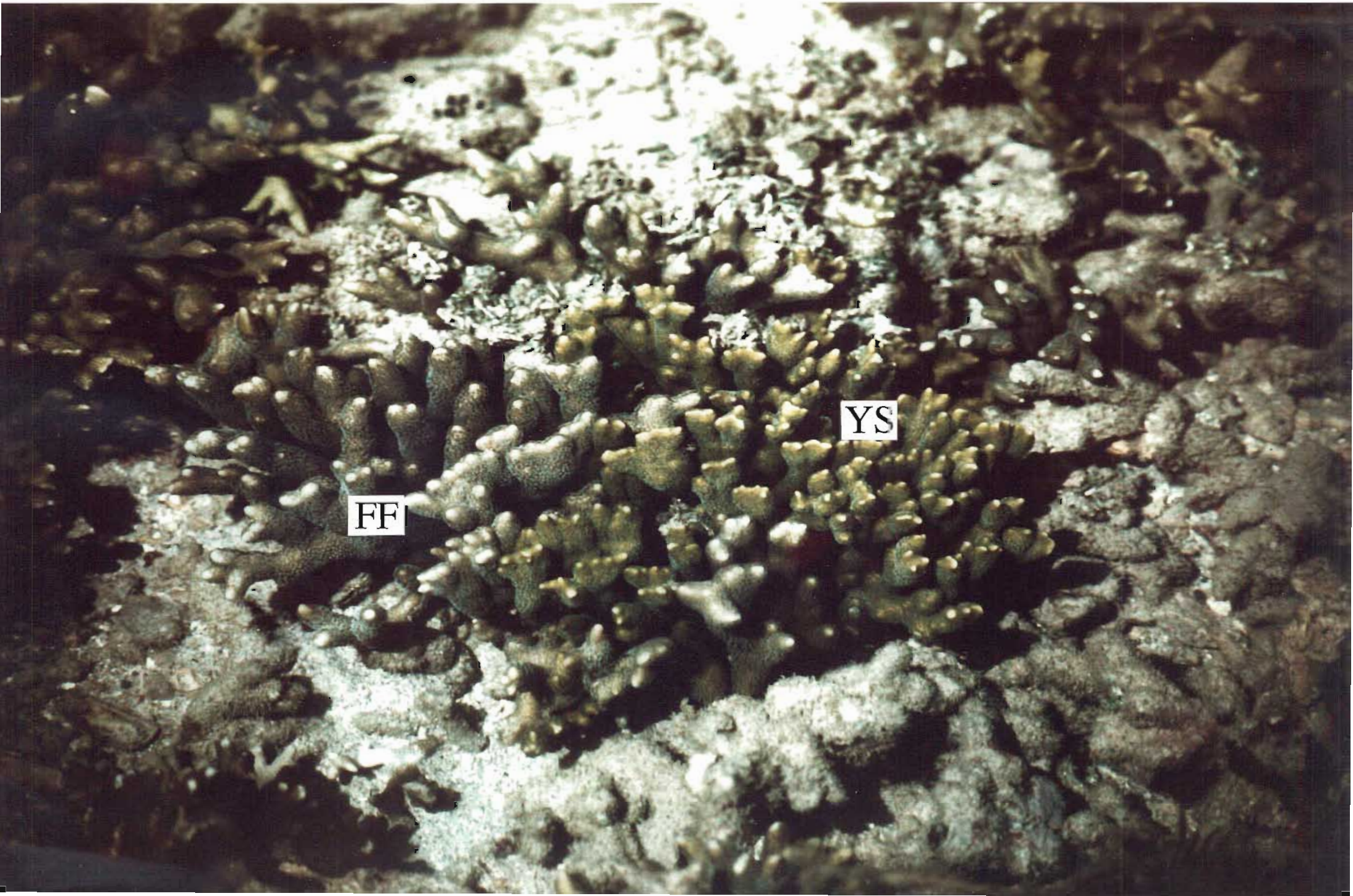
To Ann

Frontispiece

Fat fingers (FF) and yellow spatulate (YS) morphs of *Montipora digitata* (Dana 1846):

are they morphs or species?


YS
FF

Delimiting coral species using alternative techniques: *Montipora digitata* (Dana, 1846), a case study

Thesis Submitted by Ben Stobart BSc (Joint Hons) (Bangor)

in August 1994

for the Degree of Doctor of Philosophy in the Department of Marine Biology at James Cook University of North Queensland

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ABSTRACT

High levels of intra-specific skeletal variability and considerable overlap of skeletal characters between species pose problems for the application of traditional taxonomic methods. Such problems can be overcome using alternative techniques. In this study, four alternative techniques were used to investigate the species status of two sympatrically occurring morphs of *Montipora digitata* (Dana 1846) found on inshore reefs along the Queensland coast, Australia.

Allozyme electrophoresis indicated that gene flow does not occur between the two morphs, which were distinguished by one fixed gene difference at locus LT-2 and frequency differences at three other loci. These differences were consistent at three locations separated by up to 300 km. The genetic distinctiveness of the two morphs is further supported by the comparatively high values of Nei's genetic distance between morphs (0.24), in contrast to low values within populations of the two morphs (0.01). Populations of both morphs were generally in Hardy-Weinberg equilibrium verifying that sexual recruits contribute to the genetic structure of the populations. Low genetic diversity ratios (G_O:G_E), despite sampling designed to reduce the chance of collecting clonemates, indicated that asexual reproduction also occurs.

Breeding experiments demonstrated that fertilisation occurs readily among colonies of the same morph, but that there is virtually no fertilisation between morphs. Reproductive hierarchies detected within the two morphs may indicate a more complex breeding structure within each population. Gamete interaction experiments revealed that the block to fertilisation between morphs occurs before egg activation. It is suggested that incompatibility of egg-sperm binding proteins is the most likely reason for reproductive isolation between the morphs. Breeding experiments involving nine species of *Montipora* demonstrated that hybridisation occurs within the genus *Montipora in vitro*, but not at high levels, and survival of hybrid juveniles was poor. Hybridisation is therefore unlikely to play a major role in the evolution of the genus.

Univariate and multivariate morphometric analyses based on five skeletal characters revealed that there are significant differences in skeletal morphology between the two morphs. Considerable overlap in these characters, however, makes them unsuitable for species identification. Septal shape was found to reliably separate the two morphs. In view of the concurrence among genetic, breeding and morphological data, and examination of holotypes, the two morphs were renamed *M. tortuosa* (Dana 1846) and *M. digitata* (Dana 1846).

Comparison of a number of reproductive characters indicates that the two species, *M. tortuosa* and *M. digitata*, differ in their reproductive biology. *Montipora tortuosa* produced more eggs per polyp and larger testes, and a greater percentage of polyps within the colonies produced eggs. However, *M. digitata* produced larger eggs, a strategy that made the total reproductive output between the two species equivalent. The reproductive study also revealed that these two species, and several other species within the genus *Montipora*, spawn in both spring and autumn. Reproductive outputs differed between the two breeding seasons. Differences in reproductive output and contrasting environmental conditions during the two spawning seasons are discussed in relation to ultimate and proximate cues governing coral spawning.

Values for Nei's genetic distance suggest that the two species evolved 3.5 to 7.1 million years ago. Morphological and ecological similarities between *M. tortuosa* and *M. digitata* indicate that they have not diverged substantially in this time, they probably evolved in the same habitat in which they are found today, and they are most likely monophyletic in origin. It is argued that speciation of *M. tortuosa* and *M. digitata* may have resulted from stochastic changes to egg-sperm binding proteins on the egg surface. Reproductive hierarchies within the species support the existence of molecular variability in these proteins which may have led to reproductive isolation between *M. tortuosa* and *M. digitata.* Studies on mass spawning corals so far have found high levels of hybridisation suggesting that they do not conform to most species concepts. In contrast, *M. tortuosa* and *M. digitata* show most of the qualities of "good" morphological, biological, ecological and evolutionary species.

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Completion of a thesis can be a very stressful task, but can be made easier with a bit of encouragement at the correct moments: I thank my wife Sarah, John Benzie and Keith-Martin Smith for their impeccable timing.

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DECLARATION

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

Ben Stobart

General Introduction

The origins of taxonomy as a way of thinking have been lost in antiquity (Blackwelder 1967). In its most basic form taxonomy is the recognition and classification of "kinds". As a science it generally refers to the methods and principles of classification of any group of organisms (Simpson 1961). The need to classify organisms is so basic that it is claimed to be fundamental to intelligence and communication as man practices it (Blackwelder 1967), and it can be identified in other living organisms that can recognise their own kind, kinds that are dangerous to them and kinds that provide them with food.

Species are the fundamental category of the taxonomic hierarchy (Stuessey 1990), and as such have received a great deal of attention. The number of species that have been described to date using Latin binomial names is unknown, but are probably in the order of 1.5-1.7 million (Wilson 1985, May 1988). This figure is insignificant in comparison to the tens of millions of species that are suspected to inhabit the earth (May 1988). Traditionally species have been defined on structural (morphological) grounds because structural data is often the only data available for identification purposes, and it is relatively easy to obtain (Blackwelder 1969). However taxonomy is a dynamic science that often draws on physiological, ethological, genetical and ecological data, amongst other things (Blackwelder 1969, Stuessey 1990). Any technique that identifies differences between species may be employed for taxonomic purposes.

Closely associated with taxonomy is the study of systematics. Though at one time considered to be synonymous with taxonomy, systematics now has a broader meaning than taxonomy as it includes the study of evolution and phylogeny. Systematics is currently defined as "the scientific study of the kinds and diversity of organisms and of any and all relationships among them" (Simpson 1961). The aim of systematics is not just to describe pattern, but also to explain it, and as such is a natural extension that has evolved from the process of classification.

1.1 Systematics of scleractinian corals

The taxonomy of scleractinian corals is almost exclusively based on skeletal characters (Vaughan and Wells 1943, Veron and Pichon 1976), and different species

"are expected to be separated from each other by distinct morphological gaps" (Lang 1984). These "morphological gaps" form the basis of the morphological species concept and still constitute the most accessible and widely used method for identifying species (Stuessey 1990). Though the morphological approach to species identification generally works well for most animal groups, its use becomes limited when morphological characters overlap between species. Overlap of morphological characters between species has often been reported for scleractinian corals, and consequently systematists have experienced great difficulty in delineating species boundaries in some genera (Lang 1984). Perhaps one of the most important factors promoting overlap of morphological characters is that species within many genera of scleractinian corals can exhibit high levels of skeletal variability (eg. *Porites:* Veron and Pichon 1982; *Acropora:* Veron and Wallace 1984). In extreme cases corallites within a single colony are so different that they can be assigned to different species (Potts *et al.* 1993).

Skeletal variation is governed by both environmental and genetic effects. Environmental conditions are known to affect the morphology of corals, both at the colony level (Willis 1985) and the corallite level (Foster 1977). There are two mechanisms by which coral morphology may vary with different environmental conditions. Corals may either change their growth form in response to the environment (phenotypic plasticity), or, genotype-environment associations may be determined by selection at the recruitment stage, with adult colonies being unable to alter their growth form (genotypic polymorphism)(Willis 1985). Phenotypic plasticity has been demonstrated to occur in corals by means of transplant experiments (Foster 1979, Graus and Macintyre 1982, Oliver 1984, Willis 1985). It is thought that coral phenotypes can be influenced by environmental factors such as light intensity, sedimentation rate, water activity, and food availability (Foster 1979, Willis 1985).

No study has yet demonstrated that environmental factors directly affect the distribution of corals with phenotypically stable morphs, but there is little doubt that morphological variation which is genetically based does occur (Foster 1979, Willis 1985, Willis and Ayre 1985), and environmental variation governs the distribution of different morphs of a wide variety of organisms (Begon 1990). There is indirect evidence suggesting environmental variability can govern the distribution of phenotypically stable coral morphs. For example, Willis (1985) found that phenotypically stable morphs of *Pavona cactus* tended to be associated with different depths, but there was a considerable amount of overlap in depth distributions between the two morphs. This observed distribution pattern, with different morphs occupying particular areas and overlap of morphs between the areas is expected for the active

maintenance of genetic polymorphisms (Begon 1990).

Variation in coral morphology that is not associated with environmental conditions can also occur. Genotypic polymorphism can result in morphological variation that is not environmentally correlated (eg. ecological interactions can affect the distribution of clover genotypes; Turkington and Harper 1979), and hybridisation between species can produce individuals of intermediate morphology. Recent research on corals has revealed that experimental hybridisation between species occurs readily (Willis *et al.* 1992, Miller 1994). If hybridisation is also common in the natural environment much morphological variation may be attributed to hybrids.

The overlap of skeletal characters has always posed a problem to coral taxonomists. Early taxonomists were unable to cope with the extent of the skeletal variability, and consequently described every different specimen as a new species. The resulting taxonomy was extremely confused with too many species being described (Veron and Pichon 1977). Major oceanographic expeditions at the end of the nineteenth century, in particular the Challenger expedition of 1873-1876, marked a changing point for coral taxonomy. Larger coral collections became available to taxonomists, and variability of skeletal characters was recognised as a major problem (Veron and Pichon 1976). Most significantly, Quelch (1886) related intraspecific variation in skeletal characters to environmental conditions.

Awareness of the influence environmental variability can have on coral skeletons has revolutionised coral taxonomy. The term "ecomorph" was introduced to the taxonomic literature in 1976 by Veron and Pichon to describe "intraspecific skeletal variations phenotypically and/or genotypically determined in response to specific ecological conditions". Thus this phenomenon was taken into account in subsequent monographic works (Veron and Pichon 1976, Veron *et al.* 1977, Veron and Pichon 1979, Veron and Pichon 1982, Veron and Wallace 1984). These taxonomic studies greatly reduced the confusion surrounding coral taxonomy as they strived to account for phenotypic plasticity and genetic polymorphism. The final outcome was a more streamlined taxonomy in which many species were synonymised. However, inevitably some true species are likely to have been synonymised on the assumption that they were ecomorphs, or examples of genetic polymorphism (Wallace and Willis 1994). It is also likely that within currently accepted species there will be instances of sibling species or pseudo-sibling species, as there is growing evidence that these may occur in corals (Knowlton 1993, Knowlton and Jackson 1994).

Taxonomy of Indo-Pacific corals is now at the stage where many of the classification problems that earlier workers grappled with unsuccessfully have been

resolved. A much needed framework on which to build and improve has been established by Veron and his co-workers. The task now is primarily one of identifying species that may have been mistaken as "ecomorphs" and synonymised, and to identify possible sibling species. Identification of these species is not an easy task, particularly as they have been synonymised, or simply not distinguished, due to the fact that their skeletal characters overlap. As traditional taxonomic techniques have not been able to delimit these species, there is general agreement that the best way to resolve taxonomic problems is by using a combination of alternative techniques (Lang 1984, Willis 1990, Gattuso *et al.* 1991, Garthwaite *et al.* 1994). The use of alternative techniques for coral taxonomy has been reviewed by Lang (1984), and further discussed by Willis (1990). Any feature that may differ between coral species can potentially be of use for species identification, be it morphological, biochemical physiological, behavioural or ecological (Lang 1994). In recent years studies have used morphometrics (Potts *et al.* 1993, Miller in press), allozyme electrophoresis (Ayre *et al.* 1991b, Knowlton *et al.* 1993, Garthwaite *et al.* 1994), physiological characteristics (Gattuso *et al.* 1991, Knowlton *et al.* 1993), nematocyst morphology (Hidaka 1992), reproductive compatibility experiments (Willis *et al.* 1992, Miller 1994), and live tissues (Potts *et al.* 1993) to investigate species boundaries of scleractinian corals. Alternative techniques are therefore gaining popularity, and will undoubtedly play an important part in delimiting species boundaries where traditional taxonomy has problems.

1.2 The biological significance of morphological species

A major role of taxonomy is to identify biologically significant patterns of organisms (Szalay 1993). This is important as the ultimate goal of taxonomy is to systematise data on all the kinds of organism that exist, for the use of other disciplines (Blackwelder 1969). Discernment of biologically significant patterns of organisms is unlikely without an understanding of the biological mechanisms that create and maintain species (Szalay and Bock 1991). Therefore once a classification of species has been made on morphological grounds it is important to assess its biological significance. There is general agreement that species defined morphologically do correspond with species that are defined genetically (Michaux 1989, Jackson and Cheetham 1990). Traditionally it has been assumed that species are groups of reproductively isolated organisms (Mayr 1940, 1963, 1973), however, reproductive isolation is only suitable for describing species in sexually reproducing organisms, and can be unsuitable if sexually reproducing species hybridise extensively (Templeton 1989). In cases where species do hybridise extensively their identity may be maintained by demographic exchangeability, despite gene exchange between them

(Templeton 1989). It is therefore important to study the biological basis for morphological groups, as the assumption that morphologically defined species correspond to reproductively isolated species is not always valid. The study of species boundaries is also paramount to the understanding of how species come to exist (speciation), how they relate to each other over time (phylogeny), their past history (evolution) and how they interact with each other (ecology).

At present we know relatively little about the biological validity of coral species, how they are formed, and how the integrity of species is maintained. Until recently there has been a tacit acceptance that morphologically defined coral species are reproductively isolated (reviewed in Willis 1990). In many cases this assumption may be reasonable as most genetic studies have found evidence of reproductive isolation between morphologically defined species (Ohlhorst 1984, Ayre *et al.* 1991, Van Veghel and Bak 1993, Garthwaite *et al.* 1994). However, recent research has cast serious doubts on the validity of this assumption, as experimental crosses between species in three of the most common coral genera have demonstrated that levels of interspecific hybridisation can be very high (Willis *et al.* 1992). It is now thought that morphologically-defined species may not always reflect reproductive isolation, though it still remains to be demonstrated that levels of experimental hybridisation also occur in the natural environment (Willis *et al.* ms).

A further goal for coral research should therefore be to establish how widespread reproductive isolation and hybridisation is between coral species. This is important in order to establish the biological basis of morphologically defined coral species. The importance of determining the biological relevance of coral species defined by taxonomists has recently been highlighted by Jokiel (1987), who suggested that the morphological definition of *Pocillopora damicornis* is not sensitive enough to reflect evidence of divergent evolution. Comparisons of populations of the same morphological species of *P. damicornis* from Hawaii with populations from Enewetak, Panama and south western Australia showed each population was very different in ecological terms, but not morphologically. The geographic differences in biology supported a hypothesis of divergent evolution, but morpological criteria expressed no such trend. This highlights the urgent need to determine how well coral species defined by taxonomy reflect groupings of organisms that are meaningful for questions about the ecology, evolution and speciation of corals.

A major priority in the study of coral species should be to explore species boundaries using suitable alternative taxonomic techniques such as allozyme electrophoresis, crosses of gametes to test for evidence of reproductive isolation and ecological studies to determine whether different species are ecologically similar or not.

This will also provide vital information (genetic, reproductive and ecological) for the study of most other aspects of coral biology and evolution.

The coral genus *Montipora* has had a tortured taxonomic history (Veron and Wallace 1984). Taxonomic progress has been hampered largely due to small corallites, undeveloped corallite features and high levels of morphological variation typical of the genus (see section 1.3). This study aims to use alternative taxonomic techniques to clarify a taxonomic problem within the genus *Montipora*, to investigate possible species cohesion mechanisms between coral species, and to assess the biological significance of some species within this genus.

1.3 Introduction to the genus Montipora *de Blainville, 1830*

The genus *Montipora* belongs to the family Acroporidae along with three other extant genera. Members of this family are characterised by having small corallites, rudimentary or no columellae and dissepiments, simple septa in two cycles or less, synapticulotheca, and polyps that divide by extra-tentacular budding (Veron and Wallace 1984). The family Acroporidae exhibits all growth forms known for hermatypic corals. Of the four genera in the Acroporidae the genus *Astreopora* is the only one that does not conform to these features, having larger corallites and relatively well developed septae. A systematic résumé of the Acroporidae is given in Table 1.1.

Phylum Cnidaria (Coelenterata)	
Class Anthozoa	
Subclass Hexacorallia (Zoantharia)	
Order Scleractinia	
Family Acroporidae Verrill, 1902	
Genera:	*Acropora* Ogen, 1815
	Anacropora Ridley, 1884
	Astreopora de Blainville 1830
	Montipora de Blainville, 1830

Table 1.1. Systematic résumé of the family Acroporidae

Within the Scleractinia the genus *Montipora* is the second largest in terms of numbers of species, having 211 nominal species and an unknown number of true species. In Australia alone there are 38 recognised species (Veron, 1986). Colonies of this genus often contribute significantly to overall coral cover of reefs (Wood 1983, pers. obs.). Despite these attributes the genus *Montipora* is the least studied of the

major scleractinian genera (Veron 1986). This is probably due to the difficulties involved in identifying many of the species, and the fact that many of the species are inconspicuous, being encrusting or forming small plates (Veron 1986). The corallites of *Montipora* species are the smallest of all corals, which further adds to the difficulties involved in their identification.

Members of the genus *Montipora* may form leafy, encrusting, plate-like, branching, or semi-massive colonies, with numerous intermediary forms. Size varies considerably, encrusting and massive forms tending to be relatively small, whereas foliaceous and plate-like forms may produce colonies several meters in diameter. Septal apparatus are poorly developed (a feature used to distinguish this genus from *Porites*), and they have a well developed coenosteum. Calices are approximately 0.5 mm in diameter and rarely greater than 1 mm. Columellae are absent, although occasionally septae may fuse to form a central column. There are typically six primary septae in the form of vertical rows of spines, the more conspicuous primaries occasionally being either continuously or interruptedly laminate, and secondary septae are also usually present. The coenosteum is porous and often highly elaborated with spinules, its development often overshadowing that of the corallites. Coenosteal features have thus been used for species identification. The coenosteum may be plain without elaborations of any kind or it may have developed elaborations in the form of tuberculae (if larger than the corallite) or papillae (if smaller than the corallite) or a combination of these (Veron and Wallace, 1984). It must however be noted that this definition is erroneous according to the earlier work of Bernard (1897) who states quite clearly that the papillae are large and the tubercles small. No costae or dissepiments are formed in the *Montipora*.

Distribution of the genus is extensive, ranging from the Red Sea through to the Mid-Pacific region (extends East to the Marquesas, Hawaii and Pitcairn; North to Japan, the Arabian Gulf and the Red Sea; South to the Houtman Abrolhos Islands, Lord Howe Island and Durban), however this genus does not occur in the Eastern Pacific or the Caribbean.

1.4 Montipora digitata: a case study

This study concentrates almost exclusively on the taxonomic status of two morphs of *Montipora digitata*. *Montipora digitata* is a distinctive small branching coral common on inshore reefs along the Queensland coast. Its distribution is primarily limited to the reef flat zone where it is often the most abundant coral species (Bull 1982). Colonies of *M. digitata* may be divided into several morphs. Veron and

Wallace (1984) recognise three distinct ecomorphs of this species. Many additional morphs were detected in the present study but only two morphs were easily identifiable and were detectable over a wide geographic area. One morph is grey-brown with cylindrical branches and round tips, the other, greenish yellow with round or laterally compressed branches that end in frond-like (spatulate) tips (Figure 1.1). For convenience these two morphs will be referred to as "fat fingers" (FF) and "yellow spatulate" (YS), respectively. Preliminary crosses of gametes from these two morphs indicated that they may be reproductively isolated (Willis *et al.* 1992), suggesting that further study of species boundaries was required.

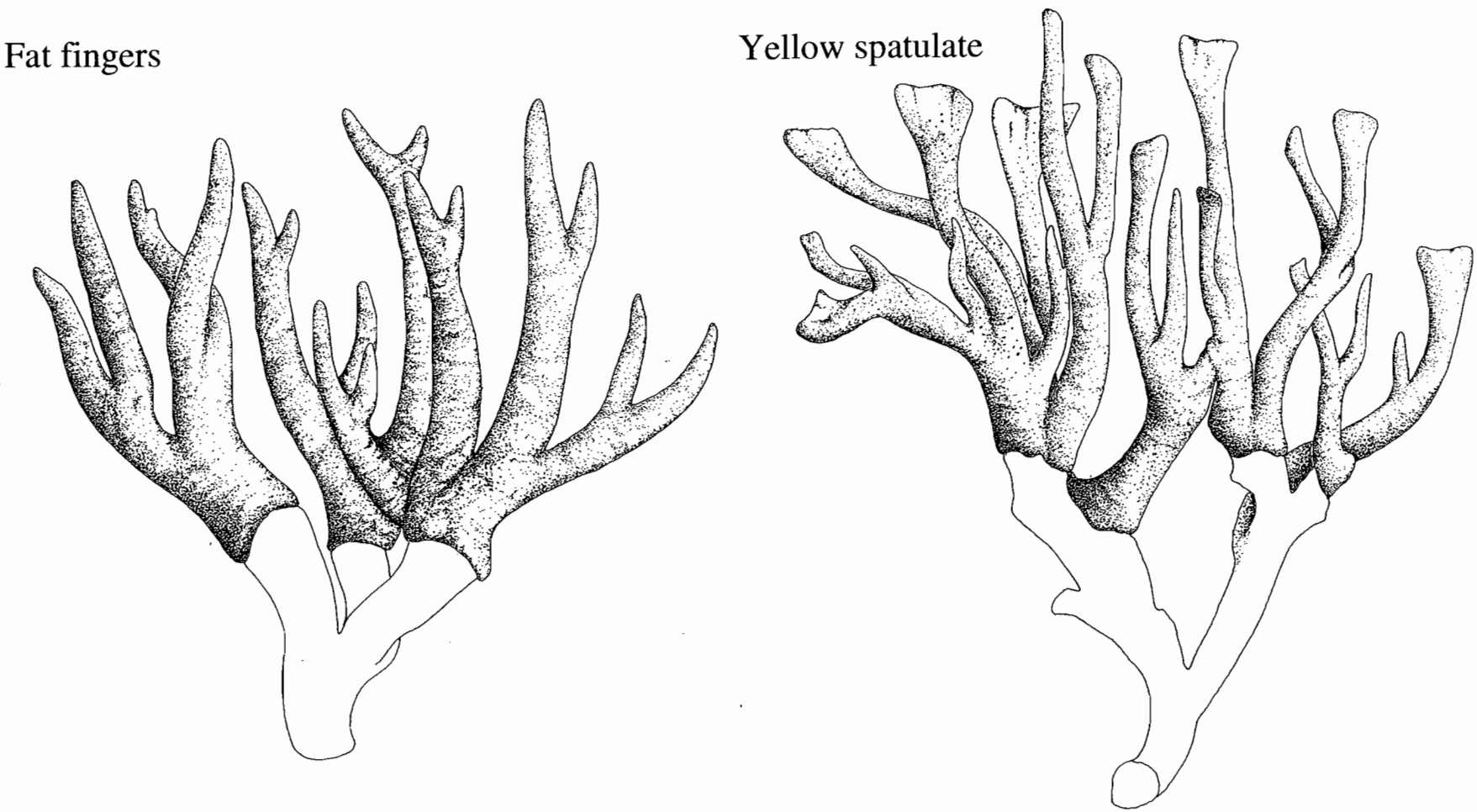


Figure 1.1. Typical morphology of fat fingers and yellow spatulate morphs of *Montipora digitata*.

1.5 Aims and Objectives

The aim of this study is to use several alternative taxonomic techniques to study the species status of two morphs of *Montipora digitata*, and to use the techniques to compare and contrast the results of traditional and alternative taxonomy. The ultimate goal is to determine the biological basis for the two morphs (or species). These aims were addressed using the following approaches:

(1) Are the two morphs different species?

Allozyme electrophoresis is first used to determine whether there is evidence of gene flow between the two morphs. This technique can be used to demonstrate reproductive isolation between species, and is particularly useful for detecting sibling species. [Chapter 2]

(2) How do they maintain their species integrity?

Breeding experiments are used as an independent test of the findings of the electrophoretic study, and then to investigate how species integrity is maintained. Breeding experiments are also used to assess reproductive compatibility both within the morphs, and between the morphs and other species in the genus *Montipora*. [Chapter 3]

(3) Could traditional taxonomic techniques be used to define the two species?

A detailed morphometric study is carried out based on numerical taxonomic techniques to determine whether the two species can be distinguished morphologically or whether they should be considered sibling species. [Chapter 4]

(4) Is there evidence the two have diverged in their reproductive ecology and biology ?

A detailed study of the reproductive ecology and growth of the two species is conducted to determine whether there is any evidence of divergence in these aspects of their life history and biology. The reproductive ecology of the two morphs is also compared to that of some other species within the genus *Montipora*. [Chapter 5]

Finally the findings of this study are discussed in relation to the evolution of coral species, mechanisms of speciation, and the ecological relevance of species described by traditional taxonomy. [Chapter 6]

Chapter 2

Genetic differentiation between two morphs of *Montipora digitata* (Dana 1846) using allozyme electrophoresis

2.1 Introduction

Due to the high levels of intraspecific skeletal variation exhibited by corals, and the overlap of morphologial characters between species, traditional taxonomic techniques are not always sensitive enough to distinguish coral species (see Chapter 1). It has been recommended that several techniques be used simultaneously to determine species limits within the Scleractinia in order to maximise the likelihood of detecting species boundaries (Willis 1990). Numerical taxonomic techniques (Wallace 1974, Foster 1984), allozyme electrophoresis (Ohlhorst 1984), DNA hybridisation techniques (McMillan and Miller 1988) and inter-specific aggressive responses (Lang 1971, Knowlton *et al.* 1992) have all been used for determining the species status of corals. There are many other techniques that can be used for taxonomic purposes, but they have rarely been used (reviewed in Lang 1984). Of all the techniques available, molecular techniques have become particularly attractive to coral taxonomists. Molecular techniques provide a powerful means of detecting sibling species (Richardson *et al.* 1986), and the results they produce are not subject to the environmentally induced variation that can affect skeletal characters (Ohlhorst 1984, Ayala 1983).

Allozyme electrophoresis is one such molecular technique that that has become extremely popular amongst taxonomists. As well as having been used extensively to detect sibling species on land (Richardson *et al.* 1986), allozyme electrophoresis has also been used for determining the species status of a wide range of marine taxa. These include sharks (Lavery and Shaklee 1991), bryozoans (Thorpe *et al.* 1978, Jackson and Cheetham 1990), molluscs (Hillis and Patton 1981, Munksgaard 1990, Morrow *et al.* 1992, Brickner *et al.* 1993, Backeljau *et al.* 1994, Yeatman and Benzie 1994), polychaetes (Grassle and Grassle 1976, Fong and Garthwaite 1994, Schmidt and Westheide 1994), crustaceans (Abdullah and Shukor 1993, Taylor and Herbert 1994) and cnidarians (Solé-Cava and Thorpe 1987, Solé-Cava and Thorpe 1992, Russo *et al.* 1994) to name a few. Allozyme electrophoresis is being used with increasing frequency to solve problems in coral taxonomy (Ohlhorst 1984, Ayre *et al.*

1991b, Knowlton *et al.* 1992, Van Veghel and Bak 1993, Brazeau and Harvell 1994, Garthwaite *et al.* 1994). Results from allozyme electrophoresis have generally supported morphological interpretations of species boundaries (Ayre and Willis 1988, Ayre *et al.* 1991b, Van Veghel and Bak 1993, Garthwaite *et al.* 1994). Instances where allozyme electrophoresis contradicts the morphological results are rare (Knowlton 1992, Miller 1994), though this may be due to the relatively few electrophoretic studies carried out on corals to date. Allozyme electrophoresis has also been used for the study of coral population genetics (Stoddart 1984a, Ayre and Willis 1988, Ayre and Dufty in press), to clarify aspects of the reproductive biology of corals (Stoddart 1983, Stoddart *et al.* 1988), to construct phylogenies (Garthwaite *et al.* 1994), for testing the accuracy of histocompatibility methods to detect clones (Heyward and Stoddart 1985, Resing and Ayre 1985, Willis and Ayre 1985), and to study relative contributions of sexual and asexual reproduction (Hunter 1993, Ayre and Dufty in press).

The aim of this study was to use allozyme electrophoresis to investigate the taxonomic status of *Montipora digitata.* Preliminary breeding trials suggested that the two morphs of *M. digitata* described in Chapter 1 were reproductively isolated (Willis *et al.* 1992). Allozyme electrophoresis offers an independent means of testing whether the populations of the two morphs are reproductively isolated. It was therefore hypothesised that sympatric populations of the two morphs were in fact sibling species, and should possess detectable fixed gene differences. A secondary objective of this study was to compare levels of asexual and sexual propagation between the two morphs. Mode of reproduction is an important life history trait that can also reflect species differences. Results of the electrophoresis study were also used to classify colonies into genetic groupings for subsequent reproductive and morphometric studies (chapters 3 and 4 respectively).

2.2 Materials and methods

2.2.1 Study sites

For convenience all study sites used during the course of this work will be described here. Two main study locations, Magnetic Island and the Palm Islands, were chosen for this study. These two locations are only separated by approximately 65 kilometres, so a third location, Low Island, 275 Km north of the Palm Islands, was also used for part of this study (Figure 2.1). These locations were selected because *M. digitata* was abundant at all of them, and they were easily accessible.

Both Magnetic Island and the Palm Islands are densely vegetated continental Islands (Hopley 1970) with fringing reefs, whereas the Low Isles are coral islands

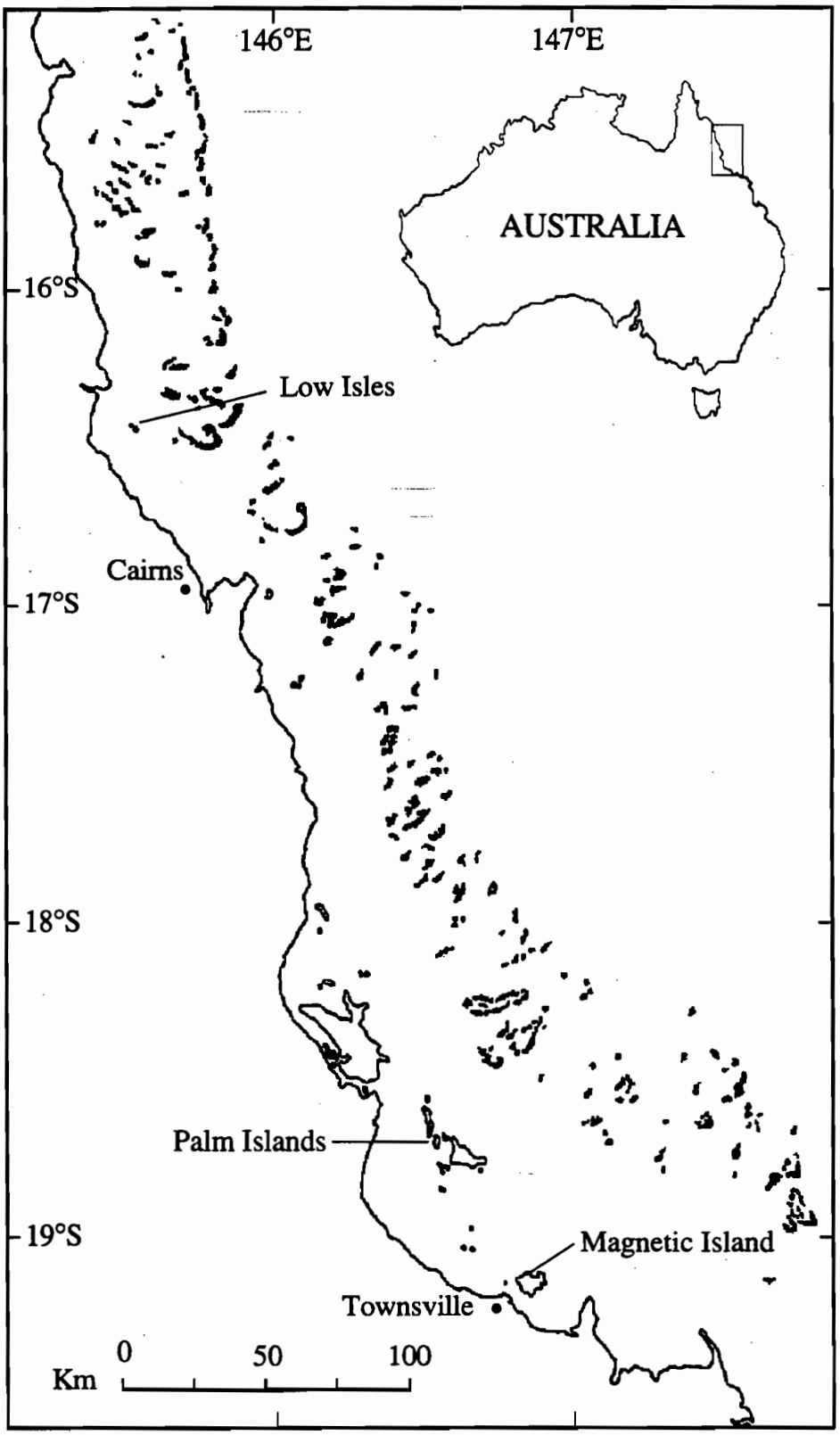


Figure 2.1. Map showing the geographical location of study sites used during the course of this project.

with extensive reef flats and large dense stands of mangrove. Two study sites, Nelly Bay (NB) and Geoffrey Bay (GB), were selected at Magnetic Island (19°10'S, 146°52'E). Geoffrey bay was the main study site (Plate 2.1). In both bays there are large stands of *Montipora digitata* on the reef flat and plate species of *Montipora* on the reef slope. The physical and biological setting at Magnetic Island is reviewed in detail by Morrissey (1980) and Bull (1982). In the Palm Islands the main study site was Orpheus Island (18°40'S, 146°30'E)(Figure 2.2a). Corals were sampled from Pioneer Bay (PB), Hazard Bay (HB) and North-East Reef (NER). *Montipora digitata* is abundant on the reef flat at Pioneer Bay and Hazard Bay, but not North-East Reef. Plate species are only found in abundance at North-East Reef. At the third site, Low Isles, corals were sampled from Low Island (Figure 2.2b) where *M. digitata* is again abundant on the reef flat. Supplementary sampling was carried out at Esk Island (Palm Island group, Figure 2.2a).

Sampling for allozyme electrophoresis was carried out at all three locations. Two sites, Geoffrey Bay and Nelly Bay, were sampled at Magnetic Island. Three sites, Pioneer Bay, Hazard Bay and the northern tip of Esk island, were sampled in the Palm Islands. And one site, Low Island, was sampled in the Low Isles.

2.2.2 Sampling and storage

Sampling was carried out between October 1991 and April 1993 with 50% of the samples being collected between March and July 1992. All samples from Low Isles were collected in February 1993. An initial sample of two small FF colonies and two YS colonies was collected in June 1991. These samples were used during the initial screening procedure to determine which enzyme systems would work, and were also retained throughout the study period for use as electrophoretic markers. A total of 263 samples were collected for this study (FF n = 130, YS n = 133). Seventy percent of the samples were collected specifically for electrophoresis (total n = 185; GB = 40, NB = 40, PB = 20, HB = 25, ESK = 20, LI = 40). For these samples equal numbers of each morph were selected from as wide an area as possible at each site. No one colony was sampled less than five meters from another unless of differing morphology. The remainder of the samples were obtained from colonies used for breeding experiments (n = 78). All colonies used in the breeding experiments were sampled for electrophoresis, these samples were integrated into the main genetic analysis, and the outcome of the analysis was used to confirm identification for the breeding experiments (see Chapter 3). Good representatives of each morph (FF n = 109, YS n = 100) as well as intermediate morphs (n = 54) were represented in the final sample.

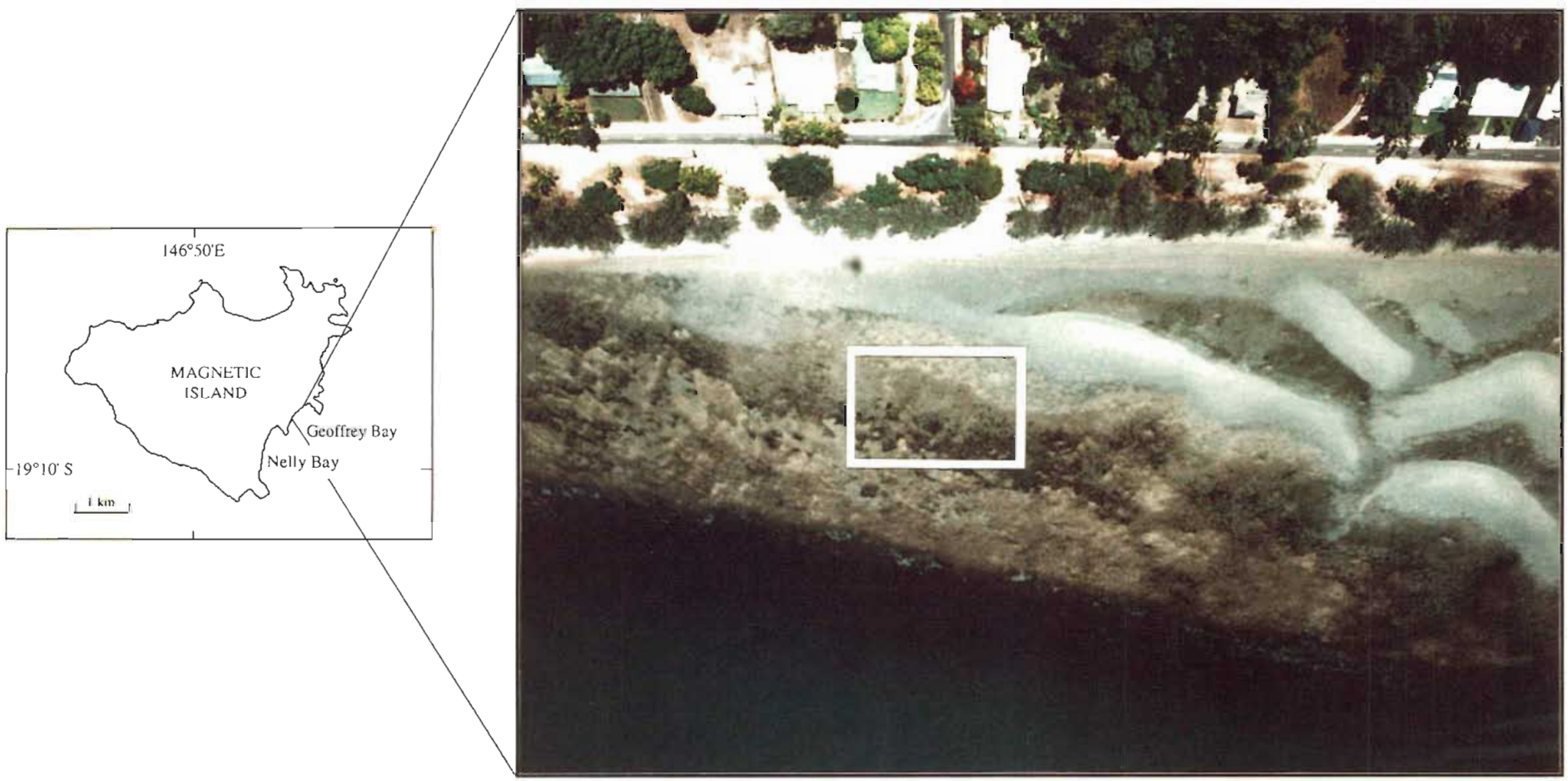


Plate 2.1. Map of study sites at Magnetic Island and aerial photograph of main study site at Geoffrey Bay. Boxed area indicates zone in which labelled colonies for gametogenic study were located.

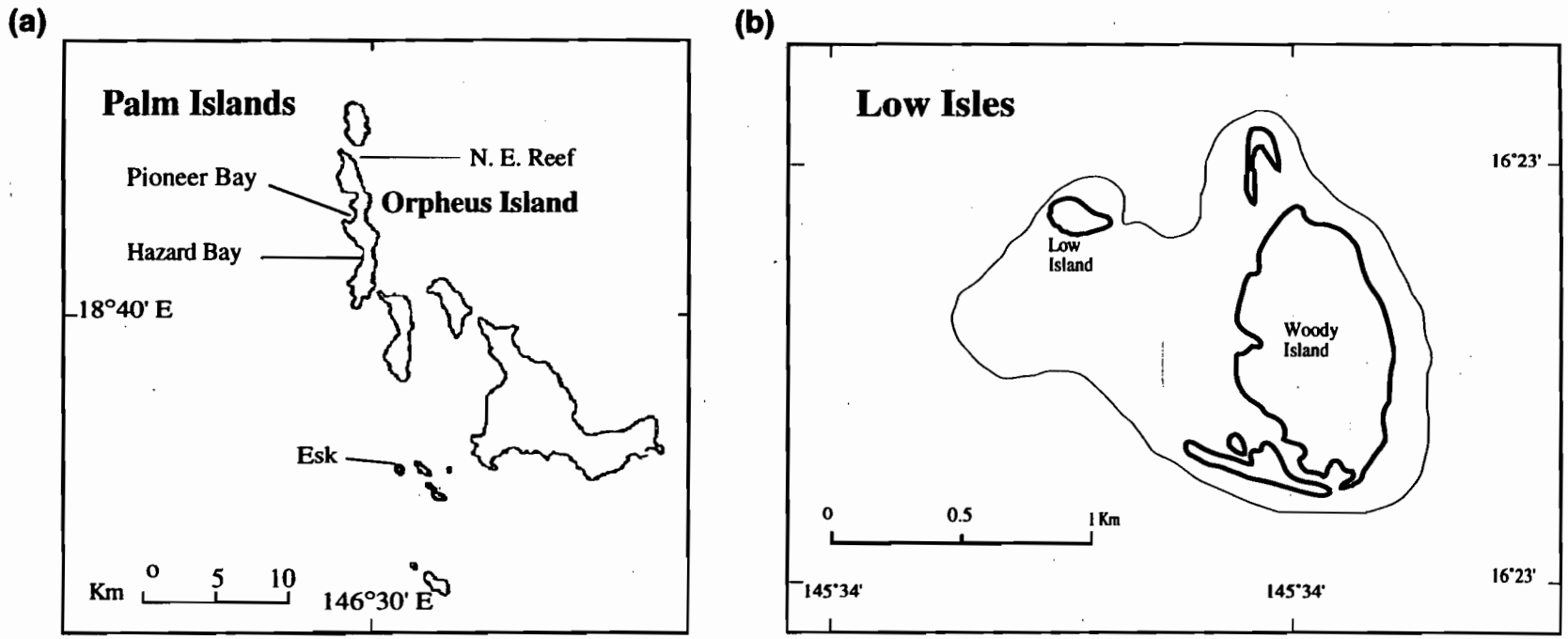


Figure 2.2. Maps of study sites in the Palm Islands (a), and at Low Isles (b).

Several branch tips were removed from each coral sampled using a pair of bone cutters. They were then placed in labelled 5 ml opaque plastic screw cap tubes (clear tubes shatter easily when frozen) and snap frozen in liquid nitrogen, or occasionally on dry ice. Branch tips were preferentially chosen as they rarely contained boring organisms, enzyme activity was good, and they were easier to crush in preparation for the loading of gels. All samples were stored in a -80°C freezer pending analysis. No sample, with the exception of markers, was stored for more than six months prior to analysis.

2.2.3 Screening for enzyme systems

Initial screening was carried out in order to determine which enzyme systems would be suitable to investigate the genetic structure of *M. digitata* morphs. The protocol used to develop such systems was based on the strategy outlined in Richardson *et al.* (1986). For initial screening both cellulose acetate (Cellogel®, Chemetron, Italy) and 12% starch gels were used. Laboratory equipment and methodology used to run cellulose acetate gels is given in Richardson *et al.* (1986), and that for starch gels is given in McDonald (1985) and Shacklee and Keenan (1986). Buffer solutions used to make the starch gels are detailed in the appendix.

Three main buffers were used for screening of enzymes using cellulose acetate gels (TM, PHOS and CP). The main buffers used for screening enzymes on starch gels were TC8, TEC and TEB. All buffer and stain recipes are given in the appendix. During screening, Cellogel was cut into small strips about 4-5 cm wide before loading the same samples onto each strip. For starch gels the samples were repeated across the gel and then it was cut into strips after samples had been run. Using small pieces of each gel was an efficient and cost-effective way of assaying many different stains. Once a potentially useful system was detected and further improvement was required, alternative buffers were tested. For a list of buffers tested see tables 2.1 and 2.2. When improving systems, a larger number of samples was added to the gels in order to establish the degree of variation in the system, and to check that the same enzyme was not being observed in several stains. In cases where activity was clearly present, but none of the buffers provided adequate resolution, several other variables were manipulated. For example, in some cases where separation was insufficient, the concentration of buffer was reduced to increase the running distance of the proteins and hopefully increase separation. Alternatively running time was varied, pH altered, or samples centrifuged to obtain a "cleaner" supernatant.

2.2.4 Sample preparation

Pieces of coral 3-5 mm long were broken from the sample and placed in a labelled ceramic depression plate along with 1-2 drops of an 0.04% aqueous solution of b-mercaptoethanol. On thawing, the samples were ground using a stainless steel grinding rod. Up to three more drops of b-mecaptoethanol were added to samples during grinding if the samples were very dry. Ceramic plates were kept on ice both prior to, and after the grinding of samples.

For loading Cellogel samples a small well was made in the crushed sample and fluid allowed to collect in it, a draftsman's pen was used to collect the sample and load it onto the Cellogel. For starch gels a square of tissue was placed on the ground samples (approximately 6 mm square piece of Kimwipes® followed by wicks made from chromatography paper; Whatman No. 3, 1.5*4mm) which were allowed to soak up the sample through the tissue. The tissue, acting as a crude filter, greatly reduced the amount of mucus on the wicks. This has been shown to reduce problems with smearing and warping (Benzie, 1990). Prior to loading starch gels wicks were blotted on a tissue and then inserted in order into a cut parallel to, and approximately one-sixth way from the gel base. A wick soaked in bromophenol blue was placed at the end of each gel in order to monitor the speed at which the samples moved through the gels. Marker samples were placed at intervals along all gels to allow cross correlation of enzyme bands between different gels.

All polymorphic systems were screened using starch gel electrophoresis as none resolved well on Cellogel. Samples were run horizontally, either along the length of the gel (n = 18), or across the width of the gel (n = 28). Running time for gels was initially 5 hours at 200-400 volts depending on the buffer, but it was found more practical to run gels overnight (running 28 samples across the gel) for 17-18 hrs at 70-120 volts. The longer running time did not reduce resolution or activity.

Following electrophoresis, gels were sliced into 2-3 layers allowing as many enzyme systems to be assayed. The sections were laid out flat on a perspex board and stained using 10 ml of stain solution and 10 ml agar. The agar was kept liquid in a 60°C oven and added to the stain solution immediately prior to pouring over the gel surface. This is essential as once certain stains are mixed with the agar it sets very rapidly. Gels were scored as soon as they could be resolved, they were never left until they had overstained. Once all gels had been scored, a second score was made to reconfirm the first. This was particularly useful for checking any stains that were not clearly resolved during the first scoring. Normally all stains used could be scored within three hours of staining.

Out of a total of thirty-nine enzyme systems surveyed, a total of six were chosen for this study for reasons detailed in the results section. The final systems screened were peptidase EC 3.4.11* using leucyl tyrosine (LT) and leu-gly-gly (LGG) substrates and a LiOH buffer, phosphoglucomutase EC 2.7.5.1 (PGM) and malate dehydrogenase EC 1.1.1.37 (MDH) using a TEC 7.9 buffer (modified from recipe 2 of Soltis *et al.* 1983 by addition of EDTA), and malic enzyme EC 1.1.1.40 (ME) and Hexokinase EC 2.7.1.1 (HK) using a TC8 buffer. Superoxidase dismutase EC 1.15.1.1 (SOD) also appeared on HK stained gels and was scored. All stain recipes are given in the appendix. Enzyme nomenclature follows the format suggested by Shaklee *et al.* (1990), and details of all enzymes used during this study are given in Table 1 in the appendix.

The actual distance travelled by proteins was scored for all gels. The marker samples were used to account for warping effects across the gels. To standardise all results the most common allele at each locus was used as the standard "100" allele (always identified relative to the marker). Migration of this allele was measured from the base of the gel, and all other alleles were then scored relative to the "100" allele by measuring their migration differences in millimeters. Scored distances were subsequently related to the standard allele as follows:

- common allele = x/x * 100 = 100
- other alleles = y/x * 100 = n

where x is the distance travelled by the common allele, y the distance travelled by the allele being related to the common allele, and n a standardised value. This procedure is necessary to account for the differences in mobility of protein bands between runs.

2.2.5 Statistical analyses

Individual colonies were first separated into genetic groups using principal coordinates analysis in the PATN pattern analysis package and applying the Gower metric algorithm (Belbin 1987), for which each allele was coded as a separate character, deleting those for which there was no variation in the total data set. Gene frequencies, basic statistics of genetic variability, tests for conformance to Hardy-Weinberg expectations, and clustering procedures were performed using the BIOSYS package (Swofford and Selander 1981). Tests for conformance of gene frequencies to those expected under conditions of Hardy-Weinberg equilibrium used the exact probabilities (Elston and Forthofer 1977) and significance values were adjusted for multiple simultaneous tests (Miller 1966). Weir and Cockerham's (1984) F-statistic was used to determine F_{IS} (within-population variation) and F_{ST} (differentiation among populations), and the statistical significance of F_{IS} and F_{ST} was calculated using equations given in Waples (1987).

Genotypic diversity expected for sexually reproducing populations (G_E) was calculated with compensation for small sample size following Stoddart (1983). This is the expected genotypic diversity under Hardy-Weinberg equilibrium and linkage equilibrium. Observed genotypic diversity (G_O) was calculated using the formula $G_O = 1/\Sigma p_i^2$ (Stoddart and Taylor 1988). The overall effect of asexual reproduction was assessed as the ratio of G_O to G_E. A sexually reproducing population should display a G_O:G_E ratio of approximately one, whereas a genetically variable population with high levels of asexual recruitment should display a lower value (Ayre 1984).

2.3 Results

2.3.1 Enzymes surveyed

Many of the enzymes exhibited good activity and resolution on Cellogel (summarised in Table 2.1), but the results were never as good as for starch gels. In an attempt to improve the resolution and separation of EST, ENOL and ACON on Cellogel, centrifuged samples were tested as well as samples run on half concentration buffer. Centrifuging did improve resolution, but reducing the buffer concentration made it worse. It was therefore decided to run all systems on starch. The activity and resolution of all enzymes tested on starch gels is given in Table 2.2, along with an indication of whether the enzyme was variable or not, the buffers tested, and the buffer that gave the best results. Details of systems not used in the final study, but which could be made to work for future studies relatively easily are given in the appendix (Table 2).

2.3.2 Details of systems used in this study

Enzyme systems LT , LGG and ME formed one-banded and three-banded phenotypes corresponding to dimeric homozygotes and heterozygotes, respectively, whereas PGM formed one-banded and two-banded phenotypes corresponding to monomeric homozygotes and heterozygotes, respectively (figures 2.3-2.6). SOD was also scored on gels stained for HK but was invariant; appearing only as one-banded homozygotes. Unfortunately HK could not be included in the final analysis as scoring was not consistent throughout the study due to problems with warping and inconsistencies in staining from day to day.

LT showed two strongly staining loci, one of which was invariant (Figure 2.3 and Plate 2.2). In FF samples there appeared to be two alleles in the region of LT-2,

ENZYME	ACTIVITY	RESOLUTION	VARIABLE?	BEST BUFFER
AAT(UV)	+	-	-	-
AAT(Fast violet)	+++	**	Y	-
ACON	+++	**	N	-
ADH	+	-	-	-
AK	+++	**	Y	CP
CK	++	*	Y	-
DIAPH	++	*	Y	-
ENOL	+++	**	Y	TM, PHOS
EST	+++	**	Y	TM
GDH	++	*	N	-
G-6PD	+	-	-	-
GPI	+++	**	Y	PHOS
HK	+++	**		
IDH	++	**	Y	TM
LDH	+	-	-	-
MDH	+++	***	Y	-
ME	+++	**	Y	-
MPI	+	-	-	-
NP	+	-	-	-
Pep(LG)	+++	**	Y	-
Pep(LP)	+++	**	Y	CP
Pep(LT)	+++	**	Y	-
PGK	+	-	-	-
PGM	+++	***	Y	-
PK	+++	**	Y	-
SOD	+	-	N	-
SDH	+	-	-	-
TPI	+	-	-	-

Table 2.1. Primary survey of all enzyme systems tested on cellogel using the buffers CP, PHOS and TM. Activity, (+): none; (++): some; (+++): good. Resolution, (*): inadequate; (**): has potential; (***): good. Variability, (Y): yes; (N): none observed.

Enzyme	Activity	Resolution	Variable?	Buffer
AAT(UV)	-	-	-	TEB, TEC, TC6, TC8, LIOH
ACON	-	-	-	TEB, TEC, TC7, POUL
ACP	+	*	-	TC6, TC8, TEB, TEC
ADH	+	*	N	TC7, TEC, TEB
AK	+	*	N	TEC, TEB, TC7
ALD	-	-	-	TEC
ALP	+	*	-	TC6, TEB
CK	++	*	N	TC7, POUL, TEC,TEB
DIAPH	++	**	N	(TC8), TC6, LIOH, TEC, TC7, POUL
ENOL	++	**	Y	(TC6), TC8, LIOH, TC7, POUL, TEC, TEB
EST	++	**	Y	(TEB, TEC), TC6, TC8
EST (fluorescent)	++	**	Y	(LIOH), TEC
FBP	+	*	-	TC8
FUM	-	-	-	TC8, TEC
GA3PD	-	-	-	TEB
GDA	-	-	-	TEB
GDH	-	-	-	TC6, TEB, TEC, TC8, TC7, POUL
GLDH	+	*	-	TEB, TEC, TC8
G3PD	-	-	-	TEB
G-6PD	+	*	-	TEB, TEC, TC8
GPI	++	**	Y	(TC8), LIOH, TC6, TC7
GPT	-	-	-	TC8
GSR	++	**	Y	TEB, TC8, TEB, LIOH
HBOH	-	-	-	TC8
HK	++	**	Y	(LIOH), TC8, TC6, TC7, POUL, TEC, TEB
IDH	++	*	Y	TEB, TEC, TC7
LDH	+	*	N	TC7, TEC, TEB
MDH	++	***	Y	(TC8), TEB, TEC, TC7
ME	++	***	Y	(TC8), TC6, LIOH, TEC, POUL, TC7
MPI	-	-	-	TC6, TC7, TC8
NP	-	-	-	TC6, TC8
Pep(LG)	++	***	Y	(LIOH), TC8, TC6, TEC, TEB
Pep(LP)	++	**	Y	(LIOH), TC6, TC7, TC8, TEB, TEC
Peo(LT)	++	***	Y	(LIOH), TC6, TC8, TEB, TEC
PGK	-	-	-	TEB, TEC
PGM	++	***	Y	(TEC), TC8, TC6
SOD(ON HK)	++	***	N	(TC8)
TPI	+	*	-	TC8, LIOH

Table 2.2. Primary survey of all enzyme systems tested on starch gels. Activity, (+): none; (++): some; (+++): good . Resolution, (*): inadequate; (**): has potential; (***): good. Variability, (Y): yes; (N): none observed . brackets indicate best buffer.

however they were extremely close together (1 mm apart) and were therefore scored as *LT-2*100*. There was additional variation for YS samples in the LT-2 region, but although separation was often good, resolution was too poor to allow reliable scoring, so all alleles in this 1-2 cm wide region on the gel were therefore scored as *LT-2*55*. The variation at *LT-2*55* never overlapped with that at *LT-2*100*.

LGG also showed two strongly staining loci, again one being invariant. Though resolution was not always good on LGG-2 there was little warping and scoring was considered reliable enough to avoid grouping alleles as for LT. Such scoring was not possible on other buffers tested as they produced considerable warp. Six allelic variants occurred at LGG-2 in the combinations shown in Figure 2.4.

PGM stained very strongly and fast so gels had to be scored promptly. There were three allelic variants on one locus (Figure 2.5, Plate 2.3). Occasionally a second locus which was not scored would appear much higher up the gel (Figure 2.5). Scoring of PGM gels was unequivocal and consequently re-runs were rarely necessary.

Both MDH and ME developed slowly and bands were often faint with poor separation of variable alleles. In both of these systems, however, the resolution was good, making scoring reliable. MDH would occasionally develop on ME stained gels above the ME locus, confirming that the two systems were not being confounded. Two allelic variants were detected on MDH-1, and three allelic variants on ME (Figure 2.6).

2.3.3 Survey results

Principal coordinates analysis of all unique genotypes detected in the populations demonstrated two distinct groupings (Figure 2.7). All colonies having an unequivocal FF morphology clustered together to the right of the PCA plot. All colonies having an unequivocal YS morphology clustered together on the left of the PCA plot. Morphologically intermediate specimens clustered at random into one of these two groups rather than forming a separate cluster, or occupying an intermediate position between the genetic groupings of FF and YS individuals. Clustering did not correlate with geographic location. There were some genotypes that separated from the main groupings of both morphs along the second PCA axis (Figure 2.7). These genotypes did not represent intermediate or identifiable morphologies and consisted of 5 unequivocal FF and 14 unequivocal YS morphotypes. Eighty-nine percent of the variation in the data series was accounted for by the first PCA axis. Only 10% was associated with the scatter along PCA axis 2, and 0.6% with PCA axis 3.

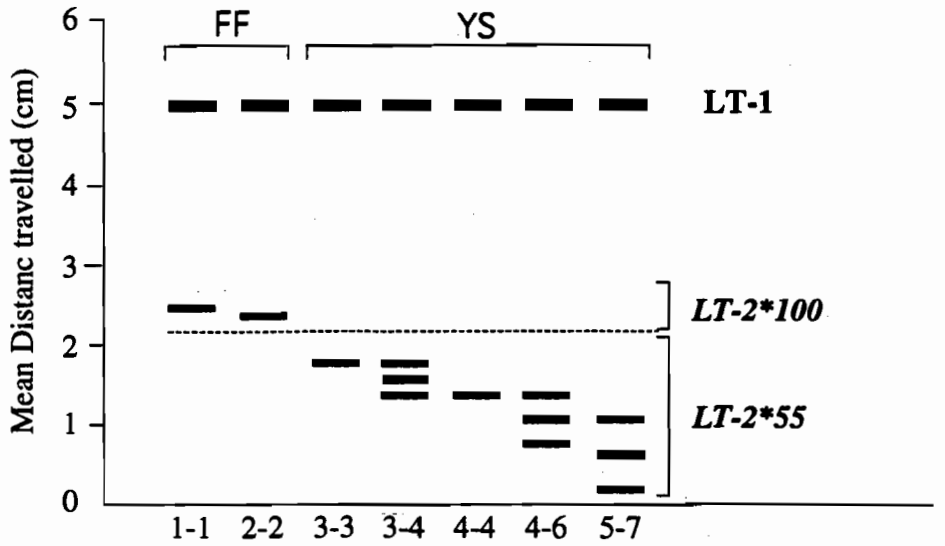


Figure 2.3. Enzyme variants observed for LT (LiOH buffer), showing the variants found for the FF and YS morphs of *Montipora digitata*, and the combinations of enzyme variants fused for these morphs as *LT-2*100* and *LT-2*55* respectively.

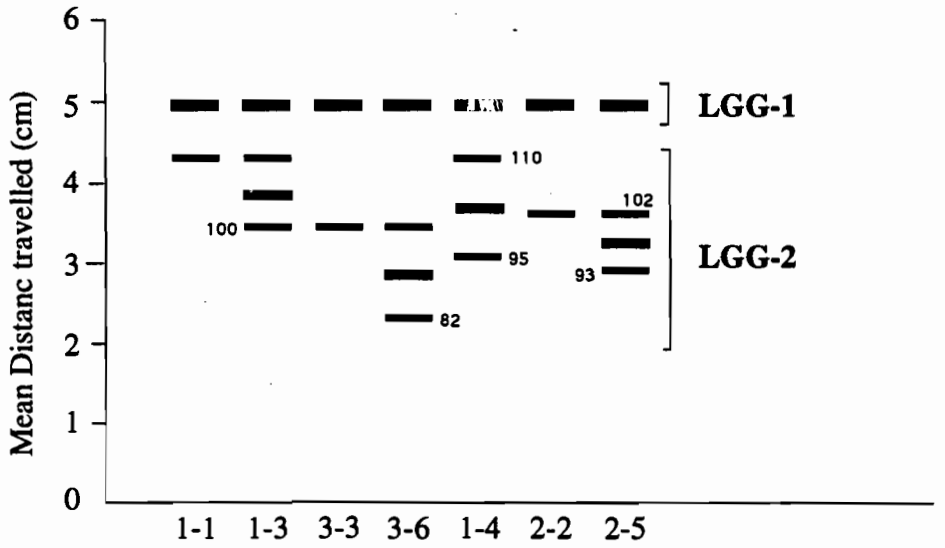


Figure 2.4. Enzyme variants observed for LGG (LiOH buffer). Numbers on diagram are standardised allele numbers.

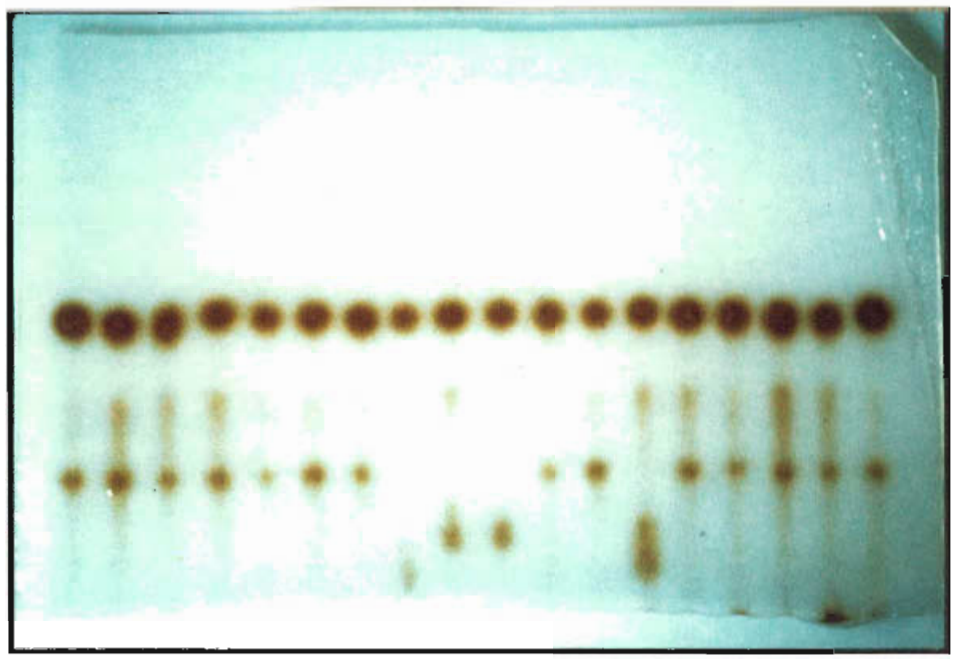


Plate 2.2. Starch gel zymogram stained for LT (LiOH buffer), showing the variants found for the fat fingers (FF) and yellow spatulate (YS) morphs of *Montipora digitata* (see Figure 2.3). The variants at *LT-2*100* and *LT-2*55* were fixed for FF and YS morphs respectively. Note variability at *LT-2*55* that could not be scored due to poor resolution.

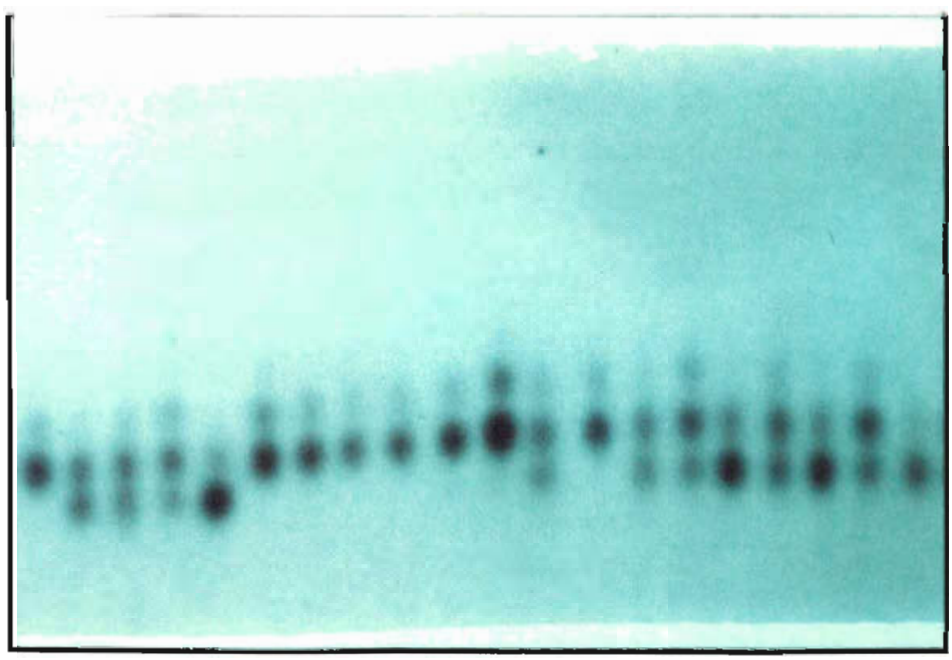


Plate 2.3. Starch gel zymogram stained for PGM (TEC 7.9 buffer), showing the variants found for the fat fingers (FF) and yellow spatulate (YS) morphs of *Montipora digitata* (see Figure 2.5). The unscored locus had not stained on this zymogram.

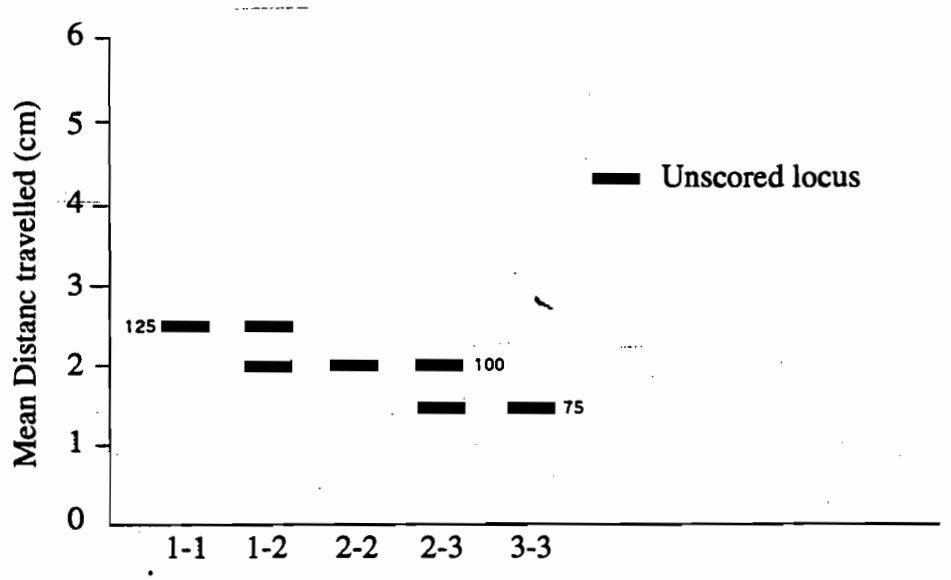


Figure 2.5. Enzyme variants observed for PGM (TEC 7.9 buffer). Numbers on diagram are standardised allele numbers.

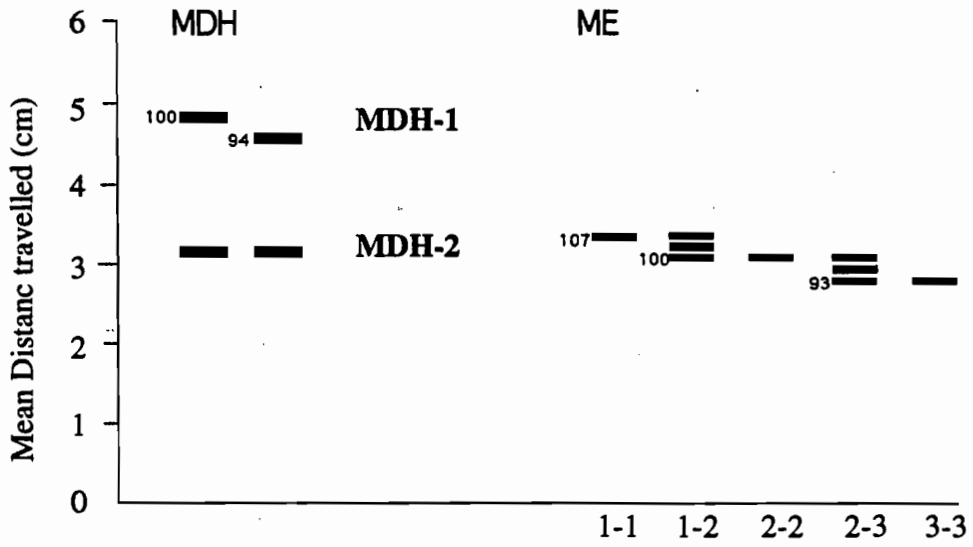


Figure 2.6. Enzyme variants observed for MDH and ME (TC8 buffer). Numbers on diagram are standardised allele numbers.

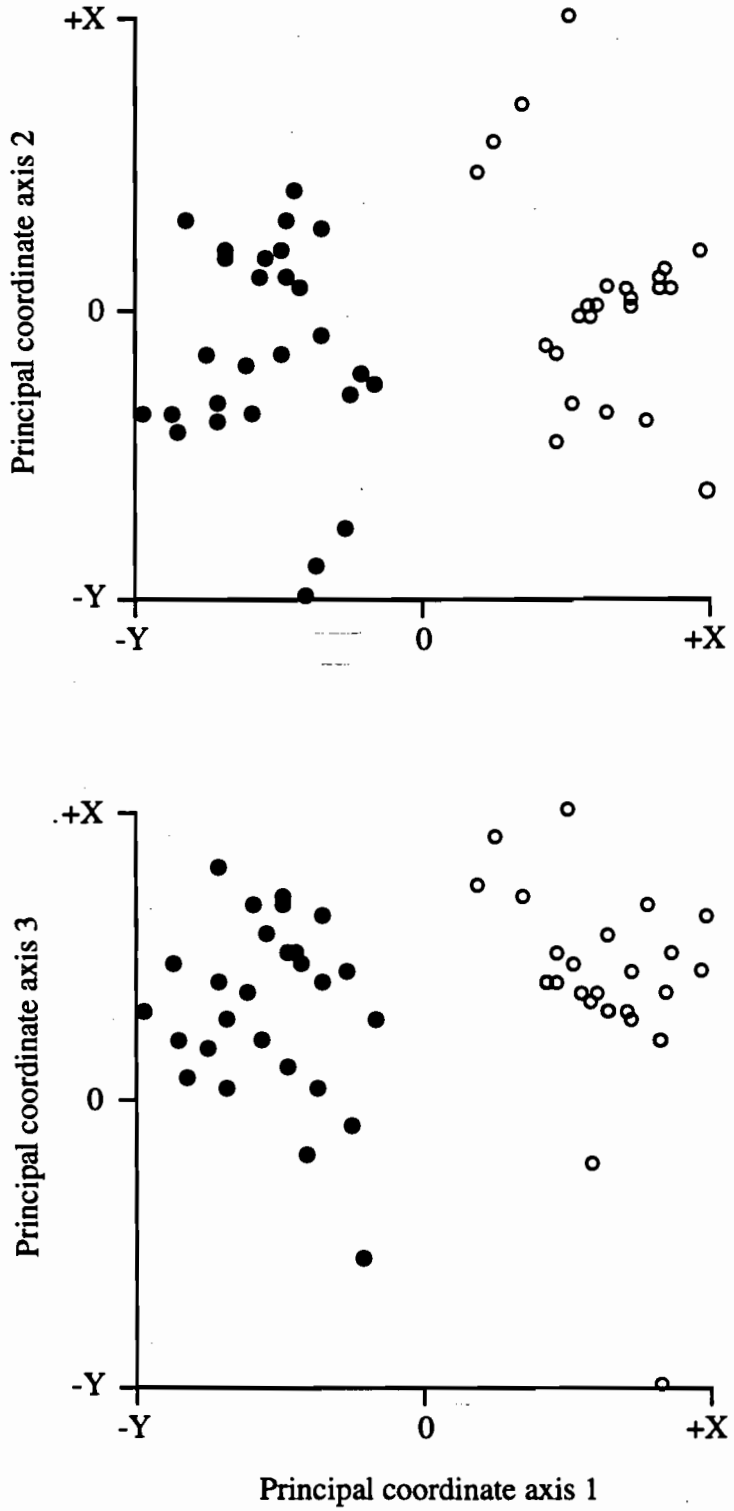


Figure. 2.7. Principal coordinates plot of all unique 5-locus genotypes observed in the *Montipora digitata* populations sampled (n = 263). The first axis accounts for 89% of the variation, the second and third account for 10% and 0.6% respectively. ● = YS, O = FF.

The two groups separated on the PCA plot were distinguished by a fixed gene difference at *LT-2** where all FF individuals had allele **100*, and all YS individuals had allele **55* (Table 2.3). There were also several major shifts in gene frequencies between YS and FF individuals. At *LGG-2**, allele **93* was the most common in YS morphs whereas allele **100* was most common in FF morphs (Table 2.3). *LGG-2*111* was present in five of the six FF populations but absent in all YS populations. For *PGM,* YS individuals had almost equal numbers of **100* and **75* alleles whereas FF individuals had almost exclusively **100* alleles. Similarly, at ME, YS individuals predominantly had **100* and **107* alleles, whereas FF individuals had **100* and **93* alleles. The rare *MDH-1*94* allele occurred only in two FF populations.

All populations showed genetic variation, with 22.2 to 44.4% of the five loci polymorphic, and an average of 1.2 to 2.1 alleles per locus (Table 2.4). Direct count heterozygosities ranged from 0.028 to 0.214 and the mean was 0.053 for FF populations and 0.142 for YS populations. Most of the observed heterozygotes had values lower than those expected under conditions of Hardy-Weinberg equilibrium, however all standard errors overlapped between observed and expected heterozygotes. More detailed tests of conformance of observed gene frequencies to those expected under conditions of Hardy-Weinberg equilibrium using the exact test showed five significant deviations (Table 2.5). Heterozygote deficits for *ME** were observed in two populations of FF (GB heterozygotes Observed = 4, Expected = 14.6; homozygotes O = 44, E = 33.4, and HB heterozygotes O = 0, E = 5.3; homozygotes O = 12, E = 6.7), and two YS populations (GB heterozygotes O = 7, E = 17.4; homozygotes O = 55, E = 44.6, and LI heterozygotes O = 1, E = 9.3; homozygotes O = 22, E = 13.7). Heterozygote deficits for *LGG** were observed in one FF population (HB heterozygotes O = 3, E = 6.2; homozygotes O = 9, E = 5.8).

Genotypic diversity (G_O) varied considerably between populations ranging from 2 to 7.8 in FF populations and from 4 to 9.9 in YS populations (Table 2.4). For FF populations the ratio of observed to expected genotypic diversity (G_O:G_E) varied between 0.61 and 1.16 with an average of 0.79, and for YS populations G_O:G_E ranged from 0.55 to 1.41 and averaged 0.86. Only two YS populations (NB and ESK) and one FF population (LI) had a G_O:G_E ratio greater than one. Populations of both morphs had low G_O:G_E ratios at GB, NB and HB (the NB yellow spatulate estimate is high but not considered valid due to the very small sample size it is based on), and values approaching one at PB. At Esk and Low Isles the ratios were quite different for the two morphs.

Locus, allele	Fat Fingers						Yellow Spatulate					
	Magnetic Island		Palm Islands			Low Isles	Magnetic Island		Palm Islands			Low Isles
	GB	NB	PB	HB	ESK		GB	NB	PB	HB	ESK	
LT-2*												
100	1.000	1.000	1.000	1.000	1.000	1.000	-	-	-	-	-	-
55	-	-	-	-	-	-	1.000	1.000	1.000	1.000	1.000	1.000
LGG-2*												
111	0.417	0.148	-	0.083	0.036	0.618	-	-	-	-	-	-
102	0.031	0.093	0.375	0.125	0.143	-	0.315	-	0.380	0.462	0.333	0.348
100	0.490	0.704	0.458	0.667	0.679	0.382	0.024	0.125	-	-	-	0.065
95	0.021	-	-	-	-	-	-	-	-	-	-	-
93	0.031	0.056	0.125	0.125	-	-	0.653	0.750	0.620	0.538	0.583	0.522
82	0.010	-	0.042	-	0.143	-	0.008	0.125	-	-	0.083	0.065
PGM*												
125	0.104	-	-	-	-	-	-	-	-	-	0.083	-
100	0.875	0.981	1.000	1.000	1.000	0.941	0.524	0.500	0.540	0.308	0.583	0.391
75	0.021	0.019	-	-	-	0.059	0.476	0.500	0.460	0.692	0.333	0.609
MDH-1*												
100	0.979	0.963	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
94	0.021	0.037	-	-	-	-	-	-	-	-	-	-
ME*												
107	0.031	-	-	-	-	-	0.169	-	0.320	0.423	0.167	0.283
100	0.813	0.963	0.917	0.667	0.964	1.000	0.831	1.000	0.660	0.577	0.833	0.717
93	0.156	0.037	0.083	0.333	0.036	-	-	-	0.200	-	-	-
(*n*)	48	27	12	12	14	17	62	4	25	13	6	23

Table 2.3. Gene frequencies at five loci for fat fingers and yellow spatulate morphs of *Montipora digitata* at three sites on the Great Barrier Reef. Magnetic Island: Geoffrey Bay (GB), Nelly Bay (NB). Palm Islands: Pioneer Bay (PB), Hazard Bay (HB), Esk Island (ESK). One sample site only from Low Isles. -: gene frequency is zero; (n): Number of individuals screened.

	Fat Fingers						Yellow Spatulate					
	Magnetic Island		Palm Islands			Low Isles	Magnetic Island		Palm Islands			Low Isles
	GB	NB	PB	HB	ESK		GB	NB	PB	HB	ESK	
Mean No. of alleles per locus	2.1	1.7	1.4	1.4	1.4	1.2	1.6	1.3	1.4	1.3	1.6	1.6
	(0.6)	(0.3)	(0.3)	(0.3)	(0.3)	(0.1)	(0.3)	(0.2)	(0.2)	(0.2)	(0.3)	(0.3)
Polymorphic loci (%)	44.4	44.4	22.2	22.2	22.2	22.2	33.3	22.2	33.3	33.3	33.3	33.3
Heterozygosity:												
Direct count	0.097	0.049	0.037	0.028	0.048	0.059	0.133	0.083	0.147	0.214	0.167	0.106
	(0.062)	(0.045)	(0.037)	(0.028)	(0.039)	(0.046)	(0.079)	(0.059)	(0.078)	(0.115)	(0.096)	(0.069)
Expected	0.131	0.074	0.091	0.112	0.065	0.067	0.140	0.115	0.162	0.163	0.165	0.168
	(0.070)	(0.052)	(0.073)	(0.074)	(0.057)	(0.054)	(0.073)	(0.077)	(0.081)	(0.082)	(0.087)	(0.086)
Nc	17	6	5	4	3	4	16	4	13	6	6	11
G_E	10.43	4.20	4.49	5.40	3.30	2.85	11.04	2.84	10.68	7.55	4.87	12.27
G_O	7.84	3.02	4.00	3.27	2.00	3.32	6.89	4.00	9.92	4.83	5.14	6.70
G_O:G_E	0.75	0.72	0.89	0.61	0.61	1.16	0.62	1.41	0.93	0.64	1.06	0.55

Table 2.4. Mean genetic variability (±SE) for the six fat fingers and the six yellow spatulate populations of *Montipora digitata* surveyed. A locus was considered polymorphic if more than one allele was detected. Expected heterozygosities are unbiased estimates following Nei (1978). Nc is the number of electrophoretically distinct 5-locus genotypes. G_O and G_E are observed and expected genotypic diversities respectively. G_O:G_E is the genotypic diversity ratio.

Population	Locus				
	LT	LGG-2	PGM	MDH-1	ME
1 FF GB	-	0.395	1.000	0.011	0.000*
2 FF NB	-	0.165	1.000	0.019	0.019
3 FF PB	-	0.005	-	-	0.043
4 FF HB	-	0.001*	-	-	0.001 *
5 FF ESK	-	0.003	-	-	1.000
6 FF LI	-	0.624	-	-	1.000
7 YS GB	-	1.000	0.201	-	0.000*
8 YS NB	-	0.143	1.000	-	-
9 YS PB	-	0.391	0.688	-	0.075
10 YS HB	-	0.006	1.000	-	1.000
11 YS ESK	-	0.152	0.394	-	1.000
12 YS LI	-	0.676	0.208	-	0.000*

*<0.05; **<0.01

Table 2.5. Tests for conformance to Hardy-Weinberg equilibrium. Statistics (χ^2 values) for all *Montipora digitata* populations calculated using exact probabilities (Elston and Forthofer 1977) are shown. Significance values adjusted for multiple simultaneous tests (Miller 1966) are shown below the table.

F-statistic analyses demonstrated highly significant differentiation between FF populations (mean F_{ST} = 0.045) but not between YS populations (mean F_{ST} = 0.015) of *M. digitata* (Table 2.6). All polymorphic loci contributed to the significant mean F_{ST} value in FF populations. There was also a highly significant genetic inhomogeneity within the FF populations (mean F_{IS} = 0.479). All polymorphic loci except *PGM** contributed to this effect, and reflected the deviations from Hardy-Weinberg discussed above as well as the lower average genotypic diversity ratio (G_O:G_E). The mean F_{IS} value was not significant for YS populations, although the value for *ME** was significant (Table 2.6).

	Fat Fingers		Yellow Spatulate	
Locus	FIS	FST	FIS	FST
*LGG-2**	0.229**	0.123***	-0.095	0.015
*PGM**	-0.099	0.047**	-0.005	0.013
*MDH-1**	1.000***	0.036*	-	-
*ME**	0.787***	0.045**	0.503***	0.160
Mean ± SE	0.479 ± 0.084 ***	0.063 ± 0.007**	0.135± 0.093	0.063 ± 0.024

*<0.05; **<0.01; ***<0.001

Table 2.6. Summary F-statistic estimates of: within population variations (F_{IS}), and between population variations (F_{ST}) for both morphs of *Montipora digitata*. *LT** is not included as it was monomorphic for a different allele in each taxa.

Genetic distances between populations of the same morph were an order of magnitude less than those between morphs (Table 2.7). Nei's unbiased genetic distance among FF populations averaged 0.014, and among YS populations averaged 0.007, while the average genetic distance between FF and YS populations was 0.237. There was no overlap between standard errors calculated for among population and between population genetic distances (e.g. Standard error for highest among population of 0.035 was 0.042, while that for the lowest between population genetic distance of 0.180 was 0.099: calculations of standard errors were made using formulae in Nei, 1987). Cluster analysis (Figure 2.8) clearly showed the separation between YS and FF populations, and greater separation among FF populations than among YS populations.

Population		1	2	3	4	5	6	7	8	9	10	11	12
1	FF GB	-											
2	FF NB	0.009	-										
3	FF PB	0.019	0.008	-									
4	FF HB	0.012	0.008	0.010	-								
5	FF ESK	0.015	0.001	0.005	0.009	-							
6	FF LI	0.006	0.019	0.031	0.035	0.026	-						
7	YS GB	0.232	0.232	0.203	0.235	0.233	0.237	-					
8	YS NB	0.222	0.218	0.205	0.230	0.220	0.222	0.003	-				
9	YS PB	0.240	0.245	0.210	0.239	0.245	0.250	0.001	0.016	-			
10	YS HB	0.285	0.294	0.256	0.288	0.294	0.297	0.013	0.035	0.004	-		
11	YS ESK	0.210	0.211	0.180	0.215	0.210	0.216	0.000	0.000	0.000	0.013	-	
12	YS LI	0.246	0.250	0.221	0.251	0.249	0.255	0.003	0.013	0.000	0.000	0.000	-

Table 2.7. Nei's unbiased genetic distance (Nei 1978) between each pair of *Montipora digitata* populations. Shaded area indicates genetic distances between morphs.

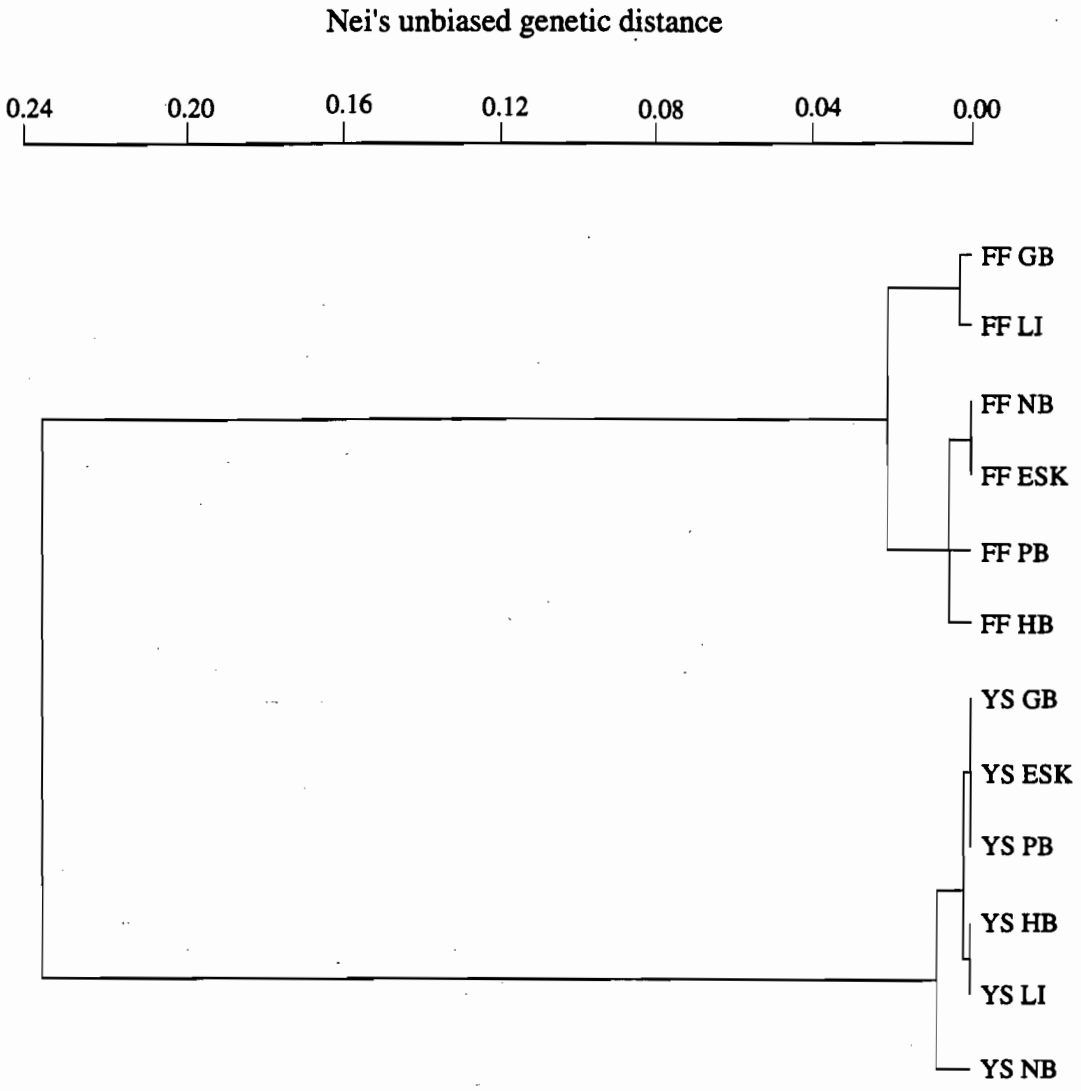


Figure 2.8. Dendrogram based on unbiased genetic distances (Nei 1978) among all 12 *Montipora digitata* populations surveyed. Values were clustered using the UPGMA algorithm (Cophenetic correlation for the dendrogram was 0.98).

2.4 Discussion

The analysis of allozyme variation demonstrated that the two morphs of *M. digitata* should be considered to be two distinct species. The existence of one fixed difference at *LT-2**, as well as allelic frequency differences at the four other loci examined, is clear evidence that the two morphs of *M. digitata* represent sympatric populations which are reproductively isolated. This is further supported by the fact that the same genetic differences were found in all of the six populations sampled. Though the occurrence of one fixed gene difference does not always prove species status, and ideally there should be a minimum of two diagnostic fixed differences (Richardson *et al.* 1986), the occurrence of strong gene frequency differences at three loci as well as the fixed gene difference provides very strong evidence of reproductive isolation.

Species status can also be inferred using Nei's genetic distance (D). Thorpe (1982), has suggested that a Nei's genetic identity (I) of 0.85 be used as a cut-off point for considering allopatric taxa to be of specific status. An I of 0.85 corresponds to a D of 0.16 (Fong and Garthwaite 1994), which is lower than the average D of 0.24 found between FF and YS populations. While the estimates of genetic identity should be evaluated with caution due to their large standard errors, the relatively high average D between FF and YS populations further suggests that the two morphs are not conspecific. It is possible that the scatter along PCA axis 2 (Figure 2.7) may represent further taxa. This is not considered likely however as: only a few colonies fell out of the main grouping; they did not represent intermediate morphologies (all of them being easily identifiable FF or YS morphs showing no unusual patterns of branch morphology or colour); and they were sampled from several different sites.

General lack of deviations from Hardy-Weinberg equilibrium for both morphs suggest that there is random mating in most of the populations examined. Sexual reproduction occurs twice a year during two major spawning events (see Chapter 3), and even though colonies are hermaphroditic there is no inbreeding (Heyward and Babcock 1986). The few observed deviations from Hardy-Weinberg may be caused by asexual reproduction, assortative matiing, or linkage disequilibrium. The mean direct count heterozygosity for YS populations was similar to the general mean of H = 0.15 which has been calculated for marine invertebrates (Ferguson 1980). The mean for FF populations was a third of this value suggesting different population structuring. The relatively low G_O:G_E ratio values found for most populations, despite

sampling designed to avoid clonemates (individuals sampled were separated by a minimum of 5 m to avoid obtaining clonemates produced by local fragmentation), indicates that asexual reproduction is occurring in both taxa. Similar deviations from Hardy-Weinberg equilibria and G_O:G_E ratio values have been associated with asexual reproduction in other corals that are known to reproduce asexually (Stoddart 1983, Resing and Ayre 1985, Ayre and Willis 1988, Hunter 1993). Clonal dispersal may therefore be occurring over distances greater than 5m. This is not surprising as storms and cyclones may distribute coral fragments over a wide area (see review by Highsmith 1982). Other studies have also found clones may be dispersed over a wide area. Ayre and Willis (1988) found *Pavona cactus* colonies with the same 4-locus genotypes separated by up to 93 m, and Hunter (1993) found *Porites compressa* colonies with the same 7-locus genotypes separated by up to 16.5 m. This recent work by Hunter also found that genetic studies may require a minimum of six to seven polymorphic loci to accurately describe genotypic diversity. The four polymorphic loci used for this study may therefore indicate that the number of clones has been overestimated. Despite this possibility, a degree of clonality is very likely in *M. digitata* populations as asexual reproduction by fragmentation is known to occur readily (Heyward and Collins 1985b, pers. obs.).

Genotypic diversity ratios varied among FF and YS populations, although no clear patterns emerge which might explain different ratios found between sites for the two morphs of *M. digitata*. Pioneer Bay and HB are both sheltered sites yet they have very different genotypic diversity ratios, and GB and NB are more exposed sites but have similar ratios to HB. The reef flat at HB is not exposed at low tide (as is also the case at NB), but it is at PB, which may account for the difference between these two bays. The situation is further complicated by the differences between the FF and YS populations at Esk and LI, these differences may be the result of differences in the life-history characteristics of the two morphs. Various factors may cause differences in the degree of clonality between sites: these include differences in the relative levels of reproduction; the degree of sexual colonisation they exhibit; and the survival of sexual and asexual recruits (Hughes 1989). Differences will thus be mediated by local environmental regimes and the physical disturbances experienced by populations (Hunter 1993). While it is possible to describe the local environmental regimes it is far more difficult to determine when physical disturbances last occurred and how intense they were. Disturbance will accelerate fragmentation of corals (Tunnicliffe 1981, Highsmith 1982), and therefore disturbed environments should exhibit greater levels of clonality. However, very high levels of disturbance will clear patches in the habitat that make room for sexually derived recruits and consequently reduce clonality (Sebens and Thorne 1985). Such a scenario has been documented for the anemone *A.*

tenebrosa (Ayre 1994), and the coral *Porites compressa* (Hunter 1993). Both of these species displayed relatively low levels of genotypic diversity where found on stable shores, and high genotypic diversity on unstable shores. At an intermediate level of disturbance clonal diversity will probably be maximised (Sebens and Thorne 1985). No particular scenario may be applied to the various populations of *M. digitata* as the sampling was designed to reduce the chance of collectig clonemates, so the true clonal structure of the population is not known.

Genotypic diversity ratios vary considerably between anthozoans (see review by Hunter 1993), which presumably reflects differences in their life-histories. For example, *P. cactus* sampled from nine sites had a genotypic diversity ranging from 0.02 to 0.9 with an average of 0.35 (Ayre and Willis 1988), and *Pocillopora damicornis* from three different sites had a genotypic diversity that ranged from 0.09 to 0.61 with an average of 0.40 (Stoddart 1984b). Asexual reproduction would therefore appear to be the major source of recruitment for these two species. In contrast Hunter (1993) found that six populations of *Porites compressa* had genotypic diversity ratios ranging from 0.43 to 0.99 and averaged 0.76 indicating that sexual reproduction contributes a significant amount to the population structure. Ayre and Dufty (in press) also found that populations of *Seriatopora hystrix* from twelve separate reefs had a relatively high genotypic diversity ratio (average 0.74), despite predictions that a large proportion of reproduction in this species should be by fragmentation. The high genotypic diversity found for *M. digitata* in this study reflects the sampling strategy used (i.e. clonemates were avoided) and is therefore not compareable to values found for *S. hystrix* and *P. compressa.*

High F_{St} values calculated for individuals across all variable loci for FF populations imply that there is some restriction in gene flow among FF populations. The low F_{ST} values for YS populations show that they were not significantly genetically differentiated. F_{ST} values for the FF populations may be influenced by asexual reproduction. Fat fingers populations had a lower mean genotypic diversity ratio than YS populations, implying that clonality was greater in FF populations. By comparing F_{ST} values calculated for individual and clonal allele frequencies it has been shown that clonality (asexual reproduction) may account for some differentiation among populations (Ayre *et al.* 1991a). However other factors may also be influencing the difference in F_{ST} values between YS and FF populations, as the difference in genotypic diversity ratio between them was not very large. Such factors include physical isolation, isolation by distance, localised selection, overlapping generations and the effective size of the breeding population (Stoddart 1984a, Ayre *et al.* 1991a). The higher F_{ST} value for FF populations is not likely to be the result of

isolation by physical factors such as currents. If physical isolation was responsible for this difference the F_{ST} value for the YS population would also be expected to show differentiation, as both FF and YS populations spawn at the same time and from the same environment. In view of the difference in F_{ST} values between the morphs it seems likely that life-history characters they do not share may be responsible for the difference. For example, the two morphs may have different lifespans, or their biology may lead to differences in the way they respond to localised selection.

Levels of genetic differentiation observed during this study for both FF and YS populations are comparable to those found for sedentary marine invertebrates with planktonic larvae such as echinoderms (Nishida and Lucas 1988, Watts *et al.* 1990) and molluscs (Johnson and Black 1984). F_{ST} values were considerably lower than those found between populations of the coral *P. damicornis* (Stoddart 1984a), and between populations of the sea anemones *A. tenebrosa* (Ayre *et al.* 1991a), *Anthopleura elegantissima*, *A. xanthogrammica* and *A. artemisia* (Smith and Potts 1987). Higher F_{St} values have been attributed to poor larval dispersal (Waples 1987, Watts *et al.* 1990). This difference in F_{St} values can therefore be explained by the difference in life histories these species exhibit. Both *P. damicornis* and *A. tenebrosa* brood their young, whereas *M. digitata* is a broadcast spawner. Brooded planulae are thought to be adapted to rapid settlement (Stoddart 1984a) which would reduce gene flow between populations, whereas broadcast spawners produce larvae adapted for widespread dispersal (see review by Harrison and Wallace 1990), and would therefore be expected to have lower F_{St} values.

In conclusion, the two presently defined morphs of *M. digitata* found on inshore reefs along the Queensland coast should be considered to be two species based on genetic evidence. Populations of both morphs were generally in Hardy-Weinberg equilibrium suggesting random mating in most of the populations examined. The genotypic diversity ratio G_O:G_E suggests that reproduction occurs both sexually and asexually in both morphs. This corresponds to the observed pattern of reproduction for this species. Genotypic diversity varied considerably between sites possibly reflecting differences in site environments and disturbance histories. There were also differences in the degree of genetic differentiation among the FF populations but not the YS populations. These differences may have been influenced by the slightly greater incidence of asexual reproduction in the FF population, however this was unlikely to be the only cause. Other factors such as localised selection and overlapping generations could have affected the degree of genetic differentiation. Relatively low F_{St} values found for *M. digitata* during this study imply that gene flow between broadcast spawning species is greater than that between brooding species. This

observation is in accordance with the hypothesis that brooded planulae are adapted for rapid settlement, whereas planulae produced by broadcast spawners are adapted for widespread dispersal.

Chapter 3

Reproductive compatibility within the genus *Montipora*

3.1 Introduction

Reproductive isolation lies at the heart of the biological species concept (Sokal and Crovello 1970, Mayr 1973, Mishler and Donoghue 1982), and is considered to be conclusive proof of species status by most taxonomists (Eldredge 1993). Though crossability in the laboratory does not prove conspecificity (Mayr 1963, p. 405), it is generally accepted that failure to cross does prove inter-specificity (eg. Miller 1982a, Palumbi and Metz 1991a, Marsden 1992). Unfortunately, the investigation of reproductive isolation is often difficult and impractical, so conventional taxonomic criteria are generally used in preference. Reproductive isolation is therefore mainly investigated when traditional taxonomic methods have not been successful (hydrozoans: Miller 1982a; polychaetes: Marsden 1992, echinoderms: Metz *et al.* 1991), or for organisms such as echinoderms, on which it is relatively easy to conduct reproduction experiments (Uehara *et al.* 1990, Metz *et al.* 1991). The study of reproductive isolation is further complicated by the fact that reproductive isolation can occur within species if reproductive hierarchies exist in which closely related members of the species are incompatible but more distantly related individuals interbreed (Solbrig 1968, Grant 1958, p. 49). The existence of reproductive hierarchies is well established in the literature on plants, and are proposed to be a mechanism for preventing inbreeding (Williams 1964, p. 211).

Until recently there has been a "tacit acceptance of the biological species concept" within the Scleractinia (reviewed in Willis 1990), and the use of "reproductive taxonomy" has been recommended for solving problems related to coral taxonomy (Hodgson 1988). Theoretically, reproductive isolation should be a valid criterion for delimiting species within the Scleractinia, as it has proven to be for other marine invertebrates (eg. echinoderms: Lessios and Cunningham 1990). However, no scleractinian coral species have been reclassified due to the discovery of reproductive isolation, though Wallace and Willis (1994) have suggested that two morphs of *Acropora millepora* may be two species based on fertilisation data. Most reproductive studies conducted on mass spawning scleractinian corals have demonstrated that hybridisation occurs readily between morphological species (eg. *Montipora*: Hodgson 1988; *Acropora:* Willis *et al.* 1992; *Platygyra* : Miller 1994). The probable causes of high levels of hybridisation within these genera have been discussed by Willis *et al.* (ms). They are putatively caused by the evolution of morphological species by

vicariance events without changes occurring to the specific gamete-recognition systems. The ocurrence of such polytypic species, consisting of several morphospecies that interbreed, is thought to be common amongst both the animal and plant kingdoms (Bremer and Wanntorp 1979). Given the high levels of hybridisation that have been reported from laboratory studies of several genera of corals, it is important that the levels of hybridisation likely to occur in the natural environment be determined.

Although much is understood about the mechanisms that lead to successful fertilisation in a wide variety of marine invertebrates (Nuccitelli *et al.* 1989), and in particular echinoderms (Metz *et al.* 1991), little work has been carried out on such fertilisation mechanisms in coral. The possible mechanisms that can lead to reproductive isolation in broadcast-spawning marine invertebrates such as corals are summarised in Table 3.1. So far research on reproductive isolation mechanisms in scleractinian corals has focussed on sperm chemoattraction, and it has been hypothesised that species-specific attractants may act to reduce the occurrence of hybridisation (Coll *et al.* 1994). Species specificity of sperm attraction has been found for other marine invertebrates such as echinoderms (Ward *et al.* 1985), hydrozoans (Miller 1982a) and ascidians (Miller 1982b). Egg-sperm interactions have not been studied at other levels in corals, and consequently little is known about interactions such as polyspermy and sperm-egg binding. Such interactions have, however, been studied in sea urchins for which polyspermy is a common event (Byrd and Collins 1975), and it is known that binding proteins that allow egg-sperm fusion are very species-specific (Palumbi and Metz 1991a). Understanding such mechanisms and how they operate is an important step towards determining how reproductive isolation occurs, and in particular it allows speculation on how such isolation evolves.

The genetic study in Chapter 2 has demonstrated that the two morphs of *Montipora digitata* are reproductively isolated and are therefore two species. However it does not provide any information on the mechanisms that are causing the reproductive isolation. Isolation between the two morphs could be mediated by any of the mechanisms listed in Table 3.1. The aim of this work is therefore to investigate the breeding compatibility of the two morphs of *Montipora digitata* in order to determine how reproductive isolation between them is being mediated. Reproductive relationships among seven species within the genus are also investigated to determine whether other morphological species within the genus are also reproductively isolated, or whether they hybridse as has been found for other coral species.

Barrier Type	Isolation mechanism	Reference	Phylum & Class
Prezygotic	Temporal (spawning at different times)	Dillon (1992) Uehara *et al.* (1990) Byrne & Anderson (in press)	Mollusca: Bivalvia Echinodermata: Echinoidea
	Spatial (Spawning in different habitats)	Lucas & Jones (1976) Lessios & Cunningham (1990)	Echinodermata: Stelleroidea Echinoidea
	Chemotaxis (Sperm not attracted to eggs)	Coll *et al.* (1994) Miller (1979) Miller (1982b)	Cnidaria: Zoantharia Hydromedusae Chordata: Ascidiacea
	Acrosome reaction (Egg jelly fails to induce reaction)	Segall & Lennarz (1979)	Echinodermata: Echinoidea
	Binding of sperm to egg (Acrosomal protein "bindin" fails to attach sperm to egg vitelline layer)	Glabe & Vacquier (1977) Glabe & Lennarz (1979) Brandriff *et al.* (1978)	Echinodermata: Echinoidea Mollusca: Bivalvia
	Fusion of plasma membranes	Metz *et al.* (1991)	Hypothetical, no example given
Postzygotic	Zygotic mortality	Uehara *et al.* (1990)	Echinodermata: Echinoidea
	Hybrid inferiority	Lucas & Jones (1976)	Echinodermata: Stelleroidea
	Partial hybrid sterility (F1 progeny more likely to be fertilised by one of parental species than other)	Strathmann (1981)	Echinodermata: Echinoidea
	Hybrid sterility		

Table 3.1. Summary of main isolating mechanisms that can act as barriers to reproduction between free-spawning marine organisms.

3.2 Materials and methods

3.2.1 Study sites

Fertilisation experiments were carried out at Magnetic Island (see Plate 2.1) and Orpheus Island (see Figure 2.2) using colonies collected from Geoffrey Bay and Nelly Bay at Magnetic Island and Hazard Bay, Pioneer Bay and North-East Reef at Orpheus Island. Experiments were conducted twice a year at Magnetic Island in spring and autumn, and once a year at Orpheus Island in spring, between March 1991 and December 1993.

3.2.2 Pre-spawning protocols

The time of spawning could be accurately predicted as members of the genus *Montipora* spawn soon after dark 2-3 nights after the full moon (see Chapter 5). Imminent spawning was confirmed by examining broken coral branches for polyps that contained pigmented eggs which could be seen with the naked eye a few days before spawning. *Montipora* eggs are characteristically brown because they contain symbiotic zooxanthellae which make them easy to see in broken fragments.

Prior to spawning suitable colonies were collected that had large numbers of eggs, and these were stored at a central holding point. In the case of *M. digitata*, colonies could be easily removed from the substrate without any tools, whereas plate forms were removed with a hammer and chisel. Every effort was made to minimise damage to the colonies, and they were stored in an area known not to be subject to excessive water turbulence or sedimentation.

All equipment used to carry out the crosses was preconditioned by soaking in seawater for 1-3 days to leach potentially noxious chemicals from the glassware and particularly from the plasticware, and promote growth of a film of bacteria which is thought to be beneficial to the health of coral embryos (B. Willis pers. comm.).

On the evening of spawning, 'sperm-free' seawater (SFS) was collected several hundred metres off the reef (or from storage tanks at Orpheus Island) in large plastic water storage containers. It was assumed that sperm from spawnings on previous nights would be unlikely to survive for 24 hours.

3.2.3 Obtaining and crossing coral gametes

The following procedure was used to obtain and cross all coral gametes during this study, unless otherwise stated. For crosses at Magnetic Island colonies were collected from holding points in Geoffrey Bay 1-2 hours before dark and transferred to buckets on the beach. At Orpheus Island colonies were transferred to raceways on the day of spawning and were held in buckets submerged in running seawater. Just prior

to dark the water in the raceways was turned off, and the water level was lowered to isolate the buckets. Occasionally colonies were held in the raceways for several days if they did not spawn on the first night. At dusk all nearby light sources were turned off, or black plastic sheeting was placed over the buckets, so as not to hinder the diurnal photoperiod cycle.

After dark, colonies were checked for spawning at approximately 15 minute intervals (more often once spawning began). Care was taken not to illuminate the colonies for long periods when checking for spawning. Spawning times were recorded and egg-sperm bundles collected as soon as possible using an adapted pooter connected to a glass pipette. Each colony was assigned an exclusive colour code, which was used for all glassware and plasticware in order to avoid contamination between colonies.

Once a suitable number of egg-sperm bundles was collected they were poured into a washing container with a plankton mesh base (65-100 μm) which was held in a bowl of SFS and gently agitated until the eggs and sperm in the bundles had separated. The washer was then removed from the bowl and the eggs washed 10 times in SFS. The sperm rich seawater from the first wash was kept for the experiments; it was stored in a beaker with aeration and washed eggs were kept in a bowl with light aeration.

To determine the concentration of the sperm in the seawater, sperm was first fixed (10 % formalin v/v) and then counted using a haemocytometer. The stock supply was then diluted using SFS to a concentration of 2.5×10^6/ml for use in crosses. This concentration has been determined to yield optimal fertilisation levels in *M. digitata* by Oliver and Babcock (1992).

Crosses were then carried out in numbered 25 ml scintillation vials using a standard grid cross with three replicates per cross (Figure 3.1). All crosses had controls (eggs incubated without sperm) to control for potential selfing or contamination during the collecting or washing procedure. Vials were filled with sperm or SFS (for controls), after which approximately 100 eggs from the apropriate colony were pipetted into each. Vials were then put into a perspex rack and the rack was suspended from a buoy in the sea to allow gentle agitation and maintenance of in *situ* temperature. Vials were retrieved after 3 hours and the percent fertilisation was estimated from the number of fertilised eggs in the first 100 counted. Stage of development, and appearance, were also noted. A second count was often made another 3 hours after the first count ended in order to determine survival of embryos.

Eggs / Sperm	*M. digitata* FF Colony 1	*M. digitata* FF Colony 2	*M. digitata* YS Colony 1	*M. digitata* YS Colony 2	Plate Colony 1	Plate Colony 2
M. digitata FF Colony 1	Self	intra-morph	inter-morph	inter-morph	inter species	inter species
M. digitata FF Colony 2	intra-morph	Self	inter-morph	inter-morph	inter species	inter species
M. digitata YS Colony 1	inter-morph	inter-morph	Self	intra-morph	inter species	inter species
M. digitata YS Colony 2	inter-morph	inter-morph	intra-morph	Self	inter species	inter species
Plate Colony 1	inter species	inter species	inter species	inter species	Self	intra-species
Plate Colony 2	inter species	inter species	inter species	inter species	intra-species	Self
Control						

Figure 3.1. Standard grid outline for crosses within and between species. Three replicate crosses were performed per grid square. Controls had eggs and SFS but no sperm.

Samples of colonies used in crosses were collected for identification the morning after all crosses were performed. Colonies of *M. digitata* were bleached for use in the morphometric study (Chapter 4). Samples of *M. digitata* colonies used in crosses were also collected for electrophoretic identification. All samples for electrophoresis were stored and processed as described in Chapter 2, and morph identification (yellow spatulate v.s. fat finger) was confirmed electrophoretically using morph specific markers at the *LT-2** enzyme locus. In some crosses additional morphological divisions were made for convenience. These included brown stumpy (colonies brown with thick anastomosing branches that generally had stunted branch tips due to exposure at low tide; BS), green pointy (colonies green with pointed branch tips; GP), and fine pointy (colonies with thin branches generally ending in points; FP).

3.2.4 Egg activation experiments

To further explore the basis of gamete incompatibility found between the YS and FF morphs of *M. digitata* (see section 3.3), an experiment was carried out to test for egg activation (the process in which an egg is triggered to start developing). The underlying assumption of this experiment was that the egg activation process is irreversible. The experiment involved crossing eggs of morph A with sperm of morph B, removing and washing the eggs after two hours, and then adding sperm from morph A. The reciprocal cross was also carried out. Simultaneously, the following series of controls were prepared: (1) eggs of each morph were crossed with sperm of the other morph but with no sperm change; (2) eggs were crossed with sperm of the same morph; and (3) eggs were incubated without sperm. All crosses were examined after 5 hours and the percent fertilisation determined. The design of this experiment is summarised below (Figure 3.2).

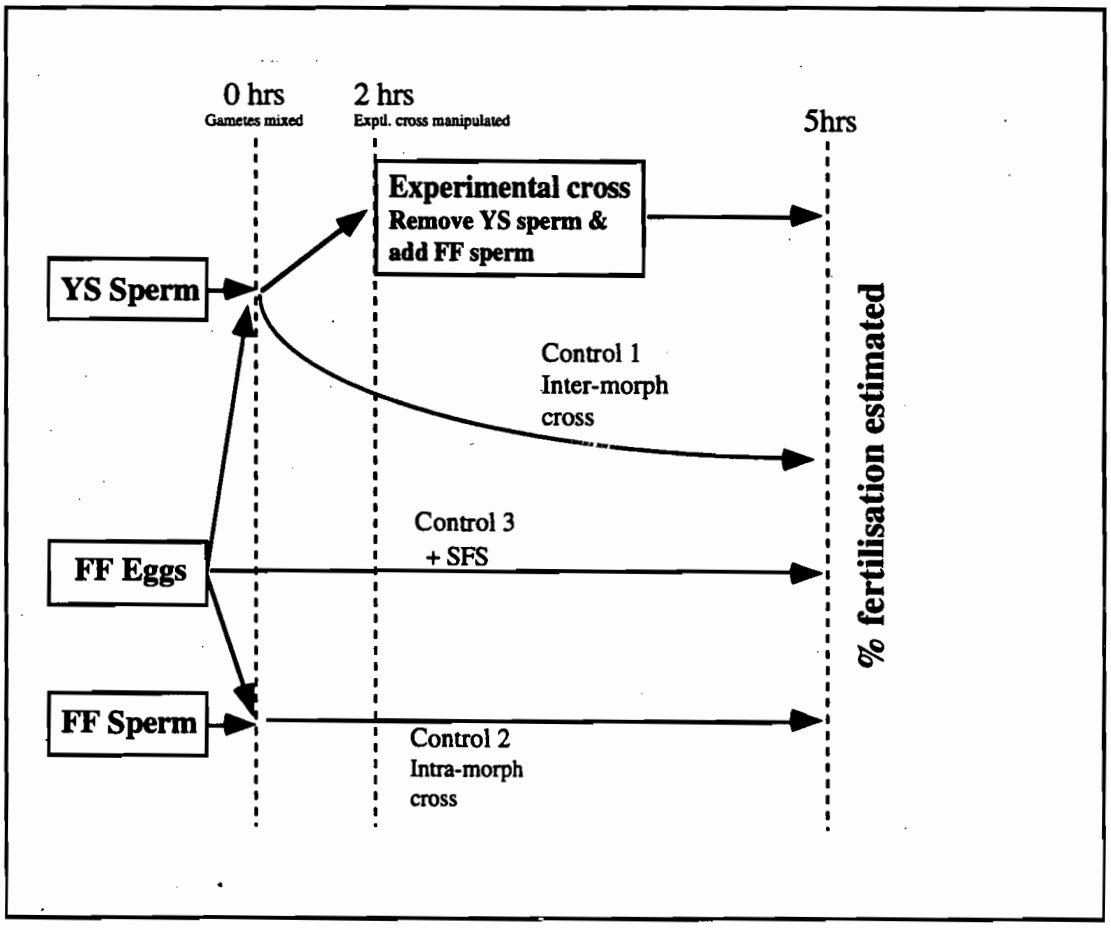


Figure 3.2. Experimental design for egg activation experiments showing gamete mixing in one direction. The reciprocal cross and controls were also performed. FF and YS are fat fingers and yellow spatulate morphs of *Montipora digitata,* respectively. SFS: sperm-free seawater.

3.2.5 Data analysis

Mean percentage fertilisation was calculated for each cross (Sum of replicate vial fertilisation/No. vials). Students' t-tests were used ($\alpha = 0.05$) to compare mean percentage fertilisation between experimental crosses and first control crosses for the egg acivation experiment. Data were tested for homogeneity of variances and Cochran's correction applied if variances were not equal. All mean values quoted represent the mean of replicate crosses (i.e. the mean of a series of crosses involving the same morphs of species where each individual cross involves three replicate vials) unless otherwise stated. The results presented correspond only to crosses that are known to be reliable. Crosses that did not work are not presented but are discussed in section 3.3.4.

3.3 Results

3.3.1 Crosses between morphs of Montipora digitata

Crosses between the two morphs of *M. digitata* showed almost complete reproductive isolation (Figure 3.3). Of a total of 90 reciprocal crosses between the two morphs, only four of them yielded fertilisation at a very low level (1, 2, 4 and 5%). Within the morphs fertilisation varied a great deal (0-100%) with average fertilisation levels of 60% and 50% for the FF and YS morphs respectively (Figure 3.3). The frequency distribution of percent fertilisation did not differ between the morphs (χ^2 0.05, 4 df = 1.317; $p>0.75$). Typical examples of results for colony crosses are shown in tables 3.2 to 3.5.

Crosses made within morphs showed that percentage fertilisation was generally similar in reiprocal crosses (tables 3.2-3.5). There was also evidence that there may be breeding types or further species within the morphological groups, as certain colonies did not cross successfully within their own morphs. For example, within the yellow spatulate morphological range FP crossed well with almost all other morphological variants, whereas BS2 only crossed with BS1 and FP, and neither YS2 or GP crossed with BS2 in either direction (Table 3.2). Similarly YS5 did not cross well with YS1 in either direction, but both of these morphs crossed well with YS2 (Table 3.3). Similar breeding inconsistencies were found among the FF colonies. For example, FF4 and FF2 gametes were incompatible, but both could fertilise successfully with gametes from colonies FF1, FF3 and FF5 (Table 3.5). Thus virtually complete blocks to reproduction seem to exist between colonies within each morphological category, but such blocks do not constitute species differences because gene flow between colonies can clearly occur by means of a third party.

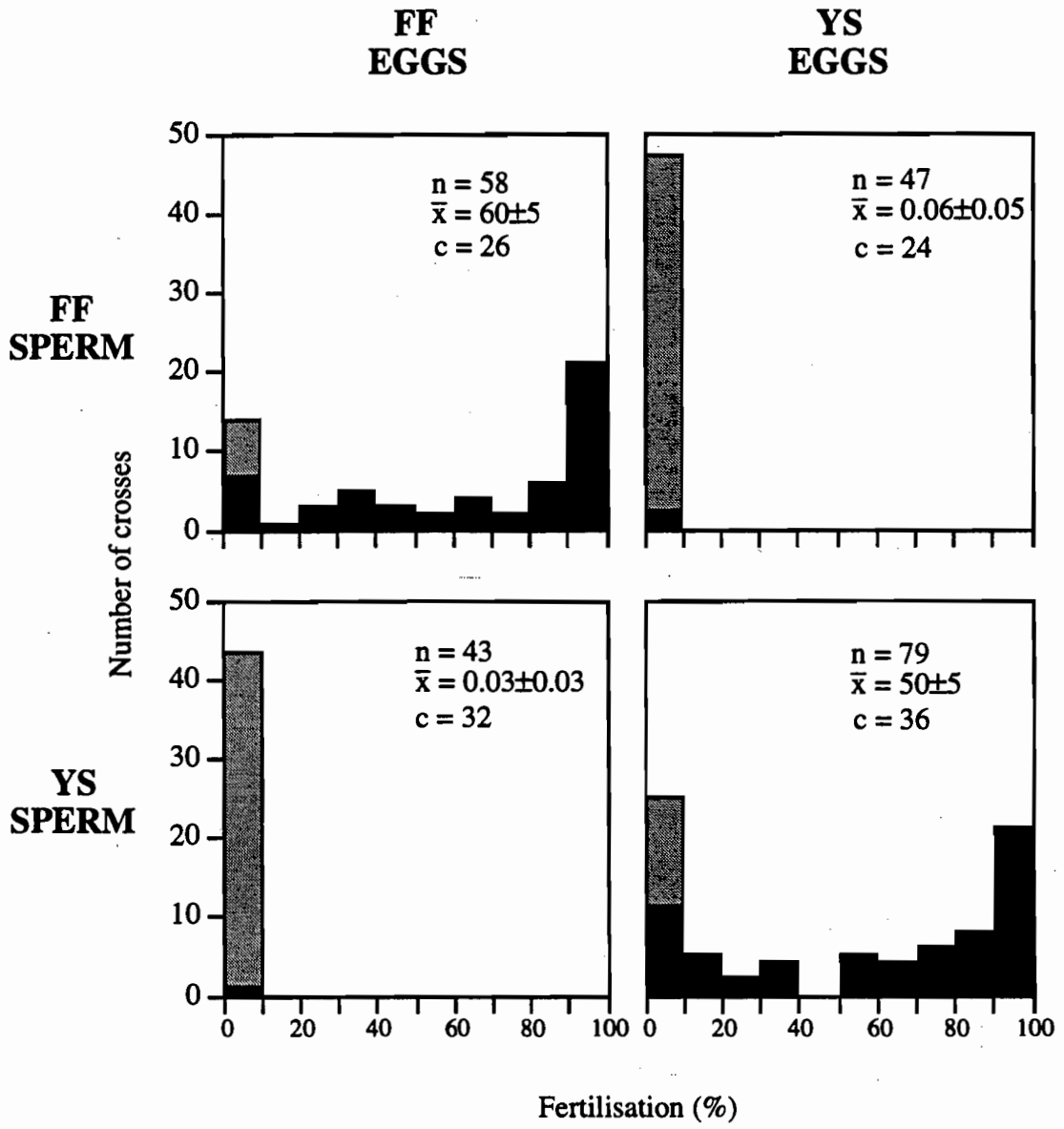


Figure 3.3. Summary of percent fertilisation between FF and YS morphs of *M. digitata*. (■): crosses with fertilisation, (▩): crosses with zero fertilisation, n: total number of colony crosses (3 replicate breeding trials per colony cross), $\bar{x}$: mean percent fertilisation, c: number of different colonies used.

♂ \ ♁	YS1	BS1	BS2	YS2	GP	FP	MP1	MP2
YS1	-	89±8	-	0.3*	11±2	98±1	-	-
(BS1)	-	-	-	-	-	-	-	-
BS2	-	99±0.3	-	-	-	98±1	-	-
(YS2)	-	10±3	-	-	-	2	-	-
GP	-	99±32	-	2*±1.6	-	100	-	-
FP	100	100	100	100	100	1	-	-
MP1	-	-	-	-	-	-	-	-
MP2	-	-	-	-	-	-	-	-
Control	-	-	-	1	-	-	-	-

Table 3.2. Example of a cross performed in Geoffrey Bay on 01/04/91 showing mean % fertilisation ±SE. The cross was performed between four tentative morphological divisions within the YS morph range for *Montipora digitata*, and *M. peltiformis*. YS: yellow spatulate; BS: brown stumpy; GP: green pointy; FP: fine pointy; MP: *M. peltiformis*; *: cross likely to be invalidated due to control contamination; -: indicates zero fertilisation. Brackets indicate suspect sperm.

♂ \ ♁	FF1	FF2	YS1	YS2	YS3	YS4	YS5	MP
FF1	-	100	-	-	-	-	-	-
FF2	92±8	-	-	-	-	-	-	-
YS1	-	-	-	36±11	18±12	50	-	-
YS2	-	-	92±1	-	87±7	6±2	94±0.3	-
YS3	1±1.3	-	10±2	33±10	-	67±6	1±0.6	-
YS4	-	-	90±6	7±1	97±1	16±4	82±2	-
YS5	-	-	6±3	77±4	4±3.6	92±2	-	-
MP	-	-	-	-	-	-	-	1
Control	-	-	-	-	-	-	-	-

Table 3.3. Example of a cross performed in Geoffrey Bay on 13/10/92 showing mean % fertilisation ±SE. The cross was performed between two colonies of the fat fingers morph (FF) *Montipora digitata*, five colonies of the yellow spatulate morph (YS) *M. digitata*, and one colony of *Montipora peltiformis*. -: indicates zero fertilisation.

♂ \ ♀	FF1	FF2	YS1	YS2	BS1	BS2	YS3	MC
FF1	-	38±17	-	-	-	-	29±3	-
FF2	43±4	-	-	-	-	-	-	-
YS1	-	-	-	53±7	30±13	78±3	-	44±4
YS2	-	-	85±9	1±0.7	58±10	34±1	92±2	-
BS1	-	-	18±2	98±1	-	-	15±8	-
BS2	-	-	33±10	99	-	-	72±12	-
YS3	-	-	61±3	85±3	89±3	-	32±10	69±6
MC	3±1.4	1	-	0.3	0.3	-	24±2	42±10
Control	-	-	-	-	-	-	13±5	-

Table 3.4. Example of a cross performed in Geoffrey Bay on 25/10/91 showing mean % fertilisation ±SE. The cross was performed between *Montipora digitata* fat fingers (FF), two tentative divisions within the yellow spatulate morphological range (YS: yellow Spatulate; BS: Brown Stumpy), and one colony of *Montipora crassituberculata* (MC). As YS3 eggs had a contaminated control, the entire column was not used. -: indicates zero.

♂ \ ♀	FF1	FF2	FF3	FF4	YS1	YS2	YS3	FF5
FF1	-	35±16	-	10±7	-	-	-	46±11
FF2	91±2	-	98±1	-	-	-	-	97±2
FF3	20±10	6±3	-	-	-	-	-	3±1
FF4	84±16	-	56±29	-	-	-	-	21±15
YS1	-	-	-	-	-	-	-	-
YS2	-	-	-	-	7±1	2±1	14±8	-
YS3	-	-	-	-	-	1±0.3	-	-
FF5	98±1	92±1	85±3	78±9	-	-	-	3±2
Control	-	-	-	-	-	-	-	-

Table 3.5. Example of a cross performed in Pioneer Bay on 13/11/92 showing mean % fertilisation ±SE. The cross was performed between fat fingers (FF) and yellow spatulate (YS) *Montipora digitata*. -: indicates zero.

3.3.2 Egg- sperm interaction

The egg activation experiments demonstrate that the block to fertilisation between the two morphs of *M. digitata* occurs before egg activation. The experiment performed in April 1993 gave the clearest result (Table 3.6 and summarised in Figure 3.4a). Eggs exposed to sperm of the opposite morph for two hours and then to sperm from the same morph (experimental cross) had high levels of fertilisation (YS 90%±1.6 and FF 61%±15.0; Figure 3.4a). High levels of fertilisation were also obtained for the first control in which intra-morph crosses were made (YS 90%±6.5 and FF 51%±11.2). Fertilisation levels in the first control did not differ significantly from the experimental cross values for either the YS cross ($t = 0.011$, 16 df; $p>0.05$) or the FF cross ($t = 0.511$, 25 df; $p>0.05$). Inter-morph crosses (control 2) showed virtually no fertilisation, with only a very low level (< 3% fertilisation) occurring between two crosses of FF sperm with YS eggs (Table 3.6). Such a low level of fertilisation is negligible in comparison with the experimental cross and control 1. The third control containing only eggs and no sperm had no fertilisation thus validating the sperm-free seawater.

The second cross performed in December 1993 generally corroborated the results of the first experiment, but overall levels of fertilisation were very low because eggs were beginning to break down as they were added to the vials. Both the experimental cross and the first control again show fertilisation, whereas the second and third controls do not (Table 3.7 and summarised in Figure 3.4b). In this case it appears that there may be some reduction of fertilisation between the experimental cross and the normal intra-morph cross (control 1), however this is an experimental artefact. Control 1 eggs received sperm immediately the cross was carried out, allowing immediate fertilisation, whereas the experimental cross eggs only received sperm capable of fertilising them two hours later, during which time they deteriorated further. The original design of this experiment incorporated a handling control to allow for such an event (i.e. intra-morph crosses were kept in SFS for 2 hours before adding the corresponding sperm), however it was decided that the extra time required to process the vials after two hours (ie adding the correct sperm) would have been more detrimental to the experiment. The more time the vials are kept out of the sea the less likely larvae are to survive due to lack of agitation and temperature fluctuation. In the case of the first experiment where eggs were in good health on addition to the vials, such a control was not necessary. The first experiment thus shows that exposure of eggs to sperm from a different morph does not reduce the ability of eggs to subsequently be fertilised by sperm from the same morph. The results obtained for the second experiment also support this if it is assumed that the low levels of fertilisation in the experimental cross reflected the deteriorating quality of the eggs.

♂ \ ♁	FF1	FF2	FF3	YS1	YS2	YS3
FF1		17±5.6	1.3±0.6	85±6.8	94±2.5	93±2.2
				-	2±1.4	-
FF2	99±0.8		99±0.6	83±2.5	79±7.2	98±0.6
				-	-	-
FF3	-	94±1.3		96±1.2	94±4	93±3
				-	2.3±2.3	-
YS1	85±12	3±3	95±2.4		99±0.3	93±0.3
	-	-	-			
YS2	9±9	-	-	97±2		96.97±3
	-	-	-			
YS3	-	-	-	M	67±33	
	-	-	-			
Control	-	-	-	-	-	-

Table 3.6. Egg-sperm interaction experiment performed in Geoffrey Bay on 09/04/93 showing mean % fertilisation ±SE (mean estimated from 3 replicates). Shading indicates experimental cross in which sperm was changed after 2 hours (and replaced by sperm of same morph as eggs). -: indicates zero fertilisation; M: missing data.

♂ \ ♁	FF1	FF2	YS1	YS2	YS3
FF1		-	4.3±4.3	-	4±0.6
			-	-	-
FF2	15±13.5		0.7±0.7	0.7±0.7	1±0.6
			-	-	-
YS1	-	-		74±6	61±19
	-	-			
YS2	-	5±4.5	-		-
	-	-			
YS3	1±1	5.7±3.8	-	-	
	-	-			
Control	-	-	-	-	-

Table 3.7. Egg-sperm interaction experiment performed in Geoffrey Bay on 01/12/93 showing % fertilisation ± SE (mean estimated from 3 replcates). Shading indicates cross in which sperm was changed after 2 hours (and replaced by sperm of same morph as eggs). -: indicates zero fertilisation.

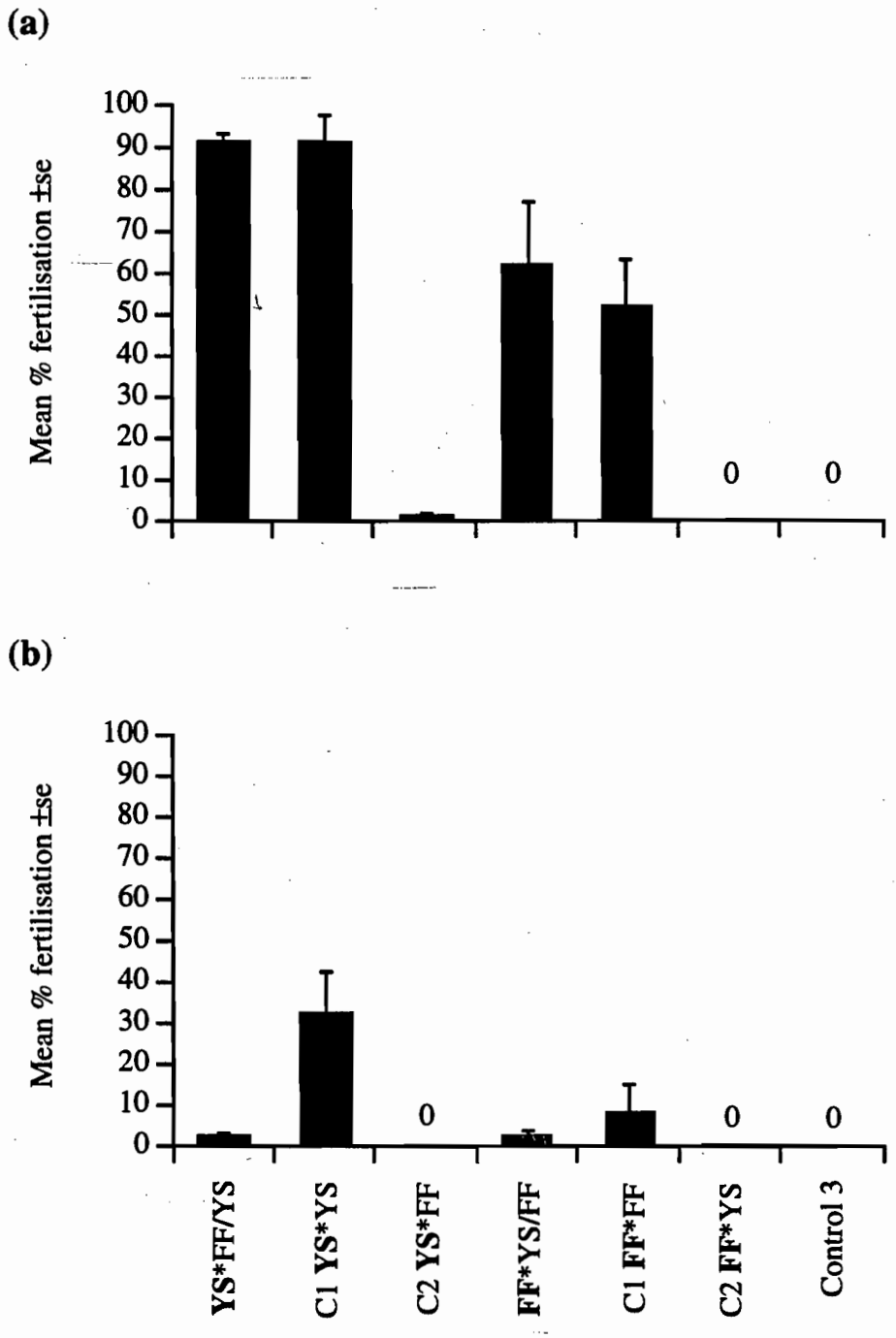


Figure 3.4. Mean percent fertilisation ±SE for combinations of fat fingers (FF) and yellow spatulate (YS) eggs and sperm (eggs in bold) for crosses made on (a) 09/04/93 and (b) 01/12/93. (*): indicates cross, (/): indicates subsequent sperm change, eg. FF/YS refers to change from FF to YS sperm. C: Control, Control 3: eggs but no sperm.

3.3.3 Between species crosses

The results of 96 crosses carried out between nine species of *Montipora* over a three year period show that hybridisation does occur between some species (summarised in Table 3.8). All species except *M. peltiformis* hybridised with at least one other species, but generally at a very low level. *Montipora stellata* was the only species to consistently yield high fertilisation rates when its sperm were crossed with eggs from other species, though the incompleteness of the grid does not rule out the possibility that other species may behave in a similar manner. Most hybrid crosses involving either eggs or sperm of *M. spumosa* resulted in low levels of fertilisation. There was no fertilisation between species in 46% of the crosses, though crosses between both morphs of *M. digitata* and *M. peltiformis* were the only ones in which fertilisation did not occur in reciprocal crosses (Table 3.8). Finally, there was evidence of moderate levels of self-fertilisation in *M. crassituberculata*, *M. aequituberculata* and *M. spumosa*.

Although few hybrid crosses provided enough larvae to follow larval viability, there is some indication that hybrid larvae may be less viable than larvae from conspecific crosses. Larvae from conspecific crosses survived in high numbers to the second count, whereas all hybrid larvae with the exception of those from the *M. spumosa* x *M. digitata* (YS) cross had low levels of survival (Table 3.9) Survival of larvae from conspecific crosses was often greater than 100% due to the fertilisation of more eggs between the first and second count, or more commonly due to fragmentation of embryos.

3.3.4 Inter-annual variability in fertilisation success

Many experimental crosses performed during this study failed to produce any fertilisation. Throughout the three-year study fertilisation success in FF and YS crosses varied greatly despite using exactly the same experimental design and handling techniques (figures 3.5 a & b respectively). Only 50% of crosses within the two *M. digitata* morphs and 46% of within-species crosses for plate species resulted in fertilisation. Crosses were unsuccessful for both morphs of *M. digitata* on approximately the same dates (Spearman's rank correlation = 0.710, n = 13, $p<0.01$). The plates never crossed well, with the exception of crosses performed on 20/03/92 and 14/10/92 (Figure 3.5a). It is noteworthy that for both morphs the percent fertilisation for crosses that did work corresponds reasonably well with the percent of crosses producing fertilised eggs (Spearmans rank correlation FF = 0.703, n = 13, $p<0.05$; YS = 0.691, n = 14, $p<0.01$; figures 3.5 a & b), indicating that whatever was preventing crosses from working was also affecting percent fertilisation in the crosses that did work.

Sperm\Eggs	FF	YS	*M. cras.*	*M. aeq.*	*M. spu.*	*M. stel.*	*M. pelt.*
M. digitata (FF)			0 n=2		9±5 n=5		0 n=2
M. digitata (YS)			0 n=8		2.5±2 n=2		0 n=4
M. Crassituberculata	1±0.3 n=4	0.05±0.03 n=13	43±20 n=5	4 n=1	0 n=1	0 n=1	
M. aequituberculata		97 n=1		23±5 n=9	99 n=1		
M. spumosa	2±1 n=7	4 n=1	1 n=2		25 n=1	0 n=2	
M. stellata	0.25 n=4	83±12 n=2	47±16 n=7	19±17 n=2	50±48 n=2	0 n=1	
M. undata				0 n=6			
M. peltiformis	0 n=2	0 n=4					
M. efflorescens				0 n=6			

Table 3.8. Summary of mean percent fertilisation ±SE for crosses between six species of *Montipora*. n: number of crosses. Highlighted cells indicate selfs. Hybrid crosses are only presented for colonies that were able to fertilise within-species.

Sperm\Eggs	FF	YS	*M. stellata*	*M. crassitub.*	*M. spumosa*
M. digitata (FF)	107±7 n=24				
M. digitata (YS)		121±7 n=64			50±23 n=2
M. stellata	10±10 n=3	0 n=9		0 n=6	0 n=10
M. crassituberculata				81±9 n=14	

Table 3.9. Summary of mean percent of embryos ±SE surviving to become larvae in second counts on crosses. Highlighted cells indicate within species crosses (not selfs).

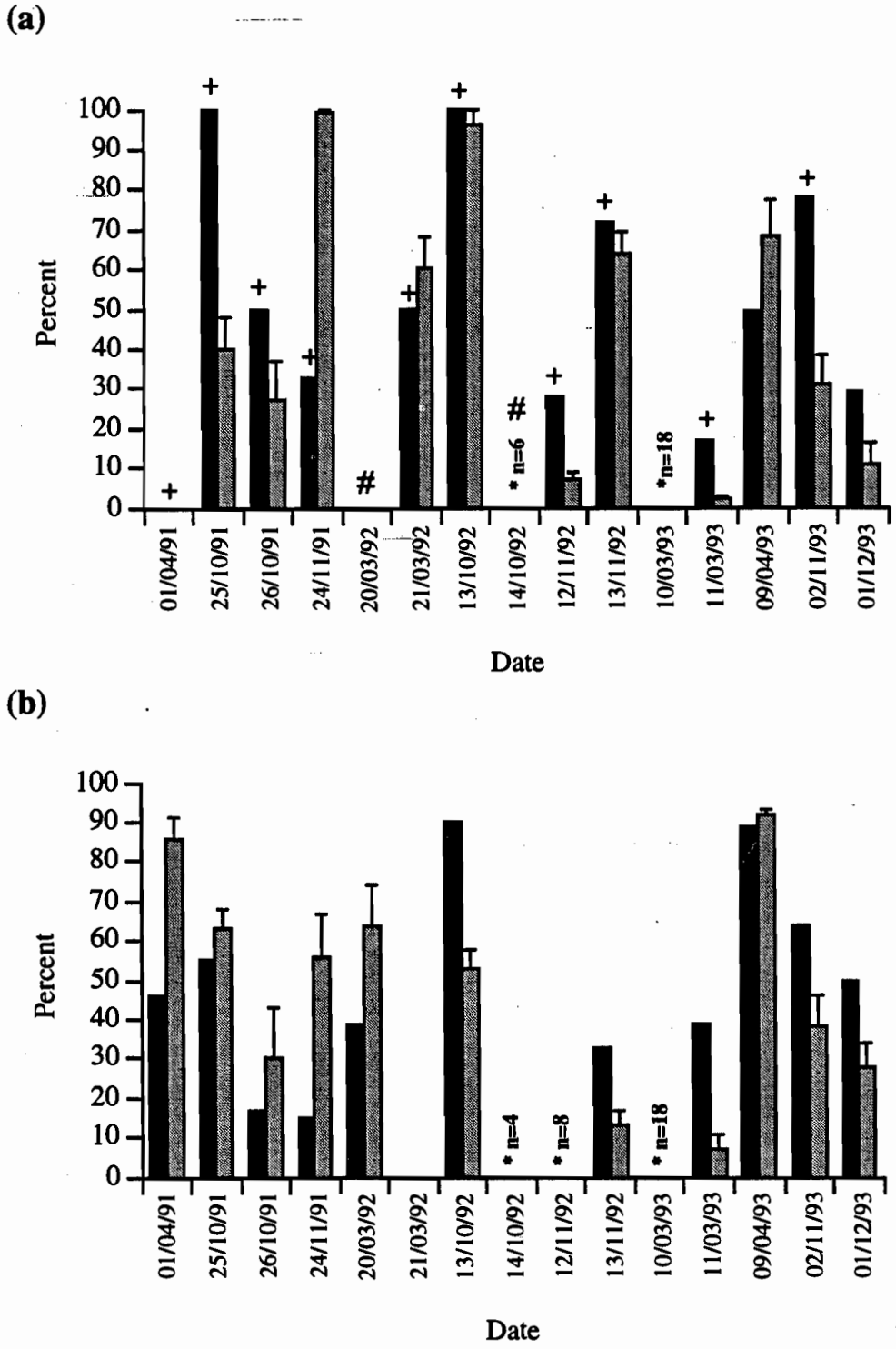


Figure 3.5. Summary of percent of crosses resulting in fertilisation (■), and the mean percent fertilisation ±SE for these crosses (▩). Crosses were between FF colonies (a), and YS colonies (b), of *Montipora digitata*. *: indicates crosses in which fertilisation was zero; n: number of crosses attempted. Information on plate *Montipora* crosses is given in (a). +: plate crosses with <50% fertilisation; #: plate crosses with >50% fertilisation.

Initially it was thought that excessive handling during the gamete seperation process might have adversely affected the gametes and accounted for some of the variability in fertilisation success between spawning periods. In an attempt to control for handling effects ten bucket controls were set up at the same time as a full cross in November 1993. Gametes were mixed directly in buckets without being processed as for the vial crosses (this crude method of crossing gametes worked very well in March 1994). Both the vial cross and the bucket controls yielded virtually no fertilisation suggesting that the methodology used for vial crosses was not at fault. This belief is further supported by the fact that crosses performed on corals from the genus *Platygyra* (K. Miller pers. comm.) and *Acropora* (B. Willis pers. comm.) during the same spawning periods using exactly the same methods and equipment have all been successful. Also crosses performed on *Montipora* species at Magnetic Island on the same nights as the crosses mentioned above, but with totally different sets of equipment, have also failed (R. Babcock (10/92), P. Harrison (10/92, 11/93), A. Heyward (11/93) pers. comm.).

3.4 Discussion

Crosses between the two morphs of *M. digitata* demonstrate that they are generally not interfertile, and that on the rare occasions when fertilisation does occur it does so at a very reduced level. Such reproductive isolation supports the findings of the genetic study in Chapter 2 and is further evidence that the two morphs of *M. digitata* should be considered distinct species. This is the first occasion on which strong reproductive isolation has been demonstrated to occur between morphologically similar coral species.

Within the morphs there was generally a high level of fertilisation, the average of around 50-60% being similar to the 44±10 % obtained for the same species by Heyward and Babcock (1986), though they did not distinguish between morphs. Fertilisation was considerably lower than the 90% fertilisation recorded for *M. digitata* by Oliver and Babcock (1992), which may reflect differences in the performance of this species from year to year as described in section 3.3.4. The same authors found natural levels of fertilisation for *M. digitata* were approximately 80 %.

There is clear evidence that a reproductive hierarchy is in operation within both morphs, as reproductive success varied a great deal depending on the colonies that were crossed. Some of this variation could be attributed to the existence of further taxa within the samples. However this is not thought to be likely as in cases where reproduction did not occur between two individuals of the same morph, they generally both reproduced with a third party indicating that gene exchange could occur (eg. see Table 3.2). Genetic evidence also supported the existence of only two species, though

there was some structuring within species (Chapter 2). Such hierarchies of reproductive compatibilities are common in both the plant and animal kingdoms (Williams 1964, Dillon 1978).

Lack of fertilisation in crosses between the two morphs of *M. digitata* demonstrates that reproductive isolation in this case is mediated by pre-zygotic mechanisms. The egg-sperm interaction experiment implies that the pre-zygotic barrier involves lack of binding of the sperm to the egg membrane, and no egg activation. This interpretation depends on the assumption that once the sperm does bind to the egg membrane it triggers an irreversible egg membrane reaction. Given that the role of the egg membrane (cortical) reaction is to prevent further penetrations by sperm (Dale 1983), this assumption appears to be valid. The failure of sperm from incompatible morphs to initiate an egg membrane reaction suggests that there is no gamete wastage. The potential for gamete wastage has been detected in other members of the genus *Montipora*, and is thought to reduce the fitness of populations as energy is invested in producing gametes that do not produce offspring (Hodgson 1988). If sperm fertilise eggs several hours after they have been exposed to an alternative source of sperm, there can have been no interaction between the eggs and the first sperm they were exposed to. Polyspermy involving sperm from both morphs is not a possibility as eggs were exposed to sperm of the opposite morphs for two hours before adding that of the same morph. Full cortical reaction would only take around 25 seconds (Byrd and Collins 1975), so sperm from both morphs would have had to be added within this time frame in order to penetrate the eggs.

It is not clear why sperm fail to bind to eggs of incompatible morphs of *M. digitata*. One possible mechanism would be species specific sperm chemoattraction which is known to occur widely in marine invertebrates (Miller 1985). Sperm chemotaxis can increase the chances of successful fertilisation not only by causing sperm to swim towards the egg, but also by preparing sperm for egg penetration or ensuring a critical angle of sperm approach for egg penetration (Miller 1982a). Sperm chemoattraction has recently been demonstrated to occur in *M. digitata* by Coll *et al.* (1994). However, the sperm attractant isolated from eggs of the FF morph attracted sperm from both morphs of *M. digitata* equally well. It therefore seems unlikely that pre-zygotic isolation is mediated by a lack of chemoattraction between incompatible colonies, especially as eggs are being held in what is probably an artificially high sperm concentration.

The most plausible explanation for reproductive isolation in this instance lies at the level of the egg-sperm interaction. If sperm meet the eggs but do not produce a cortical reaction they must be blocked at the egg surface. At this stage the most likely block to fertilisation is incompatibility between the binding protein ("bindin") on the surface of the sperm and receptors on the egg membrane. Bindin adheres the sperm to

the eggs (Glabe and Vacquier 1977), and may also activate the eggs (Gould and Stephano 1989). This protein has been demonstrated to be species-specific in echinoderms (Glabe and Vacquier 1977, Glabe and Lennarz 1979). It is thought that bindin from the sperm acrosomal vesicle interacts with glycoprotein receptors on the egg vitelline layer (Glabe and Vacquier 1977). Thus incompatibility due to differences in the bindin protein or the vitelline receptors would render fertilisation unlikely or impossible. Palumbi and Metz (1991a), have suggested that such an incompatibility may be responsible for the almost complete reproductive isolation between the closely related echinoderms of the genus *Echinometra*. They also suggest that small changes in the bindin sequence may have disproportionate effects on reproductive isolation due to the primary role of this protein in fertilisation. Thus rapid functional evolution of bindin may be associated with rapid speciation. Other biochemical events at the egg membrane occur and may be responsible for the reproductive incompatibility (see Table 2.1), but it is clear that reproductive isolation is occurring before the cortical reaction takes place.

Interspecific crosses carried out within the genus *Montipora* suggest that hybridisation does not occur readily within the genus, and that when it does occur survival of embryos may be less than that for conspecific crosses. Hybridisation levels are low despite the opportunity for hybridisation being high for synchronised mass spawning corals (Hodgson 1988, Willis *et al* ms). Hodgson (1988), found a similar result working on three species of *Montipora* from Hawaii. *Montipora verrucosa* and *M. patula* sperm fertilised *M. dilitata* eggs but the embryos did not develop beyond the four cell stage. These crosses were only carried out in one direction, and the possibility that they might be more successful in the other direction was not dismissed as asymmetrical cross fertilisation is known to occur in invertebrates (Strathmann 1981, Miller 1982a, Uehara *et al.* 1990). In this study there was no suggestion that asymmetrical fertilisation occurrs within the *Montipora* populations at Orpheus or Magnetic Island. The lack of gamete interactions between the two morphs of *M. digitata*, and possibly several other species within this genus, is of considerable significance to their reproductive ecology. It is likely that possible gamete wastage due to hybridisation will not occur when gametes of the two morphs encounter each other in the field. This means that the reproductive fitness of populations of either morph is not affected by hybrid inviability as feared by Hodgson (1988).

Poor hybridisation levels found to occur within the genus *Montipora* contrast with those found for other coral genera in which hybridisation between morphological species occurs readily (Willis *et al.* 1992, Miller 1994, Wallace and Willis 1994). Studies on hybridisation have been recommended as a useful method for examining species boundaries (Hodgson 1988, Willis 1990, Palumbi and Metz 1991b), the opportunity for hybridisation to occur being particularly good for marine organisms that

reproduce by the free-spawning of gametes (Lessios and Cunningham 1990, Byrne and Anderson in press, Wallace and Willis 1994). Scleractinian coral taxonomy is largely based on the assumption that corals do not hybridise (Hodgson 1988). This is now known not to be true for certain genera whose taxonomy is being re-examined as a result (*Platygyra*: Miller 1994, *Acropora*: Wallace and Willis 1994). Although results from this and one other study (Hodgson 1988) suggest that hybridisation does not occur readily within the genus *Montipora*, more support for this statement is required in view of the poor performance of intraspecific plate crosses during the period of this study, and the report by Willis *et al.* (1992), that *M. digitata* FF hybridises readily with *M. spumosa*.

It is not clear why the barrier to fertilisation should be so complete for the two morphs of *M. digitata*, and yet not be complete for other morphologically different species. Attempts have been made to explain differences in reproductive isolation on the assumption that natural selection can favour reinforcement of pre-zygotic isolation (i.e. as hypothesised by Dobzhansky, 1937). However there has been justifiable criticism of this theory as there is no evidence that reproductive isolation can be adaptive (Paterson 1993). If reinforcement were accepted it could be argued that the two morphs of *M. digitata* show strong barriers to fertilisation between each other, but not with plate species, because "speciation by reinforcement" predicts stronger barriers to reproduction between truly sympatric species (Dobzhansky 1937). However this theory does not hold because plate species of *Montipora* show little sign of strong reproductive isolation despite occupying the same environment on the reef slope and spawning on the same night within a few hours of each other. Strong blocks to fertilisation have been found between closely related echinoderms by Metz *et al.* (1991), and Lessios and Cunningham (1990), but others have found no correlation between fertilisation rate and taxonomic relationship of parental species (Uehara *et al.* 1990).

The variability in fertilisation success during the course of this study appears to be peculiar to the genus *Montipora*. Poor fertilisations did not occur all the time despite using the same methodology, suggesting that the reduced fertilisation levels were real. However there is reason to believe that the reduced fertilisation may still be an experimental artefact. It may be that gametes released were not ready to be spawned. It is possible that gametes of species in the genus *Montipora* may not be fully mature due to the shortness of their biannual gametogenic cycles (Stobart *et al.* 1993). For example, there was a total reproductive failure in March 1993, but fertilisation was extremely good one month later in April 1993. Thus when conditions are favourable gametes will be "ready" to spawn on time and crosses will work well, but when conditions are not favourable the opposite will apply. The implication is that gametes may be spawned before they are mature. This may not occur in the natural environment but it is conceivable that corals collected for experiments may be stressed and spawn

prematurely. Other coral genera may respond in a similar manner, but because they have a full year to undergo one gametogenic cycle their gametes are more likely to be mature .

Another possible explanation for the variability in fertilisation success is that *Montipora* gametes may be less robust than those of other species, and that the effects of varying experimental conditions from spawning to spawning (eg. temperature) are responsible for the observed patterns of fertilisation success. When carrying out crosses *Montipora* eggs appear less robust and break-up earlier than *Acropora* or *Platygyra* eggs (pers. obs.). *Montipora* eggs are unusual in that they contain zooxanthellae (Heyward and Collins 1985a, Harrison and Wallace 1990). Carrying zooxanthellae may make eggs more fragile due to the propensity of corals to expel zooxanthellae when stressed. When zooxanthellate corals are subjected to stress, particularly thermal stress, they typically expel their zooxanthellae (Jokiel and Coles 1990). There appears to be a very delicate balance between the coral host and its symbiotic algae which is easily disrupted by stress. Perhaps there is a very thin line drawn between tolerating and rejecting non-self cells. Variation in experimental conditions such as temperature and light regimes may disrupt the delicate balance, resulting in the expulsion of zooxanthellae. If this does occur the egg cell membrane is likely to be damaged, and the eggs will break-up. *Porites* is the only other genus in which eggs contain zooxanthellae (reviewed in Harrison and Wallace 1990), it will be interesting to see if crosses between *Porites* species are equally problematic.

In conclusion, there is a strong barrier to reproduction between the FF and YS morphs of *M. digitata* suggesting that they should be considered different species. This is consistent with the results of the electrophoretic study in Chapter 2, which demonstrated a lack of gene flow between the two morphs. The barrier to reproduction is pre-zygotic and involves the inability of sperm from incompatible morphs to activate eggs. This means that there is no wastage of gametes due to hybrid inviability between the two morphs, which is important for the fitness of the two morphs (species). Sperm chemotaxis is not specific for the two morphs (Coll *et al.* 1994), and thus the most likely reason for effective reproductive isolation is the incompatibility of egg-sperm binding processes between the two morphs. Although strong barriers to reproduction are evident between some species of *Montipora*, there are other species between which barriers are weak. While the formation of interspecific hybrids is likely as most species spawn on the same night, the rates of hybridisation are probably low, and larvae may not survive to adulthood.

Chapter 4

Morphometric study of two morphs of *Montipora digitata*

4.1 Introduction

Despite the taxonomic difficulties associated with describing species morphologically (eg. *Porites*: Brakel 1977, Garthwaite *et al.* 1994) traditional taxonomic techniques remain the commonest methods for delimiting coral species due to the enormous practical benefits involved (Lang 1984). Coral colonies need only be bleached before identification is possible, identification is cheap and fast (eg. molecular techniques are expensive and more time consuming), and they can then be stored indefinitely without the need for any form of preservative. Furthermore fossil corals can also be identified and compared to present day forms (eg. Budd 1990). It appears that new techniques will complement the traditional techniques, but not replace them.

Species level taxonomic work on corals is based on characters that are readily observed on upper calical surfaces, as they are convenient and easy to measure (Budd 1990). Until recently species limits have commonly been defined by qualitative rather than quantitative criteria (eg. Veron and Wallace 1984, Foster 1984, Veron and Pichon 1982). Similarly, ecomorph variability has been described qualitatively (eg. Veron and Pichon 1976). Qualitative methods are extremely useful in pioneering taxonomy as they allow a rapid exploration of the species present and are generally very accurate. Evidence of the accuracy of such methods lies in the fact that most molecular studies have confirmed the species status of species that have been defined in this manner (Willis and Ayre 1985, Ayre and Willis 1988, Ayre *et al.* 1991b, Van Veghel and Bak 1993, Garthwaite *et al.* 1994), with few exceptions (Knowlton 1992). The disadvantages of using qualitative criteria however are considerable. Qualitative criteria are not easily defined and cannot be applied objectively. They only work well for the expert that developed them, but due to their subjectivity are prone to misinterpretation by future taxonomists.

Since the 1960's taxonomic studies have increasingly made use of numerical taxonomic techniques (Rohlf and Bookstein 1990). The trend has been to move from qualitative taxonomy to numerical taxonomy in order to remove some of the subjectivity associated with traditional taxonomy. Morphometric techniques that have been applied to other organisms for many years, particularly vertebrates (eg. Bogan

1978, Carpenter *et al.* 1978, Blondel *et al.* 1984, Zelditch 1988), have been adopted for the study of morphological variation in corals (Powers 1970, Powers and Rohlf 1972, Wallace 1974, Brakel 1977, Foster 1984, Cairns 1989, Van Veghel and Bak 1993, Amaral 1994). Numerical taxonomy does require more work per species, but has the advantage that once a species has been described it is relatively easy to compare specimens objectively now and in the future. Morphometric analyses have also proved useful for investigating the extent of phenotypic plasticity within species (Foster 1977, Foster 1979, Foster 1984, Willis 1987, Amaral 1994), the relationship of corallite characters to colony shape (Foster 1983), the relationship of morphological variation to mode of reproduction (Budd 1990), and the extent to which corals have changed over geological time (Pandolfi and Burke 1989). These studies have primarily used corallite level measurements (eg. Brakel 1976, 1977, Foster 1983). Morphological features at the colony level are generally considered to be more plastic within species in response to environmental variation (Brakel 1977). However, there is also a considerable degree of environmentally-induced variation at the corallite level (Foster 1979), although the trend still remains to measure corallite-level features, because they are easier to measure accurately and objectively. Morphometric studies will probably never replace the more subjective forms of taxonomy due to the amount of effort and cost required to undertake them. They will, however, play a key role in the study of species that are not easily identifiable in the traditional manner. For example, sibling species often have minor morphological characters that individually show considerable overlap, but can be separated using multivariate morphometric techniques (Knowlton 1993).

The genetic and reproductive studies in chapters 2 and 3 established that the two morphs of *M. digitata* are two species. In view of this they shall be referred to as species from this point onwards. This morphological study had two primary objectives. The first was to determine whether there are any skeletal characters, or combinations of characters, that could be reliably used to identify the two species. The second was to formally describe the two species and quantify the degree of variation in their skeletal characters in specimens from several different geographic locations. The use of allozyme electrophoresis for *a priori* identification of species allowed a powerful assessment of which characters were species specific. Morphological characters found to be species specific allowed a study of type specimens of species synonymised with *M. digitata*, and the reinstatement of a suitable name for the second species.

4.2 Materials and methods

4.2.1 Collection of colonies

Colonies used for the morphometric analysis were collected from three main sites, Geoffrey Bay (n = 28), Nelly Bay (n = 14) and Pioneer Bay (n = 11). A further two samples each from Low Isles and Wewak (Papua New Guinea) were also included in the analyses (these were the only samples available from these sites). Site locations and descriptions are provided in Chapter 2.

Eighteen of the colonies from Geoffrey Bay were collected during coral spawning (the colonies were used in the reproduction experiments). These consisted of 8 FF and 10 YS colonies, although the identity of half of these colonies was uncertain until confirmed by electrophoresis. A further 5 FF and 5 YS colonies were collected that were "good" morphological examples of the two species. Colonies from spawning experiments were also used from Pioneer Bay (6FF and 5 YS) and Nelly Bay (4FF). The electrophoretic study revealed that most colonies collected in Nelly Bay were FF, including most of those thought to be of YS morphology. In view of this a further 10 colonies of spatulate morphology were collected from Nelly Bay (which were 6 FF and 4 YS based on electrophoresis). The FF and YS samples from Low Isles and Papua New Guinea represented "good" examples of the two species at the respective locations. In order to reduce the chance of collecting clonemates all colonies collected were separated by more than 5 m unless of differing morphology. Samples were not selected at random for this study in order to make use of already available colonies. As a consequence there is a bias towards "good" representatives of the two species, the implications of which will be addressed in the discussion.

In order to determine the species status of colonies, small samples were collected from each colony for identification using allozyme electrophoresis, except for colonies collected from Low Isles and Papua New Guinea. All samples collected for electrophoresis were treated as described in Chapter 2. The fixed gene difference at *LT-2** was used to determine the species status of the colonies. Colonies used for morphometric analysis were placed in a weak sodium hypochlorite solution until all living tissue had been digested leaving a clean skeleton.

4.2.2 Morphometric analysis

One of the most prominent features of members of the genus Acroporidae is their lack of diagnostic skeletal features (Veron and Wallace 1984, Wallace and Willis 1994). This was very apparent when selecting skeletal features to include in this morphometric study and is the reason few features were used. Further problems were

the auto-correlation of some of the characters that could be measured (for example, corallite diameter and area are clearly strongly correlated), and lack of other features such as tuberculae and papillae which are common in the genus *Montipora* but which do not occur in *M. digitata*. Attempts were made to use the colony level features inter-branching distance and branch width, however, these features were extremely variable both within and between colonies and so were discarded. Such features are also more likely to be affected by the immediate environment. Despite these difficulties both corallite-level and colony-level features were found for inclusion in the morphometric study.

Corallite measurements:

Corallite measurements were made from scanned video images. Three corallite characters were measured (Figure 4.1). These characters are defined as follows:-

Corallite diameter (DI) = the largest distance across a corallite.

Inter-corallite distance (IN) = the distance between the center of corallites.

Septa (SE) = the length of the leading (longest) septa.

A video camera attached to a binocular microscope and connected to a Macintosh IIci PC was used to generate images of the corals at 3.5X magnification. The images were "grabbed" using a Framegrabber and stored on disks. Typically there were 7-11 corallites per frame "grabbed". Images were measured using Image version 1.44 and measurements were stored directly to a spreadsheet. Calibration of the images was carried out prior to each measuring session using Mitutoyo dial calipers accurate to 0.05mm. All frames were "grabbed" at 3.5X magnification, where 81 pixels were equivalent to 1mm.

For each colony used in the study, five measurements of each variable were made from each of four branches. Branches were selected by placing a grid over the colony and selecting the branch nearest to a grid point which was selected using random number tables (Zar 1984). Only one frame was analysed per branch as all frames contained 5-15 corallites. All measurements were obtained from frames "grabbed" between 2 and 3 cm from the branch tip. This distance was decided on as it was far enough from the branch tip for corallites to be fully formed, yet not so close to the colony base that corallites may have been affected by reduced light or increased sediment loads. The first frame to come into view between 2 and 3 cm from the branch tip was used for making corallite measurements.

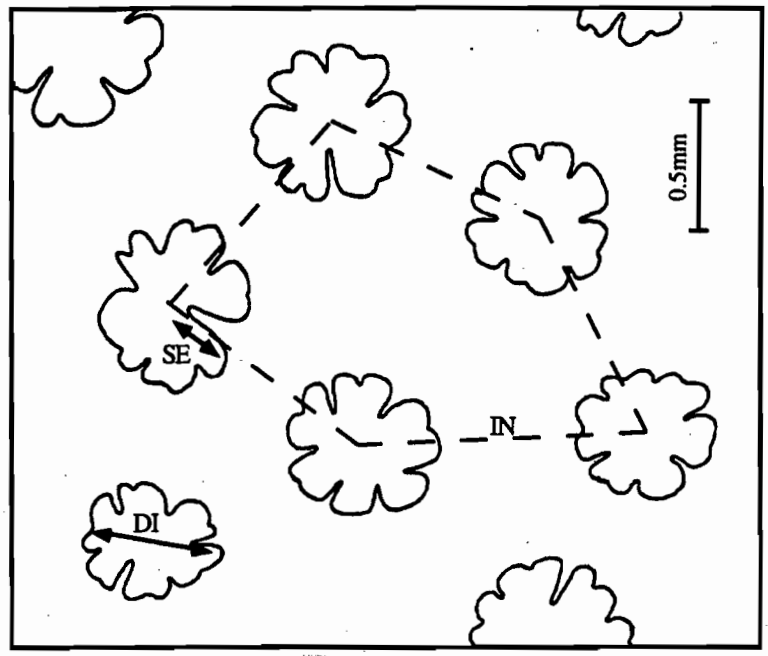


Figure 4.1. Corallite-level measurements made for morphometric analysis of FF and YS *Montipora digitata*. Inter-corallite distance IN (- - -) was measured between the five corallites forming a pentagon nearest to the frame center, corallite diameter (DI) and leading septum length (SE) were measured from the five largest corallites in the "grabbed" video frame.

Within each frame the five largest corallites were chosen for measurements of corallite diameter and leading septum length. Corallites to be measured were first labelled alphabetically in order to prevent re-measurement, and to allow re-measurement if required. Standardisation of the measurement of inter-corallite distance was achieved by selecting five corallites that formed a pentagon in the center of the "grabbed" frame and measuring the distance between each of them (Figure 4.1). This does not violate the assumption of independence of measurements as there is no reason to believe that measurements of nearby corallites will be dependent on each other. Inter-corallite distances within the pentagon were variable suggesting that they were not correlated, and selecting inter-corallite distances in this manner provided a means of quickly and consistently sampling the same feature between samples.

Both corallite diameter and septal length were measured from the largest corallites in the frame. This allowed much faster processing of the colonies. Selection of features in this manner is valid for comparison of the two species (as it is a standardised method for both of them), but it is not suitable for describing the

variability of these features. Further measurements of these two features were therefore made to account for the full extent of their variability in the two species. This was achieved by measuring all corallites within the four sampled frames from 10FF and 10YS colonies. Within each morph the two most different colonies from each site (those furthest apart in the canonical discriminant analysis) were selected for measurement (except for samples from Papua New Guinea and Low Isles where only one sample was available per morph). The most different colonies were selected to obtain a better estimate of the extent of variation in corallite diameter and septal length.

Although not included in the multivariate analysis, septal shape was also compared between the two species. The shapes of first cycle septa were classified as: 1) serrated: septa in the form of rows of spines; 2) lamino-serrated: individual septum ocurring as a combination of spines and fused spines; 3) laminar: septa consisting of continuous sheets of fused spines. Septa were counted from a total of 59 colonies (Geoffrey Bay: 14 FF and 14 YS; Nelly Bay: 14 FF and 6 YS; Pioneer Bay: 5FF and 6YS). A total of 509 FF corallites (septa n = 3059), and 374 YS corallites (septa = 2247) were examined. In each colony the shapes of all first cycle septa were recorded for five corallites from three randomly selected branches giving a total of fifteen corallites examined per colony. Coralites were examined in the zone 2-3 cm from the branch-tip used for the morphometric measurements described above. They were selected consistently by selecting the first corallite encountered within the measurement zone and then the next four below running towards the branch base.

Colony measurements:

A feature that initially distinguishes the two species of *M. digitata* is the difference in branch tip morphology they exhibit. Fat fingers colonies have rounded branch tips and yellow spatulate colonies have explanate branch tips. The shape of branch tips was incorporated into the analysis by two measurements. These were (also see Figure 4.2):-

Branch tip width (W1) = greatest width of branch tip at 0.5 cm from the tip.

Branch tip breadth (W2) = width at 90° to W1 and 0.5 cm from the tip.

The ratio of these two measurements gave an estimate of the degree of flattening of branch tips (i.e. of how spatulate they were). A total of twenty of each of the two branch tip measurements was made per colony from branches chosen randomly in the same manner as branches selected for corallite measurements. Measurements were made using a pair of Mitutoyo dial calipers accurate to 0.05mm.

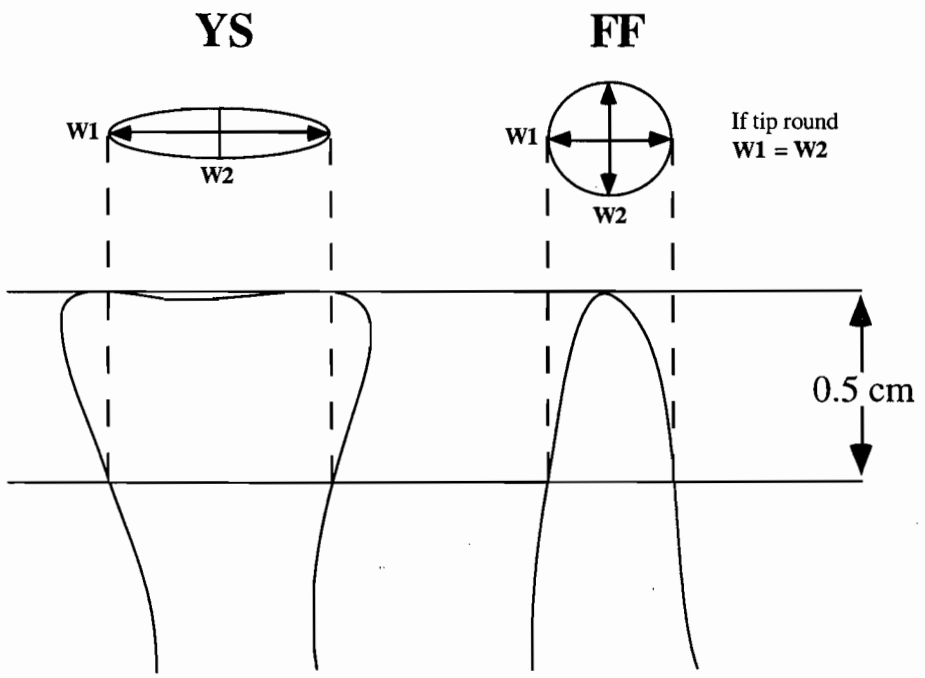


Figure 4.2. Branch-tip measurements made for morphometric analysis of fat fingers and yellow spatulate *Montipora digitata*. All measurements were made 0.5 cm from the branch-tip. The greatest width (W1), and width at 90° to the greatest width (W2) were measured.

4.2.4 Septal morphology of juvenile Montipora digitata.

During the course of this study it became apparent that septal morphology differs between FF and YS *M. digitata*. However, it was not clear why or how such a difference could occur. Newly settled juvenile corals from YS crosses and FF crosses were reared to determine whether corallite features observed in adult colonies were also present in juveniles, and to gain insights into the way in which the septa are laid down. Any differences between the two species at the juvenile stage would be significant given that juvenile corals have few reliable taxonomic features (Babcock 1992).

Juvenile corals were obtained from Orpheus Island during coral spawning periods in November 1992 and 1993. Separated and cleaned eggs and sperm from fertilisation experiments were mixed in 2.5 litre plastic pots (eggs and sperm were obtained as described in chapter 2 and mixed at similar concentrations). The pots were sealed and placed in the sea to agitate for approximately 15 hrs, after which the lids were removed and replaced with plankton mesh (100 μm). The pots were then left for two days in the sea where they were "pumped" every 6-12 hrs (this involves squeezing the pots to allow replacement of stagnant seawater within them). After three

days the contents of the pots (motile larvae about 0.5 mm long) were placed in buckets with seawater running through them. Larvae were prevented from escaping by placing a plankton mesh sock over the bucket overflow. Buckets were held in a raceway shaded from direct sunlight by shadecloth. Unglazed tiles that had been conditioned in seawater were placed in the buckets to provide a medium for larvae to settle on. After a period of 3.5-5 months the tiles were collected and placed in bleach. Juvenile corals produced by the two morphs were then coated with gold and photographed using a scanning electron microscope. A count of septa and septal shape for juveniles was made.

4.2.5 Validating septal shape as a distinguishing character

To determine the validity of septal shape as a distinguishing character, and to rule out any possibility of bias in its description, an assessment of species identity was made using septal shape only. *Montipora digitata* branches were selected from Nelly Bay, where identification of fat fingers and yellow spatulate *M. digitata* based on gross morphology is most difficult (as colonies that have spatulate branch tips are often FF based on allozyme electrophoresis results, see Chapter 2), and examined with no prior knowledge of species status. A total of seventeen branches were collected from colonies with spatulate branch tips, and a sample was taken from each for electrophoretic identification. The colonies were first identified using the septal shape as the identification criterion. Once this had been done the samples were identified using allozyme electrophoresis and the results were compared.

4.2.6 Examination of museum collections

Holotypes of species of *Montipora* synonymous with *M. digitata* were examined to determine which pre-existing name would be appropriate to assign to either the YS or FF species of *M. digitata*. Colonies from original collections by the United States National Museum of Natural History (USNM), the British Museum of Natural History (BMNH), and the Australian Institute of Marine Science (AIMS) were examined. Corallite level measurements made for colonies from the USNM (*M. tortuosa* Dana 1846 No. 310 and *M. digitata* Dana 1846 No. 312) were made using the same image analysis technique described above.

4.2.7 Statistical analyses

The differences between individual morphological characters in FF and YS colonies were first compared using one-way analysis of variance. Alpha was adjusted

to allow for multiple tests using the Bonferroni correction α/p, where p = number of tests (α = 0.0125). Data were tested for normality (using Cochran's test), and homogeneity of variances. It was found that data needed to be log10 transformed to meet the assumptions of the analysis of variance. Data for W1 did not meet the assumptions of the ANOVA and were therefore analysed using a non-parametric Kruskal-Wallis test (Chi-square approximation).

Following the univariate study a nested (colony was nested within species) multivariate analysis of variance (MANOVA) was performed using the variables diameter, inter-corallite distance, and branch-tip widths W1 and W2. Multivariate analysis was used to look for combined effects of skeletal characters. Data were tested for multivariate normality using multivariate normality plot and multivariate Levene's test. In order to conform to multivariate normality the data were log10 transformed. Septum length and branch-tip diameter (W1) were found to be correlated so septum length was omitted from the multivariate analysis. However, variable W1 was included as it did not affect the outcome of the MANOVA (there was a significant Pillai's trace whether the variable was included or not), and its inclusion was necessary to give an estimate of the amount of branch-tip flattening (this was informative when considering Nelly Bay FF colonies that had unusual branch tip morphology). MANOVA was used to determine whether species differences occurred consistently from bay to bay. The MANOVA analyses were carried out separately for each bay as there were insufficient degrees of freedom to include bays, species and colonies in a single model. Alpha values were accordingly adjusted to allow for multiple tests using the Bonferroni correction (α = 0.01). The relationship between species and sites was investigated using Canonical Discriminant Analysis (CDA). This technique finds the minimum number of dimensions that maximise the variation between *a priori* groups (in this case colonies identified using allozyme electrophoresis). Confidence limits for group centroids in the canonical discriminant analyses can be obtained using the formula (χ^2 2,.05/n) (Seber 1984). All analyses carried out for this study were performed using SAS version 6.04.

4.3 Results

4.3.1 Morphometric analysis

There are morphological differences between the two species of *M. digitata*. The five variables measured showed higher mean values for FF colonies than for YS colonies at all sites, with the exception of septum for Geoffrey Bay and inter-corallite

distance for Geoffrey Bay, Pioneer Bay and Papua New Guinea. When data from all sites was pooled FF colony means for all variables were higher than those for YS colonies (Table 4.1). One-way ANOVA showed that corallite diameter, inter-corallite distance and branch-tip diameter (W2) differ significantly between FF and YS colonies (Table 4.2). Whereas leading septum length does not differ significantly. Branch-tip diameter (W1) also differed between species (χ^2 5.55, 1 df, $0.01<p<0.025$). Despite all of these differences, no single character can be used to identify the species due to the large amount of overlap of the characters between the two species (Table 4.1)

The relationship between the two branch tip measurements recorded for colonies from Geoffrey, Pioneer and Nelly bays is shown in Figure 4.3a-c respectively. The line on the graphs represents the scenario in which all branches are perfectly round, producing a regression with a slope of one. Fat Finger colonies from Geoffrey Bay and Pioneer Bay both produced regressions with slopes closer to one than YS colonies (Table 4.3). This is consistent with the observation that fewer YS branches have round tips. In Nelly Bay the trend was reversed, with FF colonies having more explanate branch tips than YS colonies.

Multivariate analysis of variance testing for an overall species effect gave a significant Pillai's Trace when carried out for the five "good" examples of each morph from Geoffrey Bay (Pillai's Trace = 0.99, F = 90.1, df = 5, $p<0.001$), all Geoffrey Bay colonies sampled (Pillai's Trace = 0.57, F = 7.5, df = 23, $p<0.001$), and all colonies sampled (Pillai's Trace = 0.27, F = 4.7, df = 52, $p<0.01$). There was, however, no significant difference between Pioneer Bay colonies (Pillai's Trace = 0.76, F = 4.7, df = 6, $p>0.01$) or Nelly Bay colonies (Pillai's Trace = 0.57, F = 3.0, df = 9, $p>0.05$) which was probably caused by the small sample size from Pioneer Bay and the large number of FF colonies with spatulate branch tips in Nelly Bay.

Canonical Discriminant Analysis (CDA) carried out on the "good" examples of each morph collected from Geoffrey Bay shows that there is clear separation between them (Figure 4.4). The first and second canonical variables accounted for 82% and 14% of the variation respectively. Separation of the two species is primarily driven by corallite diameter (DI), smallest branch width (W2), and inter-corallite distance (IN), as shown in the bi-plot. The bi-plot is a representation of the relative contributions of each character (shown as the arrow length), and the direction in which its influence moves the points on the plot (shown by the arrow's orientation). All of these parameters were larger for fat fingers colonies. The total canonical structure for all analyses is given in the appendix (Table 3).

Variable	Statistic	GB		NB		PB		LI		PNG		Overall	
		FF	YS	FF	YS	FF	YS	FF	YS	FF	YS	FF	YS
	n	260	300	200	80	120	100	20	20	20	20	620	520
	MEAN	0.671	0.61	0.654	0.505	0.686	0.597	0.713	0.65	0.5	0.442	0.664	0.5862
DI	STDEV	0.084	0.08	0.082	0.121	0.112	0.068	0.079	0.106	0.052	0.075	0.0944	0.0987
	MIN	0.478	0.41	0.383	0.316	0.409	0.393	0.573	0.457	0.42	0.34	0.383	0.316
	MAX	0.9	0.963	0.862	0.909	0.918	0.751	0.896	0.836	0.605	0.572	0.918	0.963
	SKEW	0.196	0.446	-0.329	0.787	-0.461	-0.216	0.524	-0.388	0.747	0.211	-0.1459	-0.1437
	MEAN	1.179	1.102	1.162	0.983	1.283	1.134	1.344	1.166	1.117	0.887	1.1968	1.084
IN	STDEV	0.178	0.165	0.16	0.2	0.269	0.153	0.218	0.191	0.155	0.158	0.211	0.1805
	MIN	0.689	0.667	0.754	0.611	0.854	0.804	1.017	0.801	0.882	0.559	0.689	0.5593
	MAX	1.715	1.552	1.719	1.47	2.218	1.52	1.815	1.58	1.379	1.23	2.218	1.5798
	SKEW	0.168	0.307	0.16	0.266	0.875	0.211	0.756	0.328	0.033	0.313	0.6907	0.0732
	MEAN	0.169	0.178	0.17	0.138	0.144	0.134	0.185	0.167	0.132	0.104	0.1638	0.1602
SE	STDEV	0.035	0.035	0.038	0.048	0.043	0.032	0.039	0.052	0.03	0.036	0.0395	0.0438
	MIN	0.081	0.096	0.067	0.054	0.055	0.061	0.123	0.077	0.096	0.054	0.055	0.054
	MAX	0.28	0.336	0.272	0.252	0.302	0.227	0.259	0.286	0.218	0.177	0.302	0.336
	SKEW	0.107	0.457	0.054	0.321	0.995	0.218	0.067	0.313	1.214	0.731	0.1978	0.0724
	MEAN	0.639	0.658	0.797	0.672	0.542	0.598	0.853	0.811	0.592	0.618	0.6768	0.653
W1	STDEV	0.125	0.224	0.333	0.227	0.091	0.221	0.144	0.179	0.094	0.207	0.2336	0.2243
	MIN	0.4	0.34	0.3	0.33	0.39	0.32	0.65	0.49	0.4	0.36	0.3	0.32
	MAX	1.29	1.9	2.2	1.2	0.94	1.43	1.12	1.2	0.81	1.03	2.2	1.9
	SKEW	1.718	1.778	1.701	0.556	0.828	1.726	0.569	0.381	0.691	0.586	2.6304	1.4121
	MEAN	0.573	0.487	0.503	0.419	0.503	0.468	0.711	0.605	0.537	0.416	0.5401	0.4743
W2	STDEV	0.079	0.099	0.131	0.074	0.086	0.098	0.09	0.082	0.062	0.066	0.1094	0.1001
	MIN	0.39	0.3	0.27	0.29	0.35	0.32	0.62	0.47	0.4	0.26	0.27	0.26
	MAX	0.96	0.91	0.97	0.65	0.75	0.78	0.96	0.8	0.66	0.5	0.97	0.91
	SKEW	0.551	1.028	0.847	0.702	0.391	0.933	1.46	0.355	-0.343	-0.506	0.4215	0.9246

Table 4.1. Summary of morphometric measurements made for morphometric analysis (in mm) in FF and YS *Montipora digitata* colonies collected at: Geoffrey Bay (GB), Nelly Bay (NB), Pioneer Bay (PB), Low Isles (LI) and Papua New Guinea (PNG). (DI): Corallite diameter, (IN): inter-corallite distance, (SE): septa, (W1): greatest branch-tip width, (W2): Branch-tip width at 90° to W1. n: number of measurements.

Morphological feature	Variance source	Degrees of freedom	Sum of squares	Sum of squares	F Value	P
DI	SPECIES	1	0.890	0.890	15.159	<0.0005*
	ERROR	55	3.229	0.059		
IN	SPECIES	1	0.514	0.514	14.065	<0.0005*
	ERROR	55	2.010	0.037		
SEPTUM	SPECIES	1	0.060	0.060	0.426	>0.05
	ERROR	55	7.700	0.140		
W2	SPECIES	1	0.905	0.905	9.912	<0.005*
	ERROR	55	5.023	0.091		

Table 4.2. One-way analysis of variance comparing corallite measurements of *Montipora digitata* FF and YS colonies. DI: corallite diameter, IN: inter-corallite distance, SEPTUM: leading septum length and W2: greatest branch-tip width. Bonferroni correction $\alpha = 0.0125$, *: significant difference.

Site	Fat fingers			Yellow Spatulate		
	r^2	slope±SE	n	r^2	slope±SE	n
Geoffrey Bay	0.59	0.49± 0.025	260	0.21	0.20 ± 0.023	300
Pioneer Bay	0.65	0.75 ± 0.05	120	0.62	0.35± 0.028	100
Nelly Bay	0.015	-0.047 ± 0.032	140	0.031	0.057 ± 0.036	80

Table 4.3. Results for regression analyses of branch tip variables W1 and W2 from colonies collected in Geoffrey Bay, Pioneer Bay and Nelly Bay. n: number of branch tips.

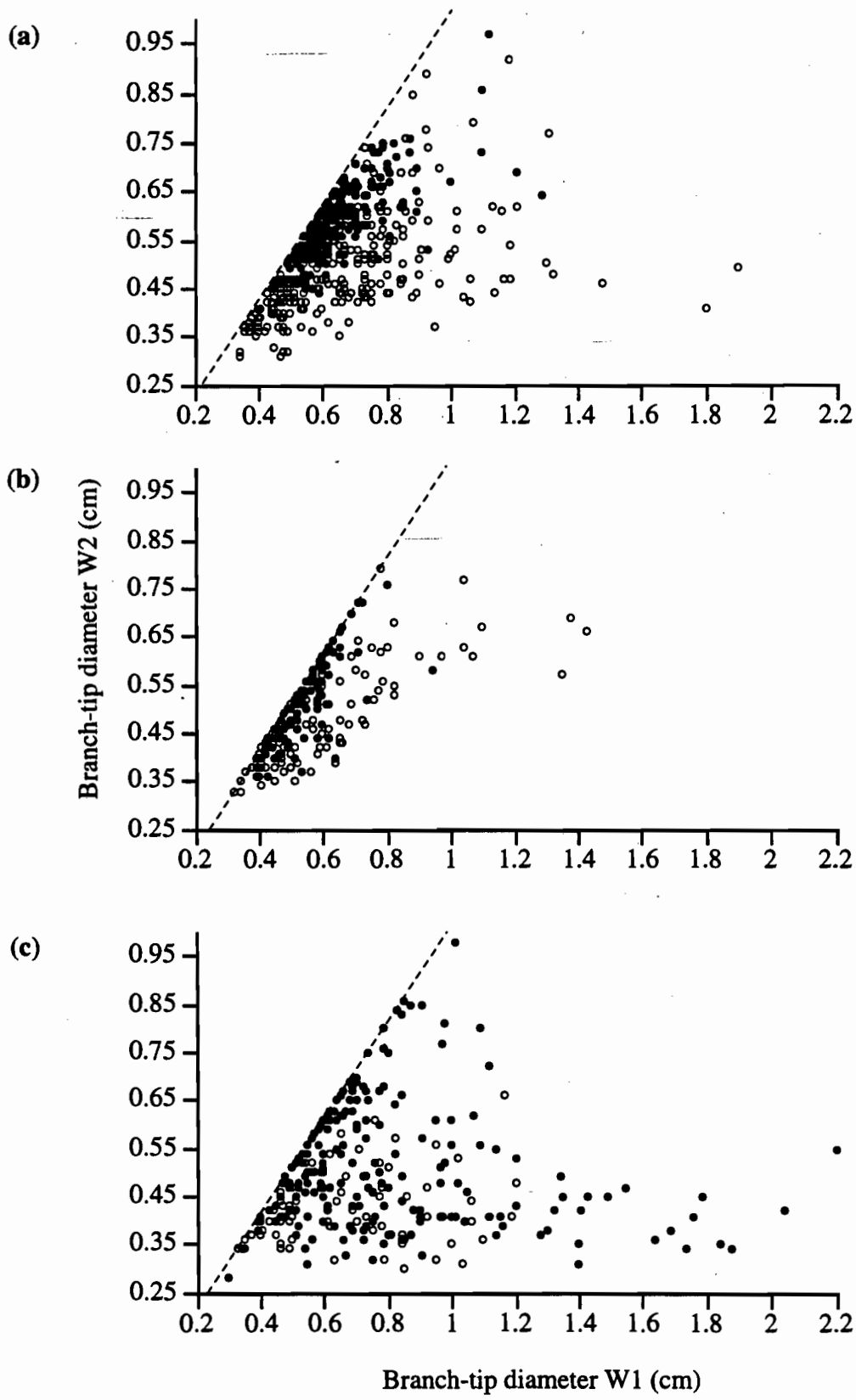


Figure 4.3. Scattter plots showing the relationship between greatest branch-tip diameter (W1), and diameter at 90° to the greatest branch-tip diameter (W2), for FF (•) and YS (o) colonies of *Montipora digitata* at Geoffrey Bay (a), Pioneer Bay (b), and Nelly Bay (c). Dashed line represents round branch-tips (i.e. W1=W2).

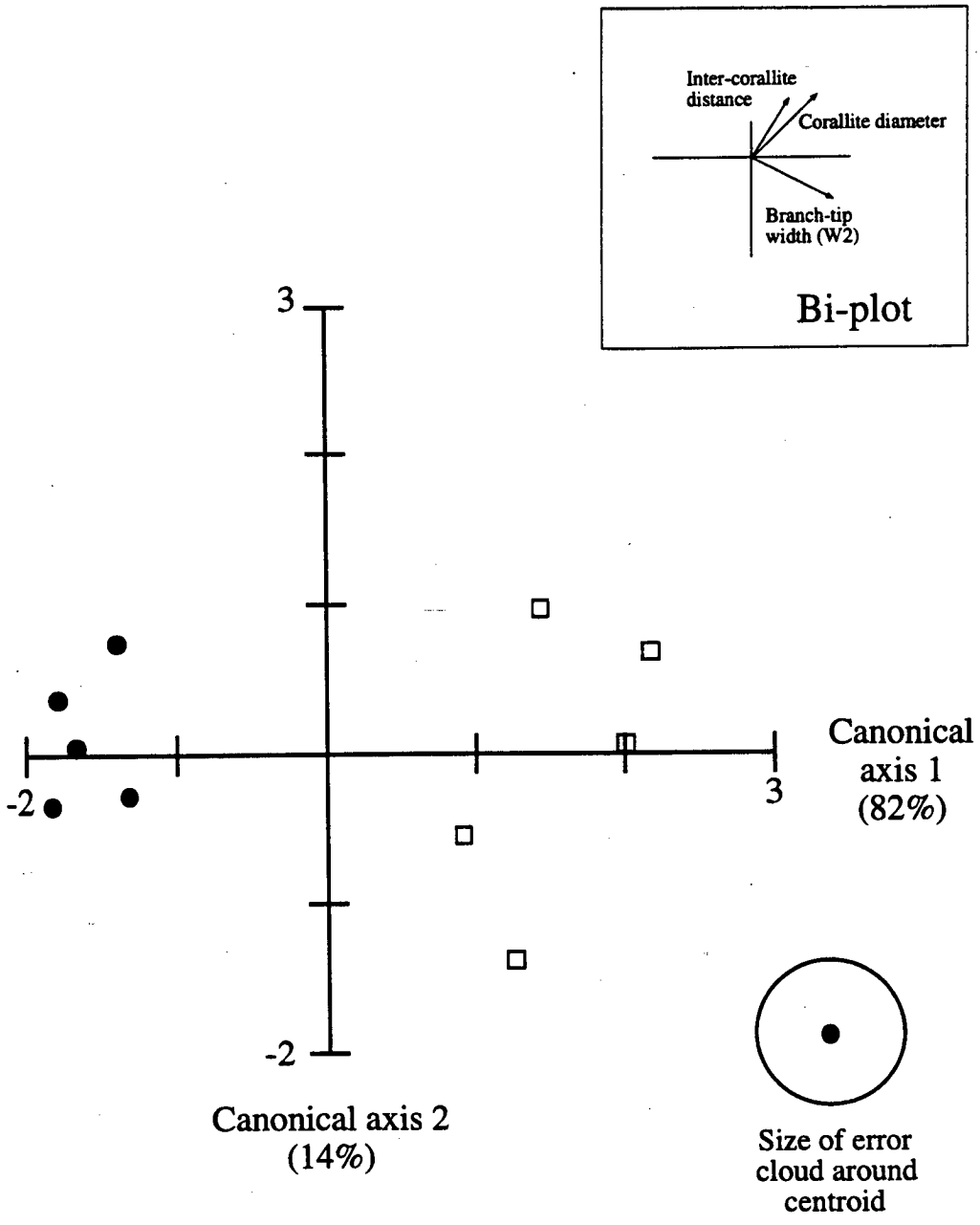


Figure 4.4. Plot of Canonical axes one and two for "good" examples of FF (□) and YS (●) *Montipora digitata* colonies from Geoffrey Bay. Symbols represent colony centroids. The bi-plot summarises the total canonical structure. Error cloud represents 95% confidence interval. Each point is derrived from 20 measurements of variables corallite diameter, inter-corallite distance, and branch widths W1 and W2 per colony.

When all colonies are analysed together the distinctness of the morphs breaks down due to overlap of the canonical variables (Figure 4.5). The first and second canonical variables account for 62% and 22% of the variation respectively. Despite the overlap, the two species still tend to cluster on opposite sides of canonical axis 1. A distinct cluster of Nelly Bay fat fingers colonies formed on the top left corner of the plot. This separation was driven by the spatulate morphology of many fat fingers colonies in Nelly Bay. Corallite diameter and the smallest branch width were the most important factors governing the separation of the species in all of these plots, as determined from the bi-plots.

4.3.2 Septal morphology

The shape of septa proved to be a distinguishing character between the two species. More than 90% of fat fingers septa examined were serrated, whereas 60-80% of yellow spatulate septa examined were laminar (total number of septa examined: FF = 3059; YS = 2247, Figure 4.6). Examples of serrated and laminar septa are given in plates 4.1a-b and 4.2a-b respectively. Lamino-serrated septa are intermediate between these two forms, each septum having both a laminar segment and spines. For FF colonies, no corallite examined (n = 3059) had more than one laminar septum, with the exception of two corallites from Pioneer Bay colonies, one of which had four, and another two. For YS colonies the trend was not as clear, thirty five corallites had less than 2 laminar septa per corallite and 8 of them had no laminar septa. The yellow spatulate corallites deficient in laminar septa were not unique to one colony. The distribution of septal shapes was similar for all three bays examined. Though the numbers of septa in the three categories were only recorded between 2 and 3 cm from the branch tip, qualitative observations suggested that the patterns of septal shape were similar throughout the colonies, including the branch tips. Furthermore, the septal difference was also evident in colonies from Papua New Guinea.

Of the seventeen colonies from Nelly Bay identified using septal shape first, followed by allozyme electrophoresis, only six were YS, and eleven were FF. The identifications based on septal morphology corresponded exactly to the allozyme electrophoresis results, indicating that septal shape generally provides a reliable way of separating the two species.

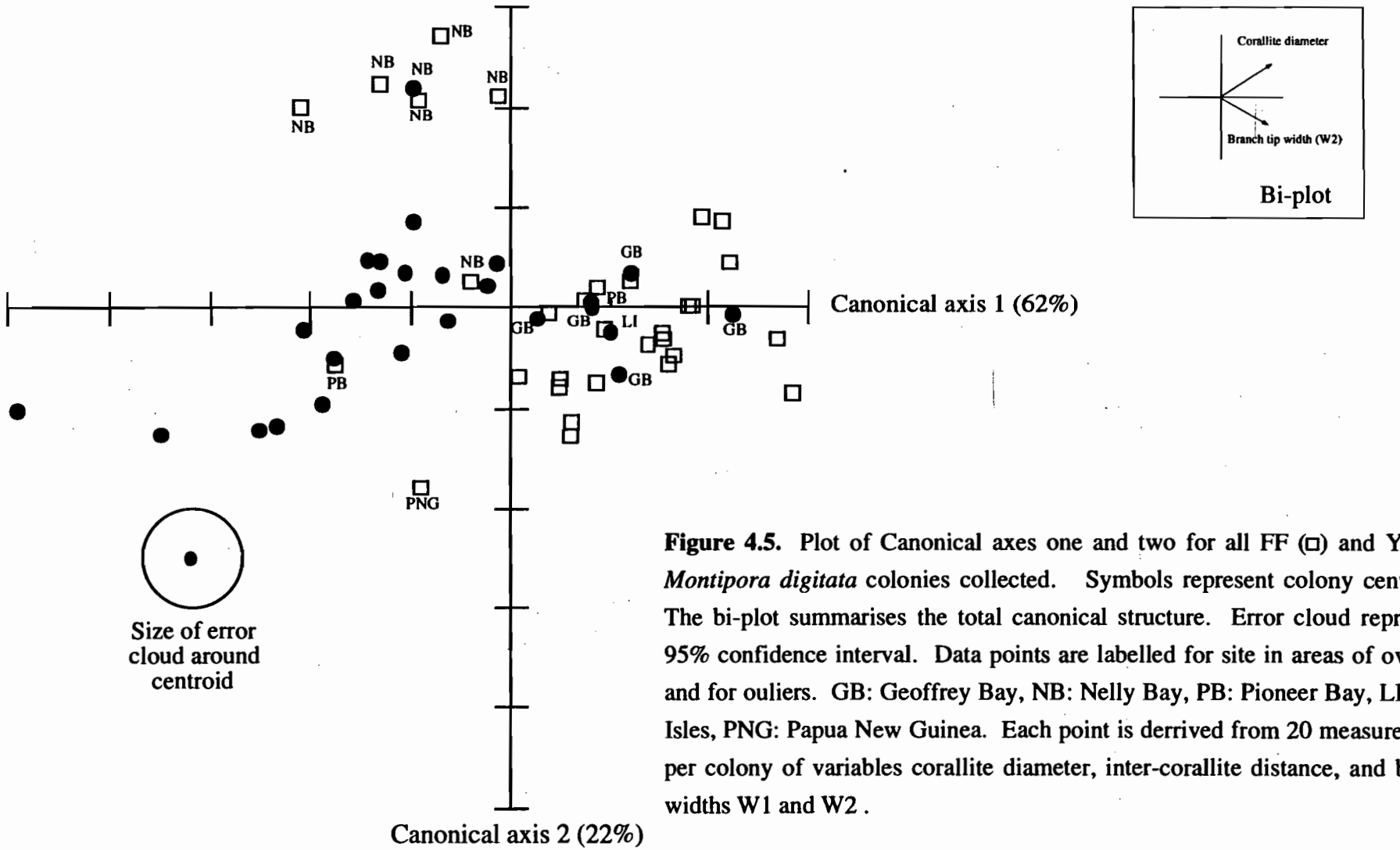


Figure 4.5. Plot of Canonical axes one and two for all FF (□) and YS (●) *Montipora digitata* colonies collected. Symbols represent colony centroids. The bi-plot summarises the total canonical structure. Error cloud represents 95% confidence interval. Data points are labelled for site in areas of overlap, and for ouliers. GB: Geoffrey Bay, NB: Nelly Bay, PB: Pioneer Bay, LI: Low Isles, PNG: Papua New Guinea. Each point is derrived from 20 measurements per colony of variables corallite diameter, inter-corallite distance, and branch widths W1 and W2 .

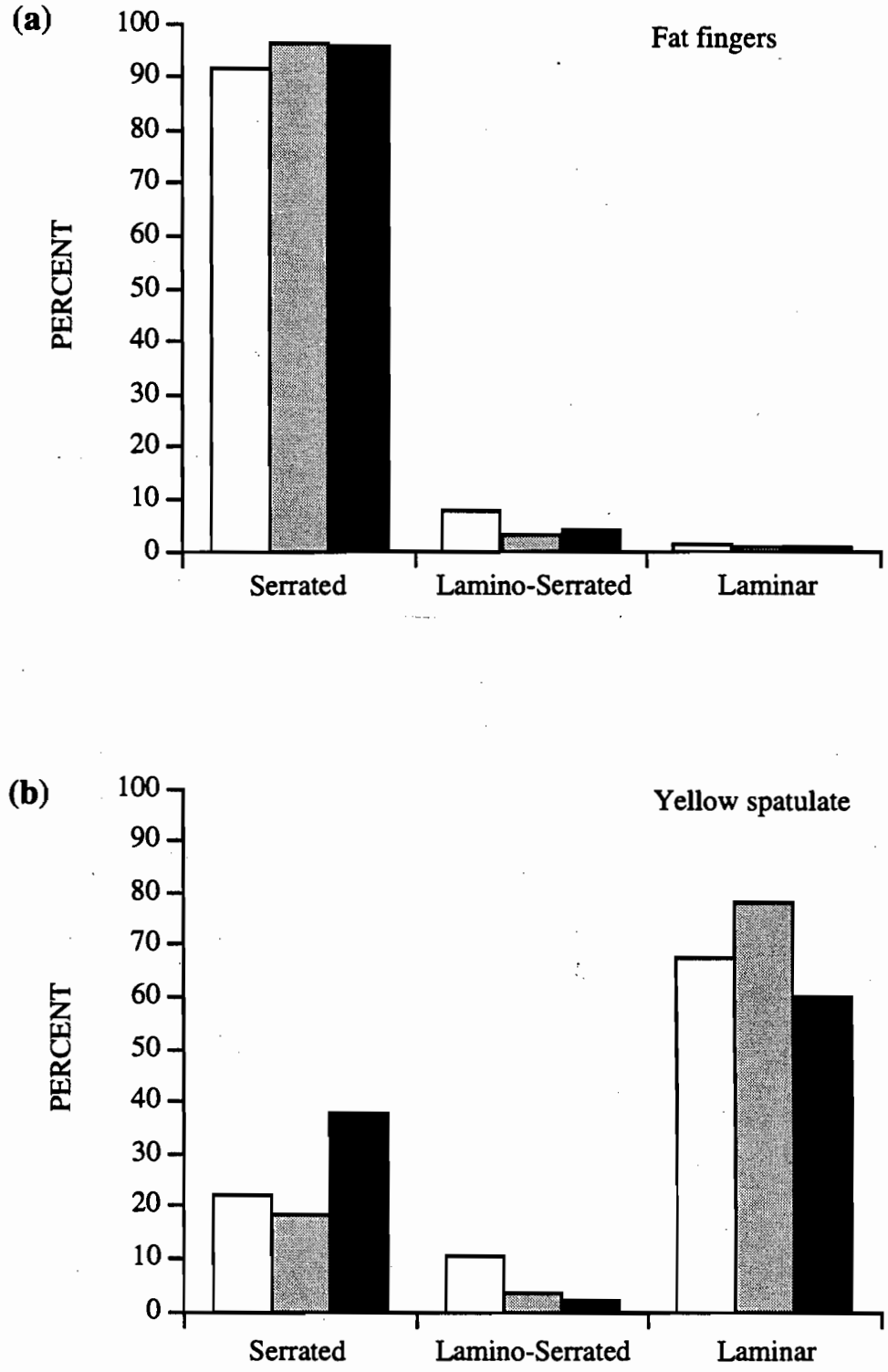
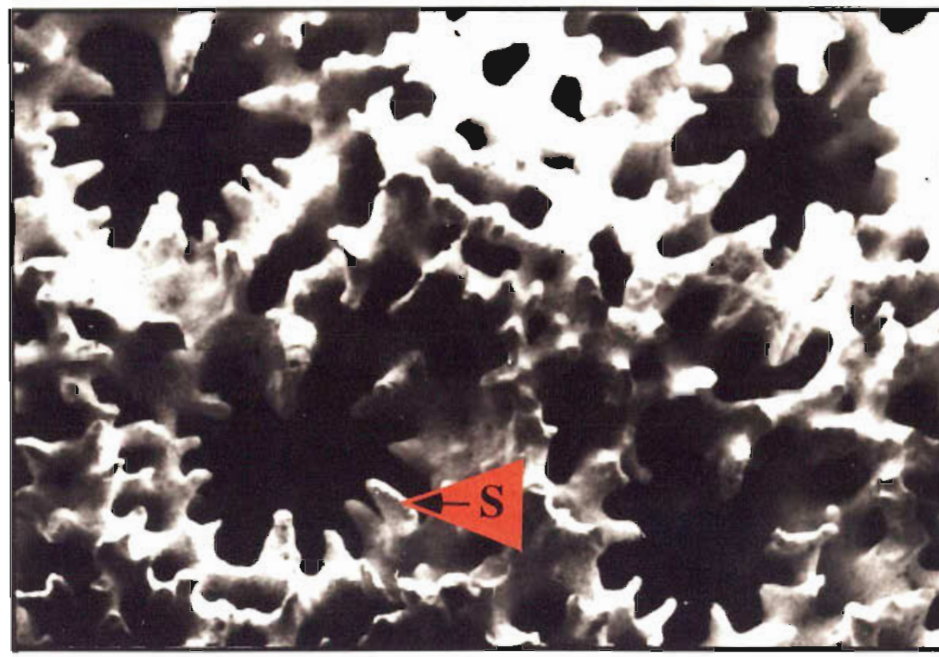


Figure 4.6. Percentage of serrated, lamino-serrated and laminar septae in FF (a) and YS (b) colonies of *Montipora digitata* from Geoffrey Bay (□), Nelly Bay (▨), and Pioneer Bay (■). Number of septae counted: Geoffrey Bay FF = 1259, YS = 1259; Nelly Bay FF = 1260, YS = 540; Pioneer Bay FF = 540, YS = 448.

(a)



(b)

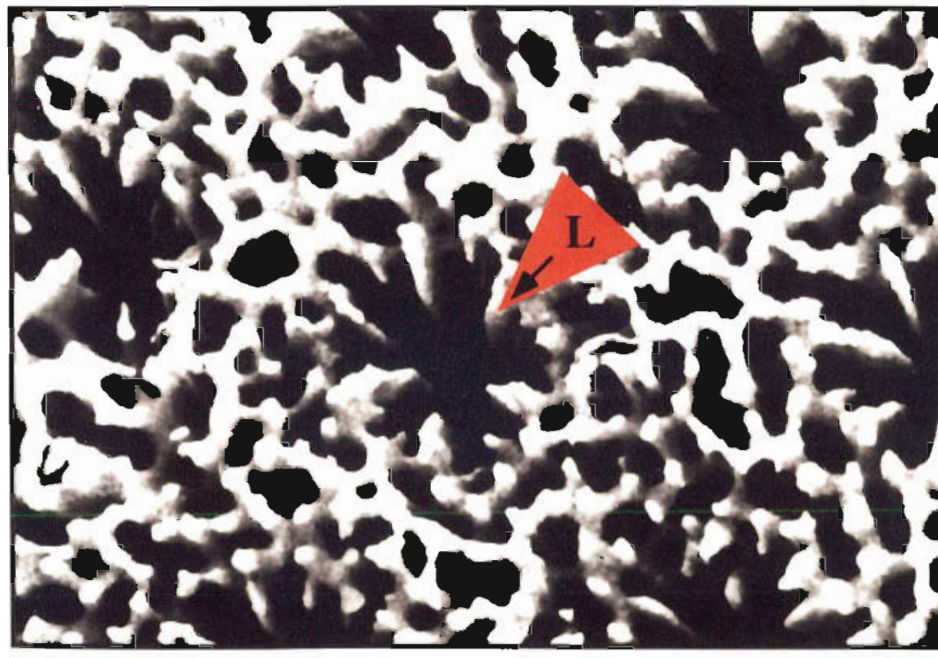
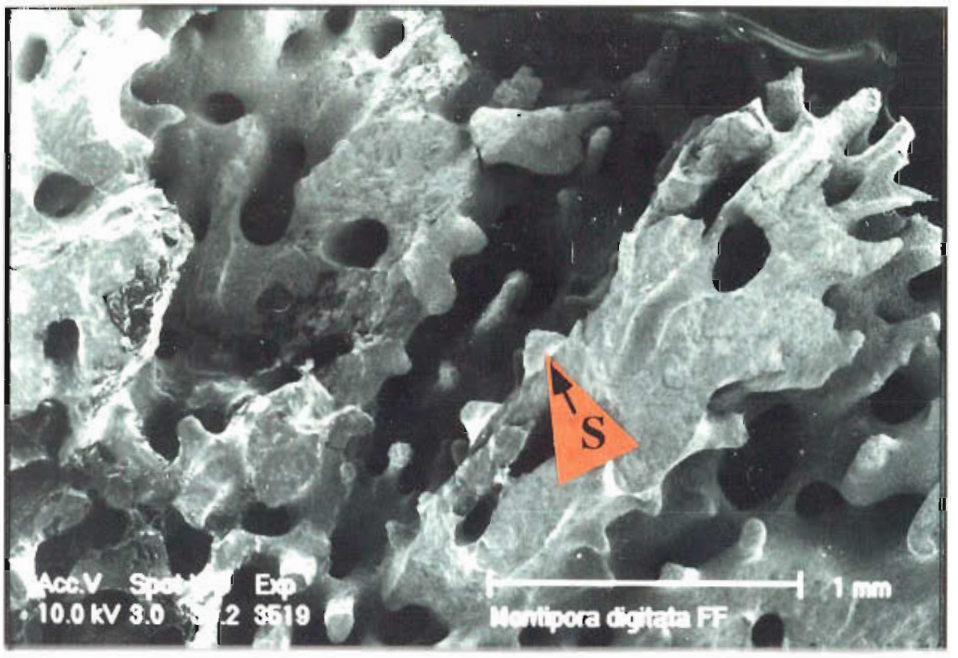


Plate 4.1. Scanning electron micrographs of the skeletons of fat fingers (FF) and yellow spatulate (YS) *Montipora digitata* showing the structure of corallites and septae. (a): fat fingers, (b): yellow spatulate. The FF morph has septae composed of a number of teeth (ie. serrated)(S), and the YS morph has laminar septae (uninterrupted sheets)(L).

(a)



(b)

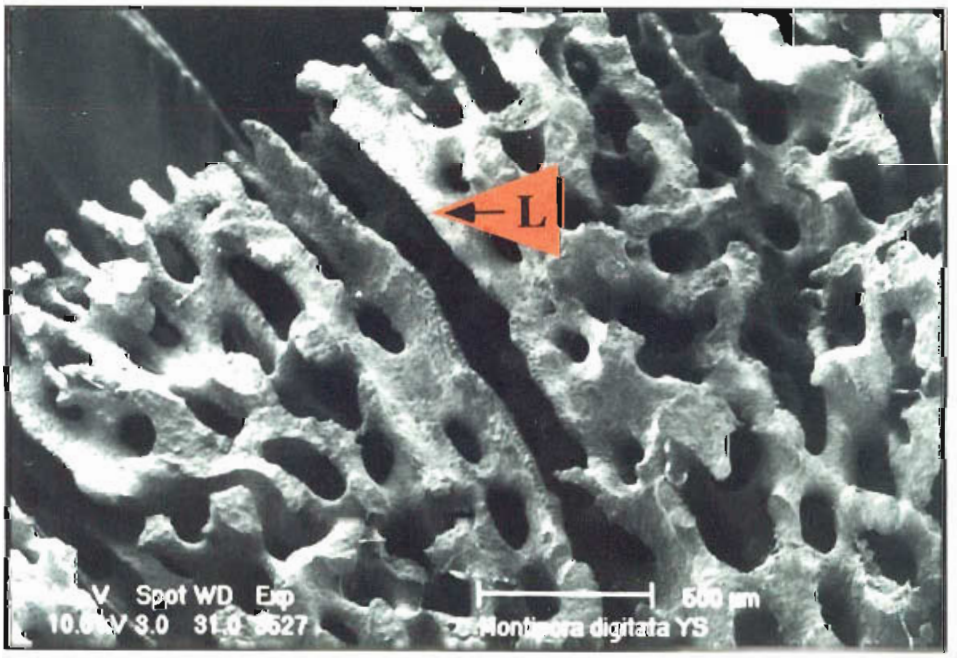


Plate 4.2. Scanning electron micrographs of fat fingers (FF) and yellow spatulate (YS) *Montipora digitata* corallites in longitudinal section. (a): fat fingers, (b): yellow spatulate. The FF morph has septae composed of a number of teeth (ie. serrated)(S), and the YS morph has laminar septae (uninterrupted sheets)(L).

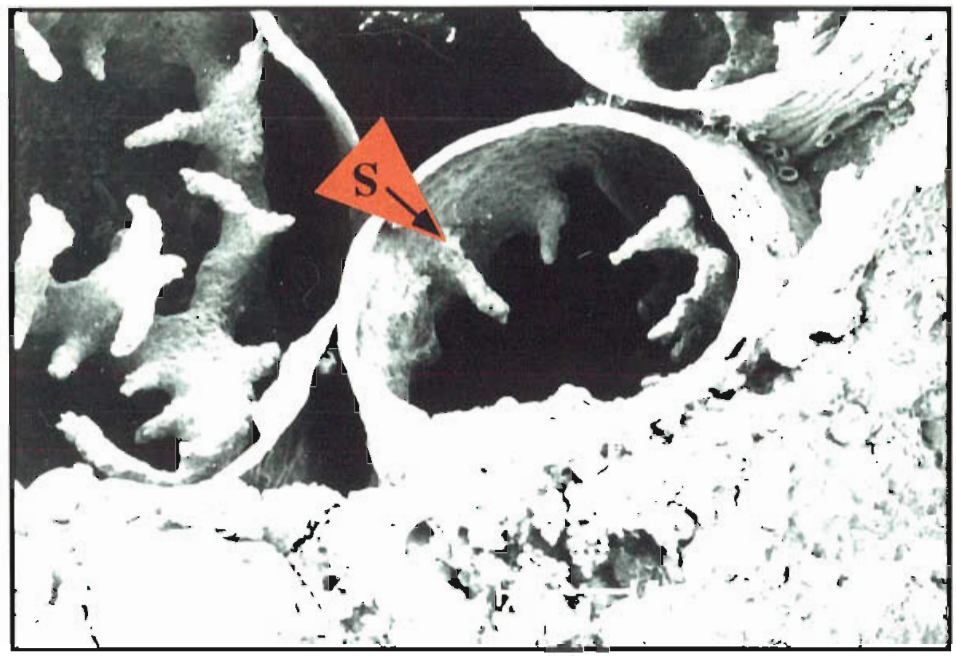
4.3.3 Morphology of juveniles

The septal morphology of juvenile corals could not be established conclusively, as conflicting results were obtained between juveniles reared in the two years . Survival of larvae from the spawnings in 1992 and 1993 was poor. The November 1992 spawning yielded 57 FF juveniles (218 serrated and 14 laminar septa), and only 4 YS juveniles (3 serrated and 12 laminar septa). This contrasted with the survival of juveniles from the November 1993 spawning, which yielded no FF juveniles and 39 YS juveniles (165 serrated septa, none laminar). Examples of juveniles with both septal forms are shown in plates 4.3a and 4.3b. Juveniles produced in November 1992 were reared for 5 months before examination, while those from 1993 were reared for 3.5 months. Most juveniles examined were still single corallites.

4.3.4 Comparison with museum specimens

Holotypes of species of *Montipora* synonymous with *M. digitata* were examined in order to determine which old names would be appropriate to assign to the YS and FF species. The shape of septa was used as the main identification character when examining museum specimens. Other features such as the shape of branch tips were also taken into consideration, though the samples were often in too poor a condition (broken branch tips) for such gross morphological features to be evaluated. The outcome of the survey is summarised in Table 4.4. It is quite clear that most of the species examined are synonymous with both species of *M. digitata*. According to the International Code of Zoological Nomenclature (Stoll *et al.* 1962) the correct names to be resurrected when synonymised species are found to be different should be those first assigned to them. From the outset of this study it was clear that a new name for one of the species would not be suitable, as both species had probably already been described. This was likely as a large number of synonymies exist for *M. digitata* (Veron and Wallace 1984). The decision of which names should be applied to the two species was not difficult as specimens of both had been collected by Dana in 1846, and this is the earliest description of the species on record. Dana's holotypes for *M. digitata*, (Dana 1846) (USNM 312), and *M. tortuosa*, (Dana 1846) (USNM 310) were borrowed from the USNM. The decision as to which morph they corresponded to was based primarily on septal shape as mentioned above, but corallite characters were also measured using the same image analysis method described in section 3.2.2, in order to compare them with the measurements obtained for colonies used in the

(a)



(b)

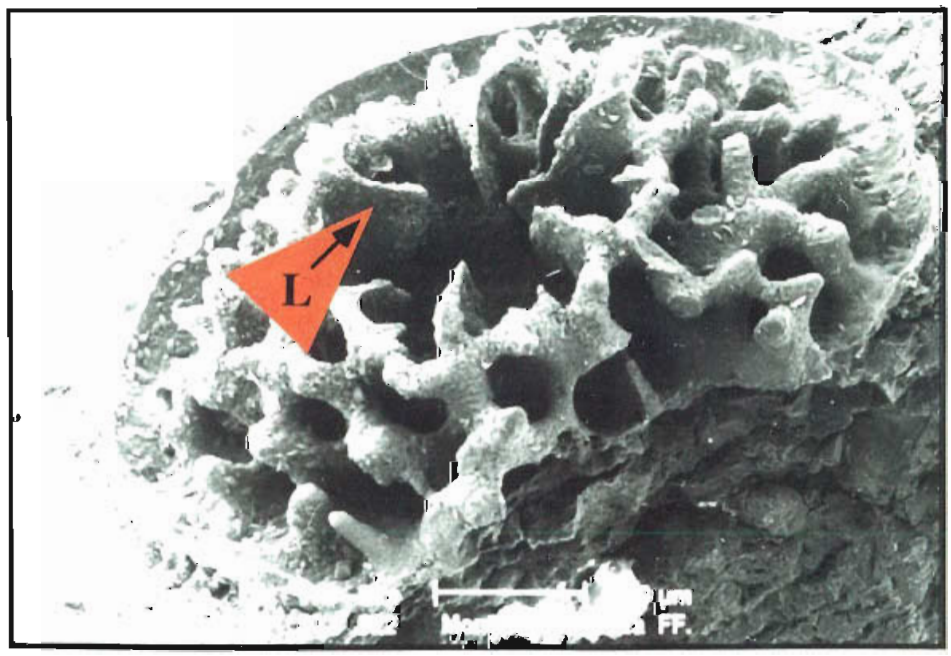


Plate 4.3. Scanning electron micrographs of 5 month old fat fingers (FF) and yellow spatulate (YS) *Montipora digitata* juvenile corallites. (a): fat fingers, (b): yellow spatulate. The FF morph has septae composed of a number of teeth (ie. serrated)(S), and the YS morph has laminar septae (uninterrupted sheets)(L).

SPECIES NAME	COLLECTED AT:	AUTHORSHIP:	LOCATED IN:	DETAILS	SPECIES
M. spatula (SH)	Warrior Is. GBR	Bernard 1892	BMNH	Sturdy colony with anastomosing branches and spatulate tips. Branches > 1cm diam. and tall. Serrated septae near branch tips but not possible to see into corallites further down as septae fuse to form columella-like structure.	FF
M. palmata	Fiji	(Dana) Bernard (1896)	AIMS	Septae serrated, spatulate tips, fine branches	?
M. tortuosa (H)	Singapore	Dana 1846	USNM 310	Septae serrated, corallites large and far apart	FF
M. poritiformis (H)		Verrill 1869	AIMS	Laminar septae protruding above corallum surface	YS
M. ramosa (H)	Gulf of Mananar	Bernard 1888	BMNH	Large sturdy colony, branches > 2cm near base. Rounded tips, serrated septae	FF
M. compressa			AIMS	Serrated septae	FF
M. indentata (H)		Bernard 1897	AIMS	Serrated septae, rounded branch tips	FF
M. fruticosa	GBR	Bernard 1897	BMNH	Fragile colony with spatulate branch tips. septae laminar	YS
M. fruticosa		Bernard 1897	AIMS	Septae poorly developed, serrated. Branches fine	FF?
M. digitata (H)	Fiji	Dana 1846	USNM 312	Septae poorly developed but many are laminar, especially leading septae. Branches relatively thin, approx. 6mm diameter.	YS
M. digitata (Sp. 365)	Hope Island	Dana 1846	AIMS	Fig 196 in Scleractinia of Eastern Australia V (S.E.A). Laminar septae, some spatulate branches.	YS
M. digitata (Sp. 365)	Broadhurst reef	Dana 1846	AIMS	Fig 195 in S.E.A., Squat anastomosing branches. Serrated septae.	FF

Continued overleaf

SPECIES NAME	COLLECTED AT:	AUTHORSHIP:	LOCATED IN:	DETAILS	SPECIES
M. digitata (Sp. 365)	Berwick Island	Dana 1846	AIMS	Fig 194 in S.E.A.. Spatulate branch tips, squat colony. Many septae serrated but also good laminar septae particularly near branch base.	YS
M. irregularis (H)	Zamboanga	Quelch 1886	BMNH	Serrated septae. Robust colony with anastomosing branches approx. 1.5cm diameter.	FF
M. marenzerelli		bernard 1897		Laminar septae	YS
M. gaimardi (SY)	Solomon Is. Australia Tongatabu	Bernard 1897	BMNH	Branches approx 1cm diameter. Laminar septae in all speciemens	YS
M. laris (SH)	Banda	Quelch 1886	BMNH	Squat colony, branches anastomose to form spatulate tips. laminar septae.	YS
M. spongilla (H)	Christmas Is.	Bernard 1897	BMNH	Robust branches 1m diam. Spatulate branch tips. Many laminar septae. Corallites close together and coenosteum smooth.	YS
M. spicata (H)		Bernard 1897	BMNH	Thin branches with spatulate or pointed tips. Anastomosing. Many septae laminar.	YS
M. fossae (SY)		crossland 1952	BMNH	Robust squat branches, rounded tips. Branc base 1.5-2 cm diameter. Many septae laminar.	YS?
M. nana (H)	Port Molle	Bernard 1882	BMNH	Squat colony with thin branches, rounded tips. Septae serrated (a few laminar near base).	FF?

Table 4.4. List of species synonymous with *M. digitata* and their classification as FF or YS morphs. For species H: Holotype, SH: Schizo-Holotype, and SY: Syntype. For location of colonies AIMS = Australian Institute of Marine Science, BMNH = British Museum of Natural History, and USNM = United States National Museum.

morphometric study. These measurements are summarised in Table 4.5, along with the measurements made for colonies collected during this study. The study revealed that the *M. digitata*, Dana 1846 holotype (USNM 312) clearly had laminar septa and therefore corresponds to a YS morphology, whereas the holotype for *M. tortuosa*, Dana 1846 (USNM 310) had serrated septa and therefore corresponds to a FF morphology. Also corallite diameter, inter-corallite distance and leading septum length were are all larger for *M. tortuosa* Table 4.5, suggesting it is a FF colony (however the holotypes were from different sites so environmental differences may account for the differences in the corallite characters measured). In view of this the two species can now be called *M. tortuosa* if they correspond to the FF description, and *M. digitata* if they correspond to the YS description.

4.4 Discussion

This morphological study has demonstrated that there are distinct morphological differences between the two species previously described as morphs of *M. digitata*. Despite the many differences found, only septal shape proved to be a reliable character for separating the two species. It was important to find a species-specific skeletal character in order to find a suitable name for either the FF or the YS species of *M. digitata*, as allozyme electrophoresis could not be used to identify museum specimens. From the study of museum specimens it was concluded that Dana's original collections of 1846 contained both FF and YS specimens of *M. digitata*. Dana had originally recognised these two species as *M. tortuosa* (FF) and *M. digitata* (YS), but they were later synonymised as *M. digitata* by Veron and Wallace (1984). Veron and Wallace (1984) did recognise three ecomorphs of *M. digitata*, but they did not distinguish between the FF and YS morphs. However Veron (in prep) does recognise that *M. digitata* is a species complex over its full geographic range. *Montipora tortuosa* (Dana 1846), and *M. digitata* (Dana 1846) are therefore the legitimate names for the FF and YS species, and they shall be referred to as such from here onwards.

Montipora tortuosa colonies have larger corallites that are further apart from each other than those of *M. digitata* colonies. The branch tips of *M. tortuosa* colonies tend to be rounded, with the exception of colonies from Nelly Bay, whereas *M. digitata* colonies tend to have spatulate branch tips. Corallite diameter has been found useful for the identification of several other coral species such as members of the genus *Platygyra* (Miller in press), and *Flavellum* (Cairns 1989). Similarly, inter-corallite distance proved useful for the study of *Montastraea annularis* morphotypes (Van

Variable	Statistic	Species			
		M. tortuosa FF	*M. tortuosa* Holotype (USNM 310)	*M. digitata* YS	*M. digitata* Holotype (USNM 312)
DIAM	N	10 (299)	1 (15)	10 (311)	1 (39)
	MEAN	0.600	0.531	0.542	0.463
	STDEV	0.127	0.043	0.134	0.071
	MIN	0.190	0.430	0.260	0.320
	MAX	0.907	0.590	0.963	0.590
SEPTA	N	10 (272)	1 (15)	10 (266)	1 (21)
	MEAN	0.162	0.131	0.145	0.114
	STDEV	0.044	0.031	0.051	0.021
	MIN	0.055	0.080	0.050	0.080
	MAX	0.302	0.190	0.336	0.150
INTCOR	N	31 (620)	1 (15)	26 (520)	1 (20)
	MEAN	1.197	1.341	1.084	1.076
	STDEV	0.211	0.174	0.180	0.157
	MIN	0.689	1.040	0.559	0.790
	MAX	2.218	1.770	1.580	1.410
W1	n	31 (620)		26 (520)	
	MEAN	6.77		6.53	
	STDEV	2.34		2.24	
	MIN	3.00		3.20	
	MAX	22.00		19.00	
W2	n	31 (620)		26 (520)	
	MEAN	5.40		4.74	
	STDEV	1.09		1.00	
	MIN	2.70		2.60	
	MAX	9.70		9.10	

Table 4.5. Summary of morphometric measurements (mm) for *Montipora tortuosa* (FF) and *Montipora digitata* (YS) colonies collected from all sites during this study, and for holotypes collected by Dana 1846 (USNM codes 310 = *Montipora tortuosa* and 312 = *Montipora digitata*). DIAM: maximum corallite diameter, SEPTA: leading septum length, INTCOR: inter-corallite distance, W1: greatest branch tip width, W2: smallest branch tip width, N: number of colonies sampled and total number of measurements taken (in brackets).

Veghel and Bak 1993), and septal features are often used for species descriptions (Veron and Wallace 1984). Despite the significant morphological differences found between *M. tortuosa* and *M. digitata* during this study, none of the skeletal features measured can be used to reliably identify the species. The skeletal features measured overlapped both when considered singly (univariate study) and when considered in combination with other characters (multivariate study), and are therfore not species-specific. Overlap in morphological characters between coral species is not uncommon (Foster 1982 cited in Foster 1984, Brakel 1977). For example, overlap in morphological characters has been found in morphometric studies of *M. annularis* (Van Veghel and Bak 1993) and *Porites* (Brakel 1977). Overlap of characters between species of *Porites* is so great that Brakel (1977) referred to inter-specific variation as being almost continuous. As a result of this continuous variation Garthwaite *et al.* (1994) have suggested that poritid taxonomy is unlikely to be resolved using morphological data alone.

Variation in morphological characters within species may be genetically programmed or environmentally induced (Brakel 1977, Willis 1985, Van Veghel and Bak 1993). Awareness of the high degree of variation within coral species led to the synonymising of species and the introduction of the term "ecomorph" to the coral taxonomic literature (Veron and Pichon 1976). The existence of coral ecomorphs has far reaching implications for their taxonomy. In particular it means that the spatial distribution of coral species should be evaluated for most taxonomic studies, in order to establish that apparent species are not ecomorphs. For example, such a distributional study has recently been carried out in a study re-examining species boundaries within the genus *Platygyra* (Miller 1994). In this study a statistical investigation of the distribution of *M. tortuosa* and *M. digitata* within sites was not carried out for several reasons. Most importantly, allozyme electrophoresis demonstrated that they are two species very early in this study. Also both species are confined to the reef-flat, and can be distinguished living together on reef flats over a thousand kilometers apart (Magnetic Island and Papua New Guinea). Finally, within the reef flat, micro-environmental differences are unlikely to be responsible for their differing morphology because the two species often grow with interlocking branches (see frontispiece), and large colonies do not vary appreciably in their morphology from one area of the colony to another.

Had *a priori* identification of species not been possible using allozyme electrophoresis, the morphological differences between *M. tortuosa* and *M. digitata* would probably not have been detected using clustering techniques such as canonical discriminant analysis due to the considerable overlap in skeletal characters. However,

selection of good examples of the two species would have produced discrete clusters. Without the independent evidence provided by the genetic and breeding data it is not surprising that Veron and Wallace (1984) synonymised *M. tortuosa* and *M. digitata,* as the variation in morphological characters appears more consistent with ecomorph status. This study therefore highlights the usefulness of allozyme electrophoresis as a taxonomic tool.

The two species also differed in the shape of their first cycle septa. Again, this feature would have been difficult to detect without an independent means of assessing species status. The occurrence of laminar septa in *M. tortuosa* colonies is so rare that lack of laminar septa alone can be used to identify *M. tortuosa* colonies. The septal difference between the two species was evident in colonies from all sites, including those collected 1500 km away in Papua New Guinea. Species identification based on septal shape also coincided with allozyme electrophoresis identifications for colonies of unusual morphology from Nelly Bay. Septal shape is therefore a stable character over a wide geographic area. The existence of this stable character is surprising in view of the general lack of good diagnostic features for species within the genus *Montipora* (Veron and Wallace 1984, Veron in prep). Also septa within the genus *Montipora* are seldom lamellate (Bernard 1897, Nemenzo 1967), and skeletal characters are not generally very stable over wide geographic areas in scleractinian corals (Veron in prep). The difference in septal shape was not apparent in the juvenile stages of both species, most septa being serrated. This suggests that the laminar nature of septa in YS colonies may be the result of secondary deposition at a later stage in the coral's life, though the fact that corallites near the branch tips of adult colonies also have laminar septa in *M. digitata* contradicts this view. It may also be that the juveniles were not allowed enough time to develop laminar septa, or were affected by rearing conditions. Juveniles from the 1993 spawning were allowed six weeks less to develop than those from the 1992 spawning. It may be that the laminar septa are the result of secondary infilling, and juveniles were not given enough time for this process to take place in 1993. Also because most of the *M. tortuosa* colonies were obtained in 1992, and *M. digitata* colonies in 1993, any difference in environmental conditions between the two years may have affected septal deposition. More juveniles of both morphs need to be reared under the same conditions to be certain that the septal shape apparent in the adults is not also typical of juveniles.

Why the septa of these two species are so different is unclear. Septa play a key role in the support and separation of the mesenteries, and they are the first skeletal structures apparent after the deposition of the skeletal plate (Wells 1967). Wells went as far as to say that "all other skeletal parts are subsequent to the septa and of

secondary importance". Differences in septal shapes found between *M. tortuosa* and *M. digitata* will have direct consequences for the polyps that produce them. Shape of septa will affect anchoring of the polyp to the skeleton, and laminar septa will reduce the amount of space available within the corallite for the polyp. This could potentially affect the polyp's feeding and reproductive abilities. There is evidence that the mesenteries of *M. digitata* colonies carry fewer eggs than *M. tortuosa* colonies, however the *M. digitata* eggs are larger so the total egg volume produced by *M. digitata* colonies did not differ from that produced by *M. tortuosa* colonies (see Chapter 5). This suggests that space is not limiting the reproductiive ability of *M. digitata* colonies. Another possible consequence of reduced space within the corallite caused by laminar septa is that they may reduce the chance of parasites such as barnacles and copepods becoming established within the corallite. These parasites were often found in samples, but their presence was not quantified.

Although morphology of colonies did vary between sites most of the variation occurred between species. There was however one unusual exception. In Nelly Bay colonies of *M. tortuosa* had spatulate branch tips, and *M. digitata* colonies were very rare. At all other sites *M. tortuosa* had rounded branch tips, and both species occurred in similar numbers (see Chapter 5). It is likely that this difference is environmentally induced, as the Nelly Bay site is different to the other sites. In Nelly Bay colonies are only very rarely exposed at low tide, and the bay is more exposed to wave action, whereas other sites are generally sheltered and colonies are exposed at low tide. *Montipora tortuosa* colonies do not have spatulate branch tips in Hazard Bay, which is also rarely exposed at low tide. However Hazard Bay is different from Nelly Bay in that it is a very sheltered site. The spatulate branch tips of *M. tortuosa* colonies in Nelly Bay parallel the shoreline offering maximum resistance to wave action. This trend is not evident in *M. digitata* colonies whose spatulas tend not to be parallel to the shore. The fact that *M. tortuosa* spatulae are parallel to the shore in Nelly Bay implies that this feature is environmentally induced. The positioning of spatulas parallel to the shore is counter-intuitive, however, explanation of this pattern is beyond the scope of this study, but would be interesting for future studies.

The identification of a species-specific morphologial character has made it possible to rename the FF and YS species of *M. digitata* as *M. tortuosa* and *M. digitata* respectively. Identification of holotypes was possible as septal shape was found to be a reliable character for distinguishing the FF and YS species. Univariate and multivariate analysis of skeletal characters demonstrated that the two species are morphologically different. They differ in the size of corallites, corallite spacing, and in the shape of branch-tips. However considerable overlap in all of these characters

means they are not definitive for identifying the species. It is significant that the more traditional method of searching a colony visually for differences led to the discovery of a character that can differentiate the two species. However, without *a priori* species identification using allozyme electrophoresis, septal shape would probably not have been identified as a "diagnostic" character. Neither would an exclusively morphometric study have identified the species, due to the considerable amount of overlap of skeletal characters. This lends support to the notion that several different techniques are best used to describe species boundaries (Lang 1984, Willis 1990), each alternative method acting as a test for the other and reducing the chance of erroneous conclusions.

Chapter 5

The reproductive ecology of *Montipora tortuosa* and *M. digitata*

5.1 Introduction

Previous chapters demonstrated that *M.tortuosa* and *M. digitata* are two species. Demonstration of species status paves the way for comparative studies to determine how different or similar species are. Comparison of species is important as the extent of differences found between them has phylogenetic implications (eg. Potts *et al.* 1993), much can be learnt about their ecology and biology, and knowledge gained provides a framework for testing theories about life-history evolution (Knowlton 1993). During this study several aspects of the reproductive biology of *M. tortuosa* and *M. digitata* are compared. Reproduction is a fundamental component of population ecology, and an integral part of life-histories. The timing of reproduction, mode of development, numbers of offspring, and the allocation of resources to reproduction are all essential components of life histories (Pianka 1983, Begon *et al.* 1990). Reproduction also has a direct effect on the genetics of populations by limiting, or allowing, gene flow (discussed in chapters 2 and 3). Reproductive characters can also be used in taxonomy (Schick 1991 p. 228), and may provide further evidence of species distinctness.

5.1.1 Coral reproductive ecology

Over the past decade a great deal of knowledge on the ways in which corals reproduce, and the timing of reproduction, has accumulated. (reviewed in Fadlallah 1983, Harrison and Wallace 1990). Corals reproduce both sexually and asexually (asexual reproduction in *M. tortuosa* and *M. digitata* was discussed in Chapter 2). Sexual reproduction in scleractinian corals occurs in four basic forms. Coral species may be hermaphroditic or gonochoric, and fertilisation and development may be external with an ensuing planktonic phase (broadcast spawners) or internal with a brief planktonic phase following release of larvae (brooders) (Harrison and Wallace 1990). Most hermatypic corals are hermaphroditic broadcast spawners. *Montipora digitata* is a hermaphroditic broadcast spawner that does not self-fertilise (Heyward and Babcock 1986).

Most species which spawn gametes for external fertilisation and development undergo a single annual cycle of gametogenesis, while brooding species have multiple or overlapping gametogenic cycles (reviewed in Richmond and Hunter 1990, Harrison

and Wallace, 1990). On the Great Barrier Reef the annual gametogenic cycle of a large number of corals culminates in a synchronous mass spawning (Harrison *et al.* 1984, Willis *et al.* 1985, Babcock *et al.* 1986). Mass spawning events have also been reported for corals in Western Australia (Simpson 1991), Japan (Heyward *et al.* 1987, Hayashibara *et al.* 1993), the Red Sea (Fadlallah *et al.* 1992) and the Gulf of Mexico (Bright 1992), though only the Western Australian and Japanese spawnings are comparable to that of the Great Barrier Reef in terms of the number of species involved. Members of the genus *Montipora* spawn during the annual spring mass spawning event on the Great Barrier Reef, but they are unusual in that they also spawn a second time in the autumn (Stobart *et al.* 1993). Study of the differences in conditions (eg. environmental, lunar and photoperiodic cycles) during the two spawning periods provides a unique opportunity to try and explain the ultimate and proximate factors that govern synchronous mass spawning. In particular, non-correspondence in conditions between periods may be used to infer they are not the important factors influencing spawning.

Coral spawning times are governed by ultimate factors, these being the evolutionary selective pressures responsible for the development and persistence of the spawning times (the underlying reason for spawning), and proximate cues, which provide the reliable timing indicators (mechanisms) necessary to synchronise spawning (Oliver *et al.* 1988). The ultimate factors responsible for mass coral spawning on the Great Barrier Reef may be ecological (eg. predator swamping), environmental (eg. restricted by temperature, tidal patterns) or the result of a genetic legacy (Oliver *et al.* 1988). On the Great Barrier Reef marked variation in physical factors, such as temperature and tidal ranges, may be responsible for the high level of reproductive synchrony within and between species (Babcock *et al.* 1986). Spawning synchrony in other invertebrate groups such as polychaetes and molluscs has also been attributed to variation in the physical factors they experience (Giese and Pearse 1974). Supporting this hypothesis, is the fact that there appears to be a lack of reproductive seasonality and synchrony, both between and within coral species, in areas where the range and amplitude of environmental variables, particularly sea temperatures and tides, are less extreme (Oliver *et al.* 1988, Richmond and Hunter 1990).

There is however evidence that contradicts the importance of environmental variation in promoting synchronous spawning. In the Caribbean, two species of *Montastrea* have been found to spawn synchronously on the same dates over a wide latitudinal range encompassing large differences in annual temperature and light cycles (Szmant 1991). Similarly, in Western Australia corals spawn synchronously over a wide latitudinal range despite large differences in temperature and tidal regimes

(Simpson 1991, Babcock *et al.* in press). The ultimate reasons for mass coral spawning therefore remain unclear (Oliver *et al.* 1988, Pearse 1990, Babcock *et al.* in press). It seems most likely that a complex combination of factors has created the selective pressure that controls coral spawning times, and thus no one factor will ever explain the patterns of spawning encountered worldwide.

The proximate cues used by corals to synchronise spawning are better understood. Both marine and terrestrial organisms generally use physical factors to synchronise spawning and reproduction, though the presence of food and chemicals may also be important in some instances (Giese and Pearse 1974). The most important proximate factors affecting marine organisms appear to be lunar periodicity (Jokiel *et al.* 1985), daily photoperiod (Babcock *et al.* 1986, McClintock and Watts 1990), tidal rhythms (Yoshioka 1989 a & b) and temperature (eg. Tranter *et al.* 1982, Van Moorsel 1983, Beauchamp 1993). Proximate control may be dominated by one of these factors or a combination of them (Giese and Pearse 1974, Naylor 1976). In the case of mass spawning corals, seasonal photoperiod and/or temperature cycles, lunar periodicity and diurnal photoperiods probably operate on progressively finer time scales to synchronise mass spawning (Babcock *et al.* 1986, Harrison and Wallace 1990).

5.1.2 The costs of reproduction

Little is known about the processes that govern the amount of energy a coral invests in reproduction. Growth and reproduction are the two most important sinks for excess metabolic energy, and there must be trade-offs occurring between them (eg. Oliver 1987, Stearns 1992). Indeed, a central question that theories of life history evolution attempt to address, concerns the timing and relative magnitude of energy allocations to growth and reproduction (Stearns 1977, 1992). The calorific content of coral eggs is very high due to their high lipid content, and therefore egg production requires a large energetic investment. Eggs of *Acropora* and *Montipora*, for example, contain 62-70% dry weight of lipid, which is almost twice the amount reported for adult non-reproductive tissues (Arai *et al.* 1993). High lipid concentration in eggs provides buoyancy required to transport egg-sperm bundles to the surface, and provides a rich food source for larval development (Arai *et al.* 1993). Sperm production also requires a large energy investment, as lipid is an important metabolite for motile sperm (Jennison 1979). Resources can be diverted to reproductive processes at the expense of growth (Loya 1985), and conversely growth can occur at the expense of reproduction (Rinkevich and Loya 1985). In some coral species both growth and reproduction can exhibit annual maxima simultaneously (Oliver 1987). Examination of such relationships in species with biannual spawning cycles may shed

light on the conditions that lead to competition for resources because contrasting environmental conditions during the two periods of gametogenesis may lead to different patterns of growth and reproduction.

5.1.3 Using reproductive traits in taxonomy

Throughout this century the general trend in taxonomy has been for standard morphological data to be increasingly supported by evidence from physiological, genetic, behavioural and ecological data (Blackwelder 1967). Any attribute by which members of one population can be distinguished from another may serve as a taxonomic character and should not be overlooked (Mayr 1969). Reproduction and growth are two potential areas where species specific differences may become evident, and therefore be useful for taxonomic purposes.

There are very few instances where reproductive characters have been used in coral taxonomy (reviewed in Willis 1990). Van Moorsel (1983) separated the *humilis* form of *Agaricia agaricites* as a new species based largely on planulation seasonality, number of planulae produced, planula size (as a volume), and maximum diameter of planulating colonies. Similarly Chornesky (1986) and Delvoye (1986) have also used reproductive criteria for defining coral species, and Harrison (1988) has demonstrated that scleractinian sperm morphology could be useful for species identification. Reproductive characters have also been used for taxonomic purposes in other cnidarians. Carter and Thorpe (1981), for example, used several characters to argue in favour of separating two varieties of *Actinia equina* into separate species. Recently Mangin (1991) used differences in the life cycle of the hydrozoan, *Samuraia tabularosa,* as a primary distinction to describe a new genus, and Brewer (1991) used differences in time of reproduction to argue that two populations of the jellyfish *Cyanea* could be separate species.

The aim of this study was to compare reproductive characteristics and rates of linear extension of branches between *M. tortuosa* and *M. digitata* to look for evidence of divergence in their life histories, and to be able to speculate on how closely related they are. A further objective of this study was to describe and quantify biannual spawning in these two species and in several other species of *Montipora* to determine how widespread biannual spawning is within the genus, and to compare the intensity of the spawning during both seasons. Differences in environmental conditions and in photoperiod and lunar patterns at the two times of spawning were examined to shed light on the ultimate factors and proximate cues that govern coral spawning. The fact that gametogenesis occurs both in winter and summer also raises questions concerning the way in which resources are allocated to growth and reproduction in these species.

It was hypothesised that the most likely time to detect competition for resources would be before the autumn spawning when gametogenic cycles are shortest and therefore require the fastest rate of development. Thus growth and reproduction were compared between two spawning seasons to address the questions concerning how these two species are able to sustain two gametogenic cycles in one year, in contrast to the single gametogenic cycle of most other species.

5.2 Materials and methods

5.2.1 Study sites

Field work for this study was carried out on the fringing reefs of Geoffrey Bay, Pioneer Bay, North-East Reef and Low Island (see Chapter 2 for location details). Sampling was carried out over a three year period between March 1991 and March 1994.

5.2.2 Comparisons of gametogenesis between M. tortuosa and M. digitata

In Geoffrey bay, ten large colonies of each of the two species were selected haphazardly over an area 50 x 50 m in the middle of the reef flat zone (see Plate 2.1), and tagged. Large colonies were selected so that they could be sampled repeatedly for over two years without excessively reducing the colony size. All colonies of the same morphology were separated by at least 3 m to minimise the likelihood of sampling clonemates. Three branches were removed from each colony at approximately monthly intervals for two years, between March 1991 and March 1993. One centimeter segments were cut from each branch 3 cm from the branch tip to avoid sampling the sterile zone described by Heyward and Collins (1985a). All samples were fixed in 10% unbuffered seawater formalin. Segments were then decalcified in a 5% HCL (v/v), 3% formalin (v/v) solution and stored in 70% alcohol prior to examination.

Ten randomly selected polyps were dissected from each branch segment, and the number of eggs per polyp was counted. The length and width of five randomly selected eggs from each of the first three polyps dissected from each branch were measured using a stereo dissector microscope fitted with an optical micrometer. All polyps and eggs were selected using random number tables (Zar 1984). An estimate of egg diameter was obtained by averaging the two measurements made for each egg. Testes were also measured for samples collected on 17/4/92 and 9/10/92. The length and width of all testes from the first three polyps selected was measured, and size was computed as the mean of the two values. Live eggs were also measured in spring 1993 (from 6 *M. tortuosa* colonies, total n = 120; 7 *M. digitata* colonies, total n = 128). The first 10-20 eggs encountered in a Bogorov tray were selected for measurement, which was carried out in exactly the same manner as for preserved eggs. Measurements of

live eggs were made to determine whether differences in fixed egg size between *M. tortuosa* and *M. digitata* were real, or caused by differences in skeletal morphology affecting the shape of fixed eggs.

5.2.3 Comparisons of reproductive output between M. tortuosa and M. digitata

Average measurements and counts of eggs and testes were used to estimate the reproductive output per unit area for *M. tortuosa* and *M. digitata* immediately prior to the October spawning in 1992. The same method was also used to calculate egg-reproductive output for both species prior to spawnings in March and October for the three years of this study. The following formulae were used to estimate reproductive output:-

Egg-reproductive output $= V_e * P_e * N_e * NP$

Testes-reproductive output $= V_t * P_t * N_t * NP$

Total reproductive output = egg output + testes output

Where V_e = Egg volume = $4/3\pi r^3$

V_t = Testes volume = $\pi r^2 h$

P_e & P_t = Proportion of polyps bearing eggs and testes respectively = Number of polyps bearing eggs or testes, divided by the total number examined

N_e & N_t = Mean number of eggs or testes per polyp

NP = Mean number of polyps per cm^2

All reproductive output estimates were calculated using mean egg size for each colony estimated from October spawnings. This was necessary as size estimates for March spawnings were affected by split spawnings. Testes output was only measured in April and October 1992. The number of polyps per cm^2 was estimated by placing a small grid with 0.5 cm divisions on each of the three branches sampled per colony, and counting all of the polyps within two $0.25cm^2$ squares. An average was then calculated from the six squares counted per colony and multiplied by four to give an estimate per cm^2. In most cases the grid occupied most of the tissue sample, so no attempt was made to locate the grid randomly on the sample.

5.2.4 Spawning patterns of M. tortuosa and M. digitata

Samples used to detect reproductive differences between *M. digitata* and *M. tortuosa* were also used to describe the biannual spawning pattern in detail, and to compare the magnitude of spawning at the two times of year. Additional samples were

collected by R. C. Babcock in April and July 1989 (No. polyps examined = 38), and February 1990 (No. polyps examined = 300). The two species were not distinguished in the latter samples.

Further sampling of *M. tortuosa* and *M. digitata* was carried out using transects in order to determine the proportion of colonies spawning in the spring and autumn at Geoffrey Bay and Pioneer Bay. Sampling was performed prior to, and where possible after, each expected spawning between March 1992 and March 1994. On each occasion three 30 m transects were run across the reef at 90° to the shore in an area of abundant *M. tortuosa* and *M. digitata* growth. The first transect was located haphazardly, and the following two were located parallel and at approximately 20 m from the first so that the whole *M. digitata* zone was sampled in a stratified manner. Colonies of the two species were sampled at 1.5 m intervals along the tape. At each interval the nearest colony greater than 10 cm in diameter at 90 degrees to the tape was sampled (one branch from each colony). A total of 20 samples were therefore collected for each species on every transect.

Samples were also collected from Low Island in February 1992 in order to determine whether the autumn spawning occurs over a wide geographic range. Additional transects were also carried out prior to the October and November full moons at Orpheus Island in 1993, a year of split spawning (*sensu* Willis *et al.* 1985) to determine how synchronous spawning is within the spring spawning season. Samples were preserved in 10% seawater formalin (v/v) and decalcified as described in section 5.2.2 above. They were then dissected and reproductive status was recorded as non-reproductive (no eggs, isolated eggs or some eggs but too small to spawn (<200μm)), or reproductive (many large eggs present).

5.2.5 Spawning observations

Direct observations of spawning of corals collected for use in fertilisation experiments (see Chapter 3) were used to compare the time of spawning between seasons, and between Magnetic and Orpheus islands. Date, time and intensity of spawning were recorded. All colonies were subjected to the minimum stress possible in order to avoid disruption of spawning times (see chapter 3 methods for handling procedure). Field observations of spawning were also recorded by divers at Magnetic Island in November 1993.

5.2.6 Spawning patterns of other Montipora species

Samples were collected from *M. spumosa*, *M. peltiformis* and *M. aequituberculata* from Magnetic Island, and *M. foliosa*, *M. undata* and *M.*

crassituberculata from Orpheus Island along transects to quantify spawning patterns. Sampling was carried out between October 1992 and April 1994. Ideally, the same species should have been sampled from both locations to allow between location comparison, however, none of the species were common at both sites. Due to the large distances between most colonies of these plate species, transects consisted of swimming parallel to the reef front at three locations along the reef, and sampling the first ten colonies encountered (>30 cm diameter) at each location. Sampling sites were chosen haphazardly, with each transect being carried out at a distance greater than 50m from the previous one. One core sample was removed from each colony at a distance greater than 5cm from the colony margin to avoid sampling the sterile zone. Samples were preserved in 10% seawater formalin (v/v) and decalcified in 5% hydrochloric acid. Reproductive status was then determined as described above in section 5.2.4. Other species of *Montipora* encountered during dives were also sampled to determine their reproductive status. Samples from five labelled colonies of *M. aequituberculata* collected in April and July 1989 by R. C. Babcock were also examined to determine the extent of egg and testes development, and the percentage of reproductive polyps.

5.2.7 Sperm morphology

Sperm size and morphology were compared between the two species using transmission electron microscopy. Egg-sperm bundles were collected from five *M. tortuosa* and five *M. digitata* (3 *M. tortuosa* and *M. digitata* from Geoffrey Bay in October 1992 and two of each from Pioneer Bay in November 1992) colonies as they spawned, and immediately fixed following Method 3 described by Harrison (1988). Fixed samples were stored in 0.1M sodium cacodylate buffered millipore-filtered seawater pH 7.3 at 4°C until embedded for sectioning. Samples were dehydrated in an ascending series of graded ethanol and then infiltrated and embedded with Spurr's resin.

For transmission electron microscopy (TEM) 'silver' sections were cut (about 70-80 nm thick). The TEM sections were picked up on copper grids and stained using acidified saturated uranyl acetate in 50% ethanol (7 mins) followed by poststaining for no more than one minute with modified lead citrate (Reynolds 1963).

Ten sperm in 'perfect' longitudinal section (LS) were then photographed from each colony, giving a total sample of 50 *M. tortuosa* and 50 *M. digitata* sperm for analysis. Sperm were classed as being in 'perfect' LS when the anterior less dense cap, proximal centriole, distal centriole and intercentriolar ligament were all visible as shown in plate 5.1 (P. Harrison pers. comm.). All sperm were photographed on to 35 mm film at 17000K during two consecutive days using the same microscope settings. Such practice reduces the risk of any microscope settings being altered, or changes in the filament emissions which can affect the accuracy of measurements.

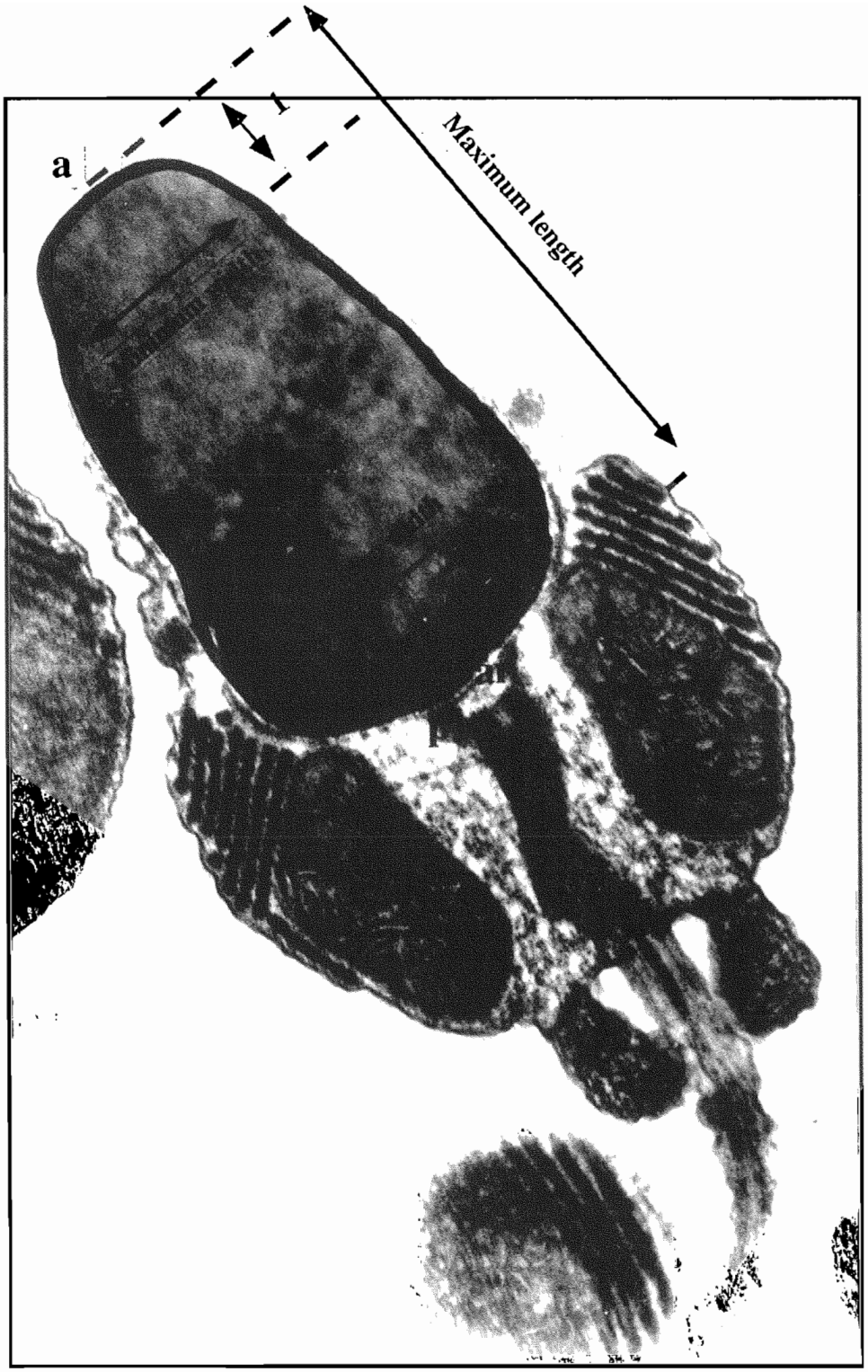
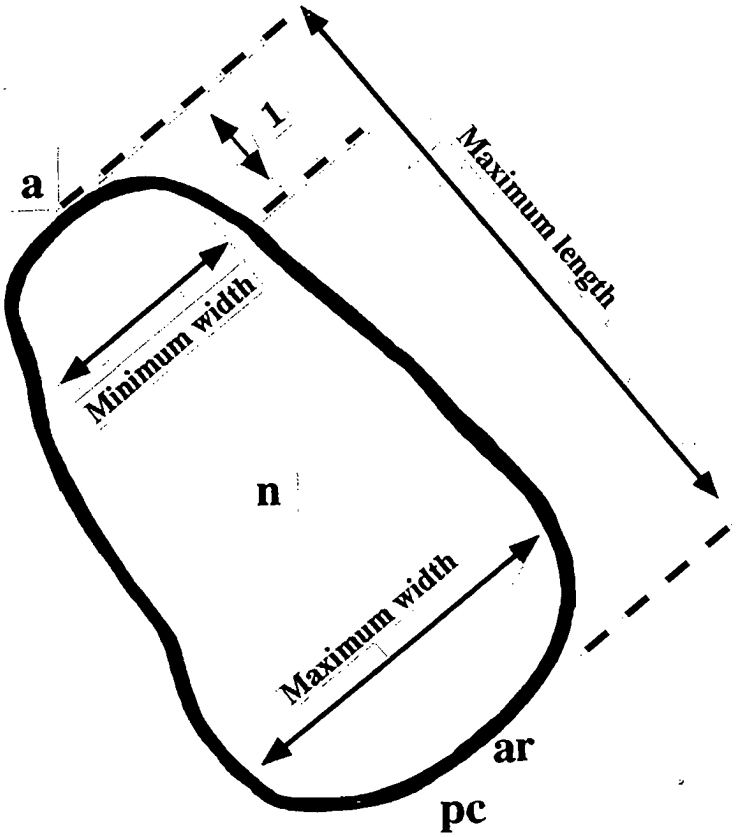


Plate 5.1. Spermatozoon of *Montipora digitata.* Overlay shows measurements made to the sperm nucleus (Minimum and maximum width, and maximum length; 1 = 1mm on negative). **a:** anterior less dense nuclear zone, **ar:** attatchment ring, **c:** cytoplasmmic collar **dc:** distal centriole, **f:** flagellum, **lg:** intercentriolar ligament **m:** mitochondria, **n**: nucleus and **pc:** proximal centriole. Magnification 17000X, 4.5cm = 1 µm.



lg

m

dc

c

f

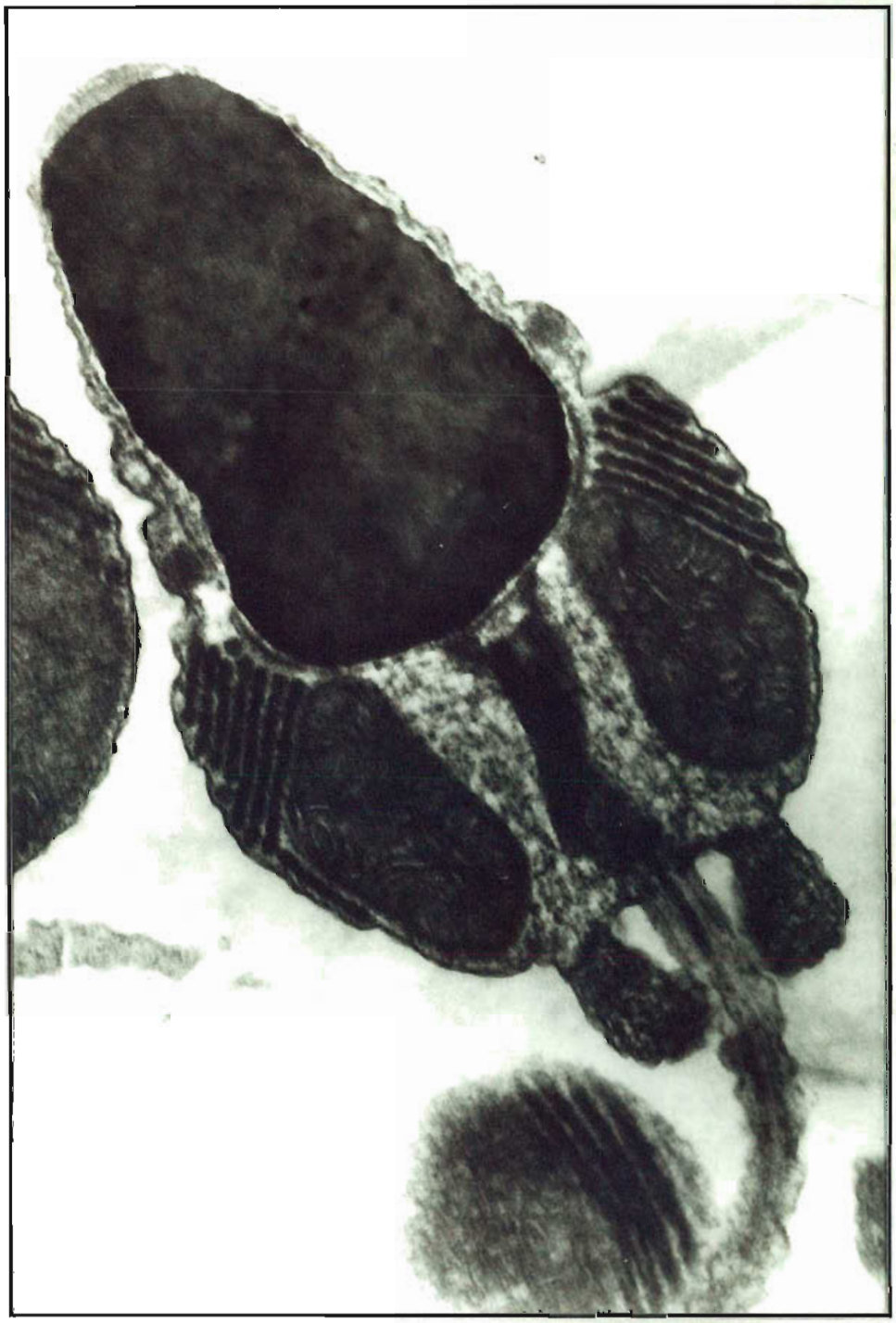


Plate 5.1. Spermatozoon of *Montipora digitata.* Overlay shows measurements made to the sperm nucleus (Minimum and maximum width, and maximum length; 1 = 1mm on negative). **a:** anterior less dense nuclear zone, **ar:** attatchment ring, **c:** cytoplasmmic collar **dc:** distal centriole, **f:** flagellum, **lg:** intercentriolar ligament **m:** mitochondria, **n**: nucleus and **pc:** proximal centriole. Magnification 17000X, 4.5cm = 1 μm.

Sperm were measured directly from the negative. Sperm nucleus length, greatest width and width 1mm from the nucleus tip were measured (Plate 5.1). No other sperm feature can be measured accurately due to small size, and the degree of error caused by section orientation (P. Harrison pers. comm.).

5.2.8 Growth differences between M. tortuosa and M. digitata

Six large colonies of each of *M. tortuosa* and *M. digitata* were collected as far apart as possible from the reef flat at Geoffrey Bay, they were all collected from, and returned to, the same depth. Colonies were collected far apart to reduce the possibility of sampling clonemates. Injuries to colonies were minimised by prying intact colonies from the rubble. Colonies were stained in buckets on the beach for approximately five hours using Alizarin red-S at a concentration of 10-15 mg/ml (Barnes 1972). After staining, all 12 colonies were placed side-by-side in a depression in the reef flat. *Montipora tortuosa* and *M. digitata* colonies were alternated as shown below so that they would be exposed to similar environmental conditions.

M. tortuosa	***M. digitata***	***M. tortuosa***	***M. digitata***
M. digitata	***M. tortuosa***	***M. digitata***	***M. tortuosa***
M. tortuosa	***M. digitata***	***M. tortuosa***	***M. digitata***

Colonies were left for 231 days between March and October 1993, after which 10 branch tips approximately 6 cm long were removed from each colony (the 10 longest branches in the center of the colony) and bleached to remove all living tissue. Branch tips were then carefully ground down to expose the alizarin stain zone using a belt sander. Each branch was measured from the old alizarin stained tip to the growing end of the branch using vernier callipers accurate to 0.01 mm.

5.2.9 Resource allocation for growth and reproduction between breeding seasons

Between April 1992 and March 1993 growth rate was quantified in the two different seasons during which gametogenesis occurs, and the reproductive output of colonies was determined for the two seasons. Growth of *M. tortuosa*, measured as branch linear extension, was quantified in Geoffrey Bay during the winter between the April and October spawning (162 days), and in the summer between the October and March spawning (142 days). Reproductive characters (egg and testes size) were also measured within the same branches used to estimate growth in order to establish whether there was a correlation between growth and reproduction.

Fifteen large *M. tortuosa* colonies were collected two to three days after the

spawning in April and October 1992 and stained for approximately five hours using Alizarin red-S (10-15 mg/ml). After staining, all colonies were placed side-by-side at a typical in situ depth on the reef flat. Colonies were left on the reef flat until 1-2 days prior to the next spawning (162 days for winter and 142 days for summer periods), at which point 10 branch tips approximately 6 cm long were removed from each colony (the 10 longest branches in the center of the colony). The branch tips were then cut into two portions: a growing end portion 4.5 cm long to measure linear extension, and the remainder to estimate reproductive charateristics. The growing end portion was bleached to remove all living tissue, while the remainder was fixed in 10% unbuffered seawater formalin. Bleached branch tips were measured as described in section 5.2.8. The portions fixed in formalin were later decalcified and parameters used to determine egg reproductive output were measured as described in section 5.2.2. For each of the ten branches removed per colony there was a measure of linear extension, an egg number count for ten randomly selected polyps, an egg size estimate for five eggs in the first three polyps examined, and a testes size estimate for all testes in the first three polyps examined. As growth and reproduction parameters were measured from the same branches the correlation between growth as linear extension, and reproductive characters, could be directly compared using linear regression analysis.

5.2.10 Environmental parameters

Temperature (accurate to 0.5°C) was measured at Geoffrey Bay (November 1991-April 1994), and Pioneer Bay (March 1992-April 1994). In Geoffrey Bay, a temperature logger was located on the seabed in the center of the bay at a depth of 5 m, whereas in Pioneer Bay the logger was attached to a mooring buoy at a depth of 5 m, in the center of the bay. Records of rainfall, windspeed and daylength at Townsville Airport were provided by the Townsville Bureau of Meteorology. Townsville airport is approximately eight nautical miles from Geoffrey Bay and 40 nm from Orpheus Island. Tidal information was obtained from Queensland Department of Transport tide tables.

5.2.11 Statistical analyses

Fully nested analysis of variance was used to compare egg numbers among branches, colonies, species and years. The same design was used to compare growth between the two species and among colonies. Analysis of variance was used to compare egg size between the two species in October, and between years. Egg sizes for March were not included in the analysis as they were often small due to spawning being split over several months at this time of the year. Reproductive output was compared between species, seasons and years using three-way analysis of variance. One-way analysis of variance was also used to compare egg-reproductive output between seasons and years, and a Tukey's Studentised Range Test was used to determine which seasons and years differed. MANOVA was used to compare the three

sperm morphology measurements made for the two species. Further details of analyses are provided in table legends. Student's t-tests were used to compare reproductive output for *M. tortuosa* and *M. digitata* immediately prior to spawning in October 1992. Level of significance for all analyses was set at α=0.05. Where multiple t-tests were performed the Bonferroni correction was applied in which significance is set at α/p (p = number of tests).

5.3 Results

5.3.1 Interspecific differences in reproductive traits

Timing of gametogenesis:

Overall patterns in the timing of gametogenesis of *M. tortuosa* and *M. digitata* were almost identical, with both species spawning biannually. Spawning was evident for tagged *M. tortuosa* and *M. digitata* colonies from a reduction of the percentage of polyps carrying eggs (Figure 5.1), a drop in the number of eggs per polyp (Figure 5.2), and a fall in egg size (Figure 5.3). Individual colonies were able to spawn biannually as tagged colonies which spawned in March-April also spawned in October of the same year (figures 5.4 and 5.5 are examples of gametogenic cycles for individual *M. tortuosa* and *M. digitata* colonies). Small (50 μm) eggs were visible 2-4 weeks after each spawning. Eggs took 5-6 months to mature, with most growth taking place in the two months prior to spawning (figures 5.1-5.3).

Percentage of egg-bearing polyps:

Montipora tortuosa colonies had a greater percentage of egg-bearing polyps than *M. digitata* colonies, only having fewer in December 1991 and May 1992. The percent difference between the two species ranged between 4 and 20 percent (Figure 5.1). There was little difference between seasons in the percent of polyps bearing eggs.

Mean egg number:

Montipora tortuosa colonies had a higher average number of eggs per polyp than *M. digitata* colonies (Figure 5.2), with the exception of March 1991 and May 1992 (Figure 5.2). Prior to spawning, the number of eggs per polyp ranged between one and six more for *M. tortuosa* than for *M. digitata*, the smallest difference between the two species occurring just prior to the autumn spawning. Egg numbers per polyp were higher in *M. tortuosa* colonies just prior to six of the seven observed spawning periods, only being lower for the poor March 1991 spawning. Fully nested analysis of variance comparing number of eggs per polyp prior to the October 1991 and October 1992 spawnings in Geoffrey Bay, shows that branch, colony and species all contribute

significantly to the variation in egg number per polyp (Table 5.1), with species accounting for 19.6% of the variation. Most of the variation was accounted for by branches (37%). The average number of eggs per polyp for spring spawning was

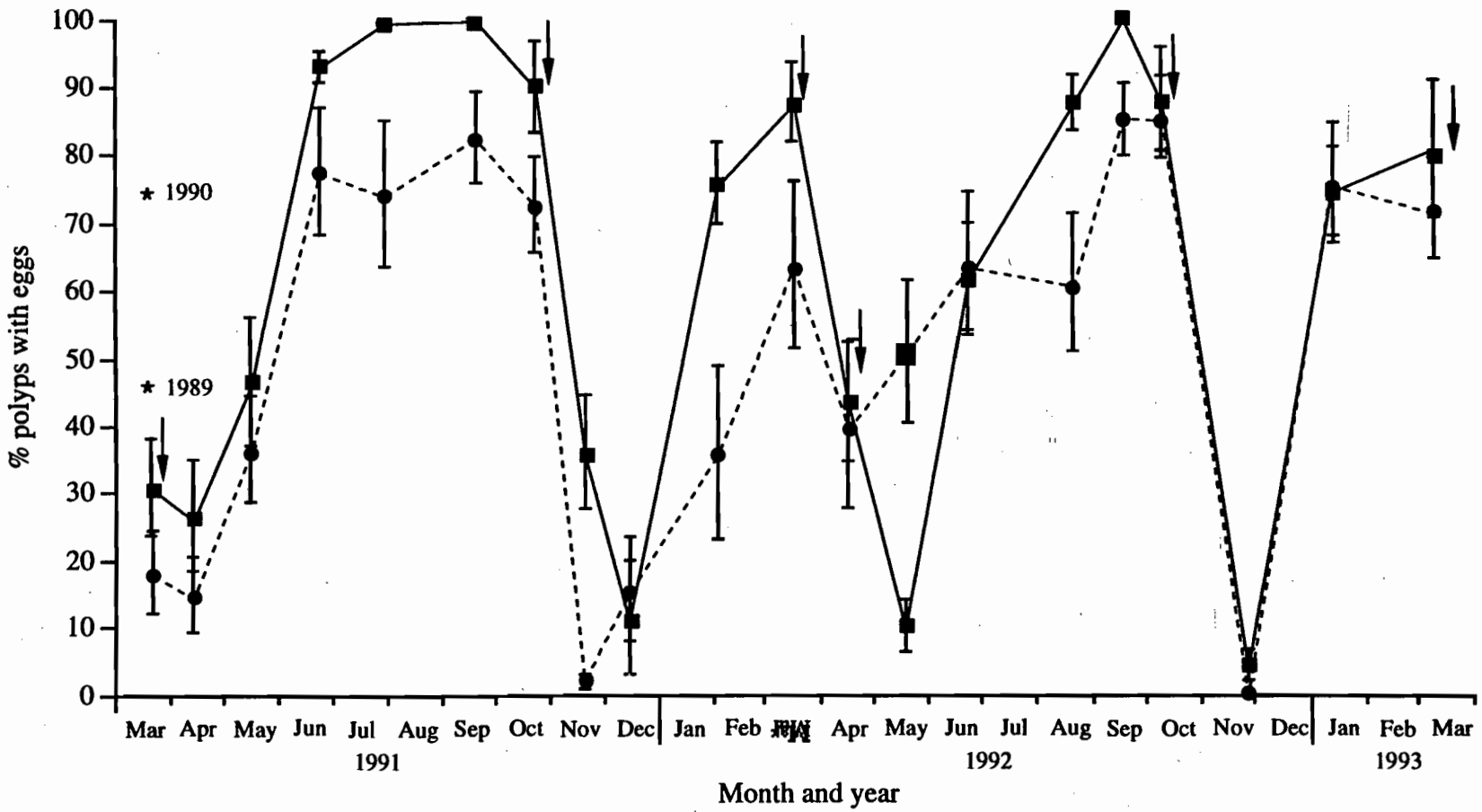


Figure 5.1. Variation in the percentage ± SE of *Montipora tortuosa* (■) and *M. digitata* (●) polyps containing eggs between March 1991 and March 1993. *: Samples from 1989 (18/04/89) and 1990 (14/02/90) collected by R. C. Babcock in which species are not distinguished. Arrows indicate known times of spawning. n = 300 per species for percentage estimate. SE calculated from colony percentage, n = 10 colonies per species.

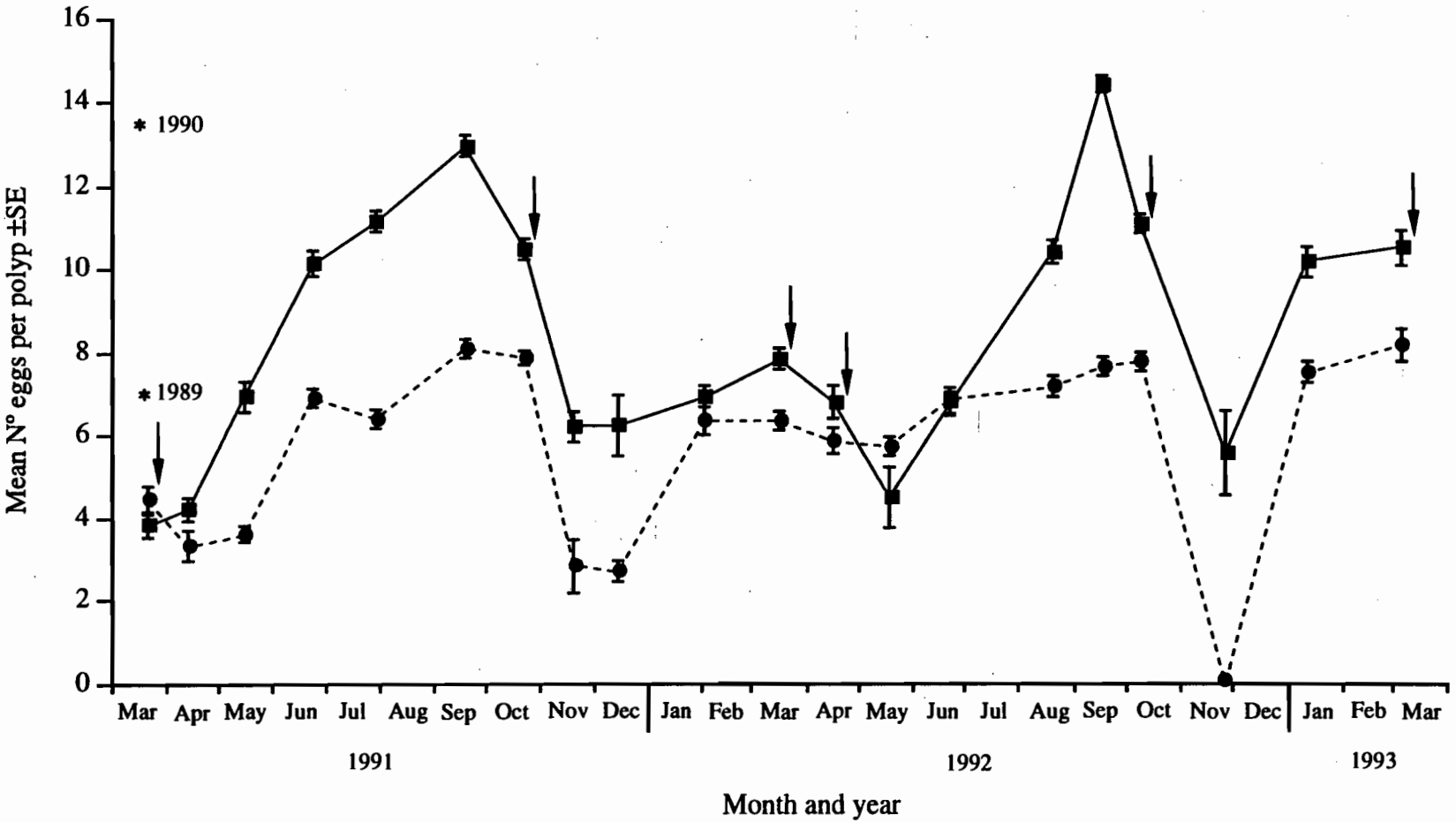


Figure 5.2. Variation in the mean number of eggs per polyp ±SE for *Montipora tortuosa* (■) and *M. digitata* (●) colonies between March 1991 and March 1993. *: Samples from 1989 (18/04/89) and 1990 (14/02/90) collected by R. C. Babcock in which species are not distinguished. Arrows indicate known times of spawning. n = 300 polyps dissected per species.

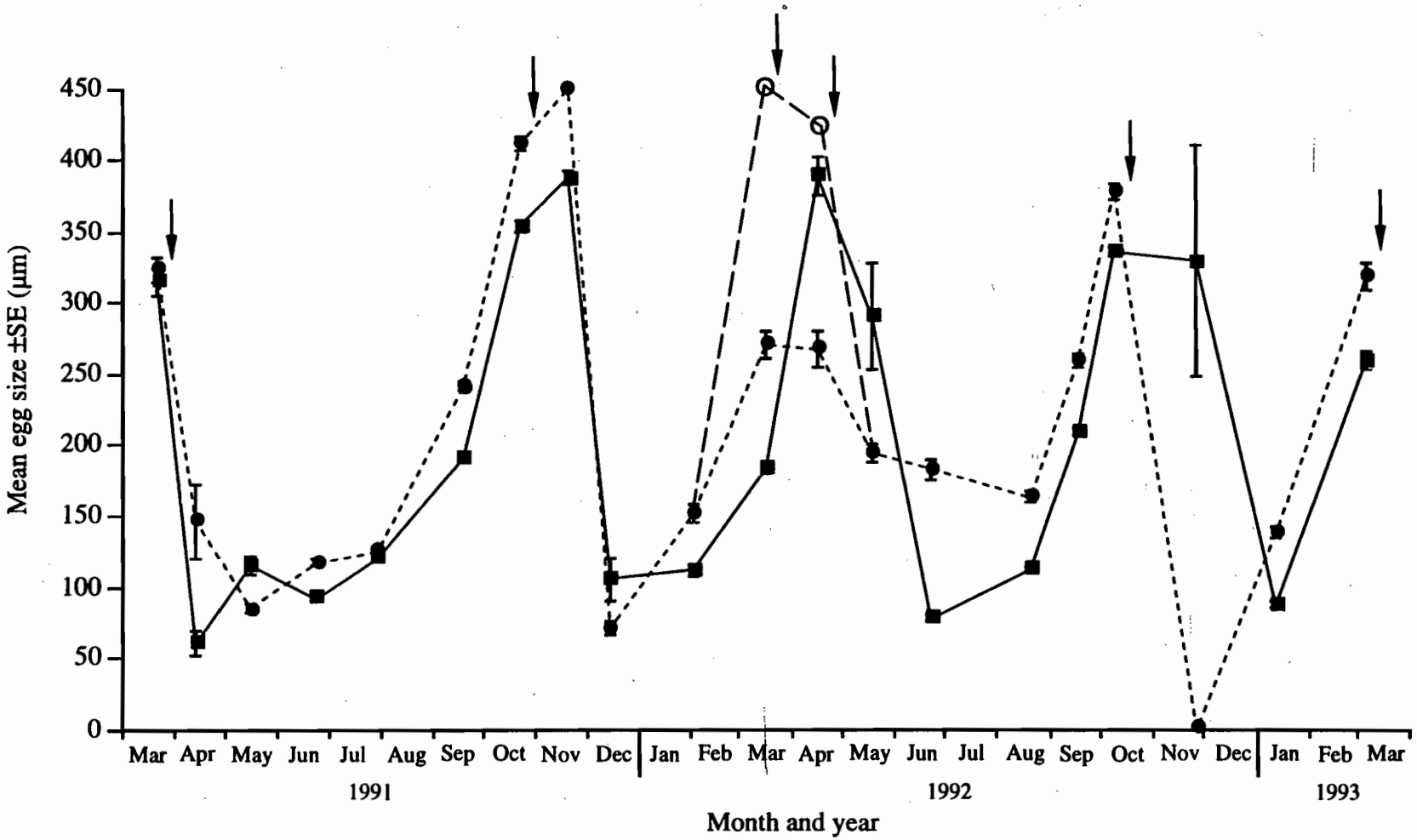


Figure 5.3. Variation in mean egg size (µm) ±SE for *Montipora tortuosa* (■) and *M. digitata* (●) colonies between March 1991 and March 1993. Arrows indicate known times of spawning, O: indicates adjusted mean to allow for split spawning in autumn 1992 (see text for details).

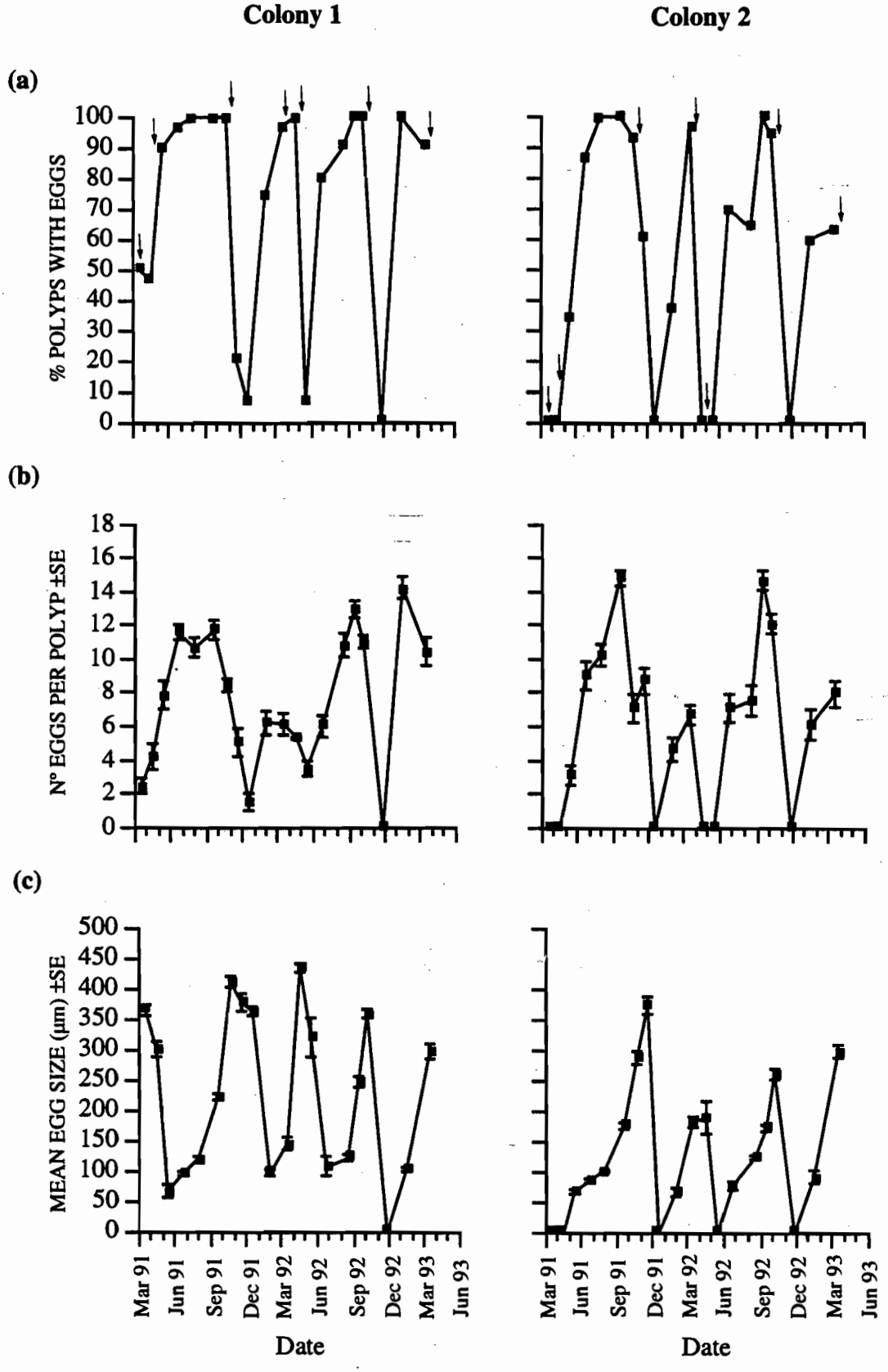


Figure 5.4. Examples of typical two year gametogenic cycles for individual *Montipora tortuosa* colonies (colonies 1 & 2) between March 1991 and March 1993. (a) percent of polyps carrying eggs, (n = 30), (b) mean number of eggs in egg bearing polyps ±SE, (c) mean egg size in egg bearing polyps ±SE. Arrows indicate dates spawning observed.

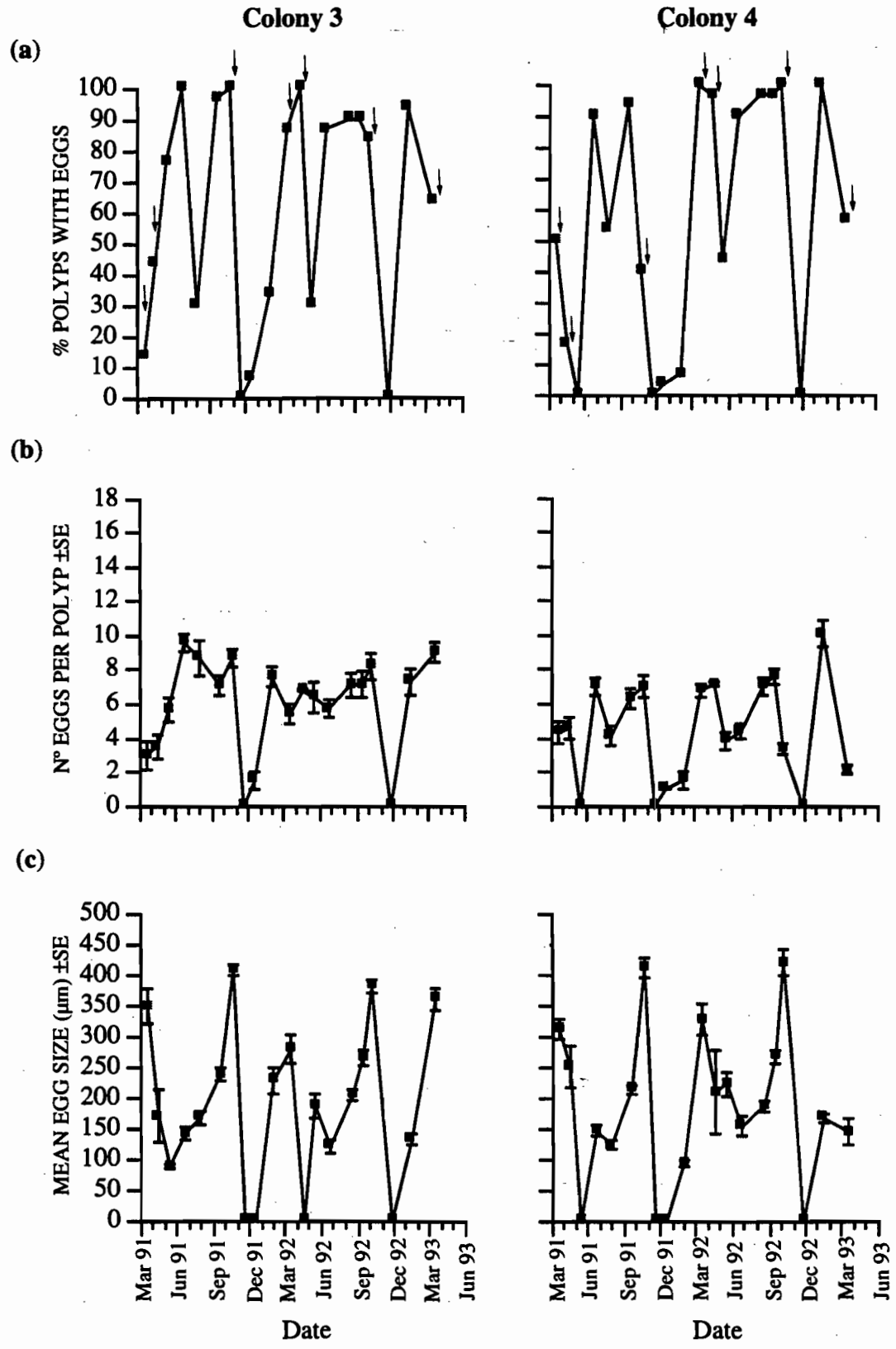


Figure 5.5. Examples of typical two year gametogenic cycles for *Montipora digitata* colonies (colonies 5 & 6) between March 1991 and March 1993. (a) percent of polyps carrying eggs (n = 30), (b) mean number of eggs in egg bearing polyps ±SE, (c) mean egg size in egg bearing polyps ±SE. Arrows indicate dates spawning observed.

11±0.6 for *M. tortuosa* and 8±0.4 for *M. digitata.* For autumn spawnings the averages were 7±0.5 and 5±0.6 respectively. Thus egg numbers per polyp were higher but more variable between the October and March spawning period for *M. tortuosa*, whereas egg numbers were lower and less variable between seasons for *M. digitata.* This trend was maintained over the three year study period, and is evident in frequency histograms of egg numbers per polyp for the two species just prior to the autumn and spring spawnings (Figure 5.6).

Variance source	Degrees of freedom	Mean square	F Value	P	Percent of total
Species	1	3519	57.2	0.017*	19.6
Year	2	61.5	0.2	0.819	0.00
Colony	36	306.2	2.66	0.0001*	21.7
Branch	80	115.3	18.1	0.0000*	37.0
Error	1080	6.4	-	-	21.7
Total	1199	25.7	-	-	100

Table 5.1. Four-factor, fully nested analysis of variance comparing the effects of species, year, colony and branch on egg number for gametogenic data collected for *Montipora tortuosa* and *M. digitata* in October 1991, and October 1992. Species was considered to be a fixed effect factor and year, colony and branch random factors. Variances were homogeneous and normal. Asterisks indicate significant difference ($p<0.05$). Note that in this case year was considered random as in this instance the analysis is only being used to detect species differences.

Mean egg size:

Whereas *M. tortuosa* had a greater percent of polyps bearing eggs and greater numbers of eggs per polyp, average egg size was consistently greater for *M. digitata* colonies prior to all spawnings except for March 1991 (see Figure 5.3). Two-way analysis of variance comparing egg size between the two species for samples collected in October 1991, 1992 and 1993 showed that egg size was significantly different between the two species, and that egg size varied between years (Table 5.2). *Montipora digitata* eggs averaged approximately 50 μm larger than *M. tortuosa* eggs (*M. tortuosa* mean egg sizes: spring = 348±2 μm, n = 971; autumn = 224±4 μm, n = 611. *M. digitata* mean egg sizes: spring = 393±4 μm, n = 665; autumn = 292±6 μm, n = 485). The size of eggs was not compared between species for March samples as spawning was not as well synchronised as it was during the spring spawning, and the existence of overlapping cohorts reduced the chance of detecting species differences in egg size. This problem is illustrated in Figure 5.7. Frequency histograms of egg size

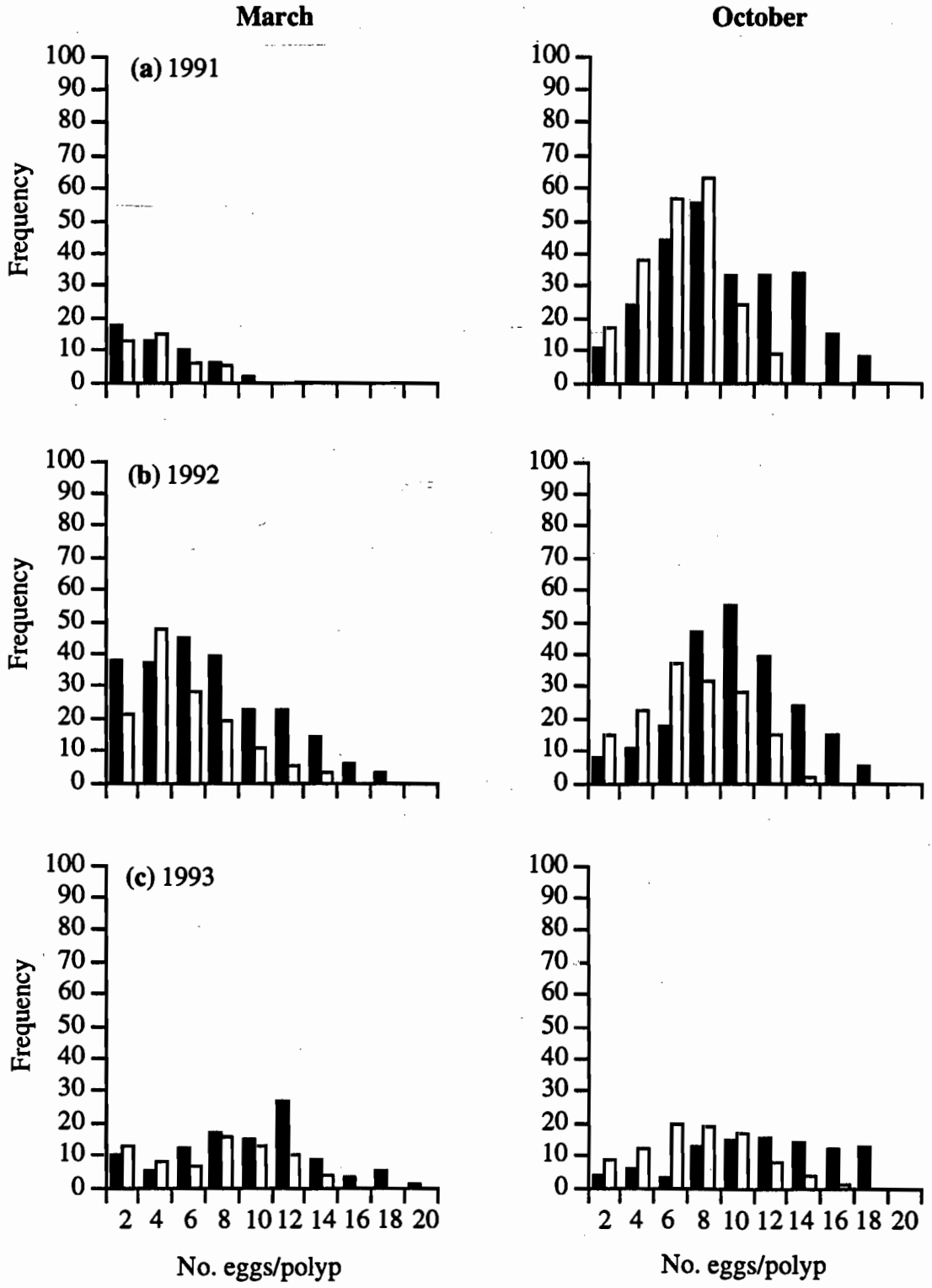


Figure 5.6. Frequency histograms showing the number of eggs per polyp in *Montipora tortuosa* (■) and *M. digitata* (□) colonies from Geoffrey Bay prior to spawning in March and October, over a three year period. Number of polyps examined per morph = 300 in 1991 and 1992, and 150 in 1993.

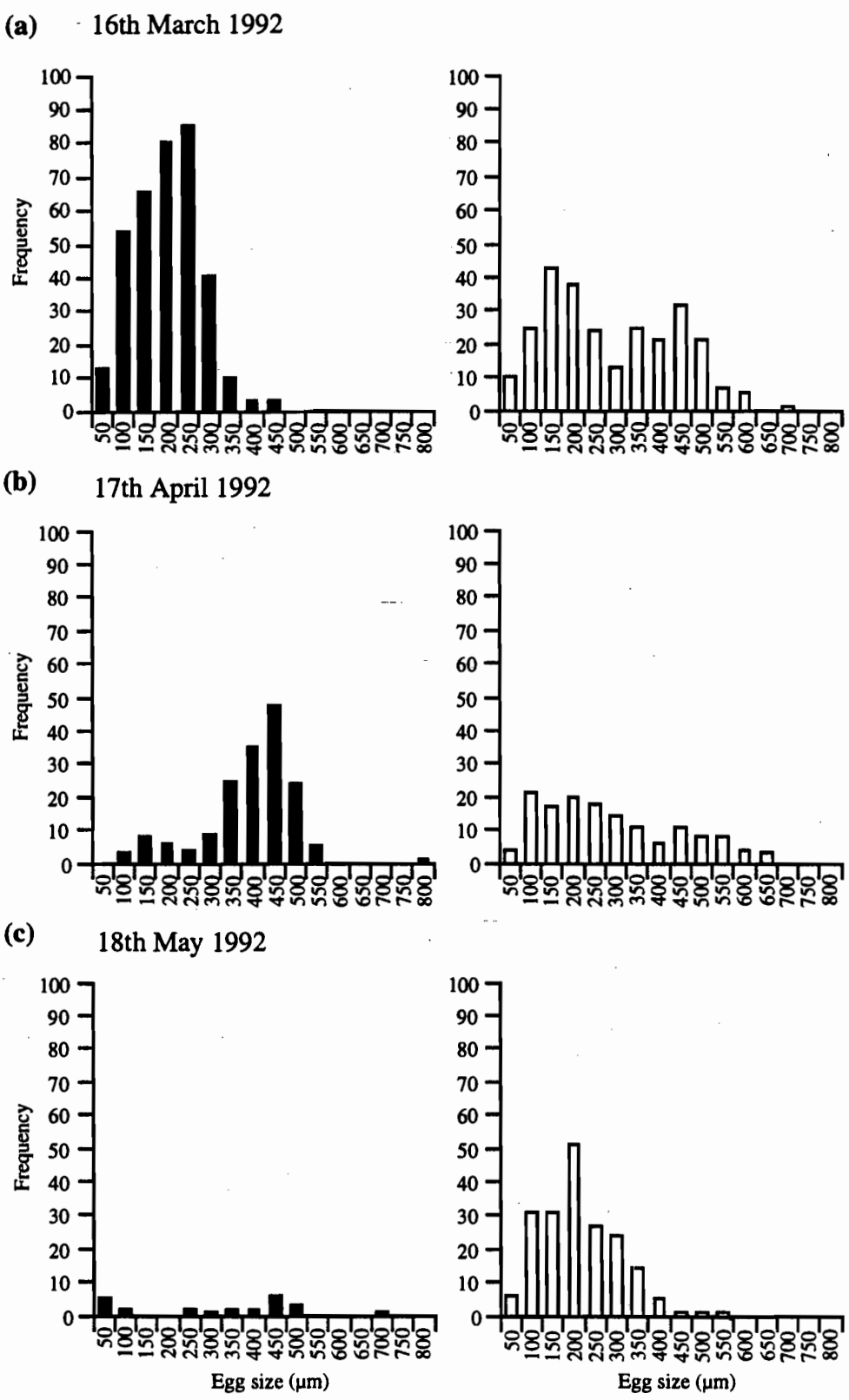


Figure 5.7. Size frequency distributions of eggs (measured in μm) for *Montipora tortuosa* (■) and *M. digitata* (□) colonies from Geoffrey Bay, showing differences in egg-size frequency distributions between the two species. Note the bimodal distribution for *M. digitata.* Samples were collected on 16/03/92 **(a)**, 17/04/92 **(b)** and **(c)** 18/05/92.

for both species prior to the March, April and May full moons in 1992 show that there were two *M. digitata* cohorts (bimodal distribution of the histogram) but only one for *M. tortuosa*. Thus in Figure 5.3 an adjusted size value is also provided for *M. digitata* to account for the overlapping gametogenic cycles. The adjusted value was calculated by excluding eggs smaller than 300 µm from the mean estimate. Overlapping gametogenic cycles suggest that spawning was split for *M. digitata*, this was confirmed by field observations. In March 1992, no *M. tortuosa* colonies with eggs were found for spawning experiments in Geoffrey Bay, but *M. digitata* colonies were relatively abundant. In April, colonies of both species were found for crossing experiments, though *M. digitata* colonies with eggs were less abundant. Again this corresponds to the observed frequency distribution in which the *M. digitata* distribution is still bimodal in April (Figure 5.7), but there are fewer large eggs remaining, whereas most of the *M. tortuosa* eggs are large enough for spawning (>300 µm). Spawning synchrony will be discussed further in section 5.3.2.

Variance source	Degrees of freedom	Mean Square	F Value	P
Species (Sp)	1	878097.01	146.2	0.0001*
Year (Yr)	2	148239.38	24.68	0.0001*
Sp*Yr	1	21715.93	3.6	0.057
Error	1631	9796435.67		

Table 5.2. Two-factor analysis of variance comparing the effect of species and year on mean egg size. n = 10 for each species on each year (1991, 1992 and 1993). Species and year were fixed effect factors. Variances were homogeneous and normal. Asterisks indicate significant difference ($p<0.05$).

Live eggs were approximately 9% smaller than fixed eggs (live *M. tortuosa* = 338±3 µm, and fixed = 348±2 µm, n = 120 and 971 respectively; live *M. digitata* = 362±3 µm, and fixed = 393±4 µm, n = 128 and 665 respectively) with *M. digitata* live eggs being significantly larger than *M. tortuosa* eggs ($t = 6.4991$, 246 df; $p<0.00001$). Fixed eggs from *M. tortuosa* and *M. digitata* were significantly larger than live eggs (*M. tortuosa* $t = 3.28$, 336 df, $P = 0.0012$; *M. digitata* $t = -6.45$, 595 df, $P = 0.0001$). Preserved eggs were often distorted due to fixation within the skeleton, which may account for the larger egg size estimated for fixed eggs.

Testes sizes:

Montipora tortuosa testes were larger than *M. digitata* testes for both the spring and autumn spawning seasons (Figure 5.8). There was also a higher percentage of *M. tortuosa* polyps containing testes (53% and 19% on 17/04/92, and 88% and 55% on 9/10/92 for *M. tortuosa* and *M. digitata* respectively). Differences in testes size between the two species were statistically significant ($t = 5.78$, 137 df; $p<0.0001$ and $t = 5.28$, 208 df. $p<0.0001$ for 17/04/92 and 9/10/92 respectively). .

Reproductive output:

The total egg-reproductive output ($t = 0.579$, 18df; $p = 0.569$) and total reproductive output ($t = 0.116$, 18df; $p = 0.909$) did not differ significantly between *M. tortuosa* and *M. digitata* in October 1992 (Table 5.3). In contrast, reproductive output estimated for testes differed between the two species ($t = 4.69$, 18df; $p = 0.001$).

Parameter	*Montipora tortuosa*		*Montipora digitata*		P
	Mean	95% CI	Mean	95% CI	
Egg Volume (mm^3)	0.022	0.0037	0.031	0.0258	0.0839[a]
Propn. E/B polyps	0.87	0.021	0.85	0.016	0.7898
No. eggs per polyp	10.6	2.49	7.6	1.65	0.029
polyps per cm^2	79.2	5.68	88.0	6.67	0.034
Testes volume (mm^3)	0.020	0.0100	0.009	0.0047	0.0071[a]
Propn. T/B polyps	0.89	0.13	0.58	0.238	0.0216[a]
No. testes per polyp	3.25	0.446	1.96	1.207	0.0025[a*]
Total egg prodn. mm^3/cm^2	16.9	1.54	19.7	2.33	0.5697
Total testes prodn. mm^3/cm^2	4.4	3.06	1.2	1.42	0.0011*[a]
Total reproductive output mm^3/cm^2	21.4	0.31	20.8	0.49	0.9087

Table 5.3. Means for reproductive parameters and 95% confidence intervals, for *Montipora tortuosa* and *M. digitata* colonies at Geoffrey Bay on 9/10/92. The means were compared using t-tests and Cochran's correction where necessary ([a]). Significance (P) was calculated from colony means (n = 10 per species). Bonferroni's corretion was applied to account for multiple t-tests, α was accordingly adjusted to 0.005. (*) = Significantly different. E/B = egg-bearing, T/B = testes-bearing. Total reproductive outputs are shaded.

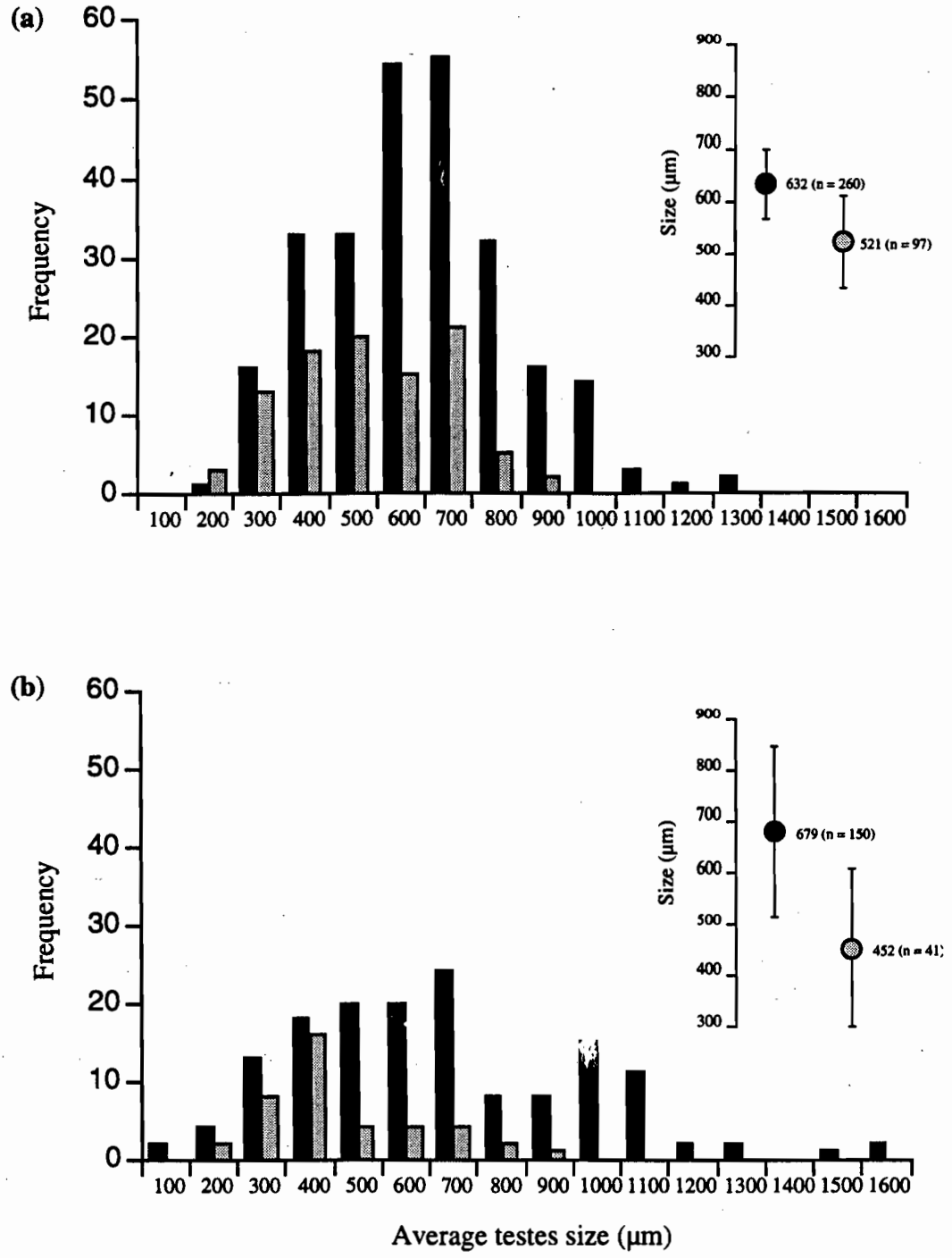


Figure 5.8. Frequency distributions of testes size (µm) for *Montipora tortuosa* (●) and *M. digitata* (◎). (a): testes size on 9/10/92 prior to the spring 1992 spawning; (b): testes size on 17/4/92 prior to the autumn 1992 spawning. Inlays show average testes size (µm) and 95% confidence intervals. n: number of measurements.

In general, testes volume, the proportion of testes bearing polyps, and the number of testes per polyp, were all greater for *M. tortuosa*. The parameters used to obtain the estimates are provided in the appendix (Table 4), and show there was a great

deal of variation in reproductive output between colonies.

During the three year study there was no difference in egg-reproductive output between *M. tortuosa* and *M. digitata* (Table 5.4). Egg-reproductive output did differ between seasons and years (Table 5.4). Egg-reproductive output was higher for the October spawning over the three years of the study, always being above 15 mm^3/cm^2 for October spawnings, and below this figure for March spawnings (Figure 5.9). However, Only the March 1991 and 1992 spawnings were significantly lower than the October spawnings (Table 5.5). There was no difference between October spawnings, but the March 1991 spawning was significantly lower than the March 1993 spawning (Table 5.5, Figure 5.9). Therefore, during the three years of this study, reproductive output varied little from year to year in spring, but did vary in the autumn, which accounts for the significant interaction between seasons and years (Table 5.4).

Variance source	Degrees of Freedom	Mean square	F Value	P
Season (Se)	1	2.6479	20.37	0.0001*
Species (Sp)	1	0.0639	0.49	0.485
Year (Yr)	1	1.1216	8.63	0.0004*
Se*Sp	2	0.0001	0.00	0.982
Se*Yr	2	1.4628	11.25	0.0001*
Sp *Yr	2	0.0116	0.09	0.915
Se*Sp*Yr	2	0.0701	0.54	0.585
Error	88	0.1300		

Table 5.4. Three-way orthogonal analysis of variance comparing the effects of season, species, and year on total egg production (mm^3/cm^2) of *Montipora tortuosa* and *M digitata.* The factors season, species and year were all fixed. Variances were homogeneous and normal after log10+1 transformation. Significant results are marked with an asterisk.

Hour of spawning:

Overall, *M. tortuosa* colonies tended to spawn an average of 18 minutes earlier than *M. digitata* colonies in buckets (Figure 5.10). This difference was statistically different when tested ($t = 5.3794$, 112 df, $p<0.00001$). There was considerable overlap in spawning time however, with *M. digitata* colonies often spawning at the same time, or before *M. tortuosa* colonies on the same night.

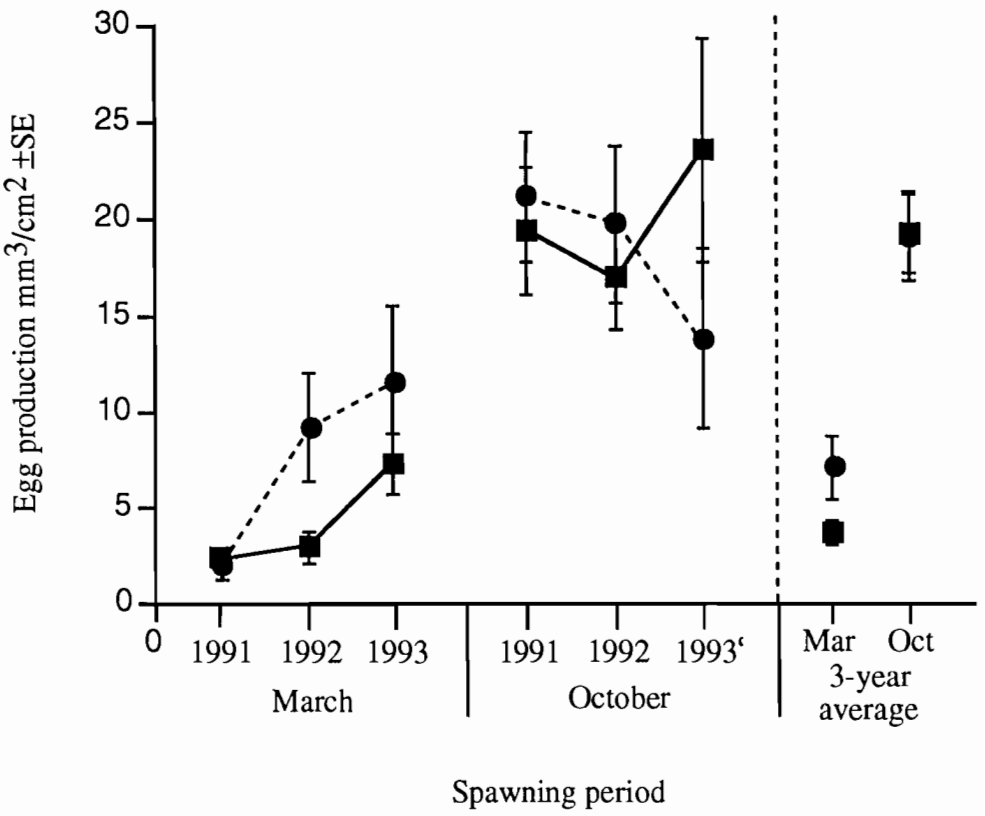


Figure 5.9. Average egg production (mm^3/cm^2) ± SE of *Montipora tortuosa* (■) and *M. digitata* (●) at Geoffrey Bay for October and March spawning periods during a three year period between March 1991 and October 1993. n = 10 colonies per species for 1991 and 1992, and n = 5 colonies for 1993.

	October 1991	October 1992	October 1993	March 1991	March 1992	March 1993
October 1991	-					
October 1992	ns	-				
October 1993	ns	ns	-			
March 1991	*	*	*	-		
March 1992	*	*	*	ns	-	
March 1993	ns	ns	ns	*	ns	-

Table 5.5. Results of Tukey's test on differences between egg production (mm^3/cm^2) over a three year period during March and October spawning seasons at Geoffrey Bay. Asterisks indicate significant differences ($\alpha = 0.05$, df = 94), ns = non-significant result.

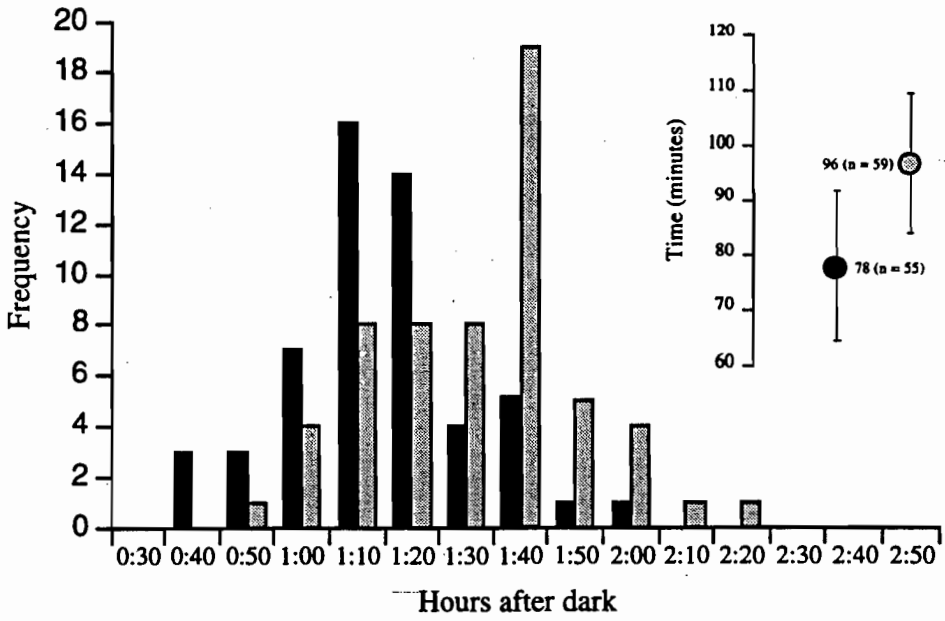


Figure 5.10. Frequency distribution of spawning time (minutes after dark) for *Montipora tortuosa* (●) and *M. digitata* (◎). Inlays show average spawning time with 95% confidence intervals, n = number of observations.

Sperm morphology:

Montipora tortuosa and *M. digitata* had similar pear-shaped spermatozoa (Plate 5.2). Spermatozoa from both species coincided well with the description given for *M. digitata* by Harrison (1988), being elongated pear-shaped and having elongated ovoid nuclei and a less dense domed cap. Dimensions of sperm nuclei for M. *tortuosa* and *M. digitata* were very similar (Table 5.6). Multivariate analysis of variance demonstrated that the sperm dimensions were not significantly different (Pillai's trace p = 0.5702).

Comparisons of growth rate between M. tortuosa and M. digitata:

Linear extension of *M. tortuosa* and *M. digitata* branches over a 231 day period did not differ between the two species (Table 5.7). Average linear extension was 0.59 cm/30 days for *M. tortuosa* colonies, and 0.53 cm/30 days for *M. digitata* colonies (SD = 0.088 and 0.087 respectively, n = 60 branches for each species). Linear extension rate was extremely variable between branches (66.7% of variation), with species and colony only accounting for 10.8% and 22.5% of the variation respectively.

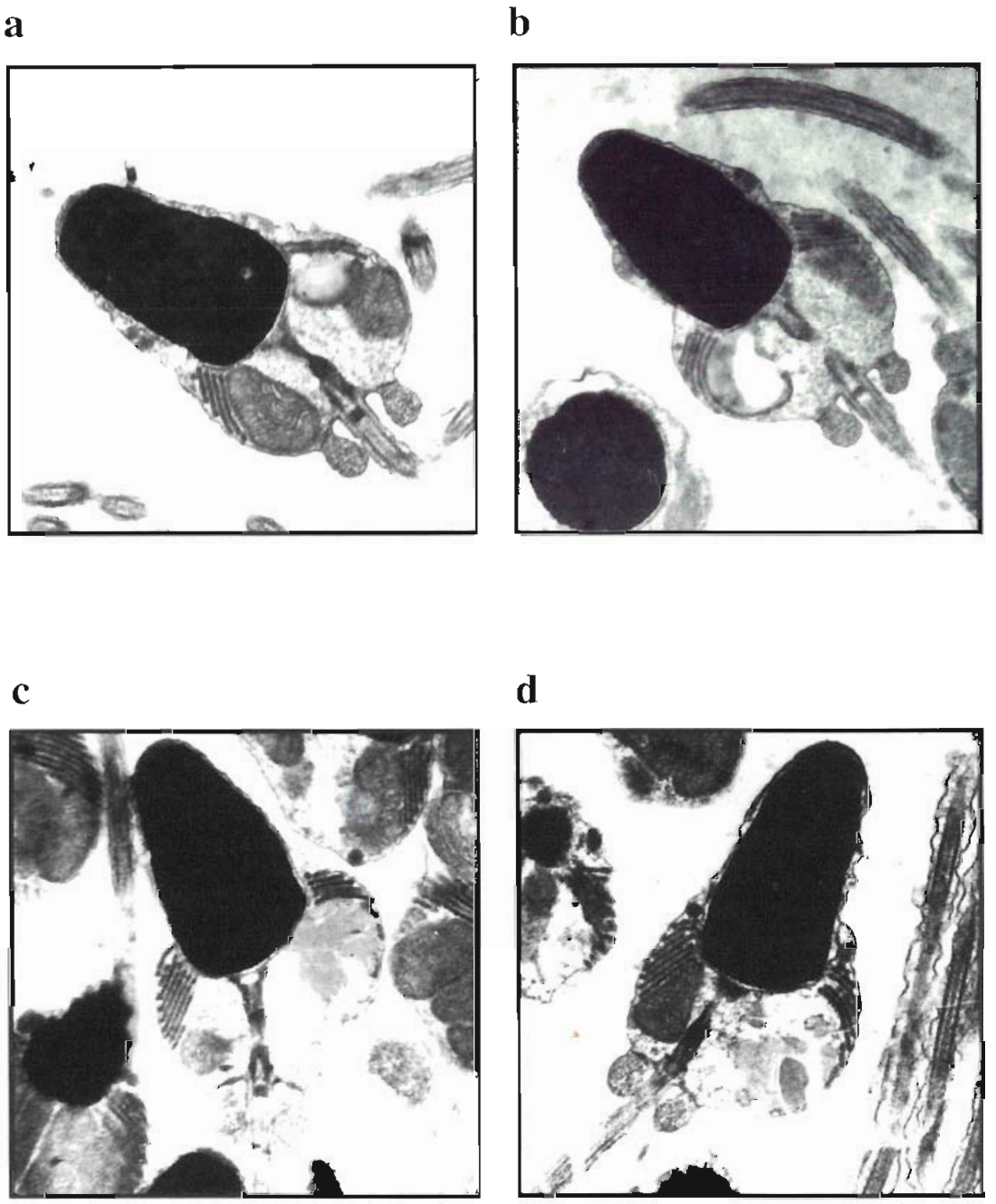


Plate 5.2. Showing the similar morphology of *Montipora tortuosa* (**a** & **b**) and *M. digitata* (**c** & **d**) sperm heads. Magnification 17000X. 2cm = approximately 1 μm.

Variable	Statistic	*M. tortuosa*	*M. digitata*
Length	MEAN	1.993	1.947
	SD	0.091	0.104
	MIN	1.812	1.788
	MAX	2.212	2.306
	SKEW	0.023	0.741
Greatest width	MEAN	1.169	1.152
	SD	0.071	0.078
	MIN	1.035	0.988
	MAX	1.318	1.294
	SKEW	-0.128	-0.031
Smallest width	MEAN	0.690	0.695
	SD	0.068	0.046
	MIN	0.612	0.588
	MAX	0.729	0.823
	SKEW	-1.732	0.002

Table 5.6. Summary statistics for *Montipora tortuosa* and *M. digitata* sperm nuclei (n = 54 for each species). All measurements are in μm.

Variance source	Degrees of freedom	Sum of squares	F Value	P	Percent of total
Species	1	0.0626	3.21	0.10346	10.8
Colony	10	0.1952	4.38	0.00004*	22.5
Error	108	0.4814	-	-	66.7
Total	119	0.7391	-	-	100

Table 5.7. Fully nested analysis of variance comparing the effects of species (*Montipora tortuosa* and *M. digitata*) and colony on growth rate over a 231 day period. Species was considered to be a fixed effect factor and colony the random factor. Variances were homogeneous and normal. Asterisk indicates significant difference ($p<0.05$).

5.3.2 The reproductive ecology of M. tortuosa and M. digitata

Reproductive seasonality:

Spring spawnings took place after the October full moon at Magnetic Island and the November full moon at Orpheus Island in all three years. In the autumn, spawnings took place after the March and/or April full moons at both Magnetic and Orpheus Islands. These observations are based on direct observations of spawning for both species during three consecutive years, with the exception of the autumn spawning at Orpheus Island, which was inferred by the disappearance of gametes from transect samples.

Comparison of breeding intensity between the spring and autumn breeding seasons:

Geoffrey Bay populations of *M. tortuosa* invested more resources in reproduction in the spring than in the autumn breeding season. This is shown by the very high percentage (90-100%) of *M. tortuosa* colonies spawning in spring during two consecutive years at Geoffrey Bay, but the relatively low numbers of colonies spawning in autumn (40-60%)(Figure 5.11a). In contrast, equivalent numbers of *M. digitata* colonies were reproductive during both seasons (56-73%).

This trend was similar at Orpheus Island, where 70-94% of *M. tortuosa* colonies were reproductive in November, but only 18-24% in autumn, in comparison to 34-60% of *M. digitata* colonies being reproductive in spring, and 22-32% in March (Figure 5.11b). A greater percentage of colonies spawned in autumn in Geoffrey Bay than in Pioneer Bay for both species (see Figure 5.11). In contrast the percentage of colonies spawning in the spring was similar at these two sites, except in November 1992, when the lower percentage of *M. digitata* colonies reproducing was probably the result of some colonies spawning a month earlier (Figure 5.11). The relative proportion of colonies of each species spawning in March was similar despite the lower numbers of colonies spawning at Pioneer Bay (Figure 5.12). The number of *M. tortuosa* colonies with eggs also dropped but to a lesser extent. Twenty eight percent and 46% of *M. tortuosa* and *M. digitata* colonies respectively sampled at Low Island in February 1993 contained mature gametes showing that biannual spawning in these two species occurs over a wide geographical area.

Spawning synchrony: month of spawning

Spawning of *M. digitata* and *M. tortuosa* was not confined to a single month in either of the two spawning seasons. During the autumn spawning season, both species spawned in March and April in all three years of this study, and a small number of colonies may also have spawned in May. Similarly there was evidence of spawning over two months in spring 1993 (Figure 5.13). Spawning over several months was expected in spring 1993 as it was a year of predicted split spawning.

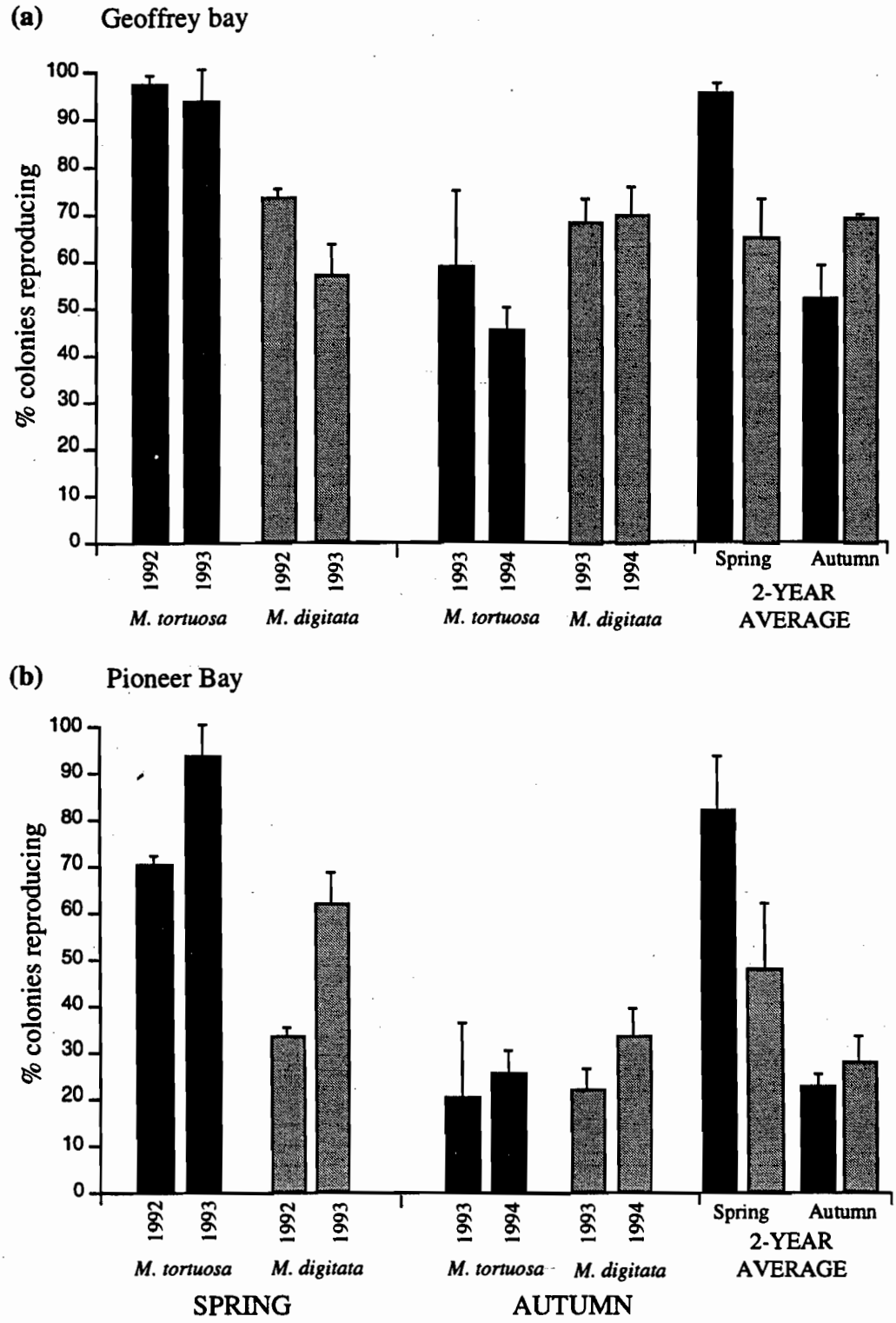


Figure 5.11. Percentage ± SE, of reproductive *Montipora tortuosa* (■) and *M. digitata* (▩) colonies at Geoffrey Bay (a), and Pioneer Bay (b) over two consecutive years during Spring and Autumn spawning periods. n = 60 for each percentage etimate (SE estimated from 3 transects, 20 colonies sampled per transect). For 2-year average SE estimated from 2 yearly averages.

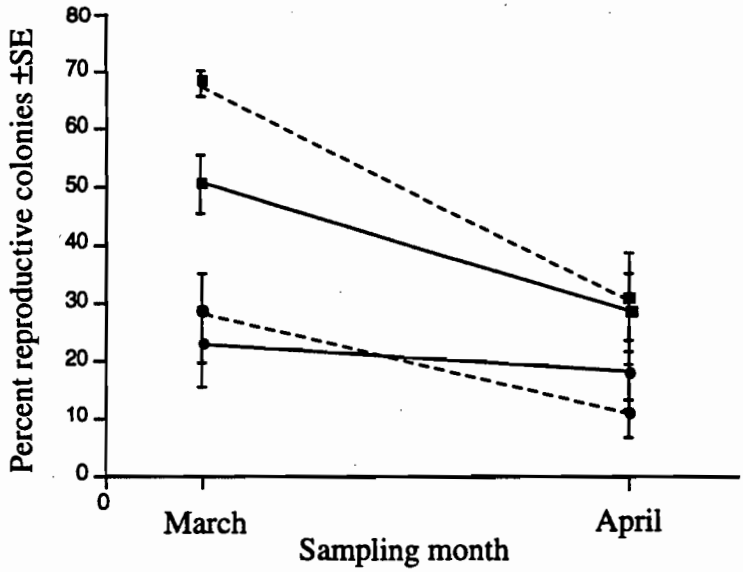


Figure 5.12. Average percentage of reproductive *Montipora tortuosa* (—) and *M. digitata* (---) colonies ±SE, prior to, and after the March spawning in 1993 and 1994 at Magnetic Island (■) and Orpheus Island(●). Each point is derived from 6 transects (3 on each year), and 60 colonies were sampled per transect.

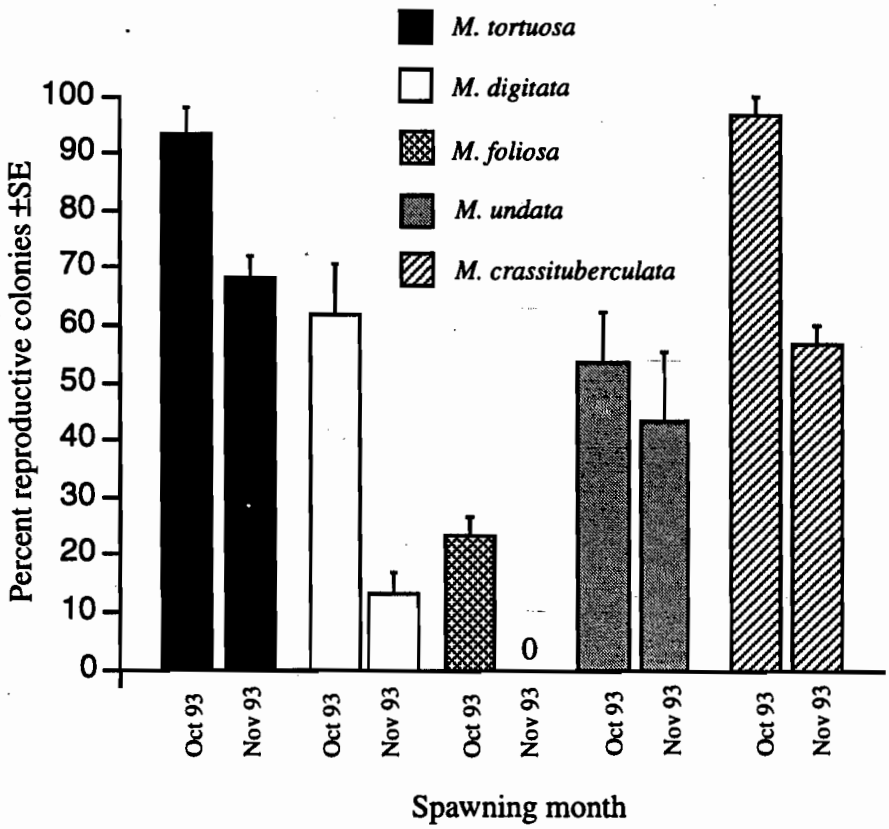


Figure 5.13. Variation in breeding synchrony (percentage±SE of reproductive colonies) among *Montipora* species at Orpheus Island in autumn 1993. Spawning was predicted to take place after the November-December full moon, this plot shows some species also spawned in October. n = 60 for *M. tortuosa* and *M. digitata*, and n = 30 for plate *Montipora* species..

The two species tended to concentrate their breeding efforts in different months when spawning took place over several months. *Montipora digitata* colonies often spawned a month earlier than *M. tortuosa* colonies. This is evident from the differences in egg size frequencies between the two species (see Figure 5.6), and the large difference in the number of colonies of these two species spawning in October at Orpheus Island (Figure 5.13). Field observations also suggested there was a difference in the month of most intense spawning, as *M. digitata* colonies with ripe eggs were easily found in March during the three years of this study, whereas *M. tortuosa* colonies with mature eggs were rare. In April this trend was reversed (see Figure 5.6).

Spawning synchrony: night of spawning

The night of spawning differed between the two sites. At Magnetic Island both *M. tortuosa* and *M digitata* spawned primarily on the second night after full moon, whereas at Orpheus Island they spawned on the third night in all three years (χ^2 = 8.57, df = 1, $p<0.005$; Figure 5.14). Night of spawning did not differ between species for colonies at Geoffrey Bay (χ^2 = 1.90, df = 2, $p>0.05$; Figure 5.14), or at Pioneer Bay (χ^2 = 0.51, df = 1, $p>0.05$; Figure 5.14).

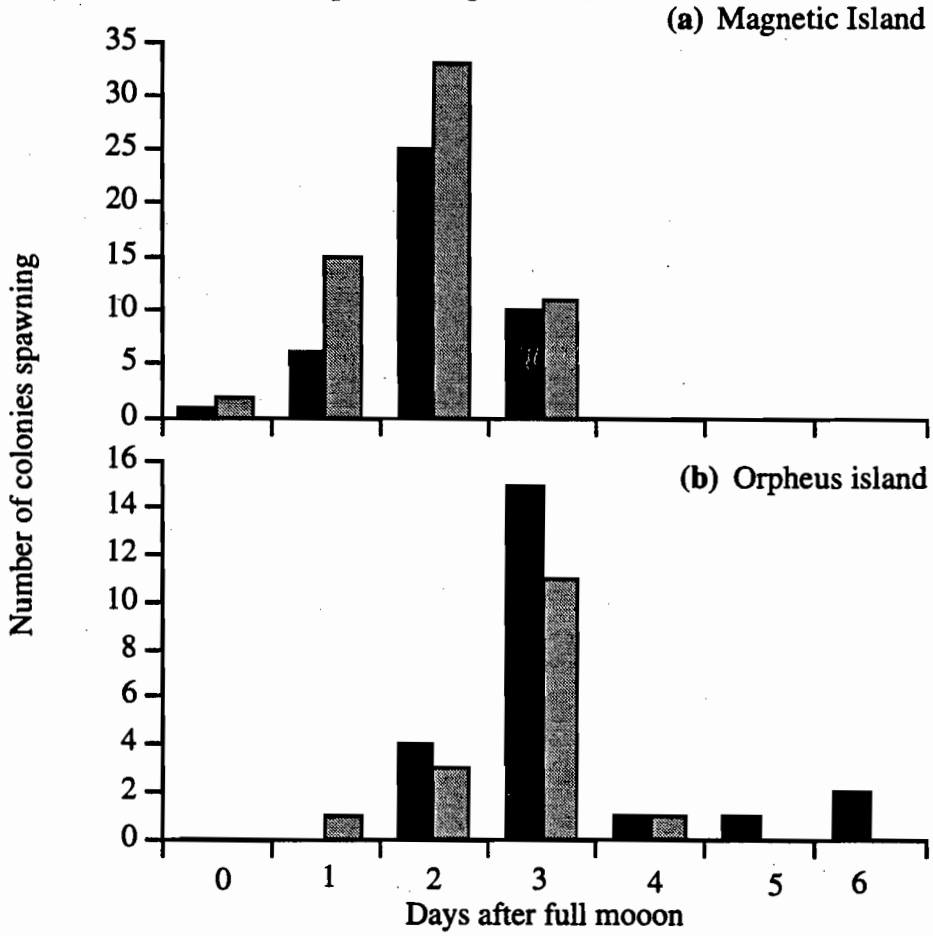


Figure 5.14. Frequency distribution for the number of *Montipora tortuosa* (■) and *Montipora digitata* (▩) colonies spawning on days after the full moon at Magnetic Island (a) and Orpheus Island (b).

5.3.3 Does M. tortuosa partition energy between growth and reproduction to sustain two breeding seasons annually?

Growth rate for *M. tortuosa* colonies measured as branch linear extension was significantly greater over the summer period of 1992 than during the winter period in 1993 ($t = 7.956$, 97 df; prob $t < 0.001$; Figure 5.15a). Summer growth averaged 0.67 cm/month (SD = 0.103, n = 49), whereas winter growth averaged 0.46 cm/month (SD = 0.109, n = 150). Growth of *M. tortuosa* colonies over the winter period in 1993 was higher than in the winter of 1992 (0.59 cm/month, see section 5.3.1). Mean number of eggs per polyp and branch linear extension during the summer period were negatively correlated ($R^2 = 0.261$, $P = 0.0002$, slope = -4.25±1.04; Figure 5.15b). There was no such relationship during the winter growth period ($R^2 = 0.001$, $p<0.05$; Figure 5.15c). No correlation was detected between branch linear extension and egg size ($R^2 = 0.002$ and 0.052 for winter and summer respectively), neither was there a correlation between branch linear extension and testes size for the winter growth period ($R^2 = 0.004$), though there was a weak correlation for the summer period ($R^2 = 0.095$, $P = 0.03$, slope = -119.4±53.9; figures 5.16a and b).

5.3.4. Spawning patterns of plate species of Montipora:

Spawning synchrony: month of spawning

Other species of *Montipora* sampled from Magnetic and Orpheus Islands exhibited similar reproductive patterns to those of *M. tortuosa* and *M. digitata*. At Magnetic Island ten species spawned both in autumn and spring, and a further three spawned in autumn, but were not checked for spawning in spring (Table 5.8). At Orpheus Island five species spawned during both seasons, and a further one spawned in autumn, but was not observed to spawn in spring (Table 5.8). A total of 14 species therefore spawned in autumn.

Of the six plate species of *Montipora* sampled between 1992 and 1994, all except *M. foliosa* had fewer reproductive colonies in autumn (Figure 5.17), in particular, reproductive activity for *M. undata* and *M. crassituberculata* was minimal. No reproductively mature colonies of *M. foliosa* were found prior to the November spawning, however sampling a month earlier in 1993 revealed that *M. foliosa* does spawn in spring, but a month earlier than most other species at Orpheus Island (i.e. 23% of *M. foliosa* colonies were reproductive before the October full moon, but none were reproductive one month later, see Figure 5.13). This was still half the number that were reproductive in March. However, it is possible that further spawning may have occurred a month earlier in September. Although species did not overlap between sites, species tended to have fewer reproductive colonies at Orpheus Island than at Magnetic Island in both breeding seasons (Figure 5.17), a pattern similar to that found

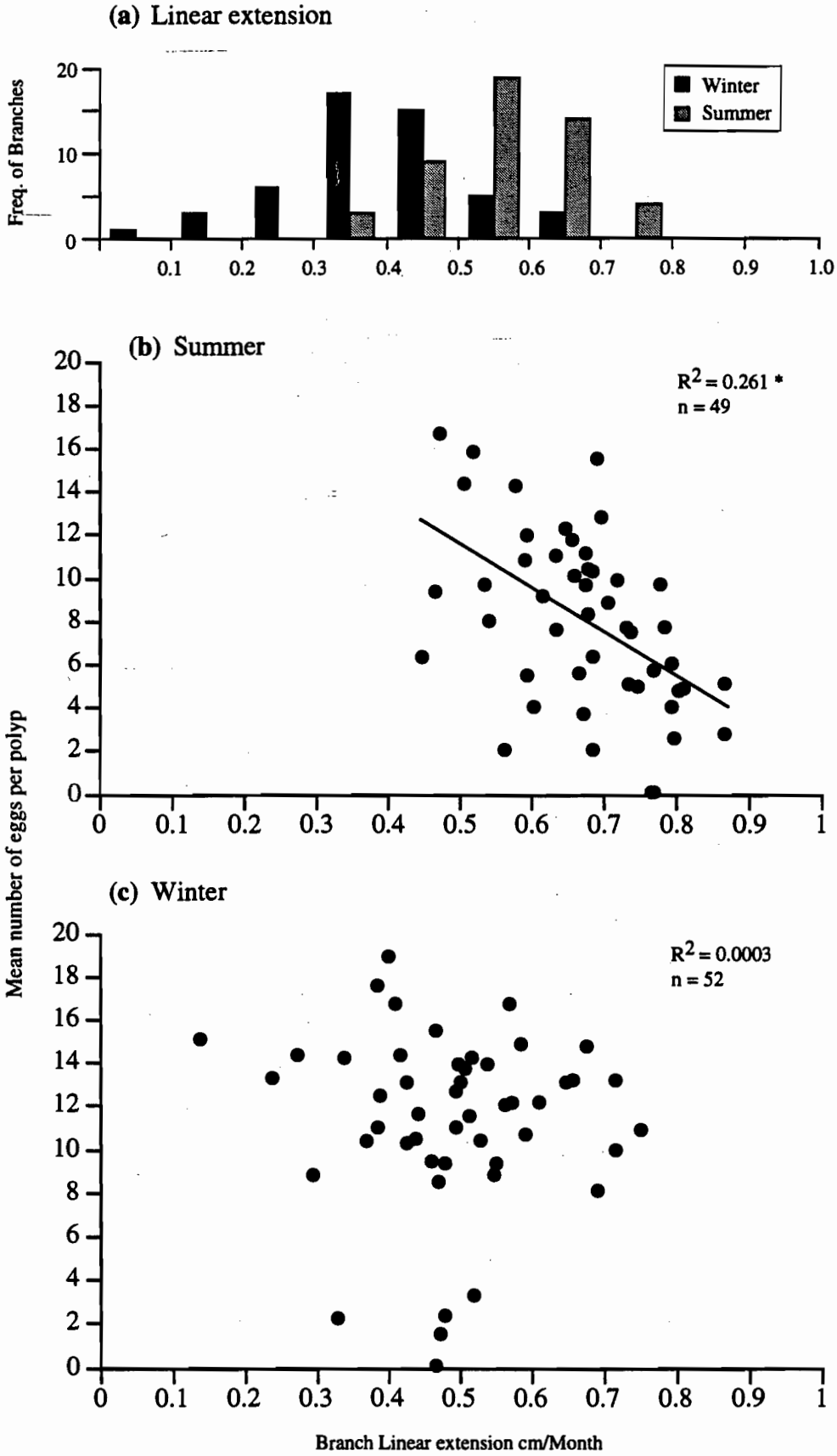


Figure 5.15. Frequency histogram of branch linear extension for *Montipora tortuosa* during summer and winter growth periods (a), and plots of egg number per polyp against branch linear extension during summer (b) and winter (c) periods. *: significant regression.

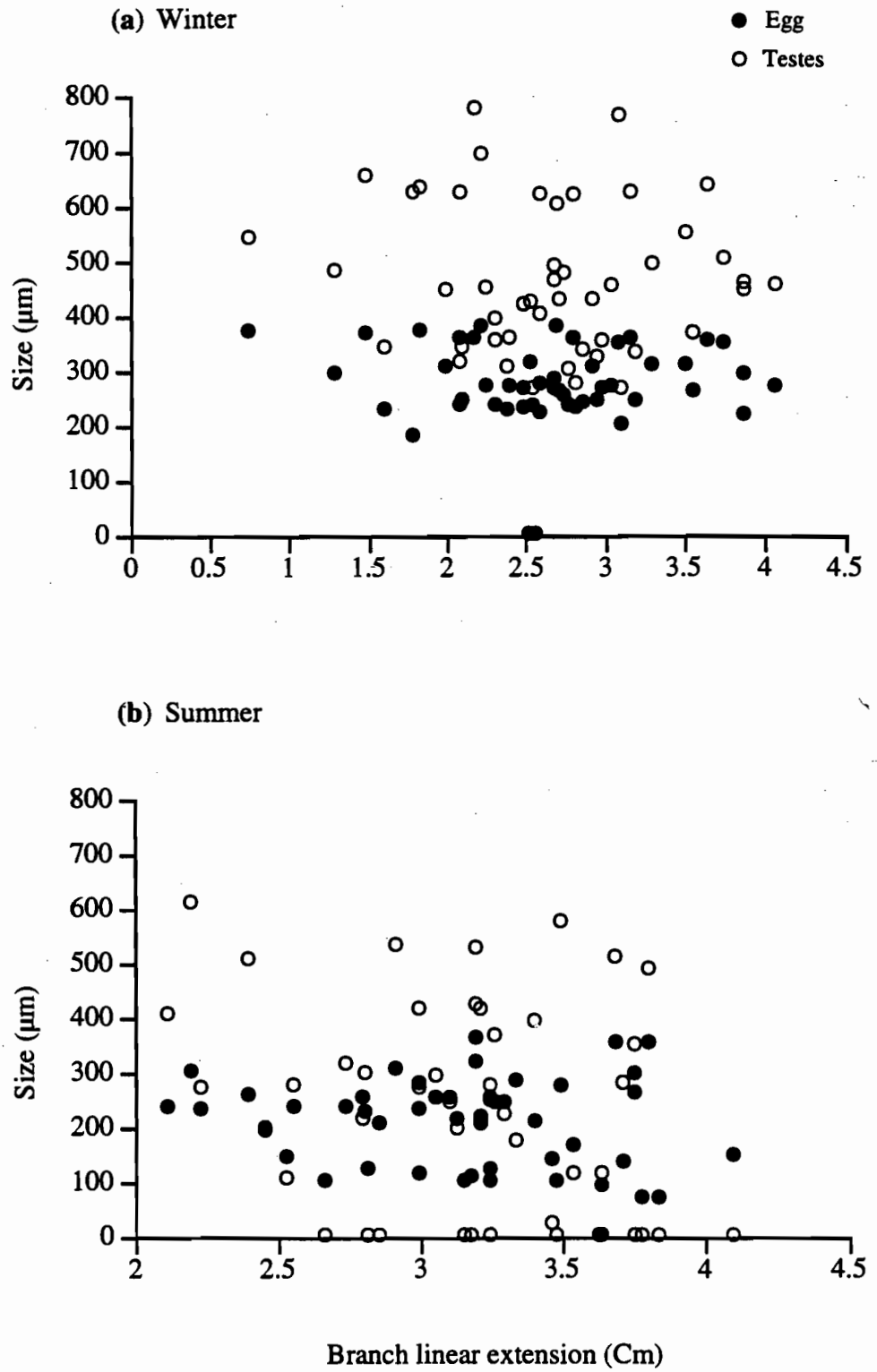


Figure 5.16. Plots of egg size (●) and testes size (O) in relation to branch linear extension for *Montipora tortuosa* colonies grown in Geoffrey Bay over the winter period (a), and the summer period (b) leading up to spawning. The only significant regression was for testes size in summer ($R^2 = 0.095$, $p>0.05$; $n = 41$).

Species	Magnetic Island				Orpheus Island			
	Spring spawning		Autumn spawning		Spring spawning		Autumn spawning	
	O	I	O	I	O	I	O	I
M. aequituberculata	**3**	**41/42**		**31/35**	**5**	**1/1**		**4/10**
M. australiensis				**5/6**				0/1
M. corbettensis				0/2				
M. crassituberculata	**4**	**1/1**	**4**	**5/5**		**31/31**		**2/36**
M. danae		**2/2**		**2/2**				0/8
M. tortuosa	**15**	**29/60**	**13**	**24/60**	**20**	**43/60**		**22/60**
M. digitata	**22**	**46/60**	**13**	**27/60**	**15**	**21/60**		**22/60**
M. efflorescens					**3**			0/5
M. floweri				0/2				0/1
M. foliosa						0/30		**12/30**
M. grisea				**3/5**				**1/4**
M. hispida	**2**	**3/3**		**1/1**	**1**	**5/5**		**1/5**
M. hoffmeisteri								0/1
M. informis		**1/1**						0/3
M. millepora		**1/1**						0/1
M. mollis		**5/5**		**4/5**				
M. monasteriata								0/4
M. peltiformis	**1**	**32/34**	**2**	**21/30**	**5**	**1/1**		
M. spumosa	**5**	**39/39**	**4**	**19/33**	**3**			0/1
M. stellata	**4**	**16/16**		**1/23**	**2**			0/2
M. tuberculosa		**1/1**						0/1
M. turtlensis				**2/2**				0/1
M. undata		**2/2**		**2/3**	**2**	**26/30**		**1/35**
Unknown		**3/3**		**1/1**				0/5
M. verrucosa					**1**			

Table 5.8. Spawning records for *Montipora* species at Magnetic Island and Orpheus Island in the spring and autumn between March 1991 and March 1994. Number of corals observed spawning in buckets (column O) and number of corals inferred to spawn by the presence of large eggs (>200μm) in decalcified samples (column I)(expressed as a fraction of number of colonies with eggs/number of colonies examined for eggs) are given. Records indicating spawning are in bold face.

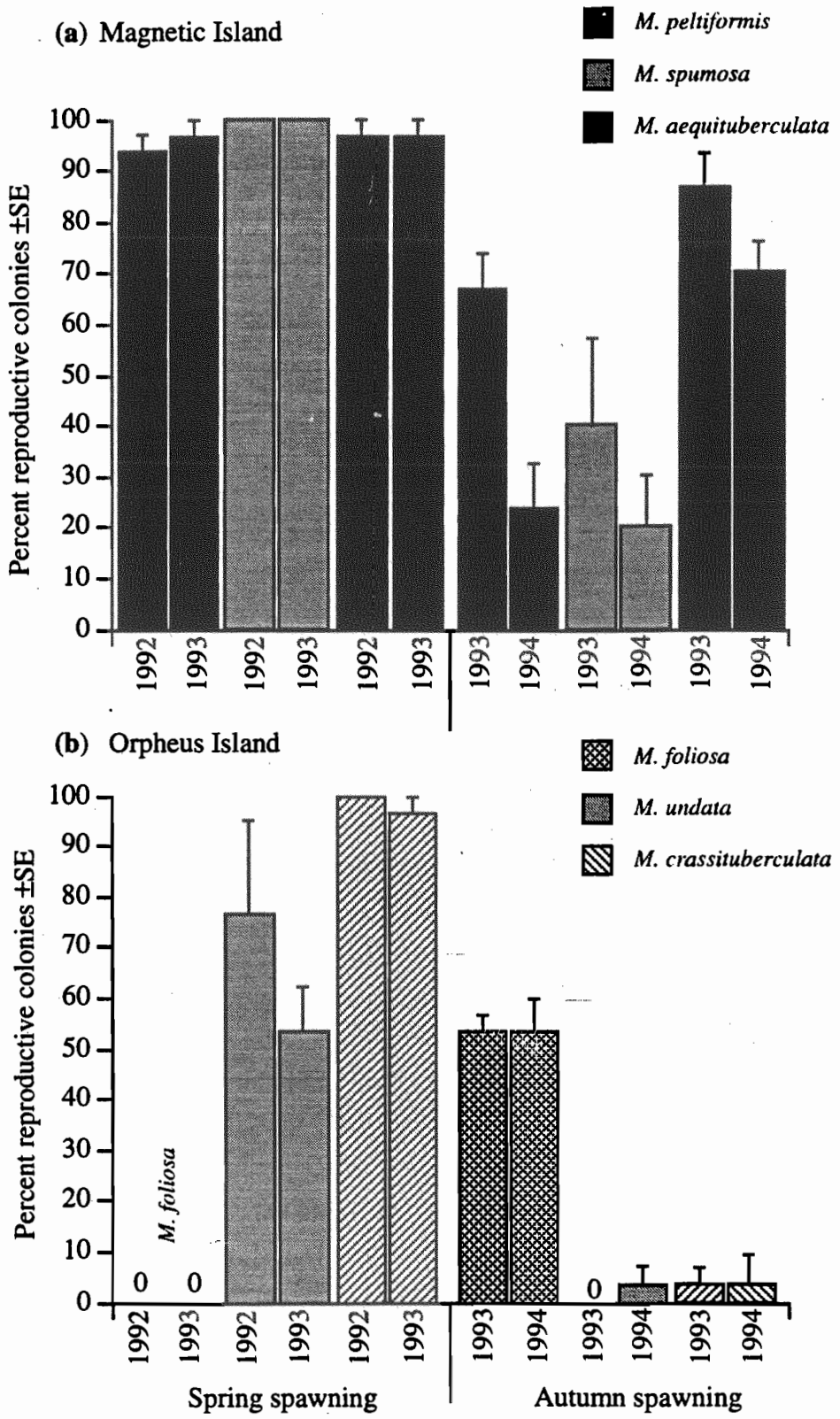


Figure 5.17. Comparisons of breeding effort between the spring and autumn breeding seasons for six species of *Montipora*. (a): Percentage ±SE of reproductive colonies at Magnetic Island in the week prior to the October (spring) and March (autumn) spawnings. (b) Percentage ±SE of reproductive colonies at Orpheus Island in the week prior to the November (spring) and March (autumn) spawnings. Number of colonies sampled per species = 30. Adjacent pairs of histograms represent records for species as ordered in each key.

for *M. tortuosa* and *M. digitata*. However, such differences between sites may reflect species differences.

There was variation in breeding synchrony among *Montipora* species in the spring of 1993. *Montipora foliosa* colonies all spawned in October 1993, whereas most *M. undata* colonies, and a large proportion of *M. crassituberculata* colonies spawned in November (see Figure 5.13). Similarly, during the autumn spawning plate species spawned over several months during the two seasons they were sampled (Figure 5.18), with some species spawning mostly in March (eg. *M. peltiformis*) and others in April or later (eg. *M. aequituberculata*).

Spawning synchrony: night of spawning

Similar to *M. tortuosa* and *M. digitata*, plate species spawned earlier at Magnetic Island than at Orpheus Island ($\chi^2 = 18.1$, df = 1, $p<0.001$; Figure 5.19). At both of these sites plate species spawned 1-2 nights after *M. tortuosa* and *M. digitata*.

5.3.5 Variation in physical parameters relative to spawning periods

The spring spawning is preceded by the dry season. During this period temperatures reach a yearly low in July-August and then rise steadily towards the summer period, and winds are moderate to strong. The autumn spawning is preceded by, and lies within, the rainy season (also the cyclone season). This period is characterised by very variable and high seawater temperatures (up to 32°C), heavy sporadic rainfall, and light to moderate winds. Considerable variation in rainfall and seawater temperature from year to year, and the occurrence of cyclones, mean that this period is much less predictable.

The spring spawning occurred approximately one month before the period of highest summer temperatures, and the autumn spawning occurred just prior to, and/or during, the period of rapid temperature drop in April at both Magnetic and Orpheus Islands (Figure 5.20). Spawning occurred within a temperature range of 26-30°C. The annual pattern of seawater temperature change was very similar at both Magnetic Island and Orpheus Island. However the temperature at Orpheus Island generally rose slower than that at Magnetic Island. At both sites, temperature dropped very rapidly around the beginning of April, the time and rate of drop coinciding remarkably well at the two sites.

Temperatures rose above 30.5°C in early 1992 and 1994 which were both years in which coral bleaching occurred at Magnetic Island (pers. obs.). In 1991 colonies of *M. digitata* on the reef flat did not bleach, but many plate species of *Montipora* on the reef slope did bleach. The pattern in 1994 was different with over 50% of both *M. tortuosa* and *M. digitata* colonies bleaching on the reef flat, but the platy *Montipora* species bleached to a lesser extent on the reef slope (pers. obs.).

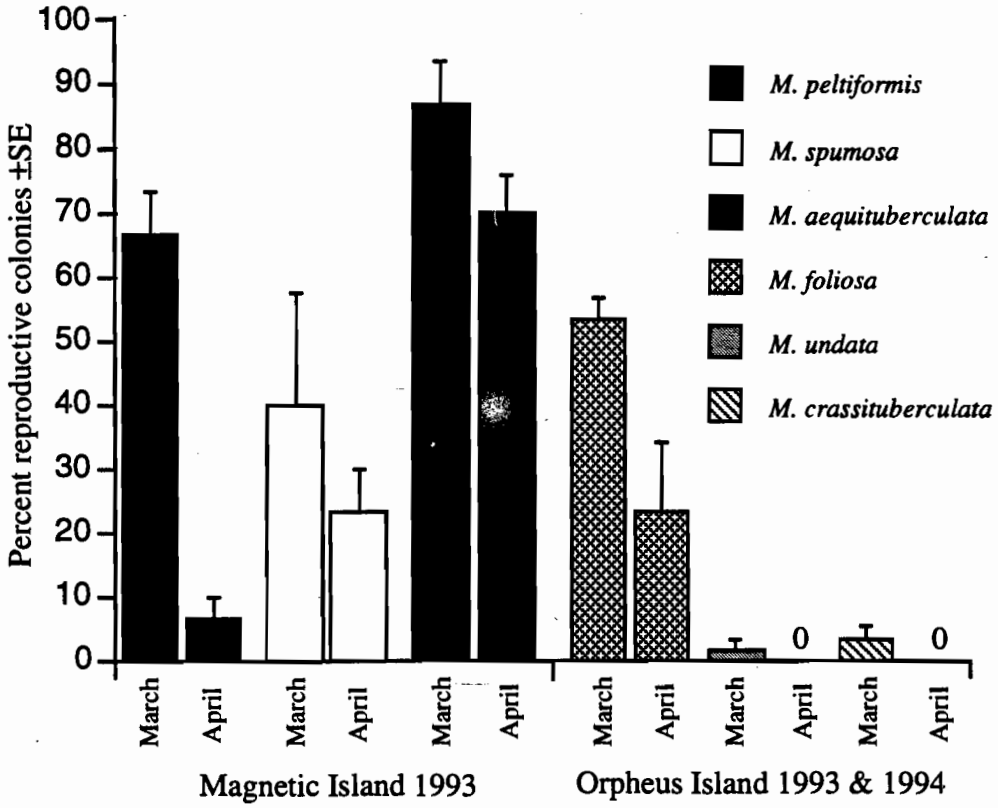


Figure 5.18. Comparisons of percentage ±SE of reproductive colonies in six plate species of *Montipora* before the March and April spawning at Magnetic and Orpheus Islands. Number of colonies sampled per species = 30. Values for Magnetic Island were only available for 1993, Orpheus Island values are estimated from 1993 and 1994 data. Adjacent pairs of histograms represent records for species as ordered in each key.

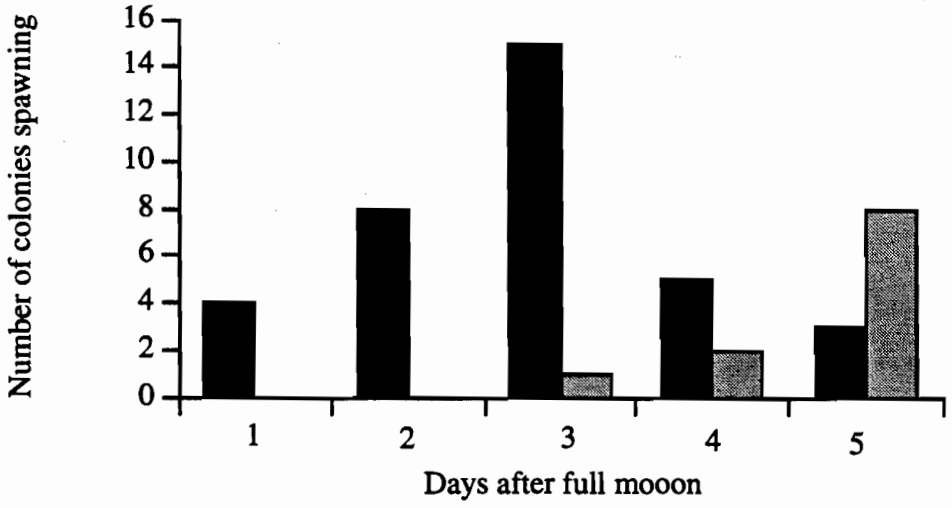


Figure 5.19. Frequency distribution for plate species of *Montipora* spawning on days after the full moon at Magnetic Island (■) and Orpheus Island (▩).

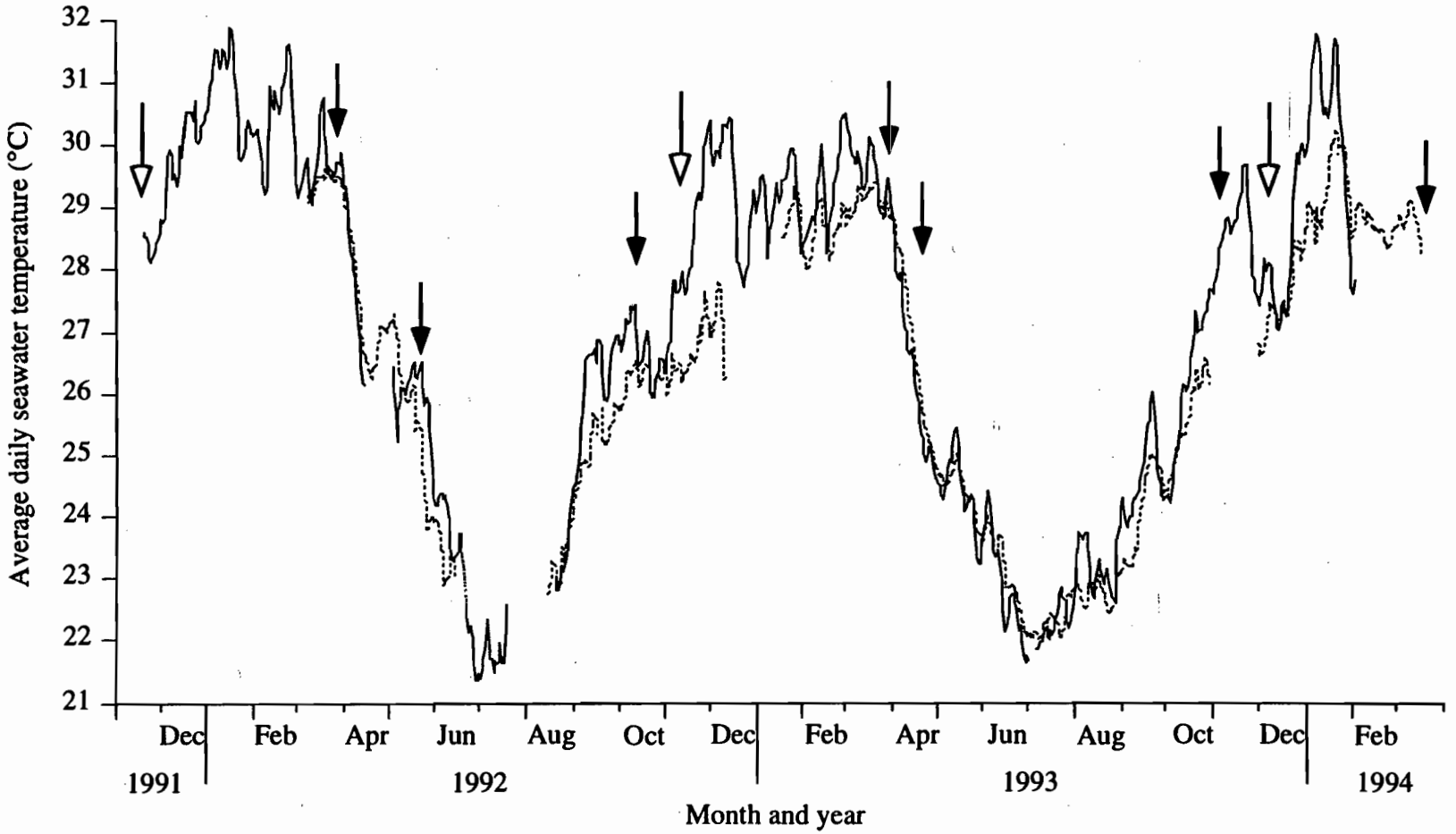


Figure 5.20. Average daily seawater temperatures (accurate to 0.5°C) for Geoffrey Bay (single line) and Pioneer Bay (dashed line) between November 1991 and March 1994. Data loggers were located at a depth of 5m (MSL) at both sites. Arrows indicate observed spawning events at Magnetic Island (closed arrows) and Orpheus Island (open arrows).

Annual average rainfall, temperature, daylength and tidal cycles relative to spawning periods are summarised in Figure 5.21. Spawning of *M. tortuosa* and *M. digitata* occurred approximately one-half of the way between the spring and neap tides at Magnetic Island, and two-thirds of the way between these tides at Orpheus Island. Plate species spawned about two thirds of the way between these tides at Magnetic Island, and on the neap tide at Orpheus Island. Spawning also occurred near the peak of daylength in the spring, and approximately halfway between the peak and trough in the autumn (Figure 5.21). Rainfall was variable from year to year, the relevant information to note in this case being that there was a very wet season in the summer of 1991-1992 (rainfall was 161mm and 565mm above average in January and February respectively), with subsequent wet seasons having much less rainfall. Windspeed was variable throughout the year ranging between 0 and 35 knots, but no season appeared to have stronger winds.

3.4 Discussion

5.4.1 Interspecific differences in reproductive traits

There are detectable differences of several reproductive traits between *Montipora tortuosa* and *M. digitata.* They differ in the number and size of their eggs, the number of polyps bearing eggs, and the number and size of testes. No differences were detected in gross sperm morphology or ultrastructure, total reproductive output per unit area, or linear extension of branches between the two species. Although reproductive criteria have rarely been used for species identification in corals, potentially they are extremely useful (Lang 1984). Irrespective of whether differences in reproductive traits have a direct effect on fertilisation success or not, they provide indirect evidence that groups of organisms may not be conspecific.

The size of eggs and testes may vary between closely related species of coral. For example Babcock (1984) found that the eggs of *Goniastrea aspera* are smaller and more numerous than those of the very similar sympatric species *G. favulus,* and Hall (1992) found the volume of *Acropora hyacinthus* eggs was one fifth larger than three other *Acropora* species. Unfortunately testes size has largely been ignored, which is reflected in the lack of size data for testes in key review papers (Szmant 1986, Harrison and Wallace 1990, Fadlallah 1983). Hall (1992) measured testes volume and number for five scleractinian corals, but only found significant differences in size and number between genera. In contrast the size and number of testes has been found to differ between two species of the same genus in this study.

The lack of differences between *M. tortuosa* and *M. digitata* sperm is surprising in view of the many other differences between the two species, particularly as it is thought that there should always be recognisable differences in size and shape between

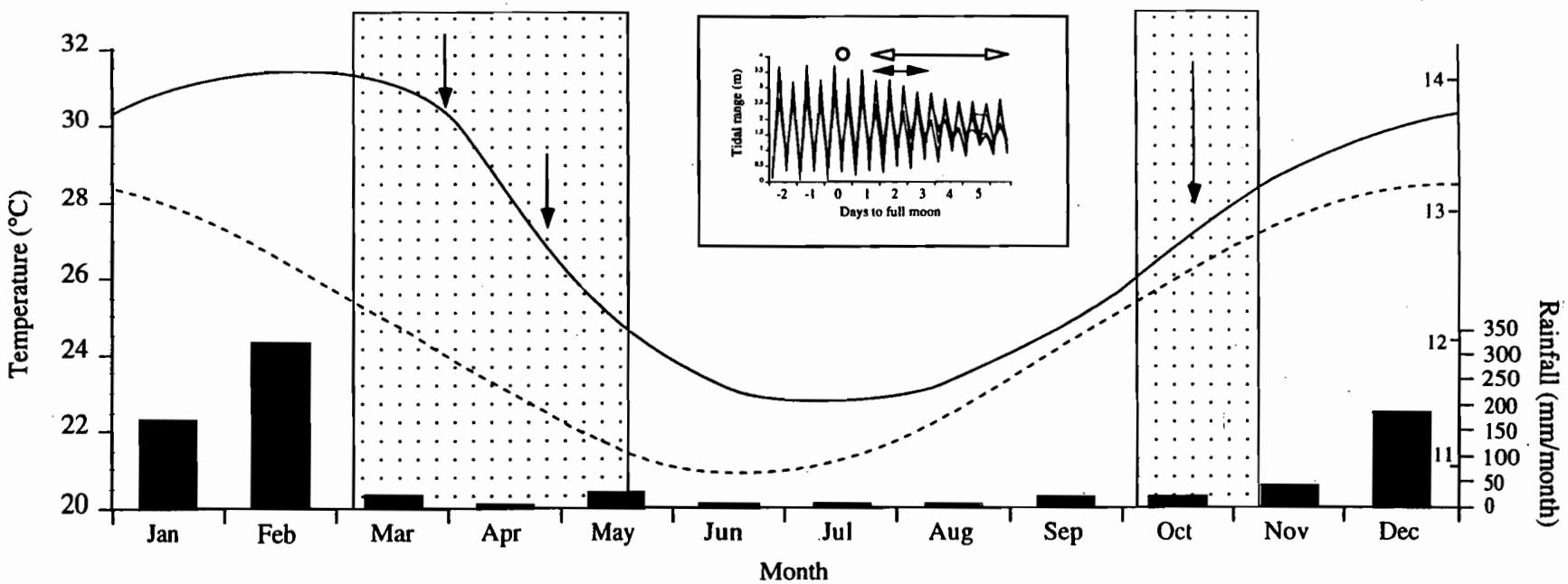


Figure 5.21. Summary of average annual rainfall (bar chart), seawater temperature (single line), and photoperiod (dashed line) between 1991 and 1993, in Geoffrey Bay. Maximum and minimum photoperiods were 13:17hrs and 10:58hrs respectively. Arrows indicate known spawning dates, and (▨) indicates tentative period in which spawning may occur. (■) Indicates rainfall. Inlay indicates average tidal pattern during spawning periods, closed arrows indicate spawning nights for *M. tortuosa* and *M. digitata*, and open arrows indicate spawning nights for plate species of *Montipora*. (○) indicates full moon.

species (Baccetti and Afzelius, 1976, Afzelius, 1979). Harrison (1988) found that sperm structure is species specific in scleractinian corals, and that differences were usually more pronounced in the nuclear region than the midpiece. However, Harrison also found that differences were often very subtle in congeneric species, which, given the relatedness of *M. tortuosa* and *M. digitata* (see Chapter 6), may explain why no differences were detected during this study. Indeed, Harrison found that sperm of closely related *Acropora millepora* and *A. pulchra* were very similar in size and structure compared to other *Acropora* species studied. While there can be little doubt that sperm morphology provides useful taxonomic characters for separating relatively distantly-related congeneric species, this may not be true for relatively closely related species. More work on sperm of other *Montipora* species to determine the extent of morphological variation of sperm within the genus would allow an assessment of whether sperm morphology can be expected to distinguish closely related species, and whether the similar sperm morphology found between *M. tortuosa* and *M. digitata* can be used to assess their phylogeny within the genus.

There was considerable evidence that within spawning seasons *M. tortuosa* and *M. digitata* populations differ in the number of colonies spawning. During the autumn spawning *M. digitata* colonies tended to spawn a month earlier than *M. tortuosa* colonies, and there was also evidence that this occurred in spring, particularly in years of split spawning. However, further proof is required to be certain they do spawn on different months. Other studies have used differences in spawning time for taxonomic purposes. For example, Richmond and Jokiel (1984) suggested that two "types" of *Pocillopora damicornis* from Hawaii may be different species based on differences in the timing of planulation. Type B *P. damicornis* planulated between the first quarter and full moon, while type Y planulated around the lunar third quarter. Brickner *et al.* (1993) has used differences in the season of reproduction of two coral-boring bivalve populations to argue that they are reproductively isolated, and consequently different species. While spawning time has rarely been used for taxonomic purposes it is potentially useful for distinguishing species as corals within the same genus may have different spawning times on the scale of hours, days, and months. Thus *Echinopora lamellosa* and *E. horrida* spawn 1:40 and 3:50 hours after dark on the 6th day after full moon (Babcock *et al.* 1986), *Montipora spumosa* and *M. undata* spawn around the 3rd and 5th nights after full moon respectively (pers obs.), and *Acropora eurystoma* and *A. scandens* spawn one month apart in the Red Sea (Schlesinger and Loya 1985).

No difference in growth was detected between *M. tortuosa* and *M. digitata*. Growth rate was fast for both species, and equivalent to that of *Acropora formosa* 'white tipped' branches which were found to grow approximately 0.44 cm/30 days by Oliver (1987). To date, growth rate has only been used in coral taxonomy on one occasion, in which vertical growth was used as corroborative "evidence" to separate

three morphotypes of *Montastraea annularis* into sibling species (Knowlton *et al.* 1993). Growth of one of the species was approximately twice that of the other two. It is noteworthy that the only instance of growth being used for coral taxonomy should have been for the supposed identification of sibling species. Sibling species are difficult to identify, so unconventional characters are often explored in the hope that they may reflect species differences. Normally such a parameter would be ignored in taxonomy as growth is not easy to estimate, is heavily influenced by environmental conditions (Dodge *et al.* 1974, Houck *et al.* 1977), and it can take a relatively long time to obtain an estimate with slow growing organisms like corals.

A variety of reproductive characters have been compared between *M. tortuosa* and *M. digitata*, and some found to be significantly different. But how should one interpret these differences? Genetic, breeding and morphometric data have already demonstrated that they are two species. The reproductive differences described in this chapter therefore provide further evidence that *M. tortuosa* and *M. digitata* are independently evolving lineages. It is noteworthy that other factors such as phenotypic plasticity, genetic isolation (from founder effects or long-term selective pressures) and local selection can also explain physiological and biochemical differences between corals (Potts 1978). However, in this case such factors can be discounted as species status of *M. tortuosa* and *M. digitata* has already been established, and the differences are not environmentally induced phenotypic characters, or a result of local selection, as both species live side by side in the same environment (see Chapter 4).

Alternative characters are becoming increasingly popular with coral taxonomists (Lang 1984, Knowlton and Jackson 1994), they are characters that are not traditionaly used in taxonomy (in this case any non-skeletal characters), and can be very useful providing they are used with caution. This is necessary as alternative characters may be redundant to taxonomy, or they may not have the same weight as classical characters (Gattuso *et al.* 1991). In this study alternative characters are used to corroborate genetic and morphometric studies, and therefore provide added evidence that *M. tortuosa* and *M. digitata* are two species. However, none of the differences described here would alone be suitable for species identification, as they are not discrete. The traditional morphological approach (Chapter 4) therefore remains the best for regular identification of species, along with the more costly genetic approach. Reproductive differences found between *M. tortuosa* and *M. digitata* also highlight the importance of distinguishing between species for obtaining accurate estimates of a wide variety of characters.

5.4.2 The reproductive ecology of M. tortuosa and M. digitata

This is the first study to document biannual spawning of broadcast spawning corals at a geographic location experiencing marked seasonality in annual sea temperature patterns. As biannual breeding appears to apply to a large proportion of

members of the genus *Montipora*, and this genus is the second most abundant in terms of species (Veron 1986), the general belief that broadcast spawners undergo a single cycle of gametogenesis (Harrison and Wallace 1990) should no longer apply. A previous study at Magnetic Island did find evidence that *Montipora monasteriata* and *M. foliosa* initiate a second gametogenic cycle (Robertson 1981), but the March spawning was not confirmed. Similarly Szmant-Froelich *et al.* (1980) found that the temperate coral *Astrangia danae* initiates a second gametogenic cycle, though no evidence of a second spawning was encountered. Harrison and Wallace (1990) suggested that gametes from the second cycle were resorbed in these cases. However, they also hypothesised that under favourable environmental conditions some broadcast spawning corals may be capable of completing more than one gametogenic cycle per year, because experimental maintenance of corals under conditions of maximum local sea temperatures and regular feeding allowed *A. danae* to spawn year-round (Szmant-Froelich *et al.* 1980). Biannual spawning has been documented for other members of the family Acroporidae on low latitude reefs. In Western Samoa, three species of broadcast-spawning corals undergo two gametogenic cycles every year, each culminating in synchronised spawning, whereas the same species only spawn once a year on the Great Barrier Reef (Mildner 1991). Evidence of two gametogenic cycles was also found for several broadcasting species at Madang in Papua New Guinea (Oliver *et al.* 1988). Therefore, it seems that at least within the family Acroporidae, corals are able to undergo more than one gametogenic cycle per year .

Differences in gamete generation times can not explain why members of the genus *Montipora* are able to spawn biannually on the Great Barrier Reef, but other genera do not. A shorter time for gametogenesis in *Montipora* species can not explain this difference, as other species produce gametes in a similar time-frame. For example, in the period leading up to the spring spawning, Babcock (1984) reported a period of gametogenesis for *Goniastrea aspera* of approximately six months, which is the same as that for *M. digitata* and *M. tortuosa* during the same time of the year. Although many species of *Acropora* have longer gametogenic cycles (Wallace 1985b), longer gamete generation times need not prevent biannual spawning. Mildner (1991) found that three *Acropora* species in Samoa were able to spawn biannually by overlapping gametogenic cycles.

It is possible that members of the genus *Montipora* are able to spawn biannually at the expense of growth. Trade-offs between growth and reproduction occur for many organisms (Begon *et al.* 1990), and most commonly growth is reduced in favour of reproduction (Stearns 1992). A negative correlation between colony growth and reproduction was detected for the autumn spawning. Though the correlation was

weak, it is consistent with a trade-off occurring during the period leading to the autumn spawning. Branches that grew most during this period had a lower reproductive output, suggesting that growth can take place at the expense of reproduction in summer. A similar trade-off at the expense of reproduction has been reported for *Stylophora pistillata* by Rinkevich and Loya (1985). For this study, growth was measured above the area used to estimate reproductive output which suggests that energy is being translocated to the growing tips, and therefore not available for egg production in the area in which egg production was measured. Alternatively growth may also have been high in the area in which egg production was measured: although growth measured using alizarin staining was minimal in this area, secondary infilling may have occurred and required energy. No trade-off was detected between egg size and growth, which is not surprising as smaller eggs would probably be less likely to survive. There was also no evidence of a trade-off between testes size and growth. Although preliminary, the results presented here show that *M. tortuosa* and *M. digitata* may be suitable species to use for the study of growth-reproduction trade-offs in scleractinian corals. The fact that no trade-off is apparent during the spring spawning emphasises the importance of studying such phenomena under a suite of different conditions as advocated by Stearns (1992). The differing environmental conditions leading-up to the two spawning periods providing contrasting regimes in which to study possible trade-offs between growth and reproduction.

Relative to other species, there is no suggestion that biannual reproduction occurs at the expense of overall colony growth, as members of the genus *Montipora* are generally fast growing (pers. obs.), and *Montipora tortuosa* and *M. digitata*, in particular, may grow 7 cm a year. This rate is about half that of the fastest growing *Acropora* species (Isdale 1977). Members of the genus *Montipora* are therefore able to grow fast while in an almost continuous state of gametogenesis, suggesting that they acquire enough energy for both growth and reproduction. Only when growth rate is extremely high does there appear to be a reduction in reproductive output. Monthly growth rate estimates obtained for *M. digitata* by Heyward and Collins (1985a), show there is little difference in growth rate between months, discounting the possibility that most skeletal growth could take place in the early stage of gametogenesis when egg growth is slowest. It is noteworthy that these authors deteted no difference between summer and winter growth, however they did not measure growth for two months over the summer and their analyses did not examine seasonal differences. A similar study by Oliver (1987) found that *Acropora formosa* is also able to grow fast and reproduce simultaneously without any apparent negative effects to either process.

Montipora tortuosa and *M. digitata* did not differ in their total reproductive outputs, but they did show some differences in reproductive strategy. *Montipora*

tortuosa has a much greater proportion of colonies spawning in October than March, whereas *M. digitata* has similar numbers of colonies spawning during both seasons. there also appears to be some difference in the amount of energy invested in egg and testes production. *Montipora tortuosa* produces eggs of smaller volume than *M. digitata*, but testes of greater volume, balancing the total volume output for reproduction. Thus investments in reproduction are equivalent for the two species, assuming that the production of eggs and testes require similar energy investments. This assumption is not unreasonable as lipid is as important an energy source for sperm as it is a metabolic reserve for eggs (Schick 1991).

Results show that egg-reproductive output for the Autumn spawning can be much lower and more variable than outputs for the spring spawning for both *M. tortuosa* and *M. digitata*. A previous year-long study of gametogenesis in *M. digitata* by Heyward and Collins (1985a) failed to detect a second spawning period in March, providing further evidence that the autumn spawning varies in intensity considerably from year to year. Such variation in breeding intensity may be environmentally induced. Environmental conditions can affect egg production by limiting food supply (nutritional state affects reproduction, Stearns 1992), which in corals may be of heterotrophic or autotrophic origin, or directly stressing the corals (Kojis and Quinn 1981, 1984, Rinkevich and Loya 1985). It is not likely that food supply will be limited during the period leading-up to the autumn spawning, as planktonic food is abundant during the summer period (James Cook University 1975), and there are more hours of daylight to fuel zooxanthellar energy production. The reduced fecundity in autumn could be the result of stress induced by the high temperatures and lowered salinity often experienced by the corals in the period leading to the autumn spawning. Such a scenario is likely as reproduction is thought to have a narrower tolerance to stress than any other life function (Gerking 1980). Lowered fecundity in corals has previously been associated with exposure to elevated temperatures (Kojis and Quinn 1984), and exposure to sedimentation or turbidity (Kojis and Quinn 1984).

The variable reproductive output of colonies during the four autumn spawning seasons spanned by this study appears to be related to variable rainfall and temperatures during the preceding summers. Egg-reproductive output of colonies during the spawning in 1990, which was preceded by a dry summer and no bleaching, was equivalent in magnitude to outputs found for the spring spawnings. During the summer of 1990-91 there was a particularly heavy rainy season with rainfalls 161mm and 565mm above average for January and February respectively. The lower autumn egg-reproductive output by both *M. tortuosa* and *M. digitata* following this period may have been caused by stress induced by low salinity. This interpretation is supported by

the comparatively high egg-reproductive output during the following two summers when rainfall was much less. Outputs remained high in 1992 despite a bleaching event associated with elevated sea temperatures in January (pers. obs.). As *Montipora tortuosa* and *M. digitata* did not bleach during this event, they appear not to have been stressed. However the elevated temperatures may have contributed to the observed one month delay in spawning. In 1993, summer temperatures did not rise excessively, rainfall was minimal and egg-reproductive output was high again. Other species of *Montipora* also varied in the proportion of colonies spawning in spring and autumn, all but one tending to spawn in spring. The failure to detect a large spring spawning in *M. foliosa* may have been because of an earlier spawning the previous month. Overall, the lower and more variable reproductive outputs for the autumn spawnings in comparison to the spring spawnings suggest that the summer months are more marginal for gametogenic development, at least partially because of variable temperatures and rainfall commonly experienced by corals during these months.

Members of the genus *Montipora* therefore appear to be unusual in that they are able to sustain biannual gametogenic cycles, although members of other genera are able to spawn biannually in other locations (Mildner 1991). Why should autumn spawning be restricted to the genus *Montipora* on the Great Barrier Reef? One hypothesis is that the autumn spawning may be a genetic legacy that has disappeared in other coral species. It is conceivable that currents flowing south to the Great Barrier Reef have carried larvae of corals that spawned in autumn from elsewhere, or that there may have been a time in the past when conditions on the Great Barrier Reef were conducive to biannual spawning for most species. As conditions changed over time, there may have been strong selective pressure against biannual spawning for most species, but not for those of the genus *Montipora*. Members of the genus *Montipora* are unusual in that their eggs contain zooxanthellae, in contrast to all other species examined so far, with the exception of members of the genus *Porites* (Kojis and Quinn 1981, Harrison and Wallace 1990). The importance of zooxanthellate eggs for biannual spawning could be tested by determining whether any *Porites* species spawn in autumn on the Great Barrier Reef. Kojis and Quinn (1981) found no evidence of biannual spawning in four species of *Porites* at Heron Island, however, this is a high latitude site with lower annual temperatures than those found in Townsville. As a consequence the spring spawning at Heron Island is in December, and a second spawning in March may not be possible. Biannual spawning may also be a recently evolved phenomenon rather than a legacy on the decline. The advantages associated with such a strategy are obvious. Spawning in autumn would allow members of the genus *Montipora* to recruit to new habitats at a time when new spaces are likely to have been opened for settlement (after the summer cyclone season), and differing predator or competitor populations may

provide different conditions for juvenile survival. Spawning twice a year also reduces the risk of catastrophic events destroying the annual reproductive effort of these species.

Just as comparisons of spawning patterns in geographic regions with differing environmental regimes provide insights into the ultimate factors and proximate cues governing coral spawning (Willis *et al.* 1985, Babcock *et al.* in press), so can differences in conditions during spawning at two different times of the year provide insights into these processes. Generally, there has been agreement that variation in temperature may exert the selective pressure required for the evolution of spawning synchrony (Schlesinger and Loya 1985, Babcock *et al.* 1986, Szmant 1986), as temperature is known to influence the reproductive activities of marine animals (Giese and Pearse 1974). This study has shown that members of the genus *Montipora* which spawn biannually do so within a similar temperature window (26-30° C), and during times of both rising and falling sea temperature. These results suggest that spawning can occur over a wide temperature range, and that the direction of temperature change is not critial to spawning. Although spawning does occur within a wide temperature window, temperature may still be a significant ultimate factor governing coral spawning. By spawning in spring and autumn members of the genus *Montipora* avoid spawning at the warmest and coldest times of the year, thus the advantage may lie in avoiding stressful extremes rather than spawning at a specific temperature. For example, a study by Jokiel and Guinther (1978) found *Pocillopora damicornis* colonies could reproduce at temperatures ranging from 23 to 30°C, however the optimal temperature for reproduction was 26-27°C. This suggests that corals can spawn at different temperatures if they have "no choice", but in areas where they have a full range of temperatures, as is the case on reefs close to Townsville, they will probably spawn within an optimal temperature range (in this case 27-30 °C).

Apart from the possibility of an optimal temperature range for spawning, temperature also clearly affects the rate of gametogenesis, and therefore the month of spawning. Differences in the month of spring spawning between Orpheus and Magnetic Islands have been attributed to a slower rise in sea temperature at Orpheus Island (Babcock *et al.* 1986). In this study the slower temperature rise at Orpheus Island was apparent during the two years temperature was recorded, and on both years most spring spawning was delayed by a month at this location even though water temperature was above 26°C by October at both sites. It seems most likely that the slower temperature rise at Orpheus Island delays gametogenesis, and eggs are not mature enough to be released by Otober. The slightly lower temperature at Orpheus Island is unlikely to inhibit spawning in October as corals within the genus Aroporidae

can spawn at temperatures as low as 23.5°C elsewhere (Simpson 1991, Babcock *et al.* in press). In autumn there is no apparent difference in the month of spawning between the two sites, and temperature drops simultaneously at these two sites during this time. In comparison with Magnetic Island, considerably fewer colonies spawn in autumn at Orpheus Island. The reason for this difference is unclear, but it may be associated with the fact that Orpheus Island colonies only have four months from the spring spawning for gametogenesis before the sharp drop in temperature that occurs with the onset of winter, whereas colonies at Magnetic Island have five months. However it is not clear how such a difference between the sites could reduce the number of colonies initiating second gametogenic cycles. The differences in spawning patterns between these two sites merit further research of spawning synchrony, in particular because several factors important for spawning synchrony such as lunar periodicity and photoperiod can be ruled out as possible causes of differences in spawning patterns, due to the proximity of the two sites to each other. Furthermore corals at the two sites are not likely to differ much genetically (Chapter 2), thus eliminating genetic legacy factors that must be considered when comparing distant populations.

Temperature undoubtedly has some effect on coral spawning seasons, however, other factors such as coinciding of spawning with neap tides to maximise fertilisation, and predator satiation, have also been suggested as possible ultimate factors controlling coral spawning (Oliver *et al* 1988, Pearse 1990). Despite much speculation there is little evidence that any such factors may exert the selective pressures necessary to synchronise spawning. It seems most likely that a complex combination of physical and ecological factors will act as ultimate reasons for spawning. Coral gametes are particularly vulnerable to adverse weather conditions at the time of spawning, as most gametes are concentrated on the sea surface at this time. Harrison *et al.* (1984) found that heavy rain during a mass spawning event destroyed large numbers of eggs. Larvae on the other hand do not appear to be affected as much by weather conditions. For example, in a recent study, larval recruitment at Heron island was not adversely affected by the passage of a cyclone (C. Mundy pers. comm.). Therefore it is possible that selective pressures for spawning time are strongly influenced by the timing of egg fertilisation at the water surface. *Montipora* species spawn during two times of the year when weather patterns are changing, these are periods of relative calm and may therefore be more favourable spawning periods. Although the autumn spawning falls within the cyclone season, cyclones are less likely to occur at the end of March and begining of April (Lourensz 1977, R. Jaycock pers. com.). Future studies of coral spawning should examine long term meteorological and oceanographic patterns as they may provide a better idea of the ultimate factors governing coral spawning.

The biannual spawning pattern does provide a clearer indication of the proximate cues that may be important in synchronising spawning within the genus *Montipora*. Photoperiod has often been demonstrated to be a vital proximate cue to synchronise reproduction in both animals and plants (eg. Mammals: Hanson 1985; molluscs: Wayne and Block 1992, Yoshioka 1989 a & b; echinoderms: Pearse and Ernisse 1982, McClintock and Watts 1990; algae: Lüning 1988; fish: Gibson 1971). Though spawning does not coincide exactly with a particular stage of the annual photoperiod cycle, spawning at both times of the year, and from year to year, does fall within a similar photoperiodic range. However possible photoperiodic control can not be separated from closely correlated temperature effects, spawning in both seasons also being correlated with temperature range (26-30°C). If either/or these factors act as proximate cues members of the genus *Montipora* must be responding to their ranges, not to the direction in which they change, as both are rising during the spring spawning and falling during the autumn spawning. In corals there is no doubt that light/dark cues are used to synchronise the hour of spawning, and that lunar periodicity can play a role in synchronising the month of spawning (Jokiel 1985, Jokiel *et al.* 1985, Hunter 1988, Johnson 1992). Both spawning periods for *Montipora* species do correspond exactly to the same number of days after full moon, so lunar periodicity is probably an important proximate cue. This is consistent with other work that has found lunar rhythms to be important in synchronising spawning (Richmond and Jokiel 1984, Jokiel *et al.* 1985).

Conclusions

Comparison of a suite of reproductive characters between *M. tortuosa* and *M. digitata* has demonstrated that there are differences between the two species. *Montipora tortuosa* has more eggs per polyp, and a greater percentage of polyps in sexually mature colonies develop eggs. However *M. digitata* produces larger eggs so that total egg-reproductive outputs are balanced between the two species. The size and number of testes differed between the two species, *M. tortuosa* having larger, more numerous testes, and therefore greater testes-reproductive output. A greater percentage of *M. digitata* colonies spawned a month earlier than *M. tortuosa* colonies, though many colonies of both species also spawned on the same month.

A trade-off between colony growth and egg production may have occurred during the 1992-1993 summer period, prior to the spawning in March 1993, the number of eggs per polyp being less in branches that grew more. The biannual spawning of a branching species of coral that is easily accessible on the reef flat provides an ideal opportunity for further trade-off studies. The differing environmental conditions prior to the two spawnings providing contrasting regimes in which to measure growth and reproduction.

Members of the genus *Montipora* are the first to be documented to spawn biannually on a coral reef with distinct seasonality in annual temperature and photoperiod. Comparisons of spawning during the two seasons demonstrate that egg-reproductive output is generally greater in spring than in autumn for both *M. tortuosa* and *M. digitata*. During the three years of this study the egg-reproductive output varied considerably between years for the autumn spawning, but not the spring spawning. It is suggested that this difference may be the result of less predictable temperature and rainfall conditions prevailing prior to the autumn spawning. Other plate species showed a similar trend, with fewer colonies spawning in the autumn.

Comparison of environmental conditions prior to the spring and autumn spawning periods did not rule out the possibility that temperature may be an ultimate factor governing spawning, but it is proposed that stable weather conditions during the two periods of spawning may also contribute to the evolution of spawning synchrony at these two times of year. Temperature and daylength are both rising during the spring spawning, and falling during the autumn spawning. 'If these factors are acting as proximate cues for spawning the corals are most probably responding to the change in both these factors which could act as a trigger for spawning. Spawning during both seasons occurs at the same point in the lunar cycle, suggesting that this is an important proximate cue. Further studies of biannual spawning may provide unique insights into the ultimate factors and proximate cues that govern coral spawning.

Chapter 6
General discussion

6.1 The species status of Montipora digitata

This study has demonstrated that the fat finger and yellow spatulate morphs of *Montipora digitata* are two species, irrespective of whether one uses a morphological or "biological" definition of species. The two morphs are genetically distinct, morphologically different, and they do not cross fertilise. In addition they differ in several aspects of their reproductive ecology. The two morphs had been classified as separate species by Dana (1846), but were recently synonymised by Veron and Pichon (1984). It is now appropriate to resurrect the original names given to these two species, the fat fingers species corresponding to *Montipora tortuosa* (Dana 1846), and the yellow spatulate morph to *M. digitata* (Dana 1846).

The genetic data alone provides strong evidence that the two morphs are separate species (Chapter 2), particularly as they live in sympatry, and morphological and genetic differences are consistent over a wide geographic area (300km). Samples for genetic analysis were not obtained from more distant locations, however in this case genetic distinctness can be inferred from morphological differences as morphological and genetic species boundaries concur. The two morphs are readily distinguishable approximately 1500 km-from the Great Barrier Reef on the north east coast of Papua New Guinea. They could also be distinguished in museum samples collected as far away as Singapore (Chapter 4). The Great Barrier Reef and the north east coast of Papua New Guinea are not directly linked by surface circulation currents, and are geographically isolated from each other. Similarly, Singapore is not directly linked to either of these two locations. The differences between the two morphs are therefore well established on a large geographical scale. Septal shape in particular remains different between the morphs at all locations, indicating it is a geographically stable taxonomic character. It is important to establish this as it is known that apparent species barriers in one area may not exist in others, i.e. certain species barriers break down over a wide geographical area (Mayr 1988).

The inference of reproductive isolation between the two morphs of *M. digitata* from the allozyme study was confirmed by the fact that they do not cross fertilise. Reproductive isolation between populations should lead to differences in a range of phenotypic and ecological characteristics as they follow independent courses of

evolution (Ayala 1982). Phenotypic differences are evident in the morphology (Chapter 4), and ecological differences in the reproductive ecology (Chapter 5) of the two taxa.

6.2 Biological significance and species concepts

Species concepts and the species debate revolve around the question "what is a species"? Although an enormous amount of energy has gone into the debate and it has lasted over a hundred years, we are still no closer to finding an answer to the question (Mishler and Budd 1990). There is now growing agreement that no answer has been reached because there is no one suitable answer (Mishler 1985, de Queiroz and Donoghue 1988, Templeton 1989, O'Hara 1993). To a degree this notion is reflected by the fact that most biological disciplines have formulated their own species concept to meet their particular requirements. Thus there are ecological (Ehrlich and Raven 1969, Van Valen 1976), evolutionary (Meglitsch 1954, Simpson 1961, Wiley 1978, Willmann 1985 cited in Mayr 1988) phylogenetic (Cracraft 1983, Willis 1981) and biological concepts (Dobzhansky 1937, Mayr 1940, 1963, Grant 1958, Paterson 1978, 1985). Templeton (1989) rightly states that "a species concept can be evaluated only in terms of a particular goal or purpose", a view also shared by others (eg. de Queiroz and Donoghue 1990, Mitchell *et al.* 1993). Due to the apparently unsolvable nature of the species debate it has been suggested that we should abandon attempts to find an answer to the species problem (O'Hara 1993), as rigid empiricism will not advance our understanding of evolutionary history very far (de Quieroz and Donoghue 1990, O'Hara 1991, 1993). It is true that species concepts can help our understanding of species and evolution, but there is also justified concern that species concepts distract attention from the true purpose of evolutionary biology (eg. Budd and Mishler 1990); to attempt to explain how the patterns we see in nature came to be.

The introduction to this thesis emphasised the importance of assessing the biological significance of species. This present study is one of the few cases where morphological species have been reassessed using biological evidence. Normally species are defined by taxonomy and then assumed to be biologically relevant. *Montipora tortuosa* and *M. digitata* are good examples of biological species (reproductively isolated species), however they also conform to ecological (they have ecological differences), evolutionary (they are reproductively isolated and therefore have independently evolving gene pools), morphological and many other species concepts. Nothing would therefore be gained by advocating one concept or another; the important fact is that *M. tortuosa* and *M. digitata* are different, and that their differences can be explained by the fact that they are reproductively isolated.

Reproductive interactions have only been studied in a relatively small number of coral species, however, evidence to date suggests that strong reproductive isolation between coral species may be the exception rather than the rule on the Great Barrier Reef, with most coral species hybridising readily. Hybridisation occurred between some members of the genus *Montipora* during this study, and breeding trials involving other members of this genus (Willis *et al.* 1992), and other genera (Willis *et al.* 1992, Miller 1994, Wallace and Willis 1994), have resulted in high levels of hybridisation. Low levels of hybridisation have rarely been reported (Hodgson 1988).

High levels of hybridisation between corals has not yet been demonstrated to occur naturally (as opposed to *in vitro*), but there is no apparent reason why high levels of natural hybridisation should not occur (Wallace and Willis 1994). The opportunity for hybridisation is certainly present as gametes of many different species are mixed at the water surface during periods of synchronous mass spawning (Harrison *et al.* 1984, Willis *et al.* 1985, Babcock *et al.* 1986). Hybridisation between corals is particularly likely, due to the absence of many of the premating barriers to reproduction exhibited by organisms that have internal fertilisation systems and behaviourally governed matings (eg. Grant 1985). Molecular level barriers are the only physical barriers to crossing between coral gametes that are released on the same night. Assuming hybridisation does occur we must ask whether hybrids are likely to survive, and if they do, whether they are able to reproduce. Crosses performed during this study suggest that *Montipora* hybrid larvae may not survive as well as larvae produced from intraspecific crosses. Similarly, Hodgson (1988), found that *Montipora* hybrids did not survive. Crosses performed in other studies detected no difference in survival of hybrids (Willis *et al.* 1992, Miller 1994), and coral hybrids were able to grow for over three years (Miller 1994). Although hybrids can survive and grow, the fertility of F1 hybrids has not yet been established. As other hybridising species almost always show some element of hybrid unfitness (Hewitt 1988), more research on coral hybrids is required before the consequences of experimentally induced hybridisation can be further assessed.

The consequences of hybridisation in animals and plants have been reviewed extensively by Harrison (1993). Hybridisation can lead to the formation of new species by hybrid speciation, as well as to the merging or consolidation of species by introgression (Grant 1981, Arnold 1992, Rieseberg and Wendel 1993). If hybridisation is common between coral species then coral evolution may be reticulate, with some species diverging, others converging, and some probably stable at present time. Reticulate evolution has long been known to occur for plant species (Grant 1981, 1985), and has been inferred to occur in animal taxa (Arnold 1992). It has been inferred in both animal and plant groups through non concordance of phylogenies

based on ribosomal and chloroplast DNA in plants, and ribosomal and mitochondrial DNA in animals (Arnold 1992). In view of the hybridisation and speciation patterns observed in corals, Veron (in prep) is proposing that reticulate evolution is probably a common process in coral. The implications of reticulate evolution are particularly relevant to the study of phylogeny, as introgression can render reconstruction of evolutionary histories more difficult (Rieseberg and Wendel 1993, Wallace and Willis 1994). Phylogenetic studies relying exclusively on chloroplast and mitochondrial DNA may be particularly susceptible to errors introduced by introgression (reviewed in Rieseberg and Wendel 1993). For example, introgressive transfer of mtDNA resets the molecular clock to zero in an introgressed lineage relative to the donor lineage (Smith 1992). Similarly if species hybridise, construction of phylogenies using shared characters may be meaningless because species with very similar characters may have gained them through introgression rather than recent divergence.

One of the main reasons so much effort has been put into finding an answer to the species problem is that species are considered to have an ontological status not shared by other taxa (Mishler 1985, Scoble 1985). Species are considered by many to be the fundamental unit of nature, and are therefore generally considered to be the unit of evolution (Stebbins 1977, Cracraft 1983, 1987, Scoble 1985). As a consequence the majority of evolutionary theory revolves around explaining how species evolve. The "unit of evolution" is poorly defined and consequently has often been used synonymously with "unit of selection" (eg. Hull 1984). However the unit of selection is the unit on which natural selection works (Lewontin 1970, Wright 1980, Dawkins 1984, 1976, Gliddon and Gouyon 1989), and should not be confused with the unit of evolution. Reproductively isolated species such as *M. tortuosa* and *M. digitata* may be viewed as independent units of evolution because their gene pools are not linked by sexual reproduction. Therefore changes occurring in either species will occur independently of the other, providing they are not coevolving (eg. Ehrlich and Raven 1964). However, units of evolution need not all be reproductively isolated because reproductive isolation may not be inherently linked to speciation (Bremmer and Wanntorp 1979). Therefore hybridising species may also be considered units of evolution providing they maintain their identity. Hybridising species may maintain their identity due to the effects of demographic exchangeability (*sensu* Templeton 1989). These subtleties must be taken into account when considering the way we delimit coral species, in particular we must bear in mind that reproductive isolation is not the only process that delimits units relevant to the study of evolution. This is in turn important because it affects the way taxonomy should deal with species that are not reproductively isolated.

There is no clear answer to the way we should classify species that are not reproductively isolated. If we decide to gauge species on reproductive compatibility alone where do we draw the line? How much hybridisation do we allow before we consider two groups of organisms to be the same species? The essence of the problem lies in the fact that in hybridising species, morphological and reproductive species boundaries are not aligned. The lack of alignment of morphologically and reproductively defined species boundaries is a well known phenomenon in both the animal and plant kingdoms (Bremmer and Wanntorp 1979). This lack of alignment lies at the heart of much of the species debate (Eldredge 1993). Theoretically there is no problem in deciding morphologically indistinguishable organisms are species when they are found to be reproductively isolated, but when the opposite applies there is no consensus as to what feature should be used to delimit the species. There does in fact appear to be a tacit acceptance that morphospecies are important. Particularly in the plant literature, morphologically defined species that hybridise extensively are still considered to be species, though collectively they are referred to as a syngameon (Grant 1981). Animal species are also not necessarily synonymised if they are found to hybridise (Harrison 1993). The problem is normally approached by gathering information on the biology and ecology of the hybridising groups and making a decision based on several criteria (Blackwelder 1969, Dillon 1978, Doyen and Slobodchikoff 1974). Such an approach appears sensible because there is agreement that "biologically significant" species can be maintained by mechanisms other than reproductive isolation (Mischler 1985, Cracraft 1987, Templeton 1989). Synonymising coral species that hybridise extensively would therefore require considerable information on their biology and ecology.

6.3 Factors mediating the coexistence of M. digitata and M. tortuosa

Despite the differences between *M. digitata* and *M. tortuosa* highlighted in section 6.1, the two species do share many similarities. Both species:

- have a similar gross morphology, both being branching species of similar size (Chapter 4);
- grow at similar rates (Chapter 5);
- reproduce asexually by fragmentation (Heyward and Collins 1985b, pers. obs.);
- reproduce sexually releasing gametes biannually into the water column (chapters 3 and 5);
- invest a similar amount of energy in reproduction (though the way in which they invest the energy differs; Chapter 5);
- occupy the same area on the reef flat at all of the sites studied (pers. obs.).

In view of their similar morphology and size, and their shared habitat, it is likely that *M. digitata* and *M. tortuosa* compete for space. The harsh nature of the intertidal habitat *M. digitata* and *M. tortuosa* occupy suggests that they are probably able to coexist due to new areas for colonisation continually arising, i.e. disturbance is preventing the assemblage reaching climax status (Connell 1978). Bare patches are commonly found on the reef flat at all of the sites used for this study. It is likely that these patches are regularly produced by storms and cyclones, and other smaller scale sources of disturbance such as predation, and are important for maintaining populations of individual species. Reef walking also causes considerable disturbance in certain areas (pers. obs.). Without regular disturbance clearing space on the reef flat one would predict that corals would eventually be restricted to the reef edge. Corals growing on the reef flat can not grow upwards beyond their tolerance to regular tidal exposure, and are therefore restricted primarily to horizontal growth. Furthermore, where branching corals become densely aggregated they promote accumulation of sand and rubble between their branches which eventually kills them (pers. obs.). Areas where this occurs are not recolonised by corals as exposure at low tide is too frequent, thus with time species would eventually be restricted to the reef edge.

Where competing corals come into contact, one should find evidence of competitive interactions such as overgrowing, overtopping or digestion (Connell 1973). Overgrowth does occur but is not extensive (pers. obs.), and overtopping cannot occur as exposure at low tide restricts corals on the reef flat to lateral growth. There is some indirect evidence that digestion may be an important form of competition between *M. tortuosa* and *M. digitata* (and also with other reef flat corals), as they both possess acontia with large β-mastigophores. Acontia have been associated with defence (Schick 1991), although they may also be used for feeding (Manuel 1991). It seems unlikely that acontia are used for feeding as both species of *Montipora* have very small polyps, and it has been suggested that corals with small polyps rely primarily on photosynthesis for nutrition (Porter 1976). There was considerable variation in the number of polyps containing acontia, and their size, in samples (collected for the gametogenic study in Chapter 5). Such variation could be related to the proximity of neighbouring colonies, but further study would be required to verify this, and to determine how much direct aggression occurs between these two species.

Speciation of *M. tortuosa* and *M. digitata* has occurred with little change in many aspects of their biology and morphology. It is possible that these two species have not diverged much due to the advantages inherent in their branching morphology. The intertidal is a particularly variable environment, species inhabiting this environment having to endure regular prolonged aerial exposure due to tides with associated temperature fluctuations, high levels of solar radiation, desiccation, salinity changes

and exposure to wave action (Done 1983). Such a harsh environment may lead to selection pressures that restrict the occurrence of certain morphologies, and have therefore reduced the likelihood of divergence between *M. tortuosa* and *M. digitata*. Corals with other morphologies do live on the reef-flat, but most are restricted to tidal pools (eg *Porites* and *Acropora*, pers. obs.), although there are a few massive species that are regularly exposed at low tide (eg. *Goniastrea*). Advantages to having a branching morphology on the reef flat may include being: 1) better suited to dislodging sediment; less directly exposed to the sun than colonies of massive and platy morphology; less prone to desiccation of inner colony branches (because branching slows down air movement); less prone to upward suction caused by water flow than will colonies of plate morphology (water flow over flat objects causes upward suction, eg. see Telford 1981), which is important in an environment where surfaces for attachment are scarce; more likely that colonies will settle in an upright position if dislodged (Heyward and Collins 1985b, pers obs). Furthermore, if branching colonies are overturned only the branch tips will die, whereas if a plate or massive colonies are overturned most of the colony will be in contact with the substrate and therefore die. Branching morphology is also conducive to reproduction by fragmentation, which is common in *M. digitata* and *M. tortuosa* (Heyward and Collins 1985b), and may be important for colonising the inner reef flat where larval recruitment may be difficult due to the heavy sedimentation and regular exposure during low tide. but they are less abundant than *M. tortuosa* and *M. digitata*, and are not able to grow as close to the shore.

6.4 Phylogeny of Montipora tortuosa and M. digitata

Montipora tortuosa and *M. digitata* exhibit morphological and ecological characteristics that suggest they are more closely related to each other than to other members of the genus *Montipora*. These two species are the only truly branching members of the genus found on the Great Barrier Reef, the remainder being primarily encrusting or plate-like. A few species, such as *M. stellata* and *M. angulata,* may have irregular branches, arising from extensive encrusting bases or plate-like bases (Veron and Wallace 1984). It is also noteworthy that the coenosteum of both *M. tortuosa* and *M. digitata* lacks both tuberculae and papillae which are features common to most other species within the genus, with the exception of a few species (eg. *M. angulata*). The indistinguishable sperm morphology of *M. tortuosa* and *M. digitata* also implies they are closely related, as variation in sperm shape can be used to construct phylogenies, the assumption being that differences in sperm morphology will increase as relatedness decreases (Harrison 1988). However the extent of sperm morphology variation within the genus *Montipora* has not been assessed, and comparisons with other species would

be necessary to establish whether the lack of detectable variation between the two species is characteristic for the genus.

Within the genus *Montipora*, *M. digitata* and *M. tortuosa* differ from other species (with perhaps the exception of *M. angulata*) found on the Great Barrier Reef in that they are the only species that appear to be able to tolerate extended periods of aerial exposure. Both species are restricted almost exclusively to the reef flat, whereas all other species of *Montipora* are located primarily on the reef slope, and those that do occasionally colonise the reef flat, such as *M. peltiformis*, are generally rare and restricted to areas that form pools at low tide.

The genetic data obtained for the two species can be used to obtain a very crude estimate of how long ago the two species diverged, if one assumes constant average mutation rates and independent evolution of loci. It has been calculated that a value for Nei's genetic distance (D) of 1 corresponds to a divergence age of approximately 15-20 million years for many organisms (Thorpe 1982, Vawter *et al.* 1980). This figure was adjusted to 29.4 million years for *Porites* and *Goniopora* by Garthwaite *et al.* (1994) who calibrated genetic data against fossil records. A very conservative estimate of 15-30 million years therefore seems reasonable for coral species in general. The average Nei's genetic distance of 0.237 found between *M. digitata* and *M. tortuosa* during this study therefore suggests that the two species diverged between 3.5 and 7.1 million years ago, somewhere between the Pliocene and Miocene. This estimate lies between the divergence times found between two *Goniopora* species (approximately 3.5 Ma) and between *Porites* species (7.6-22.3 Ma) obtained by Garthwaite *et al.* (1994). The time of divergence is also almost identical to that expected for three sibling species of *Montastraea annularis* that have Nei's genetic distances between 0.24 and 0.26 (Knowlton *et al.* 1992), corresponding to a divergence time of between 3.6 and 7.8 million years. There is no information on the time other *Montipora* species were formed, but the genus as a whole is thought to have originated in the Eocene (37-54 Ma)(Wells 1967, Veron and Kelley 1988), therefore *M. digitata* and *M. tortuosa* diverged relatively recently in relation to the origin of the genus.

The similarities between *M. tortuosa* and *M. digitata*, and the differences between these two species and other members of the genus *Montipora*, suggest that *M. tortuosa* and *M. digitata* arose from the same lineage. The alternative, that the morphological similarities exhibited by the two species are the result of convergent evolution, is unlikely because they share coenosteal features that are not likely to be the result of convergent evolution. Coenosteal features vary considerably between species within the genus *Montipora*, to the extent that they are commonly used for identification purposes (Veron and Wallace 1984). Other branching species (eg. *M. altasepta* and *M.*

capitata) that occupy the reef flat elsewhere have well developed and distinctive coenosteal elaborations, yet *M. tortuosa* and *M. digitata* from those areas have similar coenostea (pers obs). Allozyme data also suggest that these two species evolved relatively recently in relation to the origin of the genus, which further suggests that they are probably closely related. The implication is therefore that *M. tortuosa* and *M. digitata* are probably of monophyletic origin. It is also likely that they speciated in the same habitat range, as had they speciated in a different habitat gross morphological differences between them would be expected to be greater.

The divergence period for *M. digitata* and *M. tortuosa* of over three million years is in agreement with fossil data that suggests few coral species have evolved over the past three million years. In his hypothesis of "inhibited speciation and faunal stasis", Potts (1983, 1985), proposed that speciation was suppressed over this period because sea level fluctuated so rapidly that corals did not remain isolated for enough generations to form new species. This scenario is particularly likely to apply to corals, as opposed to many other marine organisms, due to the long lifespan of many coral species (Potts 1983, 1985). The widespread distribution of *M. tortuosa* and *M. digitata* (see section 6.1) also suggests that they did not diverge recently, particularly as these two species are found in areas that are not directly connected by oceanic currents (eg. Singapore and the Great Barrier Reef). The geographic limits of the distribution of *M. tortuosa* and *M. digitata* are not known, as records of distribution have previously considered them to be one species. Further studies of distribution limits for these two species may provide clues as two when and where they speciated.

Within *M. digitata* and *M. tortuosa* there is evidence of reproductive compatibility groups like those that exist in plant populations (Chapter 3), suggesting that multiple alleles are governing fertilisation success. A similar process has been suggested for echinoid species in which multiple bindin alleles have been discovered, and it has been hypothesised that rapid coevolution may be possible in males and females (Palumbi 1992). At present there is no population genetic model that adequately considers the coevolution of male and female gamete recognition loci in the context of speciation, and the nature of selective forces that act on gamete recognition loci have not been determined (Palumbi 1992). However, it is not difficult to envisage a situation in which stochastic changes upset the balance of compatibility groups and lead to speciation. Small changes to recognition systems between gametes could lead to divergence due to a runaway process similar to that expected for sexual selection (Kirkpatrick and Ryan 1991), and pleiotropy/hitchhiking (Rice and Hostert 1993). Speciation of this form could occur both in allopatry or sympatry (Rice and Hostert 1993). In echinoderms small changes to egg-sperm binding proteins may have a

rapid speciation. In view of the strong prezygotic barrier to fertilisation between *Montipora tortuosa* and *M. digitata* it seems possible that a similar process may have taken place, and that speciation occurred at the molecular level due to stochastic changes to the egg-sperm binding process. Although the processes may not be related, it is also interesting to note that there are strong barriers to selfing in *M. digitata* (Heyward and Babcock 1986). In some plants reproductive barriers between species have been suggested to be pleiotropic effects of genetic systems whose real function is to limit selfing (Palumbi 1992). However, there is no evidence that this may be the case for corals as corals that hybridise extensively such as those in the genera *Platygyra* and *Acropora* also have strong barriers to selfing (Willis and Wallace 1994).

6.5 Conclusions

This study has used traditional and alternative taxonomic techniques to investigate the species status of fat fingers and yellow spatulate morphs of *Montipora digitata*, and has compared the reproductive ecology of these morphs and other members of the genus *Montipora*. Genetic, reproductive, morphological and ecological data all support the classification of the two morphs as separate species. Differences in septal shape have allowed recognition of the two species in holotypes synonymised with *M. digitata*, and as a result the fat fingers species has been renamed *M. tortuosa* (Dana 1846), and the yellow spatulate species has been renamed *M. digitata* (Dana 1846). Because the two species can be distinguished morphologically they fit the description of pseudo-sibling species (*sensu* Knowlton 1993). In the case of *M. tortuosa* and *M. digitata,* the traditional morphological approach to delimiting species boundaries is congruent with species boundaries based on reproductive isolation.

There is evidence that other species within the genus may hybridise, although it is not known whether hybrids can survive to adulthood, or whether the F1 generation will be able to produce offspring. Hybridisation has far reaching evolutionary implications, particularly due to the fact that it might lead to reticulate evolution. Further study of the potential for hybridisation within the genus *Montipora*, and other genera is needed to increase our understanding of coral species and the way in which corals have evolved. Study of species that do not hybridise is also desirable in order to understand why so many coral species do hybridise, a phenomenon that is generally considered to be the exception rather than the rule in the animal kingdom.

The many similarities between *M. tortuosa* and *M. digitata* suggest that they are monophyletic, and that they probably evolved in the habitat they are still found today. A divergence time of 3.5-7.1 million years for these two species was estimated from

Nei's genetic distance. This is relatively recent in relation to the origin of the genus *Montipora* some 40 million years ago (based on fossil evidence), and therefore further suggests the two species are closely related. It is suggested that *M. tortuosa* and *M. digitata* emerged as species as a result of a stochastic change in the egg-sperm binding process.

This study has highlighted the value of using multiple taxonomic techniques to identify coral species, and in particular for identifying species that present a problem for traditional taxonomy. Although morphometric, breeding and genetic techniques were useful for species identification, allozyme electrophoresis proved to be the most simple, cost effective and powerful technique. However it must be stressed that the usefulness of these techniques will vary depending on the species. If a species hybridises extensively a combination of morphometric and ecological studies would be required to delimit species boundaries, as in such cases reproductive isolation is clearly not suitable. Most importantly, the taxonomy of scleractinian corals should be carried out on a case-by-case basis, as generalisations on the nature of species boundaries can not be made.

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Appendix

1.1 Buffer recipes for starch gel electrophoresis (Chapter 2)

LiOH (elec pH 8.1/ gel pH 8.4).

Elect. stock solution (5 X Elect. buffer):

12.6 gm LiOH, 118.9 gm Boric acid to 2 litres.

Gel stock solution:

54.5 gm Tris, 15.1 gm Citric acid.H_2O, 200 ml electrode stock solution. To 1 litre.

TC8 (pH 8.0)

Stock solution (5 X Elect buffer):

208.0 gm Tris, 82.03 gm Citric acid.H_2O to 2 litres.

TC7 (pH 7.0)

Stock solution (5 X Elect buffer):

163.5 gm Tris, 90.4 gm Citric acid.H_2O to 2 litres.

TC6 (pH 6.3)

Stock solution (5 X Elect buffer):

133.3 gm Tris, 86.4 gm Citric acid.H_2O to 2 litres.

TEC 7.9 (pH 7.87)

Stock solution (5 X Elect. buffer):

163.5 gm Tris, 67.25 gm Citric acid.H_2O, 15.2 gm Na_2EDTA to 2 litres.

TEB (pH 8.4)

Stock solution (5 X Elect. buffer):

181.67 gm Tris, 12.42 gm EDTA (Na_2 salt), 72.57 gm Boric acid to 2 litres.

Poulick (elec pH 8.2/ gel pH 8.7)

Elect. stock solution (5 X Elect. buffer):

185.5 gm Boric acid, 24.0 gm NaOH to 2 litres.

Gel stock solution:

184.2 gm Tris, 21 gm Citric acid.H_2O to 2 litres.

1.2 Stain recipes used for starch gels.

Malic Enzyme (ME).

Stock solution: As for MDH.

Stain: 5 ml stock soln., 5 ml 0.1 M Tris-HCL pH 8, 2 drops $MgCl_2$, 2 mg NADP, 1 ml MTT, 1 ml PMS.

Malate Dehydrogenase (MDH).

Stock solution: 13.4 gm DL-Malic acid, 49 ml 2M Na_2CO_3, water to 100 ml and adjust to pH 7 with Na_2CO_3

Stain: 5 ml stock solution, 5 ml 0.1M TRIS-HCL pH 8, 3 mg NAD, 1 ml MTT, 1 ml PMS.

Peptidase (LGG & LTT).

Stain: Peptidase 10 mg

L-Leucyl-glycylglycine (LGG)

L-Leucyl-Tyrosine (LTT)

3 mg D-Dianisidine, 3 mg L-amino Acid Oxidase, 3 mg Horseradish peroxidase, 10 ml 0.1 ml Phosphate buffer pH 7.

Phosphoglucomutase (PGM).

Stain: 10 ml 0.1M TRIS-HCL pH 8, 40 mg Glucose-1-phosphate, 2 mg NADP, 2 drops $MgCl_2$, 1 ml MTT, 1 ml PMS, 5 µl G6PD.

Hexokinase HK).

Stain: 10 ml 0.1M TRIS-HCL pH 8.5, 100 mg glucose, 13 mg ATP, 20 mg EDTA Na_4, 2 mg NADP, 2 drops $MgCl_2$ solution, 1 ml MTT, 1 ml PMS, 5 µl G6PD.

1.3 Buffer recipes for cellogel (to 1 litre)

TM (pH 7.8)

6.06 gm Tris, 2.32 gm maleic acid.

CP (pH 6.4)

3.58 gm Na_2HPO_4, 0.53 gm Citric acid.

PHOS (pH 7)

4.15 gm $Na_2HPO_4.12H_2O$, 1.31 gm $Na_2HPO_4.2H_2O$

No.	Abbreviation	Enzyme name	Enzyme commission Number
1	AAT	Aspartate aminotransferase	2.6.1.1
2	ACON	Aconitase	4.2.1.3
3	ADH	Alcohol dehydrogenase	1.1.1.1
4	AK	Adenylate kinase	2.7.4.3
5	ALD	Aldolase	4.1.2.13
6	CK	Creatine kinase	2.7.3.2
7	DIAPH	Diaphorase	1.6.*.*
8	ENOL	Enolase	4.2.1.11
9	EST	Esterase	3.1.1.1
10	FBP	Fructose diphosphatase	3.1.3.11
11	FUM	Fumarase	4.2.1.2
12	GA3PD	Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.2
13	GDA	Guanine deaminase	3.5.4.3
14	GDH	Glutamate dehydrogenase	1.4.1.3
15	GLDH	Glucose dehydrogenase	1.1.1.47
16	G-3PD	Glucose-3-phosphate dehydrogenase	
17	G-6PD	Glucose-6-phosphate dehydrogenase	1.1.1.49
18	GPI	Glucose-phosphate isomerase	5.3.1.9
19	GPT	Glutamate-pyruvate transaminase	2.6.1.2
20	GSR	Glutathione reductase	1.6.4.2
21	HBDH	Hydroxybutyrate dehydrogenase	1.1.1.30
22	HK	Hexokinase	2.7.1.1
23	IDH	Isocitrate dehydrogenase	1.1.1.42
24	LDH	Lactate Dehydrogenase	1.1.1.27
25	MDH	Malate dehydrogenase	1.1.1.37
26	ME	$NADP^+$ dependent MDH (Malic enzyme)	1.1.1.40
27	MPI	Mannose phosphate isomerase	5.3.1.8
28	NP	Nucleoside phosphorylase	2.4.2.1
29	Pep(LG)	Peptidase	3.4.11.*
30	Pep(LP)	Peptidase	3.4.13.9
31	Peo(LT)	Peptidase	3.4.11.*
32	PGK	Phosphoglycerate kinase	2.7.2.3
33	PGM	Phosphoglucomutase	2.7.5.1
34	PK	Pyruvate kinase	2.7.1.40
35	SOD	Superoxidase dismutase	1.15.1.1
36	SDH	Sorbitol dehydrogenase	1.1.1.14
37	TPI	Triose-phosphate isomerase	5.3.1.1

Table 1. Enzyme abbreviations and commission numbers for stains used during this study (after Shaklee *et al.* 1990).

Enzyme	Variation observed	Optimum buffer tested	Comments
DIAPH	Dimer	TC8	Resolution good but activity poor
EST	Dimer	TEB, TEC	Resolution good but often activity was poor. Very difficult to interpret, staining bands inconsistent.
ENOL	Dimer	TC6	Activity good but resolution poor.
GPI	Monomer	TC 8, TC 6	Resolution good but activity often poor. Stain often did not work.
HK	Monomer	LIOH	Good activity & resolution but subject to much warp.
LP	Dimer / Tetramer	LIOH, TC8	Poor resolution but good activity.

Table 2. Systems that were not used for the study but showed potential for future studies. Both Heterozygotes and homozygotes were observed in all of the above systems.

SAMPLE ANALYSED	Character	CAN 1	CAN 2	CAN 3
"Good" colonies	DIAM	0.662	0.639	-0.155
	INTCOR	0.380	0.604	0.518
	W1	0.094	-0.238	0.780
	W2	0.842	-0.423	0.295
	Eigenvalue	2.737	0.479	0.082
	% Variance	0.814	0.142	0.024
	Cum. % variance	0.814	0.957	0.984
All colonies	DIAM	0.814	0.522	-0.153
	INTCOR	0.514	0.306	-0.304
	W1	0.122	0.308	0.927
	W2	0.778	-0.441	0.442
	Eigenvalue	2.702	0.956	0.477
	% Variance	0.621	0.220	0.110
	Cum. % variance	0.621	0.841	0.951
Geoffrey Bay	DIAM	0.797	-0.585	0.047
	INTCOR	0.408	-0.268	0.132
	W1	0.169	0.260	0.942
	W2	0.786	0.542	0.288
	Eigenvalue	2.337	0.434	0.208
	% Variance	0.747	0.139	0.066
	Cum. % variance	0.747	0.886	0.952
Pioneer Bay	DIAM	0.9381	-0.188	-0.111
	INTCOR	0.508	-0.148	0.843
	W1	0.340	0.940	0.005
	W2	0.631	0.541	-0.148
	Eigenvalue	1.804	0.603	0.538
	% Variance	0.594	0.199	0.177
	Cum. % variance	0.5945	0.7934	0.9706

Table 3. Total canonical structure for canonical discriminant analyses. Correlation coefficients are given between canonical variables 1 to 3, and skeletal charaters DI (corallite diameter), INTCOR (inter-corallite diatance), W1 (greatest branch-tip diameter), and W2 (branch-tip diameter at 90° to W1). All analyses use individual colonies as the class. Eigenvalues for the first three canonical variables are also given along with the proportion of the total structure that they represent.

Species code	Mean egg volume mm^3 ± SE	n	Proportion of egg-bearing polyps	Mean N° eggs per polyp ± SE	n	Mean N° polyps per cm^2 ± SE	Mean testes volume mm^3 ± SE	n	Proportion of testes-bearing polyps	Mean N° testes per polyp ± SE	n	Egg production mm^3 per cm^2	Testes production mm^3 per cm^2	Total reproductive output mm^3 per cm^2
FF1	0.0134±0.00095	45	0.967	14.0±0.8	29	88.8±6.1	0.0141±0.00133	24	0.778	3.43±0.3	7	16.11	3.33	19.43
FF2	0.0254±0.00140	45	1.000	11.0±0.4	30	82.0±4.7	0.0184±0.00165	24	1.000	2.67±0.3	9	22.93	4.03	26.96
FF3	0.0109±0.00114	45	0.933	12.1±0.6	28	70.8±2.2	0.0066±0.00059	23	0.778	3.29±0.4	7	8.73	1.20	9.93
FF4	0.0209±0.00161	45	1.000	10.8±0.4	30	84.8±1.7	0.0182±0.00227	26	0.889	3.25±0.4	8	19.05	4.45	23.50
FF5	0.0275±0.00206	44	1.000	8.8±0.6	30	76.8±3.3	0.0149±0.00189	30	1.000	3.33±0.3	9	18.65	3.82	22.47
FF6	0.0277±0.00464	16	0.467	2.4±0.3	14	66.0±2.5	0.0308±0.00604	34	1.000	3.78±0.2	9	2.07	7.69	9.77
FF7	0.0256±0.00138	44	1.000	14.1±0.6	30	72.8±2.4	0.0144±0.00123	23	0.889	2.88±0.3	8	26.37	2.68	29.05
FF8	0.0205±0.00328	19	0.367	10.5±1.1	11	84.0±2.5	0.0387±0.00519	16	0.556	3.20±0.6	5	6.67	5.78	12.45
FF9	0.0271±0.00160	45	1.000	12.4±0.4	30	76.8±4.0	0.0273±0.00316	33	1.000	3.67±0.2	9	25.80	7.69	33.49
FF10	0.0256±0.00205	45	1.000	9.8±0.4	30	89.2±5.7	0.0173±0.00237	27	1.000	3.00±0.4	9	22.40	4.64	27.04
MEAN ±95% CI	0.022±0.0037		0.87±0.021	10.6±2.49		79.2±5.68	0.020±0.0.0100		0.89±0.130	3.25±0.446		16.9±1.54	4.5±3.06	21.4±0.31
YS1	0.0318±0.002568	39	0.833	8.2±0.7	25	93.2±3.3	0.0102±0.0013	27	0.889	3.38±0.2	8	20.28	2.85	23.13
YS2	0.0477±0.005551	34	1.000	3.4±0.3	30	87.2±5.2	0.0079±0.00061	23	1.000	2.56±0.3	9	14.00	1.76	15.76
YS3	0.0268±0.003016	31	0.867	7.1±0.7	26	73.2±3.0	0.0147±0.002193	7	0.444	1.75±0.2	4	12.11	0.84	12.94
YS4	0.0420±0.0035	45	1.000	10.9±0.4	30	94.8±4.2	0.0127±0.001031	16	0.667	2.67±0.5	6	43.24	2.14	45.38
YS5	0.0432±0.003558	45	1.000	8.1±0.4	30	86.0±2.9	0.0089±0.000968	9	0.444	2.25±0.2	4	30.10	0.77	30.87
YS6	0.0377±0.005051	24	0.567	8.9±1.1	17	93.2±4.5	0.0111±0.000887	3	0.222	1.50±0.5	2	17.68	0.34	18.03
YS7	0.0098±0.001798	19	0.600	5.2±0.6	18	101.2±9.7	0.0	0	0.000	0.00	0	3.10	0.00	3.10
YS8	0.0322±0.002455	45	1.000	9.1±05	30	80.8±7.5	0.0058±0.00058	15	0.778	2.14±0.3	7	23.68	0.78	24.45
YS9	0.0345±0.002042	44	1.000	9.2±0.4	30	94.8±10.1	0.0107±0.001412	17	0.889	2.13±0.4	7	30.21	1.92	32.13
YS10	0.0084±0.000916	30	0.600	6.1±0.5	18	75.2±5.6	0.0051±0.000492	5	0.444	1.25±0.3	3	2.32	0.21	2.53
MEAN ±95% CI	0.031±0.0258		0.85±0.016	7.6±1.65		88.0±6.67	0.009±0.0.0047		0.58±0.283	1.96±1.207		19.7±2.33	1.2±1.42	20.8±0.49
P	ns		ns	0.029		0.034	0.0071		0.0216	0.0025*		ns	0.0011*	ns

Table 4. Egg and testes reproductive output values ±SE for individual tagged colonies of *Montipora tortuosa* (FF) and *M. digitata* (YS) at Geoffrey Bay sampled 3 days before spawning on on 9/10/92. n = 30 unless otherwise shown as a seperate column next to values on table. Figures for the two species are compared using multiple t-tests, α has been accordingly adjusted to 0.005 using the Bonferroni correction. *: significant difference.

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