the coral diversity is similar to that at the Great Barrier Reef. Last year, with clearer waters off the Raffles Lighthouse island, he was lucky to spot "little bundles of eggs". This year, however, his usual research site is off-limits because of security concerns.

At Pulau Hantu, however, the divers were able to find a patch of spawning coral by following the path of the eggs floating to the surface. The marine enthusiasts make the excursion out of interest. But, Mr Guest had doubts over the future of marine tourism here. High sedimentation from reclamation works result in poor visibility, suffocate coral and in turn, affect marine life populations on the reefs.

Coral spawning off the Raffles Lighthouse island last March. Pink eggs burst from the coral and float to the surface to be fertilised by sperm from male coral.
– Photo courtesy JAMES GUEST

"I don't think the next generation will see any reef life," he said. "Anyone who has dived in Singapore waters would know that the fish life is not as healthy as it could be."

"Singapore's reefs have a breeding population of corals ... They could be used as a potential resource for scientific research," said Mr Guest.

Developing an interest in coral aquaculture could be another alternative, he added. "It is possible to start up a coral aquaculture business to provide coral to aquarists in Singapore using local corals as initial brood stock," he said.

But, for many conservationists, people need to know how our natural environment is being abused. "Most Singaporeans I have spoken to are not aware that there are coral reefs here," Mr Guest said.

Fig. A4.3 The second news article was published in the Today newspaper around the time of the 2003 coral spawning (thankfully no pictures of me this time!) (Frances 2003).

A3.3 Publications

Guest JR, Baird AH, Goh BPL, Chou LM

Multi-specific, synchronous coral spawning in Singapore.

Coral Reefs 21:422-423

Accepted: 10th May 2002 / Published online: 16th October 2002

Multispecific, synchronous coral spawning in Singapore

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There are very few reports of multispecific, synchronous coral spawning from low-latitude reefs (e.g. Baird et al. 2001), and it has been suggested that the 'mass spawning' phenomenon may be absent or significantly reduced close to the equator (Oliver et al. 1988). Singapore is a small, industrialised and heavily populated island, located approximately 1° north of the equator.

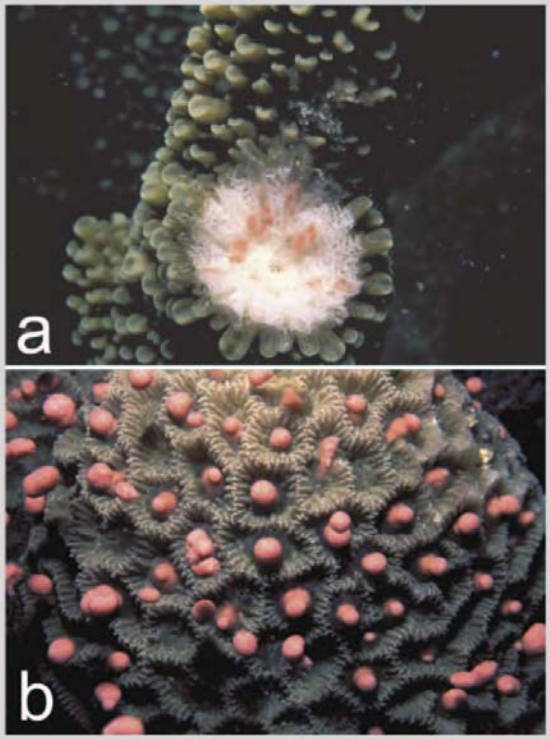

Fig. 1 **a** A broken branch of *Acropora intermedia* clearly revealing brightly coloured, mature eggs prior to the spawning event. **b** A *Platygyra verweyi* colony releasing egg sperm bundles at night

Reef sites

Despite there being high levels of sedimentation and turbidity in the coastal waters, reasonably diverse coral communities can be found around some of the islands to the south of the mainland. Sampling of *Acropora* species to determine their reproductive state was conducted at the southern-most reef off Singapore (Raffles Lighthouse, 1°10′N, 103°45′E), on 21 March 2002 (8 days before the full moon). From 22 different *Acropora* species, 15 (68%) had at least one colony with mature eggs, 48.5% of colonies had mature eggs (pigmented) (Fig. 1a), 10% had immature eggs (unpigmented), and the remaining 41.5% had no visible eggs ($n = 113$). On the 3rd, 4th and 5th nights after the full moon between 8 and 10 p.m. we observed synchronous spawning of corals. At least 18 different coral species from ten genera and five families (Acroporidae, Faviidae, Merulinidae, Oculinidae and Pectiniidae) were observed releasing gametes over the three nights (Fig. 1b). This report documents multispecific spawning of corals in Singapore for the first time and demonstrates that this remarkable phenomenon can indeed be a characteristic of equatorial coral reefs.

References

Baird AH, Saddler C, Pitt M (2001) Synchronous spawning of *Acropora* in the Solomon Islands. Coral Reefs 19:286
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Reef sites

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Appendix 5

Goniopora experiment

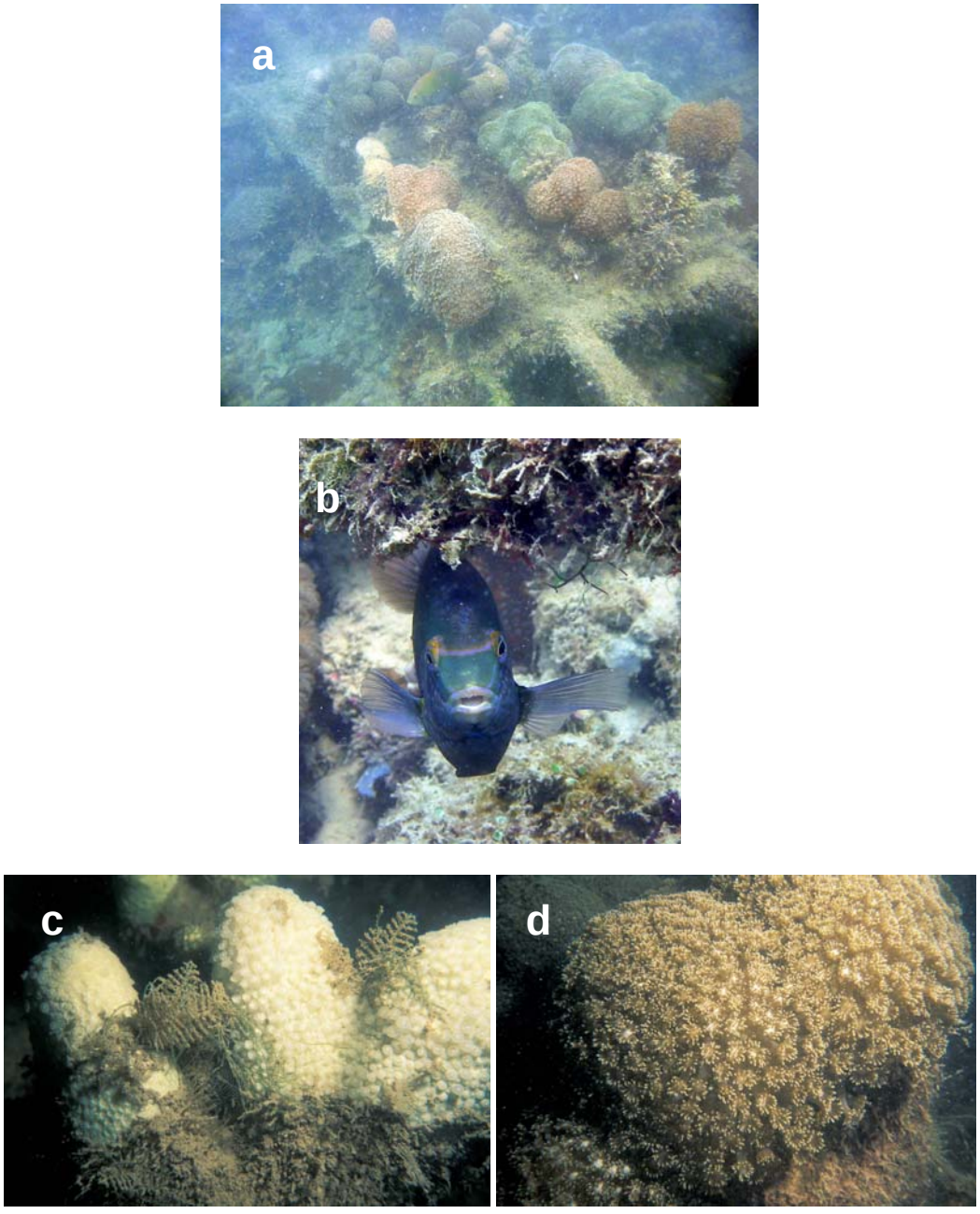

Fig. A5.1 a) The aluminium frame at Pulau Hantu shallow; b) numerous Honey-head damselfish (*Dischistodus prosopotaenia*) inhabited the frames at Pulau Hantu shallow, and may have helped to control overgrowth by macro-algae, they also regularly attacked me every time I dived to check on my corals!; c) a bleached coral fragment on the deep frame at Pulau Hantu, compared to d) a healthy fragment on the frame at Pulau Hantu shallow.